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Dynamic Hematological Responses in Endurance Horses: Unraveling Blood Physiological Markers of Exercise Stress and Recovery

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Abstract

This study aimed to investigate the effects of endurance exercise on erythrogram parameters and identify stress and inflammation markers that could serve as reliable indicators for assessing recovery in endurance horses. The study involved 26 Arabian endurance horses (4 stallions and 22 geldings) aged between 8 and 12 years, each completing a race (10 horses in 80 km, 10 horses in 120 km, and 6 horses in 160 km). Blood samples were collected at six different time points: at rest (T0), immediately after the race (T1), 3 hours after the race (T2), and 3, 7, and 14 days after the race (T3, T4, T5). The hemogram analyses included several hematological indices such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), red blood cell distribution width-to-platelet ratio (RDW/PLT), hemoglobin-to-red blood cell distribution width ratio (Hb/RDW), hemoglobin-to-platelet ratio (Hb/PLT), systemic inflammation index (SII), systemic inflammatory response index (SIRI), leukocyte shift index (LSI), and adaptation intensity index of L. Harkavy (AI). The findings revealed that some indices, such as NLR, PLR, SII, SIRI, MLR, RDW/PLT, and LSI, were sensitive to acute physiological changes related to the endurance race. These indices showed significant variations immediately after the race, indicating a stress and inflammatory response. In contrast, the ELR and AI indices displayed delayed and more prolonged responses, suggesting their utility in monitoring the post-exercise recovery phase. Overall, this study offers insights into applying hematological markers to assess endurance horses' stress, inflammation, and recovery. These findings could contribute to improved training and recovery strategies, promoting the health and welfare of equine athletes during and after intense physical exertion. Further research is recommended to explore these indices in larger samples and other equine sports.

Keywords

Cellular dynamics; endurance horses; hematological indices; immune responses; post-exercise monitoring

1. Introduction

Endurance racing, an increasingly challenging discipline within equestrian sport, has witnessed a surge in demands, particularly in terms of speed [1]. This escalation places metabolic, musculoskeletal, and cardiovascular stress on the participating athletes. A comprehensive understanding of these demands is imperative for optimizing performance, mitigating injury risks, and preventing overtraining.

Assessing fitness and recovery in endurance athletes often involves various methods, with serial blood count evaluation commonly utilized. However, interpreting hematological changes in the context of exercise presents distinctive challenges. Endurance races impose substantial physiological demands, resulting in transient alterations that can be challenging to differentiate from pathological shifts. Dehydration, splenic contraction, and exercise-induced changes in plasma volume further complicate the interpretation of hematological parameters [2].

Research has demonstrated that exercise triggers inflammation in response to local tissue damage in humans and horses [3–6]. The immune system orchestrates this response to restore tissue integrity and maintain homeostasis. In fit and healthy athletes, immune system imbalance and acute inflammation represent temporary states, considered the initial steps in the recovery process to restore tissue function [7]. However, unresolved acute inflammation poses a risk for chronic inflammation, a significant factor in various pathological conditions.

The innate immune system plays a pivotal role in the body and is crucial for combating infections and other stressors [8]. Intense exercise has been associated with a temporary weakening of the immune system, increasing the risk of respiratory disease in humans and horses, according to the "open window" hypothesis. Nevertheless, there is ongoing debate about the applicability of this theory to horses [9].

A study proposes [10] using established cellular inflammation markers in exercise immunology, advocating for data analysis solely from blood count in conjunction with classical blood count analyses and flow cytometry. The authors posit that blood-based inflammatory indices could serve as integrative, cost-effective, and time-efficient markers to detect cellular immune changes.

Our hypothesis posits that integrative cellular indices could serve as valuable parameters for evaluating effort and, particularly, recovery in horses subjected to endurance exercise. This study investigates the impact of endurance exercise on erythrogram parameters and platelet changes over 14 days following a competition. Additionally, we seek to identify potential stress and inflammation markers that can serve as reliable parameters for assessing recovery from inflammation after a race in endurance horses.

2. Materials and Methods

Twenty-six Arabian and cross Arabian horses that had completed an FEI (Fédération Equestre and Internationale) endurance race and had cleared the last veterinary inspection (hydration status, rectal temperature, gut sounds, and lameness exam) were included in this study. Six horses completed a 160 km race, ten completed a 120 km race, and ten completed an 80 km race. As the FEI categories require a minimum of two years of training experience, the horses included in the study had at least two years of training.

Due to the previously reported differences in hematological parameters between male and female athletic horses [11,12], we have opted to exclusively include males in this study, as they constitute the majority among endurance horses. The study enrolled 26 horses, consisting of 4 stallions and 22 geldings, aged between 8 and 12 years and weighing 400-440 kg.

These horses were owned by endurance riders and housed in their farms and private training centers in São Paulo. A day before the race, all the horses were transported to the competition venue and kept under similar stall conditions.

Blood samples were collected via jugular venipuncture in vacuum tubes with EDTA (ethylenediaminetetraacetic acid) to conduct a hemogram. Leukocyte differential slides were prepared on-site and stained with the May-Griinwald Giemsa method. The blood count was immediately processed using an automated hematology analyzer (BC 2800Vet, Mindray, USA) in the laboratory at the event's premises. Figure 1 shows the time samples, which consisted of three collections at the race venue (T0: at rest, before tacking; T1: immediately after the final vet check, and T2: 3 hours after T1) and three collections at the horse's origin farm (T3: 3 days after the race; T4: 7 days after the race, and T5: 14 days after the race).

Using absolute blood cell counts, we calculated several integrated hematological indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), eosinophil-to-lymphocyte ratio (ELR), red blood cell distribution width-to-platelet ratio (RDW/PLT), hemoglobin-to-red blood cell distribution width ratio (Hb/RDW), and hemoglobin-to-platelet ratio (Hb/PLT). Additionally, we calculated two indices that consider three cellular types:

The systemic inflammation index (SII):

$$SII = \frac{(Neutrophlis\ x\ Platelets)}{Lymphocytes}$$

The systemic inflammation response index (SIRI):

$$SIRI = \frac{(Neutrophils\ x\ Monocytes)}{Lymphocytes}$$

The Leukocyte Shift Index (LSI) evaluates changes in the count of neutrophils and lymphocytes following a stress event such as exercise:

$$LSI = \frac{(Post-exercise\ neutrophil\ count - Post-exercise\ lymphocyte\ count)}{(Pre-exercise\ neutrophil\ count - pre-exercise\ lymphocite\ count)}$$

The adaptation intensity index of L. Harkavy (AI) was calculated using the differential cells according to the following formula:

$$AI = \frac{Lymphocytes}{Segmented\ neutrophils}$$

The open-source Jamovi statistical platform (version 1.2.1.1) was used for data analysis. It was retrieved from https://www.jamovi.org. The Shapiro-Wilk test was used to evaluate the distributions of the traits before conducting further statistical analyses. Non-parametric repeated measures were analyzed using the Friedman test and compared using the Durbin-Conover pairwise test. The significance level was set to $p < 0.05$ for all statistical analyses.

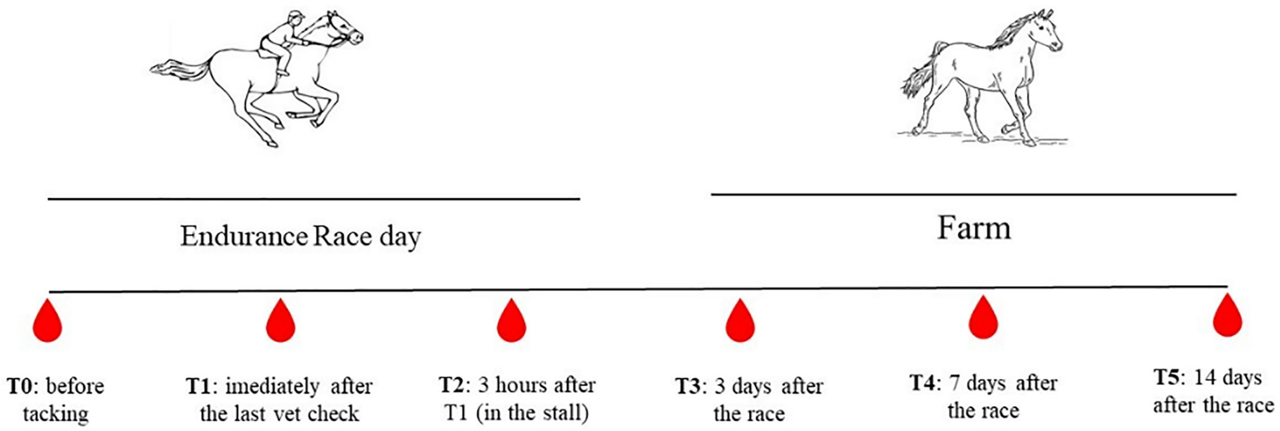

Figure 1: Blood sampling times in endurance horses that completed 80 km, 120 km, and 160 km events. Blood samples were collected before the event (pre-exercise), immediately after the event (post-exercise immediate), 3 hours post-event at the same location, and on subsequent days (3 days, 7 days, and 14 days post-event) at their respective stables.

3. Results

All the horses in the study passed the veterinary inspection before and after the race and completed the required distance. The average speed of the horses was 17.12 (±1.06) km/h for 160 km, 17.88 (±1.20) km/h for 120 km, and 19.20 (±1.88) km/h for 80 km. However, due to the small number of horses in each class, their ranking positions could not be analyzed or reported.

The current study has found alterations in various blood parameters such as red blood cell count, hemoglobin, mean cell hemoglobin concentration, hematocrit, neutrophils, monocytes, platelets, and plateletcrit on the day of the competition (T1 and T2). However, these parameters returned to normal or lower levels after three days (T3). Lymphocytes and eosinophils showed alterations on the day of the competition but showed higher levels after T3, as demonstrated in **Table 1**.

Following the ride, there was an increase in hematocrit levels during T1 and T2 compared to before the ride (T0). This shows that there was a concentration of red blood cells during the exercise. The levels of red blood cells and hemoglobin increased after the ride, which is a normal response to the increased need for oxygen during exercise. Immediately after the ride, there was a significant increase in neutrophils and a decrease in lymphocytes, indicating a stress response. There was an increase in lymphocytes from T3 to T5. Monocytes showed a noticeable increase after the race (T1 and T2) and decreased from T3 to T5. On the race day, there was a distinct decrease in eosinophils, followed by higher levels after T3. The levels of platelets increased significantly after the ride, which may be related to the increased demand during exercise. The values for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were generally stable across the time points with no significant changes, indicating no major alterations in red blood cell size, hemoglobin content, or distribution width. PCT increased at T1 and T2, then returned to basal levels from T3 to T5.

After the competition, there was a significant increase in NLR, suggesting potential inflammation, as shown in **Table 2**. However, there was a significant decrease in NLR at T3 and T4. Similarly, PLR increased post-ride but decreased over the subsequent days. SII and SIRI indicated the systemic inflammatory response, significantly increasing post-ride. A decrease followed these indices in successive days. Furthermore, MLR increased post-ride, which reflects potential stress or inflammation responses, and there was a decrease in ELR at T1 and T2 and an increase at T3.

After a horse has undergone endurance exercise, it enters an immediate post-ride period where signs of acute stress and inflammation are evident. This is reflected in increased NLR, PLR, SII, and SIRI. However, subsequent days (T3 and T4) indicate a resolution of inflammation, as evidenced by decreasing levels of NLR and PLR.

Monitoring the LSI and AI indices over time provides insights into the balance between exercise-induced stress and adaptive responses. There was an increase in LSI and a decrease in the AI values at T1 and T2, which corresponded to the conclusion of the competition, indicating stress. However, at T3, T4, and T5, the values were within the range considered "quite an adaptation," returning to the adaptive threshold only 14 days post-competition.

Table 1: Median values (Friedman test) of hematological parameters measured in endurance horses at different times: before the race (T0), immediately after the race (T1), three hours after the race (T2), three days after the race (T3), seven days after the race (T4), and fourteen days after the race (T5).

	Sample time						
	T0	T1	T2	T3	T4	T5	*p*
Eritrogram							
PCV (%)	36.0 (35.5)^{B}	43.5 (44.0)^{A}	41.0 (41.8)^{A}	34.3 (33.8)^{B}	32.2 (32.0)^{B}	33.8 (34.0)^{B}	<.001
RBC ($10^3/mm^3$)	13.5 (7.59)^{B}	18.6 (8.87)^{A}	17.3 (8.25)^{A}	14.0 (7.39)^{B}	14.7 (8.00)^{B}	14.4 (8.20)^{B}	<.001
Hemoglobin (g/dL)	13.6 (13.3)^{B}	16.0 (16.1)^{A}	20.1 (14.8)^{A}	13.6 (12.9)^{B}	12.5 (12.4)^{B}	13.0 (12.4)^{B}	<.001
MCV (fL)	51.7 (51.4)	52.2 (51.2)	51.2 (50.5)	51.2 (51.0)	51.2 (51.0)	51.9 (52.0)	0.785
MCH (pg)	18.6 (18.7)	18.4 (18.7)	18.7 (18.2)	18.1 (17.7)	18.9 (17.8)	18.7 (18.2)	0.830
MCHC (g/dL)	35.9 (35.8)^{B}	37.9 (36.0)^{A}	37.8 (36.1)^{A}	35.5 (35.5)^{B}	35.3 (35.0)^{B}	36.4 (34.7)^{B}	0.002
RDW (%)	17.5 (17.3)	17.4 (17.5)	17.7 (17.6)	16.7 (17.0)	16.7 (17.0)	16.5 (16.7)	0.332
Leukogram							
Neutrophils ($10^3/mm^3$)	5900 (5650)^{B}	11632 (11400)^{A}	12691 (12850)^{A}	4514 (4350)^{C}	5200 (5000)^{B}	4823 (4800)^{B}	<.001
Lymphocytes ($10^3/mm^3$)	2077 (1650)^{C}	2350 (1600)^{C}	1591 (1500)^{D}	2832 (2400)^{B}	3536 (2800)^{A}	2573 (2950)^{B}	<.001
Monocytes ($10^3/mm^3$)	443 (418)^{C}	833 (709)^{A}	586 (600)^{B}	486 (401)^{C}	469 (400)^{C}	354 (320)^{D}	<.001
Eosinophils ($10^3/mm^3$)	183.2 (90.0)^{C}	49.1 (0.0)^{D}	63.2 (0.0)^{D}	735.5 (675.0)^{A}	749.5 (450.0)^{A}	230.0 (190.0)^{B}	<.001
Platelets							
Platelets ($10^3/mm^3$)	153 (156)^{C}	305 (321)^{A}	214 (201)^{B}	162 (168)^{C}	171 (158)^{C}	144 (134)^{D}	<.001
MPV (fL)	6.73 (6.70)	6.70 (6.60)	6.57 (6.55)	6.76 (6.75)	6.66 (6.70)	6.58 (6.50)	0.736
PDW (%)	15.8 (15.8)	15.9 (15.9)	15.9 (15.9)	16.1 (16.0)	16.0 (16.1)	16.1 (16.0)	0.170
PCT (%)	0.103 (0.104)^{C}	0.231 (0.147)^{A}	0.174 (0.160)^{B}	0.097 (0.101)^{C}	0.125 (0.107)^{C}	0.098 (0.105)^{C}	<.001

Different letters in the same line mean statistical difference ($p < 0.05$). PCV = packed cell volume; RBC = red blood cells; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume; PDW = platelet distribution width; PCT = plateletcrit.

4. Discussion

The endurance race causes notable alterations in several blood parameters, reflecting the significant physiological demands placed on the horses [13]. Immediately after the race, we observed a sharp increase in Packed Cell Volume (PCV), Red Blood Cell Count (RBC), and Hemoglobin (Hb), indicating hemoconcentration likely due to dehydration and splenic contraction. This increase in red blood cell parameters reflects the body's adaptation to maintain oxygen delivery during intense physical exertion. During exercise, a horse's spleen contracts to expel reserve amounts of blood, increasing the number of red blood cells in circulation and ultimately enhancing oxygen delivery. This is possible due to the spleen's large storage capacity, per the previous findings [14]. This study observed an increase in RBC, PCV, hemoglobin concentration, and MCHC in T1 and T2, and these levels returned to normal beyond T3.

Horses may exhibit an increase in MCV following high-intensity exercise, attributed to the release of larger erythrocytes from the spleen. The RDW index measures RBC distribution generated by a hematological automated machine. It reflects the heterogeneity in erythrocyte size and is the most sensitive indicator for determining the degree of anisocytosis. There was no significant alteration in these parameters in the horses of this study at any collection time, aligning with the findings reported in the literature [15–18].

Platelets exhibit significant variations during exercise, crucial in hemostasis and immune modulation. A previous study [2] highlighted the importance of MPV, PDW, and PCT. The increase in platelet count and PCT during T1 and T2, followed by normalization beyond T3, suggests a dynamic response. MPV and PDW remained unaltered, emphasizing the nuanced platelet dynamics during exercise.

Plateletcrit (PCT) changes during and after the endurance race suggest significant physiological adaptations to exercise-induced stress. Immediately following the race, PCT increased dramatically, indicating a surge in platelet activity likely driven by splenic contraction and exercise-induced stress. This initial rise is consistent with the body's response to potential microtraumas and preparation for hemostasis and tissue repair [19]. As the recovery progressed, PCT levels gradually decreased, reflecting a return to normal hemodynamics and a resolution of the acute stress response. This decline could be attributed to rehydration and hemodilution as the horses regained lost fluids and decreased inflammation as the body's immune response stabilized. The decrease in PCT also suggests that the horses recovered efficiently, with their physiological systems returning to baseline. These findings highlight the importance of monitoring PCT in endurance horses to assess their recovery status. A rapid return to baseline levels of PCT can indicate successful recovery, while prolonged elevation may signal ongoing stress or a slower recovery process. Understanding these patterns can guide training adjustments and inform recovery strategies, contributing to endurance horses' overall health and performance. To develop comprehensive recovery protocols, further research could explore the relationships between PCT, other hematological markers, and endurance performance.

Assessing inflammation and immune status due to exercise remains challenging but pivotal. Studies delve into cytokine level changes, activating monocytes and cells to produce proinflammatory cytokines [5,6,20]. This study aligns with such findings, observing neutrophilia and lymphocytopenia in early recovery, akin to responses observed in pathological conditions. Leukocytes in the peripheral blood system can be a universal indicator for assessing the body's overall homeostasis. Prolonged periods of intense physical activity can lead to significant leukocytosis in endurance horses, which may last up to 48 hours. This happens due to an increase in the release of cortisol, which leads to neutrophilia. At the same time, hypercortisolemia can cause eosinopenia, but this does not affect the value of the Ne/Ly ratio because of the predominant increase in the number of neutrophils [20]. The observed decrease in neutrophils and the increase in lymphocytes during the recovery period, specifically from T3 (three days after the race) to T5 (14 days after the race), indicate a shift from the acute stress response to a more stable, recovery-focused immune state. This pattern reflects the body's efforts to return to homeostasis following the intense physiological demands of endurance racing. This pattern can be an essential marker for trainers and veterinarians, suggesting that horses move from the acute stress phase to a more adaptive, healing state.

Monocytes are critical components of the immune system, acting as phagocytes that help clear pathogens and cellular debris. They also play a role in signaling to other immune cells [21]. In this study, monocytes showed significant changes in response to the endurance race, indicating their involvement in the immune and inflammatory processes following intense exercise. Immediately after the race, monocyte counts increased significantly, suggesting an early immune response. This monocyte surge could be linked to the body's need to address tissue damage and initiate repair mechanisms following the physical exertion of the endurance race. Given their role in the immune system, the elevated monocyte levels might also indicate a transition from acute stress to recovery and repair. However, as the recovery phase progressed, monocyte counts gradually decreased. Monocyte levels began to normalize three days post-race, aligning with the overall reduction in other stress markers, such as neutrophils. This trend suggests that monocytes are involved in the early stages of inflammation and recovery, then taper off as the body's immune response stabilizes.

Inflammation markers are commonly used in human performance settings to measure the impact of exercise and the recovery process. The response of leukocytes to physical exertion is essential in objectively assessing the body's degree of adaptation. By analyzing individual recovery kinetics using these markers, exercise programs can be adjusted to better suit the individual's needs [22–24]. The current study aimed to introduce the established cellular immune inflammation markers in equine exercise physiology.

A prior study [25] explored various inflammatory markers across athletic disciplines, including those derived from red blood cells (such as RDW/PLR, Hb/RDW, and Hb/PLT) and the eosinophil-to-lymphocyte ratio, among other established indicators.

The stability of MCHC, Hb/RDW, and Hb/PLT during and after the endurance race indicates that intense exercise did not affect red blood cell morphology and oxygen-carrying capacity. The decrease in RDW/PCT suggests a temporary shift towards platelet activation, possibly as a physiological response to stress and hemostatic needs during the race. These findings underscore the complex adaptive mechanisms that endurance horses use to maintain physiological balance during and after intense exercise.

The NLR has long been used as a stress marker in horses, reflecting the relationship between specific and non-specific immunity. According to various studies, it effectively predicts exercise stress and fitness [20,23,24]. The hormone cortisol mediates the increase in neutrophils after long-duration exercise, and its effects can last for several hours or even days after the race. On the day of the competition (T0-T2), there was an increase in NLR, PLR, SII, SIRI, and MLR. These indices are calculated using cells that participate in and orchestrate the acute inflammatory response, such as neutrophils, lymphocytes, monocytes, and platelets. Therefore, they provide a reliable recovery scenario as they returned to baseline values from T3 onwards.

The Monocyte-to-Lymphocyte Ratio (MLR) index describes the balance between the effector and afferent chains of the immune response. It has been found that an imbalance between the different subpopulations of leukocytes, especially between neutrophils and monocytes and between lymphocytes and monocytes, can indicate overtraining in men [23]. Monocytosis was observed at T1 in the horses of the present study, with values decreasing at T2 and normalizing by T3. The monocyte-to-lymphocyte ratio (MLR) mirrored the dynamics of monocytes, exhibiting a similar pattern across the observed time points.

During a competition day, the PLR, SII, and SIRI indexes were also found to be sensitive. When horses exercise, their platelet count increases as the spleen, bone marrow, and lungs release fresh platelets to aid in the healing of microtrauma caused by exercise [26]. Platelets are crucial in primary hemostasis and possess various proinflammatory properties, making them useful as an inflammation marker. However, limited information is available regarding the hemostatic changes that occur after exercise in horses. Some studies have suggested that exercise-induced thrombocytosis in horses leads to changes in the clotting mechanisms, a physiological adaptation of the hemostatic system to exercise [27–29]. All horses in the study showed a significant increase in platelet count, SII, SIRI, and PLR at T1 ($p < 0.05$) and a decrease at T2, albeit different from the baseline levels, with normalization after three days.

Table 2: Median values (Friedman test) for key hematological indices in endurance horses measured at various time points: before the ride (T0), immediately after the ride (T1), three hours after the ride (T2), three days post-race (T3), seven days post-race (T4), and fourteen days post-race (T5).

	Sample time						
Indices	T0	T1	T2	T3	T4	T5	*p*
NLR	3.85 (3.92)^{B}	8.20 (8.42)^{A}	9.81 (8.98)^{A}	1.90 (1.89)^{C}	2.20 (2.03)^{C}	2.34 (1.60)^{C}	<.001
PLR	0.097 (0.090)^{C}	0.219 (0.207)^{A}	0.169 (0.135)^{B}	0.078 (0.072)^{C}	0.080 (0.055)^{C}	0.076 (0.056)^{C}	<.001
SII	478 (503)^{B}	2548 (2286)^{A}	2156 (1815)^{A}	307 (334)^{B}	414 (292)^{B}	329 (236)^{B}	<.001
SIRI	945 (858)^{B}	6226 (5835)^{A}	5509 (5664)^{A}	855 (814)^{B}	824 (766)^{B}	840 (710)^{B}	<.001
MLR	0.26 (0.23)^{B}	0.54 (0.42)^{A}	0.43 (0.42)^{A}	0.26 (0.17)^{B}	0.18 (0.16)^{B}	0.18 (0.12)^{B}	<.001
ELR	0.103 (0.073)^{B}	0.041 (0.000)^{C}	0.024 (0.000)^{C}	0.355 (0.274)^{A}	0.327 (0.172)^{A}	0.113 (0.072)^{B}	<.001
LSI	0.18 (0.00)^{C}	2.43 (2.30)^{A}	2.90 (2.60)^{A}	0.44 (0.00)^{B}	0.43 (0.00)^{B}	0.59 (0.12)^{B}	<.001
AI	0.35 (0.00)^{B}	0.22 (0.00)^{B}	- 0.12 (- 0.08)^{C}	0.62 (0.40)^{A}	0.68 (0.52)^{A}	0.54 (0.42)^{A}	<.001
RDW/PLT	0.19 (0.12)^{A}	0.06 (0.05)^{B}	0.06 (0.09)^{B}	0.12 (0.10)^{A}	0.12 (0.10)^{A}	0.14 (0.12)^{A}	<.001
Hb/RDW	0.78 (0.75)	0.82 (0.82)	0.89 (0.83)	0.81 (0.78)	0.75 (0.75)	0.79 (0.76)	0.788
Hb/PLT	0.09 (0.08)	0.07 (0.05)	0.09 (0.07)	0.09 (0.08)	0.08 (0.07)	0.09 (0.08)	0.891

Different letters in the same line mean statistical difference ($p < 0.05$). NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; SII = systemic inflammation index; SIRI = systemic inflammation response; MLR = monocyte-to-lymphocyte ratio; ELR = eosinophil-to-lymphocyte ratio; LSI = leukocyte shift index; AI = adaptive response index; RDW/PLT = red cell distribution width-to-platelet ratio; Hb/RDW = hemoglobin-to-red cell distribution ratio; Hb/PLT = hemoglobin-to-platelet ratio.

After the competition, the horses exhibited a significant decrease in eosinophils. However, all horses in this study showed an increase in eosinophils on the third day, which persisted until the seventh day after the competition. These findings are consistent with other research conducted on endurance horses [30]. Eosinophils are cells that generate various cytokines, some of which help maintain a healthy balance in the body, while others contribute to inflammation and release proinflammatory mediators that can damage tissues. Both endurance and strength exercises in humans can activate eosinophils through non-allergic means, as these cells also play a role in the regeneration of injured muscles by modifying the production of glycosaminoglycans by fibroblasts [31].

The complement system (C3, C4, and C5) may be activated in injured muscles, which can then activate eosinophils. A study conducted on human ultramarathon runners found elevated serum concentrations of eosinophil cationic protein (ECP). Previous research [31,32], observed that ECP (eosinophil cationic protein) levels and the number of eosinophils remained abnormal for 72 hours after an event. This is consistent with the findings of the current study. However, in horses, eosinophilia (an increase in eosinophil count) was observed for up to seven days, possibly due to their larger muscle mass compared to humans. The eosinophil-to-lymphocyte ratio (ELR) reflected the changes in eosinophil levels and remained high for a week. Since eosinophils have non-allergic functions and the ELR remained elevated for several days following the competition, this indicator is considered appropriate for evaluating the recovery of horses.

The Leukocyte Shift Index (LSI) is a measure that helps assess changes in the distribution of different types of white blood cells, such as neutrophils and lymphocytes. The movement of these cells depends on the intensity and duration of exercise. Studies have investigated the relationship between leukocyte counts and physical fitness, revealing an inverse relationship between the total leukocyte count and physical fitness [33]. However, the available search results do not specifically discuss the adaptation of the Leukocyte Shift Index to exercise.

According to research conducted on human athletes [34], Adaptation Intensity (AI) values are utilized to identify different types of Adaptive Reactions (AR). These AR types include stress (0.3 and below), orientation (0.31 - 0.5), quiet adaptation (0.51 - 0.7), re-activation (0.71 - 0.9), and increased activation (0.9 and above). The values of the Adaptation Intensity Index (AI) declined at T1 and T2, corresponding to the conclusion of the competition, indicating stress. However, at T3 and T4, there was an increase, with the values falling within the range considered "quite an adaptation," and returning to the adaptive threshold only 14 days post-competition. The most significant discrepancy between neutrophil and lymphocyte values, as observed by the Leukocyte Shift Index (LSI), also occurred post-competition (T1 and T2) and stabilized from the third day post-competition (T3). This observed stabilization in T3 indicates a more balanced state between neutrophils and lymphocytes, suggesting that the initial stress response was subsiding, and the horses were entering a recovery phase.

Based on the obtained results, it can be concluded that specific hematological indices, such as NLR, PLR, SII, SIRI, MLR, RDW/PLT, and LSI, are sensitive to acute changes associated with physical exertion during equine competition. These changes were observed on the day of the event, indicating the ability of these indices to reflect the immediate physiological response to exertion. On the other hand, the ELR and AI indices exhibited delayed and persistent responses, making them more specific indicators of the post-exercise recovery phase. The persistence of these changes beyond the third day suggests that these indices could provide valuable insights into prolonged adaptation and recovery in equine athletes.

An integrated approach that considers indices sensitive to the acute phase of exertion and those indicative of prolonged recovery can provide a more comprehensive and accurate evaluation of equine physiological performance in sporting environments. A thorough understanding of these blood parameter changes and indices offers a robust framework for assessing endurance horses' stress, inflammation, and recovery, which can help trainers and veterinarians develop optimal training and recovery protocols to enhance equine athletes' overall health and performance. However, further research is necessary, including animals in training across multiple sports, to determine precise cutoff points for horses.

5. Conclusion

Our study on endurance horses investigated hematological and inflammatory responses following prolonged exercise, providing valuable insights into blood cell dynamics. The utilization of NLR, PLR, SII, SIRI, RDW/PLR, MLR, and LSI as markers on the day of competition, along with ELR and AI as delayed recovery indicators offers a promising approach. These integrated cell indices can guide training and medical care for endurance horses, emphasizing the significance of monitoring strategies for performance improvement and equine well-being.

Authors' Contributions

Conceptualization: RFS and WRF; methodology: RFS and WRF; data collection: RFS; data analysis: RFS; writing—original draft preparation: RFS; writing—review and editing: RFS and WRF. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data supporting the findings of this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

The Ethics Committee approved this study for the Use of Animals of FMVZ-USP, protocol n. 2606/2012.

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A Preliminary Assessment of Shade and Shelter Use in Paddock-Kept Horses in Australia: A Pilot Study

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Abstract

There is increasing evidence to support the recommendation of providing horses with adequate shade, either natural or man-made, to safeguard their welfare. Weather conditions, particularly extreme temperatures, influence horses' shade-seeking behavior and ability to thermoregulate. However, there is limited research on other factors that may influence equine shade-seeking behavior. The aim of this study was to investigate the influence of weather conditions and shade type available on horses' shade-seeking behavior in non-extreme conditions, i.e. not in the intensity of summer, using animal-based behavioral indicators. Shade-seeking behavior of 19 university-owned horses was observed over a period of six days. Horses were kept in groups (n = 7 to 12 individuals) in paddocks with free access to an artificial shelter (AS) and natural shade (NS). The location of each horse was recorded at hourly intervals during the day along with the time of day, ambient temperature, and prevailing weather conditions. Shade use was not related to the time of day, and horses spent most of their time in non-shade (85.5%), compared to natural shade (10.2%) and artificial shade (4.3%; $H_2 = 187.85$, $p < 0.0001$). However, horses were more likely to seek artificial shade in foggy or cloudy conditions and natural shade when it was partly cloudy ($Chi^2_{12} = 30.14$; $p < 0.05$). Horses preferred natural to artificial shade, spending 78% of their shade-time in natural shade. Although horses spent the majority of their time not in the shade in this study, it does not mean that shade is not important for the thermal comfort and wellbeing of the horse. Horses still displayed shade-seeking behavior during conditions not deemed extreme, for example, in fog and reduced visibility conditions. Further research could be beneficial to understanding shade-seeking behavior in horses in order to determine what constitutes acceptable shade provision to optimize horse welfare.

Keywords

Equine; behavior; welfare; natural shade; artificial shelter; weather

1. Introduction

The modern-day domestic horse lives under largely controlled conditions and therefore relies heavily on humans to provide an appropriate environment in which to live. Horse health, welfare, and more recently 'wellbeing' have become increasingly important due to public awareness of shortfalls. This has contributed to a potentially challenged social license to operate to the extent that demands for improvement in how horses are managed are global [1,2]. The Five Domains Model framework enables the assessment of an animal's welfare, either positive or negative, using four physical domains (nutrition, environment, health, and behavioral interactions) and the fifth domain, mental state [3–5], to determine the likely quality of life and can be applied to assess horse management practices [6,7].

It is widely agreed that as non-human animals, horses are sentient beings and as such, their mental wellbeing should be considered in detail [8]. While horses cannot fully avoid negative experiences such as inclement temperatures, this can be managed in such a way that it does not unduly compromise individuals' quality of life. As Mellor [9] pointed out, it is the balance between positive and negative experiences an animal has over an extended period of time that is important.

Furthermore, the ability of individual animals to exercise agency and make choices to control their environment (for example, whether they stay out in the open or secure shade) is also becoming increasingly recognized as an important contributor to positive wellbeing [9,10].

Shelter for horses can be broadly divided into natural, such as that provided by trees and hedges, and artificial, e.g., man-made structures. Proops *et al.* [11] demonstrated that equids seek shelter under a variety of environmental and climatic conditions. Therefore, it is necessary to provide an environment that affords protection from extreme and less favorable weather conditions. This can promote subjective experiences contributing to positive equine wellbeing [5]. This is particularly important for horses that live outdoors for the majority, or all, of the time [12].

Australian animal welfare legislation is state-based and deems the failure to provide adequate shelter (shade) an act of cruelty [13–19]. However, while what is considered 'adequate' shelter remains underdefined, anecdotal observations suggest that practice is inconsistent and often to the detriment of horse welfare. The majority of research investigating the importance of shade to horses has focused on extreme cold or heat as well as wet weather conditions. There is a paucity of research into the importance of shade provision in temperate climates, including where horses are exposed to high temperatures over consecutive days.

The horse's thermoneutral zone (TNZ) is 5-25°C [20] with minor variations due to age, breed, body condition, diet, season, and climate [21]. Horses deploy heat accumulation and dissipation strategies to maintain a core body temperature of 37-40°C [22]. When the ambient temperature exceeds the equine TNZ, physiological responses occur including increased respiration rate and sweating to dissipate heat through evaporation to eliminate excessive heat and stabilize core body temperature [23,24]. Behavioral responses such as flared nostrils, head nodding, and apathy may also be exhibited [25,26]. When ambient temperatures drop below the TNZ, horses seek shelter and stand in close proximity to each other and may shiver [27,28]. In cold, windy, wet conditions, horses protect themselves by turning their heads away from the wind with their tails facing the wind or seek shelter in lieu of the prevailing wind.

Equine shade-seeking behavior (SSB) is notably influenced by weather conditions [29,30]. In hot conditions, access to shade can help with the maintenance of core temperature. Horses frequently exhibit SSB during hot, sunny conditions [29,31,32]. However, shade and shelter use are not only associated with high temperatures. Indeed, Jørgensen *et al.* [30] reported that horses were more likely to seek shelter (either natural or artificial) when ambient temperatures are outside of the equine TNZ (i.e., above or below the TNZ). Interestingly, the effect reported by Jørgensen *et al.* [33] indicates an apparent inherent 'need' for shelter, given that horses wearing rugs to help them more easily maintain their core body temperatures in cold conditions still sought shelter. Similarly, significant increases in shelter occupation have been reported by Snoeks *et al.* [21] during rain, cold (between -4°C and 3°C), and windy conditions [34].

Limited research exists directly comparing the benefits of natural vs. artificial shelter types for horses. Heleski and Murtazashvili [34] suggested that some horses preferred to stand next to artificial shelters rather than in them, possibly to avoid certain horses and/or maintain an unobstructed range of vision. In contrast, Snoeks *et al.* [21] reported a 25.6% greater use of artificial shelters compared to natural shelter in extreme temperatures and weather conditions.

Animal-based indicators are particularly important when assessing the adequacy of environmental provisions [8,27]. Therefore, understanding equine SSB in countries such as Australia that regularly experience an extreme range of temperatures, frequently exceeding 40°C for many consecutive days in the summer and well below 0°C in winter in the southeast and mountainous regions [35], is fundamental to determine what constitutes adequate shelter provision for horses.

The aim of this study was to investigate the influence of weather conditions and shade type available on equine SSB in non-extreme conditions. Individual horse behavior was used to assess natural shade and artificial shade use by paddock-kept horses in Australia.

2. Materials and Methods

2.1. Animals

Nineteen Thoroughbred and Standardbred horses belonging to Charles Sturt University were observed on six days between June 29th and July 8th, 2020. These dates fall within the Australian winter period (June 1st to August 31st). Data collection days were determined by COVID lockdown-related considerations, not by prevailing weather conditions. All horses were in good health and lived in established herds in paddocks for the entire observation period (i.e., not stabled). The horses were not regrouped for the purposes of this study. Group 1 comprised four mares and three geldings (mean age 8.86 ± 2.12 years), while Group 2 comprised 12 geldings (mean age 8.00 ± 2.76 years).

2.2. Materials

2.2.1. Weather Monitoring

Ambient temperature and wind speed were monitored using the Weatherzone application, which sources information from the Australian Bureau of Meteorology on an iPhone (iPhone 8, iOS 13.5.1). The study location was set to Estella. Weather conditions (cloudy/windy) were determined through direct observation at the beginning of each recording session.

2.2.2. Paddocks and Shelters

Groups 1 and 2 were observed in Paddock 1 (0.375 Ha - native grasses) and Paddock 2 (0.67 Ha sown with grazing oats), both at 0.05 Ha/horse stocking density. Both paddocks contained trees and bushes >2m on the boundaries, providing natural shade, along with a two-sided artificial shelter **(Figure 1)**.

Photograph by the authors (2020)

Figure 1: Horses (in Group 1) utilizing artificial shade, natural shade, and non-shade at Charles Sturt University.

2.3. Data Collection

Time of day, ambient temperature (°C), weather conditions (cloudy– sun not visible, fog, fog/cloud, partly cloudy – sun partially visible, partly cloudy/wind – greater than 10 km/h, sunny, sunny/wind), paddock being observed, total number of horses in the paddock, number of horses in natural shade, number of horses in artificial shelter, and number of horses in non-shade areas (sun) were manually recorded onto a Microsoft Excel spreadsheet.

Scan-sampling was conducted on the hour from 09:00 h to 16:30 h over three consecutive days in one week and a further three days the following week, from outside of the paddock. Horses were observed in a set order during all observation periods. The location of each horse was identified and recorded before moving on to the next horse. No other behaviors were recorded.

Horse location was categorized as 'Natural shade' if more than 50% of their body was in the shade. When the sun was temporarily obstructed by clouds, horses were classified as standing in natural shade if they were standing adjacent to trees/bushes that would ordinarily cast natural shade if the sun was not obstructed.

Horses were identified as standing in 'Artificial shade' if they were either standing in the shelter or standing in the shade produced by the shelter, with at least 50% of their body shaded.

2.4. Data Analysis

Horse location data were collated and analyzed in Microsoft Excel 2018 (Version 16.16.26). Anderson-Darling tests conducted in GraphPad online determined the distribution of the frequency of horses displaying SSB and shelter/shade occupancy percentages. As neither were normally distributed (AD = 12.4; $p < 0.001$ and AD = 12.4; $p < 0.001$, respectively) non-parametric Kruskall-Wallis tests were applied to assess shade type use. Chi-squared tests of association were then applied to determine the relationships between shade type preference, shade type, and weather conditions.

3. Results

A total of n = 96 observations of horses' locations and shade use were obtained over the two-week period.

3.1. Shade Type Preference

There was a significantly greater use of non-shaded areas (median = 7.5 horses; range 1–12) compared to both natural (median = 0 horses, range 0–5) and artificial shade (median = 0 horses, range 0–3), and more use of natural shade than artificial shade (Chi-squared = 33.152, $p < 0.00001$). However, horses overall made significantly greater use of natural shade than that afforded by artificial shelters (Mann Whitney; $p < 0.001$; **Figure 2**).

3.2. Weather Conditions

Throughout the observation periods, the mean temperature was 11.6°C ± 3.3°C (ranging from 4.5°C to 15.5°C). Weather conditions influenced the type of shade used ($Chi^2 = 30.14$; $p < 0.05$; **Table 1**).

4. Discussion

During this study, the 19 paddock-kept horses in Australia in winter spent the majority of their time in non-shade areas, compared to natural shade (NS) and artificial shade (AS). Although AS and NS were not utilized for the majority of the time during this study, it does not mean that they are not important for thermal comfort and consequently for promoting positive equine welfare.

4.1. Shade Type Preference

Horses spent a significant amount of time in non-shaded areas compared to NS and AS. There may be a number of reasons why horses in this study preferred being in the non-shaded areas, such as pasture availability (as observed by Heleski and Murtazashvili [34]) and the horses spent more time grazing rather than seeking shade/shelter; the mild temperatures and weather conditions did not provoke them to seek shade/shelter.

In this study, horses spent a greater percentage of time occupying NS as opposed to AS. This is contradictory to Snoeks *et al.* [21] finding that horses preferred artificial over natural shade in extreme temperatures (below 7.1°C or above 25.2°C) and weather conditions (rain and/or wind), with a 25% greater use of artificial compared to natural shade. Horses rarely utilized NS in cold conditions, suggested to be due to NS not sufficiently providing protection against cold and precipitation. In the current study, however, horses showed a preference for NS, although during foggy/cloudy conditions sought AS. It is possible that in thick foggy conditions the AS which had solid sides afforded the horse greater protection from the 'wet' that accompanies the fog than the natural shelter, bearing in mind the horses in this study are never rugged.

Table 1: Standardized residuals for Chi-squared test to determine the influence of weather conditions and shade type use.

	Weather Condition						
	Cloudy	Fog	Fog + Cloudy	Partly Cloudy	Partly Cloudy + Wind	Sunny	Sunny + Wind
Natural shade SR	-0.21	-0.3	-1	1.06	-0.18	-0.2	0.86
Artificial shelter SR	0.42	0.79	3.35	-0.57	-0.47	-0.01	-0.53
Non-shade SR	0.01	0.05	0.01	-0.09	0.03	0.02	-0.07

Cloudy – sun not visible due to cloud cover. Partly cloudy – sun partially visible due to cloud cover. Wind – >10 km/h from any direction. SR = standardized residual.

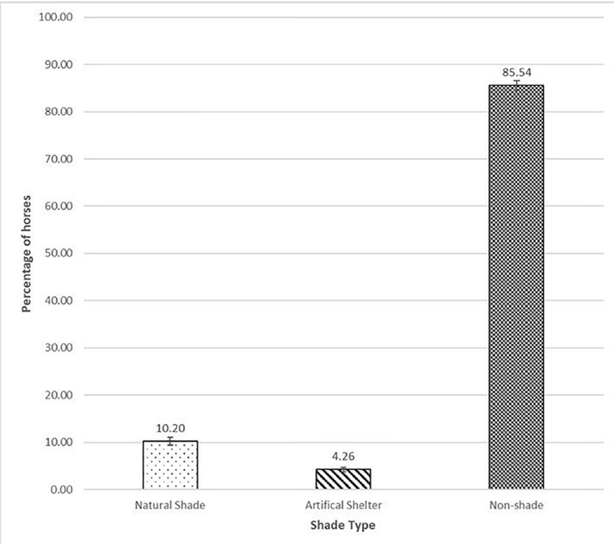

Figure 2: Outcome results for shade type preference of horses.

4.2. Weather Conditions

The temperatures observed during the current study were typical for midwinter in Wagga Wagga **[36]**. Although these temperatures (11.6°C ± 3.3°C) were mild compared to the extreme temperatures observed in other studies **[11,28,30,33]**, horses still displayed SSB and a relationship between weather conditions and SSB was evident **(Table 1)**. Since it did not rain during the current study, rain could not be a contributing factor to SSB as highlighted in other studies **[21,28,30,33,34]**.

Interestingly, horses sought AS when the sun was occluded by clouds or fog. This was during day four of the study, which was also when the lowest temperatures were recorded. Although it did not rain during this study and the temperature was not as cold compared to other studies **[21,28,30,33]**, the condensation of the fog may have created sufficient moisture for the horses to exhibit SSB. AS, which had solid sides, was preferred during foggy conditions with reduced visibility.

This is supported by the observation that during partly cloudy conditions, horses were more likely to seek natural than artificial shade.

In this study, horses were offered a choice of shelter type, i.e., artificial or natural, as well as non-shaded areas. Contemporary animal welfare assessment emphasizes the importance of agency to individual animal welfare. By being provided with a 'choice' of locations within the paddock enabled the horses to display agency **[37]**, therefore providing an environment which could go some way towards optimizing equine welfare **[5,9]**.

5. Limitations

It was noted that the horses in the larger Paddock 2 did not occupy AS throughout the study. There may be two reasons for this. First, the shelter was located at the far end of the paddock away from the gate to which horses gravitated at particular times of the day through previous conditioning to the arrival of feed during times of the year when supplementary feeding is required **[38]**. It is worth noting that during this study, horses did not receive supplementary feed due to sufficient pasture growth and feed availability. The orientation of the shelter (artificial shade location) may also influence occupation as its open sides face away from the gate to the paddock, and due to its orientation, make it difficult for occupants to maintain full sight of the herd, as highlighted by Heleski and Murtazashvili **[34]** as influential.

Other factors that may contribute to SSB, but were outside the scope of this study, include relative humidity, solar radiation, wind speed, temperature inside the artificial shelter, temperature under shade, the presence of insects **[11,39,40]**, core body temperature, and time post feeding. While the impacts of these factors on horse SSB have been reported, commonly individually, for groups of horses in various locations worldwide, a funded longitudinal study of equine SSB taking into account all of these factors, over all four seasons in Australia, would be valuable, where horses typically live out on a permanent basis and are exposed to extreme weather events and variations on a daily basis.

Despite the identified potential limitations of this study, baseline data collecting using similar methods to the published studies cited in this paper are provided for further investigations of shade/shelter use by horses in Australia and countries with similar climates and fluctuating weather conditions.

6. Conclusion

Although artificial shelter and natural shade were not utilized the majority of the time in this proof of concept study of paddock-kept horses in Australia, they are important for the thermal comfort of the horse. Environmental conditions during this study were mild compared to other studies, possibly indicating that during temperate/mild weather conditions, shade or shelter may not be as essential. However, horses still displayed SSB during conditions deemed non-extreme, such as foggy conditions, indicating the importance of providing horses with the option of shade/shelter regardless. The use of equine behavior (namely individual horse location within a paddock) in this study has yielded data which contribute to understanding both the importance of providing suitable shelter and the relevance of SSB in horses to equine welfare.

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Authors' Contributions

Marina Douglas co-designed the study, collected and analyzed the data and wrote and edited the paper. Hayley Randle co-designed the study, assisted with data analysis, and assisted with writing and editing the paper.

Data Availability

The data supporting the findings of this study are available on request from the corresponding author.

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This paper has not received any funding.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

Approval for this study was granted by Charles Sturt University's Animal Ethics Committee (Authority A20079). The study has followed the guidelines of the Declaration of Helsinki.

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Review Article Open Access

Are We on the Same Page? A Review of Horse Training Approaches, Terminology Use, and Method Reporting within the Scientific Literature

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Abstract

It is vital that the impact of different horse training approaches (TAs) is studied to ensure the methods employed are effective, ethical, and do not compromise equine welfare. While a range of TAs are referred to within the scientific literature, no research has explored whether the way these are applied, described, and reported is consistent across existing studies. This is problematic as differences in training application and method reporting may alter study outcomes, limit the potential for inter-study comparison, and impede effective scientific communication. A systematic search of the published literature from three online databases (Scopus, Web of Science, and PubMed) was used to identify studies that apply horse TAs within their methodology. A description of the training protocols was extracted from each paper and used to categorize the training approach(es) employed, identify their defining characteristics, and assess consistency within TA description. A total of 75 studies published between 1992 and 2021 were reviewed using a mapping review method, within which ten distinct TA categories were identified. Six of these aligned directly with the principles of learning theory; however, distinct differences in their application were identified. The four remaining categories were less clearly defined, with a wider range of terms used to describe them. Limited information provided within some methodologies would render accurate study replication impossible. This study highlights a need for more consistent and detailed reporting of horse TAs within the scientific literature, and subsequently, some initial recommendations to promote this have been made. This would facilitate communication between researchers and further enable comparisons to be made across studies, ultimately improving understanding of modern horse training practices and their welfare impact.

Keywords

Horse training; terminology; equine welfare, science communication

1. Introduction

Animal-centered industries are continuously striving for the implementation of more 'welfare-friendly' and socially acceptable management and training practices to help ensure their continuation by obtaining and maintaining 'legitimacy' in the public eye [1,2]. This is particularly true of equestrian disciplines, where a series of 'bad press' incidents have prompted considerable public uproar surrounding the use of horses in modern sports [3,4]. One area that appears to be of the greatest concern to both spectators [5,6] and industry stakeholders [7,8] is the continued use of training practices considered to be highly aversive, involving high levels of punishment or that conflict with the horse's learning capabilities. Consequently, there is a growing need for more ethical and socially acceptable horse training approaches to be utilized, while still ensuring that the approaches are effective in meeting training aims and minimizing the risks to safety associated with horse riding and handling activities [9,10].

The term 'training' is typically used to mean *'the intentional modification of the frequency and/or intensity of specific behavioral responses'* [11], which can be achieved using a variety of different training approaches (TAs), applied together or independently. For example, desirable behaviors can be rewarded to increase their frequency, or undesirable behaviors reduced through the application of punishment. For horse training to be both effective and ethical, it is vital that TAs are carefully selected to ensure they align with the underpinning cognitive principles of animal learning [12–14] with careful consideration given to their limitations and potential welfare implications. For this to be achieved, a comprehensive and unbiased understanding of different TAs must first be established. This relies on robust, well-reported, and peer-reviewed research being conducted to compare different TAs, investigate their effect on the horse, and assess their potential to influence welfare, to ultimately optimize horse training practices.

Over the last 30 years, the number of published studies that focus on horse training has rapidly increased as equitation science continues to be a growing area of research interest [15,16]. While this is a positive step toward improved understanding and subsequently more informed decision-making in this area, the nature of equine research means that physical and financial constraints often limit the scale to which research can be conducted by a single research team [17]. Consequently, studies involving small and often heterogeneous samples make up the body of work in this area, which may contribute to reduced result credibility when studies are considered in isolation. Maximizing opportunities for inter-study comparison and method replication is, therefore, a vital step to increase confidence in the findings and subsequently facilitate their real-world implementation [16–18]. To achieve this, existing methods must be applied consistently across different research groups and the terminology used to describe them universally understood to facilitate clear communication both within the scientific community and between researchers and individuals who are directly responsible for training horses [19,20]. The need for this is particularly pressing, given that poor understanding of horse training terminology is frequently highlighted as an area of concern within both horse-owner and practitioner populations [21–24] and may further hinder research interpretation and continued application at the industry level.

While attempts to review the equitation science literature have been made [17,25], none aim to identify which TAs are receiving the greatest research focus and assess methodological and terminology consistency across existing work. A comprehensive review of existing TAs and their application is critical to informing the development of future study methodologies and facilitating consistent reporting of equine training protocols within the literature. This would further enable comparisons to be drawn across multiple studies, increasing confidence in their findings and ultimately improving understanding of modern horse training. Consequently, this study utilizes a mapping review [26] approach to answer the following questions: (1) Which horse training approaches have been applied within the published scientific literature? (2) Which terms are used to describe the TAs identified? (3) Are inconsistencies in terminology use or study reporting that may limit inter-study comparison present within existing work?

2. Method

2.1. Study Identification and Retrieval

In December 2021, a literature search for published research articles written in English was conducted using three online databases (Scopus, Web of Science, and PubMed). Variations of the following search terms were applied: ***("training method" OR "training" OR "punishment" OR "reinforcement") AND ("horse" OR "pony" OR "equidae" OR "equine") AND NOT "exercise."*** The search terms were intentionally broad to capture as widely as possible. The term 'exercise' was excluded to minimize the use of the term 'training' to mean physical conditioning, rather than a means of behavior modification. Despite this, the search still yielded many studies that were unrelated to the topic of interest, leading to a considerable number being excluded **(Figure 1)**. The literature was exported to Mendeley Desktop referencing software for sorting, and later to Microsoft Excel for data extraction. Titles and abstracts were initially screened by one author (EB) and were excluded from further review if they (1) did not refer to the species of interest, (2) did not describe the application of (non-physical) training method/s, (3) were not original primary research, or (4) were published more than 30 years ago. Studies where the training method was not directly applied (e.g. survey-based studies) were also removed, as these methodologies typically rely on horse owners reporting on their own training approach and are therefore subject to a greater level of bias [27] or may lack sufficient detail in their description to facilitate review. Studies that focused on other equids (e.g. donkeys) as the main training subject were also excluded, as it is generally recommended that horse training strategies are not directly applied to donkeys [28] given the differences in their behavioral response to threats and potential differences in spatial reasoning [29]. The remaining articles were retained for full-text review.

2.2. Coding of Studies and Data Extraction

A description of the training protocols applied within each study was extracted verbatim from their methodology section. These descriptions were then reviewed to identify the defining characteristics and learning theory principles that underpinned each of the techniques used to modify horse behavior within these protocols. This information was then used to group the techniques into individual TA categories. As some studies involved applying more than one TA, the aim was not to categorize each article but rather each training approach described within an article. The descriptions of training and the terminology used were recorded and later assessed for consistency against other work that involved the same TA. To increase the reliability of the assessment process, 15 of the studies (representing 20% of those included) were randomly selected and assessed by two authors (EB and EJB), with the latter blinded to all other study information. Inter-assessor agreement was assessed using IBM® SPSS® Statistics (V29) software, and any discrepancies between assessor coding were recorded and later discussed until a consensus was reached. Agreement between assessors across 20% of the sample was almost perfect ($\kappa = 0.952$; $p < 0.001$) [31], with only a minor difference seen in relation to 'habituation' and how this should be coded. These discrepancies were easily resolved during a follow-up discussion where a consensus was reached.

The following additional information was also extracted from each study: (a) bibliographic information (e.g., authors, publication date, and publishing journal); (b) sample size; (c) study aim and variables; (d) the reasons for applying the training; and (e) additional notes about the training (for example, 'type of reinforcer' given, where appropriate). Any areas within the text that had the potential to limit accurate study interpretation or replication were recorded for further discussion.

3. Results and Discussion

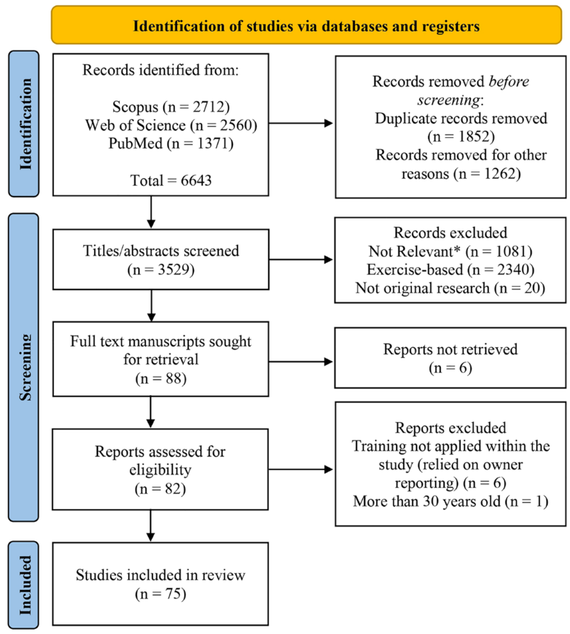
3.1. Overview of Studies

A total of 75 studies were included in this review **(Supplementary Materials)**. Only six studies were published before the year 2000 **(Figure 2)**, which may reflect the growing interest in equitation science over the last 20 years, perhaps in response to the increasing recognition of animal welfare and sentience **[32,33]** resulting in greater consideration for the ethical nature of horse training **[34,35]**. It may also have been driven by the establishment of the International Society for Equitation Science in the early 2000s, which advocates for the integration of science within equitation **[15]**.

Authors' reasons for applying training within each study loosely fell into one of three categories: (1) 'training approach comparison,' (2) 'training approach application,' or (3) 'equine cognitive research.' 'Training approach comparison' studies involved applying more than one TA and comparing the outcomes associated with each. 'Training approach application' studies applied one training approach and assessed their viability when applied to horses or documented the outcomes associated with the approach (without comparison to other approaches). 'Equine cognitive research' studies were those where the primary aim was to investigate factors associated with equine learning, memory, or cognitive abilities. Any training applied within these studies was only used to facilitate data collection (e.g., teaching horses to touch a specific shape to enable memory testing) and was not the focus of the research.

3.2. Training Approach Categorization

Following a review of the training protocols described within each study, ten unique TAs were identified by the assessors **(Table 1)**. Six of the TA categories ('positive reinforcement,' 'negative reinforcement,' 'combined reinforcement,' 'positive punishment,' 'combined positive punishment and negative reinforcement,' 'habituation') were considered to align directly with associative or non-associative learning principles **[14]**, while the remaining four TAs either involved utilizing a combination of different learning theory principles, were underpinned by concepts that do not align with learning theory, or were reported in a way that made it not possible to definitively identify the learning principles associated with them.



*Titles/abstracts were completely unrelated to the topic or about a different species (not *Equidae*).

Figure 1: The number of articles included and excluded at each stage of the retrieval process. Adapted from the updated PRISMA reporting guidelines **[30]**.

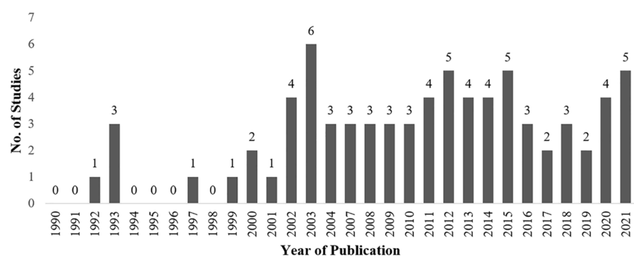


Figure 2: Studies included in this review (n = 75) displayed by year of publication.

Along with identifying which TAs have been studied within the scientific literature, a secondary aim of this study was to evaluate the way in which the methodologies of these studies were reported. This evaluation included exploring terminology use and identifying areas within the text that could limit accurate study interpretation, replication, or reduce the impact of the findings in practice. This assessment resulted in four key areas for discussion being identified: (1) the use of inconsistent terminology and poorly described methodologies; (2) within-approach variation in method application; (3) lack of information provided in some studies limiting readers' ability to identify/replicate the methods applied; and (4) misrepresentation/mislabeling of learning principles that underpin an approach. These points will be further discussed, with examples of each provided. Further discussion around the fact that 'negative punishment' was not mentioned by any authors, and the limited recognition of 'combined positive punishment and negative reinforcement' as an approach to modify horse behavior is also provided.

Table 1: Key characteristics of horse training approaches identified within the published scientific literature.

The training approach category identified within this review *(Alternative terminology used within the scientific literature)*	Number of studies that involve applying the training approach	Key characteristics of the training approach	Overview of studies and additional comments
Positive Reinforcement *('clicker training,' 'differential-reinforcement-of-other-behavior,' 'food reinforcement,' 'food reward,' 'reinforcement,' 'reward learning,' 'reinforcement training')*	41	'Positive reinforcement' (PR) was defined as 'the addition of pleasant stimuli after a behavior is performed to increase the likelihood that it is repeated.' If used in conjunction with NR, this was instead classified as 'combined reinforcement.'	11/41 (26.8%) studies referred to the learning process that underpinned this approach as only 'reinforcement,' which does not differentiate it from negative reinforcement. None of the studies mentioned 'negative punishment,' even though both assessors considered it to be a key feature of the training applied in at least six of the PR studies. 40/41 (97.6%) used food as the reinforcer (some also used tactile reinforcement alongside this), while the remaining study used 'scratch' to train only foals. A secondary reinforcer was used in 22/41 (52.7%) studies (there was some debate as to whether 'the sound of food landing in a bucket' constitutes a secondary reinforcer (e.g., **[36]**, although a decision was ultimately made to exclude this). Within these, the most commonly used was a 'clicker' (10/22), followed by the use of the word 'good' (7/22), a buzzer sound (2/22), 'electric chime sound' (1/22), and one study simply referred to the use of a 'tone.' None of the studies that focused on applying positive reinforcement involved training any behaviors associated with ridden work.
Negative Reinforcement	20	'Negative reinforcement (NR)' was defined as 'removal of aversive stimuli after a behavior is performed to increase the likelihood that it is repeated.' In all NR studies, pressure was applied until the horse showed the desired response. It was also common for this pressure to first be used to obtain the behavior (for example, applying pressure to the horse's side until they take a step away) and later become the cue. If used in conjunction with PR, this was classified as 'combined reinforcement.' If used with PP, this was considered 'combined positive punishment and negative reinforcement.'	All (100%) of studies referred to this approach as 'negative reinforcement.' In three studies, assessors felt that another approach (e.g., positive punishment or positive reinforcement) would also have played a role in shaping the horses' response, although this was not mentioned by the study authors. All but one study (19/20, 95%) applied only 'contact' negative reinforcement, while one used a combination of 'contact' and 'no-contact' pressure *(see discussion for definitions of these)*. 6/20 (30%) did not state whether any pressure applied was escalated or maintained until the desired response was shown by the horse. 13/20 (65%) stated that the pressure was escalated. Only one study clearly said that the pressure applied was kept consistent until the desired behavior was seen. 5/20 (25%) studies involved training behaviors associated with ridden work.
Positive Punishment	1	'Positive punishment (PP)' was defined as the 'addition of aversive stimuli after a behavior is performed to reduce the likelihood that it is repeated.' If used with NR, this was considered 'combined positive punishment and negative reinforcement.'	Only one study clearly described the use of positive punishment. The aversive applied in this study was the delivery of 'immediate and ventral pressure on halter lead.'
Combined Reinforcement *('blended positive and negative reinforcement')*	3	'Combined reinforcement (CR)' involves the use of both negative and positive reinforcement together to reinforce the same behavior.	In all studies, NR was to elicit the performance of a desired behavior at which point any pressure applied is released (NR) and food reinforcement delivered (PR). 2/3 (66.6%) studies called it 'combined reinforcement' while one never used this term, but just referred to 'the addition of positive reinforcement to negative reinforcement.' All studies applied 'contact' pressure for the NR element and food reinforcement for the PR part of the protocol. One study used a secondary reinforcer (the word 'good') to mark a correct response. No others reported the use of secondary reinforcers. Only one study involved training behaviors associated with ridden work (shaping a halt response on long lines). This was the only study within this review that involved using PR for ridden behaviors. They used a 'telemetrically operated reward device' to remotely deliver a reinforcer (molasses water) directly to the horse's mouth through the bit. This may support the idea that positive reinforcement is (or is perceived to be) difficult for the rider to deliver in training **[37,38]**.
Combined Positive Punishment and Negative Reinforcement *('avoidance learning,' 'avoidance conditioning')*	2	'Combined positive punishment and negative reinforcement' involved the combined use (applied sequentially) of positive punishment, followed by negative reinforcement.	Both of these studies referred to their approach as 'avoidance conditioning/ learning.' In both studies, trainers preceded the desired behavior with a noise cue, if no response was given and an aversive was applied (PP) which only ceased when horses performed the desired behavior (NR). 'Electric shock' and 'puff of air' were the aversives used.
Habituation	4	Habituation was defined as 'repeated exposure to stimuli that do not result in any reinforcement or punishment, resulting in a decreased response to the stimulus.'	It was difficult for assessors to separate out habituation as its own TA, as it was frequently combined with techniques based around associative learning and was considered to play a role in shaping equine behavior even when trainers were not consciously applying it. For this reason, it should be noted that >4 studies would have involved the use of this approach, however, only 4 specifically outlined a protocol that highlighted habituation as a key feature.

The training approach category identified within this review *(Alternative terminology used within the scientific literature)*	Number of studies that involve applying the training approach	Key characteristics of the training approach	Overview of studies and additional comments
Conspecific Model *('round pen technique,' 'Join up,' 'Monty Roberts technique,' 'natural horsemanship,' 'natural training,' 'Parelli Natural Horsemanship,' 'round pen technique,' 'sympathetic training,' 'training')*	14	Emphasis is placed on horse-human interaction/ communication through the use of body language. Attempts to replicate horse-horse communication were a key theme throughout. It was common for the early stages of the training process to involve teaching the horse to look at/ approach/spend time near the trainer. This was not mentioned in relation to any other TAs. This was most commonly achieved with the use of NR, although some also describe using PP or PR (never food, only tactile reinforcement). Much more reliance on the use of 'non-contact pressure' than any of the studies that were classified as using purely NR (which all involved making contact with the horse). 'Desensitization of the horse to touch and equipment' also appeared to be a common feature of this TA. However, details of how this was achieved were lacking in many of the studies. Some 'sensitization' to pressure cues was also alluded to. Elements of this method appeared to be unique to the trainers themselves or reliant on trainer skill (which may be why many of the studies failed to outline a 'step-by-step' protocol, as it relies on the trainer observing, interpreting, and responding to each individual horse and adapting their approach accordingly).	Learning theory principles mentioned by study authors did not fully align with those identified by assessors in any of the studies (see **Supplementary Materials**). In 10/14 (71.4%) studies, the assessors felt it was not possible to reliably determine which learning principles were being applied within the study methodology. Habituation, negative reinforcement, and positive punishment were most frequently identified by assessors. 9/14 (64.3%) studies aimed to compare the outcomes associated with the 'conspecific model' to those of different training approaches. It was compared against NR (n = 1), PR (n = 2), and conventional training (n = 6). 9/14 (64.3%) studies involved training behaviors associated with ridden work.
Conventional Training ('traditional,' 'European training method')	9	It was not possible to consistently identify key features of this approach, as its meaning appears to vary between studies, and in most instances was not described. The term was typically used to refer to methods considered to involve habituation, NR, and some PP. It was less likely to involve adding reinforcement (PR) or placing emphasis on attempting to replicate equine communication. More focus was placed on detailing the tasks horses were required to complete (e.g., lunging, accepting a rider) rather than outlining the methods used to achieve this.	In 7/9 (77.8%) studies, the assessors felt it was not possible to reliably determine which learning principles were being applied within the study methodology. This also meant that it would not be possible to accurately replicate these studies. 6/9 (66.7%) studies involved training behaviors associated with ridden work.
T-touch Equine Awareness Method	1	Only one study that reported using this approach was included in this review, which ultimately means that it is not possible to identify common features across multiple studies. The only feature that differentiated this from other TA categories was the addition of 'bodywork' as part of the training session. Other than this, assessors considered the approach described to simply involve applying NR, and at times PR, to influence horse behavioral response to handler cues when being asked to work over/around obstacles. Interestingly, the study authors report that this training approach '*does not use force or physical pain to motivate the horse to comply*' and described it as a 'non-aversive' technique, despite the fact that NR features quite heavily as part of this approach.	n/a (only one study)
Imprinting ('Imprint training')	3	'Imprint training' involves exposing foals to a range of different stimuli and handling techniques shortly after they are born. The way in which this training is applied varied across studies but always included rubbing the foal all over its body and exposing it to novel procedures (e.g., electric clipper, plastic bags, and water spray) while restraining the foal so that it could not evade the treatment applied.	One study began 'imprint training' within 10 minutes of foals being born. Both other studies trialed applying imprint training at various time points within 72 hours of a foal's life.

3.3. Inconsistent Terminology and Poorly Described Methodologies

While only ten unique TA categories were identified within this review, 29 different terms were used by study authors to describe the training applied within their studies **(Figure 3)**. Some of these terms represent well-defined, pre-existing scientific terminology (e.g., relating to operant and classical conditioning), while others were more reminiscent of subjective descriptors (e.g., 'Sympathetic,' 'Natural') that seem to reflect the intentions behind the way in which horses were trained, but do not provide information about the strategies applied to modify horse behavior. Additionally, several terms used to describe the training (e.g., 'conventional' or 'traditional') were specific to the countries/regions within which the studies were conducted.

The use of these more subjective or location-specific terms to describe the training applied would not have been so problematic if these were accompanied by accurate descriptions of what this training constitutes. However, in many cases, this was lacking, with the apparent assumption being made that these terms alone were sufficient to communicate the way in which training was applied. This renders the reader unable to understand how the training was applied or reliably replicate the results. Instead of clear protocol descriptions, broad statements such as *"the horse was taught to accept the saddle"* and *"getting the horse accustomed to the whip's movements over its head"* were commonplace in the reviewed studies. Statements of this nature do not specify how these steps were achieved, the extent to which stimuli were applied, whether the horse was systematically desensitized, whether the horse was restrained, or if any punishment/reinforcement was applied to facilitate this process.

Variation in the terms used to describe training was most evident in TAs considered to fall within the 'conspecific model' category, with nine different terms used when describing these **(Table 1)**. This raised the question of whether these should be further categorized as different approaches. This was initially attempted; however, it was not possible to identify distinctive features that clearly differentiated between these approaches based on the descriptions alone. A common feature across these studies was the reported basis on how horses communicate with other horses in a naturalistic setting, with trainers applying a 'herd-leader' premise to explain human-to-horse attachment **[39]**. The term 'conspecific model' **[40]** was used. Several terms used to describe training within this category relate to the people (e.g., 'Monty Roberts,' 'Parelli') who developed or popularized their own approach. Given this, it was initially assumed that defining features of these would have been clearly evident and render them sufficiently different to classify as separate approaches. However, this was not possible, and even though some training techniques were trademarked (e.g., Join up®), other studies that reported using a very similar 'round-pen technique' which (based on the, often limited, description given) appeared to follow the same process, but did not specifically attribute this to the trademarked method.

Ultimately, the reliance on poorly defined and often subjective terminology in many of the reviewed studies highlights considerable concerns about the quality and value of the research being produced. This not only limits opportunities for study replication but also hinders accurate result interpretation and reduces the real-world utility of the findings.

‘Avoidance-conditioning’
‘Avoidance-learning’
‘Blended positive and negative reinforcement’
‘Clicker training’
‘Combined reinforcement’
‘Conventional’ (3)
‘Differential-reinforcement-of-other-behavior (DRO)’
‘European training method’
‘Food reinforcement’ (4)
‘Food reward’
‘Habituation’ (4)
‘Imprinting’ (3)
‘Join up’
‘Monty Roberts’
‘Natural horsemanship’ (2)
‘Natural training’ (4)
‘Negative Reinforcement’ (20)
‘Negative reinforcement + positive reinforcement’
‘no-reinforcement’
‘Parelli Natural Horsemanship’
‘Positive punishment’
‘Positive reinforcement’ (25)
‘Reinforcement’ (8)
‘Reward learning’
‘Round-pen-technique’ (3)
‘Sympathetic’ (5)
‘Tellington-Touch Equine Awareness Method’
‘Traditional’ (4)
‘Training’ (2)

Figure 3: Phrases used to describe the training approaches applied within the studies (n = 75) reviewed. Those used in more than one study are accompanied by a number to illustrate how many studies used this phrase.

3.4. Variation in Method Application

Even when the wording used to describe TAs was consistent across studies, this did not necessarily mean that the way in which the methods were applied was comparable. In several instances, differences in TA application were identified even within studies that reportedly used the same approach. Differences in application will again limit the potential for inter-study comparison and could alter how horses respond to training, thus reducing the ability to generalize study outcomes to one specific approach. For example, the application of 'negative reinforcement' (NR) involves removing something aversive (e.g., pressure) to reinforce behavior and is the most commonly used method in horse training **[14,41]**. However, there are a variety of ways in which NR can be used in practice. This review highlighted that the type of aversive stimuli applied during NR protocols fell into two distinct categories; one involving the trainer or training equipment making physical contact with the horses' body (e.g., pressure is applied through a lead rope or reins), the other involving no physical contact, with the trainer instead using their body position, posture, or equipment to direct the horse's movements within an enclosed space (e.g., stable, round pen). Further consideration needs to be given to whether or not NR involves making physical contact with the horse, and work conducted to compare the effects of each on equine learning and welfare. If substantial differences are seen, it may be beneficial to consider these as two separate methods in future work – for example, referring to them as 'contact' or 'no-contact' negative reinforcement, rather than using one term to refer more generally to both. This further distinction between the approaches may also be relevant when the welfare implications of NR are discussed, as it will likely influence the way in which the approach is perceived. Anecdotally, training approaches that involve 'no-contact' NR are often viewed or marketed as being 'force-free,' thereby implying that it is more 'ethical' or has less potential to compromise welfare, which could result in psychological distress being overlooked.

Another observation made when reviewing studies that involve applying NR was that several did not clearly report whether the trainer waited for the full desired behavioral response to be performed before releasing the aversive applied, or whether they reinforced progressive steps towards the desired behavior ('shaping'). Not only is this problematic in the sense that it would not facilitate study replication, but both [42] and [43] suggest this difference in application may have implications for horse learning success, and thus has the potential to alter study findings. In addition, many studies failed to report whether the pressure exerted was escalated or simply maintained following its application. It is logical to assume that this difference may influence the outcome of studies that use 'completion time' or 'latency to perform desired behavior' as a measure of TA success and may ultimately influence how horses perceive and respond to the training.

The application of positive reinforcement (PR) was similarly subject to variation in application across studies. While the majority used food-based reinforcers **(Table 1)**, the type of food given differed between studies, from perceived 'low-value reinforcers' such as *'handfuls of the horses' usual hay,'* to those that are unlikely to be given in large quantities within the horses' normal diet, such as the *'pieces of carrots, apples, vanilla wafers, sugar cubes, crackers, bread, and sweets'* used in another. While the impact of reinforcers' perceived 'value' on horse training performance has not been explicitly studied, reward preference and volume (which was also not standardized across equine studies) have been shown to predict performance outcomes in dog training [44,45]. It is therefore possible that these differences in reinforcer value could influence equine performance-related outcomes and subsequently prevent reliable cross-study comparison. Furthermore, some studies reported that horses were food deprived (for as much as 8 hours in one instance), or that their diet was altered prior to training in an attempt to increase food motivation. This, in itself, may be considered ethically questionable and highlights a need to acknowledge the wider welfare implications associated with different TAs [46,47] that may exist outside of formal training sessions. The way in which food restrictions were reported was inconsistent between studies, or not mentioned at all, meaning that it was not always possible to reliably determine whether or not horses' diet had been altered prior to training.

3.5. Lack of Information about Study Methodologies Provided

Within the scientific community, it is widely accepted that the 'method' section of a research study should be written in sufficient detail to enable replication [48,49]. However, this was not always seen within the horse training studies reviewed. Authors frequently failed to provide the level of detail needed to fully understand or replicate the methods used. An example of this seen across many of the studies was the fact that information about the individuals applying the training was limited or not provided. In some studies, specialist trainers were recruited to apply the training approaches, while in others, it appears to be the researchers carrying out this part of the methodology themselves. However, this was not always made clear as the amount of information given about the trainers was highly variable, with many studies failing to provide sufficient (or at times any) information about the trainers, their ability, or experience **(Table 1)**. Very few stated which, if any, qualifications trainers possessed or applied any objective means to assess their ability, with many providing only vague and subjective statements, such as *'trainers were highly qualified.'* This is concerning given the fact that trainer experience has been shown to influence equine stress response during training [50,51], and correct timing when applying techniques that involve reinforcement or punishment is considered to be vital [14]. There is also some evidence to suggest that handler sex, which is another factor that was not widely reported within the studies, may influence equine behavioral response [52]. One study within this review mentioned that they specifically chose to use two 'inexperienced trainers,' another split the sample horses between one experienced and one inexperienced trainer, and one study involved the horses' owners applying the training. While potentially more representative of industry practice, the use of inexperienced trainers calls into question the reliability of the findings, as any reported effects may have been due to the way in which these individuals applied the technique, rather than a consequence associated with the TA itself. Furthermore, it has been shown that horses can differentiate between humans and, while they do not seem to show a preference for a familiar individual [53], there is evidence to support the idea that they associate handlers and trainers with the valence of past experiences [54–56]. Thus, any previously learned association with the trainer also has the potential to influence the horses' response to a TA. Furthermore, studies rarely stated whether trainers were naïve to the research hypothesis. Horses have been shown to use human pointing and body position cues during object-choice tasks [57,58] and respond to attentional cues that include the human gaze [59]. Knowledge of the research question or study variables being measured could, therefore, have led to trainers inadvertently (or deliberately, given that in several instances trainers were professionals whose livelihood was reliant on the popularity of their training approach) altering the outcome. It is possible that many of the trainers within these studies were, in fact, blinded to the research question, and this was just not acknowledged in the final published report. This is disappointing given that this would be a relatively simple way of increasing experimental rigor, a feature of equine research that is often criticized [20].

3.6. No Reported Use of Negative Punishment

A key finding that warrants further discussion is the fact that none of the research articles reviewed reported using 'negative punishment' to modify horse behavior, despite the fact that both of the assessors in our study agreed that this approach was utilized by some. Negative punishment (NP) is one of the four operant conditioning quadrants and involves the removal of something appetitive to reduce the occurrence of a behavior. Speculations can be made as to why the authors did not mention NP. It's possible that it is simply an error on the authors' part, and they may genuinely be underestimating or failing to understand the role that this quadrant plays in training. If this is the case, it may be wise for the scientific community to place as much emphasis on ensuring their own members have a clear understanding of learning theory as they do on investigating lay horse owners and trainers. Alternatively, authors may intentionally be underemphasizing the role of NP in their study as ethical approval for research that does not claim to involve using any punishment may be more likely to be granted.

Discussion surrounding NP, its role in horse training, and whether PR can even be used without it coming into play, continue to be had amongst equitation science researchers, so the fact that its use appears to be under-reported by academics themselves adds a new dimension to this area of discussion. Further work is ultimately required to better understand perceptions and knowledge of NP within the equestrian sector, as failing to acknowledge its role in training may further contribute to the use of punishment in horse training, or the use of NP mislabelled as PR.

3.7. A Note about Combined PP and NR

Unlike 'combined reinforcement' which is a term that has become relatively commonplace in relation to horse training **[14]**, the use of combined PP and NR appears to have received less attention. These two operant condition quadrants are often considered to be linked in the sense that poorly timed NR can easily become PP (similarly, PR and NP are also considered to be linked in this way) **[14,41]**, yet their combined use is not widely discussed. The apparent lack of regard for this is problematic as we posit it is likely reflective of regular training practice within the industry. For example, if a rider cues their horse to walk on and the horse does not respond in the desired way, the rider may 'give the horse a kick' or 'tap with the whip' (PP - as this occurs after the undesirable behavior occurs) and then continue to apply strong leg pressure which only ceases when the horse walks on (NR). It is vital that the application of multiple and prolonged (and potentially escalating) aversives in these instances is not overlooked or mislabelled as solely involving NR. Doing so may increase the likelihood that equine welfare is compromised, through increased use of aversives and reduced trainer awareness of their own actions. This subject was also raised by Henshall **[60]**, who suggests reconceptualizing 'positive punishment' and 'negative reinforcement' in horse training as a single continuum, rather than considering these separate training modalities, to better reflect how horses experience their application. Ultimately, greater consideration regarding the way in which 'combined PP and NR' is defined and discussed within the equestrian sector is warranted to encourage more effective and ethical training.

3.8. Limitations

It is likely that the search terms and study retrieval process used will not have captured every paper that involves horse training, and instead should be considered to provide an overview of existing horse training research published in English, rather than an attempt to document all work in this field. Furthermore, the ratio of TAs implemented within this research does not appear to be representative of real-world practice, as PR-based methods were most commonly applied, despite NR being recognized as the predominant method for training horses **[41,61]**. This is likely due to the inclusion of equine learning studies, where PR is commonly used **[62,63]**, and may also be partly attributed to the fact that research studies are likely to trial more novel methods to assess their potential for real-world use, rather than well-established practices. Additionally, methods using high levels of punishment may be less likely to be granted ethical approval or accepted for publication. It was also found to be common for individual researchers to publish multiple papers, which is likely to have a biased representation of how the different TAs are described. For example, while five studies referred to the training they applied as 'sympathetic,' the same author was involved with three of these.

3.9. Recommendations for Future Researchers

As researchers continue to investigate the impact of different TAs on training efficacy and equine welfare, it is vital that greater consideration is given to the way in which these studies are reported. Accurate and detailed reporting of a study's methodology is essential to provide background context, aid result interpretation, and facilitate accurate replication in the future if deemed necessary. Robust method reporting also increases the perceived scientific and societal 'value' of a study, further justifying the inclusion of animals and increasing the extent to which the research can be considered ethical **[49]**. To facilitate improved study reporting in the biomedical research field, regular reviews of existing research are carried out **[48,64,65]**. When inconsistencies or problems within this are identified, efforts can be made to mitigate future issues, for example through the development of guidelines like 'Animal Research: Reporting of In Vivo Experiments' (ARRIVE) **[66–68]**. There is a distinct lack of similar reporting guidelines for animal research that sits outside of the biomedical field. Given the vast number of horse training studies emerging, it is vital that greater attention is paid to the way in which equitation science research is conducted and reported to ensure the scientific integrity of research in this area is not compromised.

Some initial points for future researchers to consider prior to submitting research are provided in the form of a checklist **(Table 2)**. However, a more formal approach to standardize equine training research reporting across the industry is warranted. The development of more comprehensive guidelines (similar to those used in other industries e.g., ARRIVE guidelines developed for the biomedical field) by a group of industry professionals would likely be beneficial. Similarly, the publication of work that aims to standardize terminology within this specific field, similar to **[69]**, should be encouraged. The promotion of a collaborative approach towards equine training research, for example through the development of a research consortium similar to that of 'ManyDogs' (https://manydogsproject.github.io/) **[70]** aims to conduct reproducible cognition research relating to their target species, would also aid in establishing more consistent and robust equine research practices.

Table 2: Checklist for future research reporting.

Points to consider when reporting horse training-related research
1. Has a full description of the training protocol that is sufficiently detailed to enable study replication been provided? 2. Has information about the individual/s carrying out the training, including their skills level/experience, and their relationship to the horses used in the study, been provided? 3. Has detailed information about the study sample - including horse age, breed, sex, status (*'in foal,'* etc.), how they are usually managed, and the horses' training history (e.g., have they been exposed to a specific training approach before?) - been provided? 4. Have any changes to horses' usual management for the purpose of this study (e.g., whether horses were food deprived or their diet changed prior to training) been clearly stated and explained? 5. Have you outlined how any aversive stimuli have been applied? This includes detailing at what point they are applied, and explaining whether the intensity of the aversive is escalated or maintained throughout its application. Were there any attempts to standardize the way in which aversive stimuli were applied (e.g., use of a pressure gauge)? 6. Where reinforcement techniques were applied, is it clear whether attempts to perform the desired behavior made by the horse were reinforced ('shaping'), or is only the full performance of a desired behavior reinforced? 7. Have you considered whether any other learning principles (e.g., positive punishment, negative punishment) are playing a role in altering the horses' behavior – if so, ensure this is acknowledged within your protocol. 8. If positive reinforcement is being used, has the type and amount (if applicable) of reinforcer been clearly reported? 9. Was the reinforcer assessed to ensure its efficacy in reinforcing behavior (preference/feeding motivation test)? 10. Was a secondary reinforcer used? Was the secondary reinforcer 'pre-conditioned?' 11. Where multiple or 'combined' training approaches are used – has the timing of each approach's application (particularly in relation to the other approaches used) been clearly explained?

4. Conclusion

Ultimately, it is extremely concerning that inconsistencies, and at times potential inaccuracies, were identified within the scientific horse training literature. Not only does this reduce the credibility of the findings which could potentially hinder their translation from research into practice and perpetuate the use of ineffective or unethical horse training approaches, but it may be considered to reduce the overall scientific value of the research conducted. This is a topic that has been extensively discussed, and subsequently regulated, in the animal biomedical research field, but appears to be somewhat overlooked in companion animal behavior and training research despite there being little reasoning to justify why this same level of scrutiny should not be applied to all animal research. Academics in this area frequently highlight 'poor understanding of learning theory terminology' or 'incorrect TA application' as an area of concern within the lay horse-owning community. Yet this review highlights that it would be beneficial for work produced by the scientific community to be viewed with an equally critical eye. Despite the findings of this study, the vast amount of research being conducted in this area is undoubtedly a positive step towards the aim of promoting more ethical equitation and retaining the equestrian industry's credibility and social license to operate. Doing so is essential not only to promote improved human and horse safety during training but also to enable the equestrian industry to demonstrate its commitment to improving equine welfare.

Supplementary Materials

The **supplementary materials** include the categorization of studies within this mapping review.

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Authors' Contribution

All authors jointly conceptualized the study. E.B. developed the methodology, conducted the investigation, collected the data, conducted data analysis, and wrote the paper. E.J.B., J.H., and L.J.C. edited the paper and supervised the study.

Data Availability

The data supporting the findings of this study are available within the article and its supplementary materials.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical Approval

The nature of this project meant that ethical approval was not required as it was a purely desk-based review of existing published work.

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Temporal Trends in Equine Sperm Motility and Semen Volume: A Retrospective Analysis from a Single UK Breeding Facility

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Abstract

The equine industry preferentially selects sires based on pedigree, performance, and conformation, with little concern given to fertility. Increasing evidence supports the theory of geographic-sensitive declines in a range of semen quality parameters, yet the horse is underrepresented within this field. Data presented here retrospectively investigates trends in semen quality from a population of stallions at a single UK breeding facility (from 2001 to 2020). Data on stallion sperm motility (10,686 ejaculates, 984 stallions) and semen volume (11,122 ejaculates, 1,030 stallions) were collected from records during the years 2020 and 2021. Data were analyzed as isolated variables in a linear mixed model (REML). Fixed effects included significant covariates (year of collection, age, and abstinence period). Random effects included stallion and sample numbers. Overall trends indicated that motility has declined over the past 20 years ($p < 0.001$; overall decline: 12.19%). Motility declined similarly in both prime and senescent stallions, confirming trends are not age-specific. Trends in volume ($p < 0.001$) varied over time but typically increased (5.70 mL overall; 0.28 mL annually). Results suggest stallions could be at risk of perturbed reproductive health and function in the future, with serious implications for the economic status of breeding stallions and the health and welfare of breeding stock.

Keywords

Equine; fertility; horse; motility; sperm; semen volume; temporal trends

1. Introduction

Reproductive function, including male factor fertility, is fundamental for the sustainability of a population. Epidemiological studies in humans indicate that sperm quality parameters have declined over recent years [1,2]. In southern India, sperm motility of infertility patients declined significantly between 1993 and 2005 [3]. In a population of human fertile sperm donors from France, where semen analysis methods were consistent, progressive motility declined between 1976 and 2009 [4]. Sperm concentration and normal morphology have also been shown to decline by 13.1% and 46.2%, respectively, between 1970 and 1985 in a population of Holstein bulls [5]. Retrospective research in the dog sentinel model collected from one laboratory indicates a 30% decline in sperm motility between 1988 and 2014 [6].

The equine industry preferentially selects sires based on pedigree, performance, and conformation, with little concern given to fertility [7,8]. A systematic review and meta-regression analysis in global equine populations indicates that sperm progressive motility has declined significantly between 1984 and 2019, with stronger trends reported in western regions [9]. Results from meta-analyses are often heavily scrutinized due to differences in laboratory protocols between semen collection facilities [10,11]. Knowledge of trends in equine sperm quality from a single population is limited. Previous research in France analyzing secular trends in Breton Draught stallions (in the period 1981 to 1996) reported a yearly 1.8% decline in semen volume, although no change was observed in sperm count, and there was a yearly 2.8% increase in sperm concentration [12]. Equivalent findings have been reported in Anglo-Arab Thoroughbred stallions between 1985 and 1995 [12]. An increase in concentration and decrease in volume could be due to their inverse relationship, given that sperm count remained consistent. Subsequent research has reported mean equine seminal volumes below the recommended artificial insemination referencing range of 60–120 mL [12,13], which raises concern over the reproductive health and function of this population.

Semen quality in equine studies to date is impacted by a range of factors, which are not accounted for within previous analyses that determine reproductive trends [12]. Many stallion factors including age [14], inbreeding [13,15], genetics [16], discipline, and exercise intensity [17] impact stallion semen quality. Additional factors include seasonality [18], testicular heat stress [19], abstinence period [20], and nutrition [21]. To develop a robust understanding of semen quality trends, confounding factors must be accounted for within statistical models.

The research presented here builds upon the current methodological limitations associated with evidence syntheses that are reported in previous literature [11], by aiming to explore select equine semen quality parameters through controlled methodological approaches within a single UK-based population. Here, retrospective data on semen quality (motility and volume) was collated during the years 2020 and 2021 to assess reproductive trends within a large population, while accounting for alternative variables such as age and abstinence period that could impact sperm parameters. It is hypothesized that sperm motility and volume will have declined over time, as observed in alternative species.

2. Materials and Methods

2.1. Ethics

The research was approved by the Hartpury University Ethics Committee (ETHICS2019-52). For data collection from breeding records, a site permission form was signed by the facility's owner before data collection. All stallion data were fully anonymized throughout the research process.

2.2. Semen Collection and Analyses

Retrospective data on fresh stallion semen quality was collected from one DEFRA (Department for Environment, Food and Rural Affairs) approved breeding facility in the UK between the years 2020 and 2021. All samples were collected as part of routine breeding management practices (n = 11,722 ejaculates). Stallions utilized within the study were those for fresh collection in standing livery for the stud season between the years 2001 and 2020. Stallion feed and forage were designed to help gain the best semen quality, and stallions were housed in indoor 15ft × 15ft rubberized stallion boxes, with access to stallion paddocks for daily turnout.

Semen collection was carried out using a Missouri Complete artificial vagina (Elite Reproduction Supplies, UK), fitted with a nylon mesh filter to separate the gel-free sperm-rich fraction of the ejaculate from the gel-containing sperm-poor fraction. A teaser mare, phantom, or via ground collection approach was undertaken, as required, to obtain an ejaculate sample, with the collection method factored into the statistical model. Stallion handling and semen analysis methodology were standardized across the study period by one out of two managerial personnel at the facility.

Although the data presented here was collected retrospectively, the approach for analysis was as follows: following the successful collection of the sperm-rich fraction of each ejaculate, which contains the most spermatozoa of the ejaculate, semen was immediately analyzed at the same facility within 30 minutes. The volume of fresh ejaculate (sperm-rich fraction) was calculated by weighing the samples, using the standard conversion of one gram to one milliliter [22]. Before 2012, ejaculate was analyzed neat; however, post 2012, ejaculate was diluted using approximately 20 mL pre-warmed commercial extender, INRA 96 (Stallion AI Services, Shropshire, UK), accepting most stallions have an average concentration of 150-200 million sperm/mL, resulting in an average density of 10×10^6 million sperm/mL. A 10 µl sample of extended semen (sperm-rich fraction) was placed onto a pre-warmed slide (37°C) and covered with a 22 × 22 mm cover slip before being analyzed by phase contrast microscopy for motility (microscope; x100 magnification), subjectively assessing the percentage of sperm progressively moving forwards. While fertility assessment is multifactorial, only sperm motility and volume were assessed here. Supplementary data presents information on sperm concentration. Although sperm concentration data was available, the analysis method changed from a SpermaCue (2001–2012) to a NucleoCounter SP-100 (2013–2020). Low sample numbers, as seen in **Supplementary Table 1**, also supported this data not being presented in this report; however, the figure can be viewed in the supplementary data for reader interest **(Supplementary Figure 1)**.

2.3. Categorization of Variables

Stallions were allocated a numerical code and an ejaculate number as separate variables to account for multiple collections per stallion. Data regarding the date of collection, stallion date of birth, breed, collection method, country of birth, and discipline were collected from corresponding stallion breeding documents and competition records. Breeds were grouped as warmbloods, hotbloods, coldbloods, mixed breeds, and pony types, a recognized method of categorization in equine reproductive research [23]. The reproductive history of the stallions used in this study was not known. Raw data on the 'date of collection' was used to calculate the 'season of collection' and was used in tandem with the 'stallion date of birth' to calculate the 'age at collection.' Samples displaying haematospermia or urospermia were excluded from the dataset due to the detrimental impacts of blood and urine on sperm quality [24,25]. Extreme data outliers were defined as those greater than three times the interquartile range and were removed from the datasets [26]. Data on 11,722 samples from 1,041 stallions of mixed ages and breeds were obtained from records between the years 2001 and 2020. Following outlier removal, the datasets for sperm motility and volume consisted of 10,686 and 11,122 samples from 984 and 1,030 stallions, respectively. The overall sample numbers for stallions and ejaculates included for each year of collection are provided in **Table 1**.

2.4. Statistical Analysis

Data were analyzed using GenStat 17th edition (VSN International Ltd, Hempstead, UK) and graphically interpreted on GenStat and GraphPad Prism 9 (GraphPad Prism version 9.0, GraphPad Software, California, CA, USA). Data were analyzed using a linear mixed model (restricted maximum likelihood; REML). The model assumed that missing data were randomly distributed, enabling the inclusion of all data and preventing bias in estimated values. Stallion and ejaculate codes were included as random effects for all analyses. For fixed effects within the REML model, significance was sequentially investigated. Variable significance and the Akaike information criterion (AIC) value were interpreted to determine inclusion within the final refined model. If $p < 0.05$ and the AIC value did not change appreciably by the variables removal, then the parameter was included in the model [27]. Variables included for each parameter and significance within the statistical model are presented in **Table 2**.

Table 1: Overview of the number of stallions and subsequent ejaculates per parameter, per year.

Sperm Motility					
Year of collection	2001	2002	2003	2004	2005
Stallion/sample number	4/17	31/375	4/23	16/69	52/409
Year of collection	2006	2007	2008	2009	2010
Stallion/sample number	82/660	88/660	94/789	75/559	54/390
Year of collection	2011	2012	2013	2014	2015
Stallion/sample number	82/850	71/581	60/591	89/684	81/762
Year of collection	2016	2017	2018	2019	2020
Stallion/sample number	74/696	93/783	94/788	100/823	30/177
Volume					
Year of collection	2001	2002	2003	2004	2005
Stallion/sample number	4/17	33/388	30/206	54/464	56/433
Year of collection	2006	2007	2008	2009	2010
Stallion/sample number	82/664	89/657	93/760	73/548	54/394
Year of collection	2011	2012	2013	2014	2015
Stallion/sample number	81/832	70/559	58/571	90/674	81/734
Year of collection	2016	2017	2018	2019	2020
Stallion/sample number	73/685	92/757	95/785	100/815	30/179

Table 2: Final variables included within the fixed effects of the REML model for sperm motility and volume.

Parameter	Fixed model
Sperm motility	Year of collection; abstinence period; age; extender; breed; season of collection; country of birth
Volume	Year of collection; abstinence period; age; country of birth; season of collection

The mean (± SEM) was predicted for each year of collection, accounting for covariates included in the statistical model. Predicted means were plotted for each parameter, and a simple linear regression produced to determine the slope. The equation $y = mx + c$ was utilized to determine the overall decline over time. The yearly decline was then calculated by dividing the overall decline by the number of collection years [28]. While means predicted from the REML model accounted for age, further analyses of age-restricted time trends ensured that results were not reflective of aging stallions across the study period. Means plots for motility and volume, against age, were produced. Graphs showed age-based trends in semen quality for each parameter, which were visually interpreted. Based on the variability or trend observed, stallions were grouped as reproductively prime or senescent on an individual parameter basis. For the age range at which the semen quality parameter remained consistent, stallions were grouped as reproductively prime. For those presenting more variability, or a decline in the semen quality parameter based on increasing age, stallions were subsequently defined as reproductively senescent. Isolated REML analyses were then utilized to determine differences in time trends between age groups. A p-value of < 0.05 was considered statistically significant, and a 95% confidence interval was assumed for all analyses.

3. Results

The predicted mean (± SEM) outputs for motility and semen volume, for each year of collection, are presented in **Table 3**. These data were predicted from the REML model.

Table 3: The predicted mean (± SEM) outputs for sperm motility and semen volume, for each year of collection. Data were predicted from the REML statistical model.

Sperm Motility (%)					
Year of collection	2001	2002	2003	2004	2005
Predicted mean	47.64 ± 2.98	48.84 ± 2.63	49.29 ± 4.95	51.54 ± 2.75	47.76 ± 2.23
Year of collection	2006	2007	2008	2009	2010
Predicted mean	51.49 ± 2.18	51.95 ± 2.13	54.57 ± 2.11	58.14 ± 2.09	55.79 ± 2.17
Year of collection	2011	2012	2013	2014	2015
Predicted mean	52.01 ± 2.05	50.36 ± 2.03	47.25 ± 2.02	45.17 ± 1.98	41.55 ± 1.97
Year of collection	2016	2017	2018	2019	2020
Predicted mean	42.02 ± 1.96	41.98 ± 1.93	37.25 ± 1.93	37.43 ± 1.92	42.83 ± 2.17
Volume (mL)					
Year of collection	2001	2002	2003	2004	2005
Predicted mean	38.98 ± 5.04	43.17 ± 3.57	40.68 ± 3.47	35.17 ± 3.16	36.93 ± 3.14
Year of collection	2006	2007	2008	2009	2010
Predicted mean	35.43 ± 3.08	39.08 ± 3.00	38.68 ± 3.01	33.21 ± 2.98	33.62 ± 3.17
Year of collection	2011	2012	2013	2014	2015
Predicted mean	39.36 ± 2.93	44.33 ± 2.90	46.10 ± 2.89	44.17 ± 2.83	50.96 ± 2.82
Year of collection	2016	2017	2018	2019	2020
Predicted mean	42.56 ± 2.80	45.57 ± 2.74	46.32 ± 2.75	44.06 ± 2.74	39.03 ± 3.36

3.1. Time Trends in Mean Sperm Motility between 2001 and 2020

Motility varied over time ($p < 0.001$), declining between 2001 and 2020, with a more substantial decrease detected from 2009. Applying a trend line, sperm motility declined by 12.19% over the entirety of the study period, with a yearly decline of 0.61% **(Figure 1a)**. Focusing on the trends from 2009 to 2019 where there was a rapid decline, motility fell from 56.20% (2009) to 35.20% (2019), suggesting an overall decline of 1.90% per year (20.93% over the 11 years). Prime and senescent age groups were defined based on the variability of data (Prime: 2–17 years; n = 8,374 samples; n = 735 stallions and 'Senescent': 18–31 years; n = 1,211 samples; n = 79 stallions). This resulted in two categorization groups for sperm motility. Stallions aged between 2–17 were analyzed as prime, and those aged 18–31 analyzed as senescent. Overall, motility declined in both age groups (prime: 14.34%; senescent: 24.13%) with a yearly decline of 0.72% (prime) and 1.21% (senescent) between the years 2001 and 2020 **(Figure 1b)**.

3.2. Time Trends in Mean Semen Volume between 2001 and 2020

Means (± SEM) predicted from the model indicated that semen volume increased by 5.70 mL between 2001 and 2020, with an annual increase of 0.28 mL (REML: $p < 0.001$; **Figure 2a**). Following the analysis of time trends in volume, age-restricted trends were determined. Prime and senescent age groups were defined based on the variability of data. This resulted in the following categories for volume whereby stallions aged between 2-25 were analyzed as prime (n = 9,757 samples; 818 stallions), and those aged 26–31 were analyzed as senescent (n = 79 samples; 7 stallions). When assessing the overall trend, volume increased for prime stallions between the years 2001 and 2020 (**Figure 2b**; 10.13 mL overall). While volume declined for senescent stallions (17.85 mL overall), distinct variability in this parameter was noted across the study period.

4. Discussion

This retrospective study contributes significant data towards the equine breeding sector and supplements the debate on adverse trends across species. To our knowledge, this is the first retrospective study to present results on fresh equine sperm motility trends, displaying findings that could have distinct implications for the global equine industry. It is essential to account for specific industry and species-specific factors when determining semen quality trends in sentinel species. From an industry practice perspective, horses are used for sporting and competition purposes, the intensity and discipline of which may influence semen quality and other reproductive parameters. Failure to include such variables within analyses is a primary source of critique in human-based evidence syntheses in reproductive trends **[10]**.

Sperm motility is a fundamental parameter for the assessment of the fertilizing capabilities of semen samples in humans **[29]** and forms part of the stallion breeding soundness examination **[30]**. Furthermore, sperm motility is reported to be the most correlated kinematic parameter with equine per-cycle pregnancy rate **[31]**. In cooled semen, the threshold values of sperm motility for embryo recovery rate are > 65% **[32]**. According to earlier research, stallions with high and low fertility have been defined as those with fresh motility values of 73 ± 11% and 63 ± 17%, respectively **[33]**. All predicted motility values within the current study were below the threshold for both high and low fertility individuals, raising concern over the fertilizing capabilities of the current and future equine population. Given this study is retrospectively assessed, pregnancy data were not analyzed but future work should assess this association. Given the lack of standardization across the industry, accepted thresholds for stallion semen quality parameters including motility make it challenging to predict the true implications of values presented within this study. While findings show an overall decline in motility, from 2009 this decline was substantial and the 20.90% drop over 11 years presents a distinct cause of concern. The reason behind this increased drop is unknown. Research in other species suggests external factors to be a cause as the observed change is too sudden to be a result of genetic mechanisms **[6]**. Our hypothesis that sperm motility will have declined over time is supported from these findings.

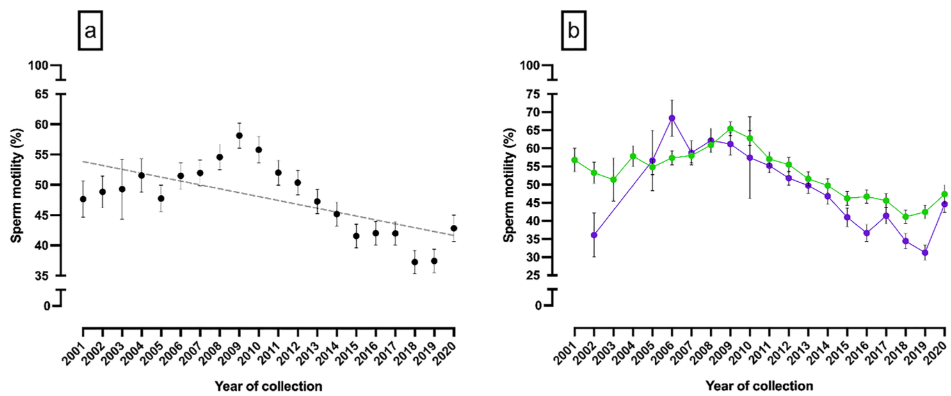

Figure 1: Time trends in mean sperm motility parameters between 2001 and 2020. (**a)** Sperm motility across all samples (%); (**b)** Sperm motility, age restricted (%). Each point represents the mean predicted value for that year. Error bars = ± 1 SEM. The black line in denotes regression slope (simple linear) of predicted means **(a)**. **(b)** Green points denote 'prime ages' and purple points denote ages classed as 'senescent.' Graphs produced on GraphPad Prism version 9.0, GraphPad Software, California, CA, USA. The equation y = mx + c determined overall trends; **(a)** y = -0.6421X + 1339 (2001-2020; graphically plotted), y = -2.093X + 4261 (2009 – 2019): **(b)** y = -0.7553X + 1572 (prime); y = -1.270X + 2605 (senescent).

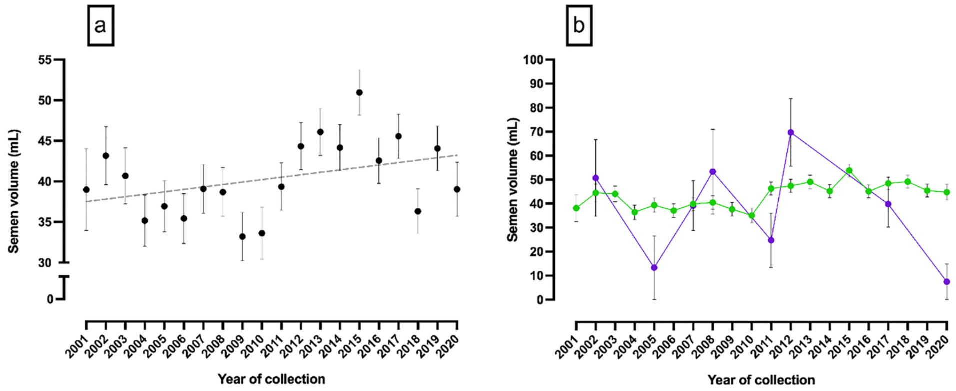

Figure 2: Time trends in mean ejaculate volume between 2001 and 2020. **(a)** Ejaculate volume across all samples (mL) **(b)** Ejaculate volume, age restricted (mL). Each point represents the mean predicted value for that year. **(b)** Green points denote 'prime ages' and purple points denote ages classed as 'senescent.' Error bars = ± 1 SEM. The black line denotes the regression slope (simple linear) of predicted means **(a)**. Graphs produced on GraphPad Prism version 9.0, GraphPad Software, California, CA, USA. The equation y = mx + c determined overall trends; **(a)** y = 0.3003X – 563.4 **(b)** y = 0.5329X - 1028 (prime); y = -0.9925X + 2032 (senescent).

Research presented here is comparable to the canine sentinel model, which also found declining motility over time in fresh sperm samples. A yearly decline of 1.2% was reported in a UK population of dogs between 1988 and 2014 **[6]**. Semen quality declines in carnivorous and omnivorous species were considered more prominent compared to herbivorous populations. In human populations, yearly declines in motility are reported between 0.66% and 1.37% **[34,35]** subject to geographical location.

When considering the method of analysis of motility within the current study, the parameter was assessed utilizing subjective microscopy. While standardization in training for semen analysis was carried out across the study period, subjective analysis methods could introduce a level of variability into the readings provided, a limitation of the current study. Employing a computer-assisted sperm analysis-based approach with standardized settings could have accounted for this potential confounding factor; however, this was outside the scope of this retrospective study. Advancements in semen collection and treatment methods, such as the use of specific semen extenders, may have impacted the results presented, and it is noted that this, in addition to advancing analysis methods, is an inherent limitation of analyzing semen quality trends across time.

Poor semen quality can have significant implications for the economic value of sires within the breeding industry and the ability to maintain desirable heritable traits in the gene pool **[14]**. In certain equine breeds such as the thoroughbred, semen quality may be at risk of the effects associated with inbreeding, given industry selection pressures behind performance and conformation **[36]**. While breed was factored into the statistical model, including five breed categories based on 71 individual breeds, inbreeding was not directly investigated here. Further research analyzing semen quality trends in stallions accounting for differential inbreeding coefficients is suggested, to determine to what extent this factor could influence the trends presented. While etiological causes of declines remain to be determined, the adverse motility trends reported here raise substantial concern over the reproductive health and breeding potential of stallions.

The quality assurance and consistency in semen volume analysis is reassuring, indicating that over the past two decades semen volume has increased, rejecting our original hypothesis that semen volume would have decreased over time. Of note, however, is that the artificial insemination referencing range in the equine industry is recommended at 60 to 120 mL **[13]**. All predicted means for volume fell below the lower bracket of this threshold value. Suboptimal semen volumes, as assessed by volume and not weight, have been reported previously in equine studies **[12,13]**. Employing the concept that 1 g is equivalent to 1 mL, as undertaken within this report **[22]**, results, together with prior publications, could indicate that reproductive aberrations resulting in low volume exist in the wider equine population. While volume is not a direct measure of testicular function, in humans, low volume can be an indication of androgen deficiency, obstruction to the ejaculatory duct, or poor development of the seminal vesicles **[29]**; all reflective of poor reproductive health. Given the findings in human studies, low volume could therefore be concerning for equine fertility; however, this remains to be investigated. Continual monitoring of semen volume is required to maintain a current understanding of trends within this parameter. Given that semen volume is impacted by a number of collection factors, research must standardize collections where possible and include a range of covariates within analyses to produce robust indications of trends in semen volume.

While this study included many variables within the statistical approach, there are some inherent limitations. The time of collection and length of time required for the collection of an ejaculate was not collated. In bulls, it is reported that a greater amount of ejaculate is obtained following increased teasing and morning collections **[37]**. Furthermore, regarding ejaculate collection, a filter approach was undertaken for collection rather than an open-ended artificial vagina. This does mean that it could have been possible for the gel proportion of the ejaculate to mix with the sperm-rich portion, impacting motility. Within this study, we refer to ejaculate volume; however, this was obtained by weighing the samples. As this study was performed retrospectively, we did not assess the specific gravity of equine sperm. The direct relationship between weight and volume hinges on the density of the semen being 1 g/mL **[38]**. Human semen studies report that weight should be an accurate index of volume.

While there are limitations and areas for further study, this comprehensive retrospective cohort study provides fundamental data on temporal trends in sperm motility and semen volume specific to a UK based equine population. Given the high economic importance of stallion fertility, the findings from this study are concerning as sperm motility and ejaculate volume were below recommended industry thresholds. The reproductive histories of the stallions used here are unknown, but it must be acknowledged that poor reproductive function could have significant industry implications, influencing the economic status of breeding stock. Reduced fertility potential is likely to result in additional costs associated with managing stallions with poor semen quality, such as the need for an increased number of collections, coverings, and inseminations required to achieve a successful pregnancy. It is the responsibility of the equine breeding sector to implement practices to optimize semen quality, including integrating fertility into selective breeding programs, enhancing standardization of analysis, and investing in further research determining the effects of external factors upon equine reproductive health and function.

Supplementary Materials

Data on sperm concentration can be found in the **supplementary materials**.

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Authors' Contributions

Conceptualization, I.T.H., R.N.B., K.N., and A.P.; Methodology, I.T.H., R.N.B., and P.H.; Formal analysis, I.T.H., R.N.B., and D.S.G.; Data curation, I.T.H. and R.N.B.; Writing—original draft preparation, I.T.H.; Writing—review and editing, I.T.H. and R.N.B.; Supervision, R.N.B., K.N., and A.P. All authors have read and agreed to the published version of the manuscript.

Data Availability

The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

The study was approved by the Hartpury University Ethics Committee (ETHICS2019-52, 17th March 2020).

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Original Article Open Access

Radiographic Texture of the Trabecular Bone in the Proximal Phalanx of Horses

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Abstract
Trabecular bone is highly dynamic in response to external and internal stimuli, and changes in its structure can be quantified through fractal analysis. However, fractal analysis is still an incipient technique in equine research. This study aimed to evaluate the complexity, heterogeneity, and density of the trabecular bone of the proximal phalanx (P1) of healthy adult horses of different breeds and sexes by measuring the values of fractal dimension (FD), lacunarity, and bone area fraction (BA/TA) in 65 radiographic examinations of the metacarpophalangeal joint and evaluate the agreement between the *BoneJ* and *FracLac* plugins for measuring FD. Regions of interest of 50 × 50 pixels were manually selected on the trabecular bone in the proximal epiphysis of the P1. No differences were observed for FD, lacunarity, and BA/TA between horses of different breeds and sexes ($p > 0.1$). The *BoneJ* and *FracLac* plugins showed no agreement when measuring FD ($p < 0.01$). Therefore, the radiographic texture of the trabecular bone of the P1 in horses had no influence depending on the analyzed breed or sex. The *FracLac* plugin measured higher FD values, and hence standardization using the *BoneJ* plugin is recommended. Further studies are required to evaluate other breeds, age groups, and training levels.

Keywords
Fractal analysis; fractal dimension; bone area fraction; lacunarity; trabecular bone; horses

1. Introduction
Trabecular bone has a complex structure that is difficult to measure accurately [1]. Its fractal nature has been described for decades and has been the target of several studies carried out in humans, mainly to evaluate bone changes resulting from diseases such as osteopenia and osteoporosis [2,3]. Trabecular bone has a higher metabolism and remodeling rate than cortical bone, which makes it more dynamic in response to variations in the magnitude and direction of mechanical loads [4]. This plasticity of internal bone morphology makes trabecular bone a good indicator of changes in bone structure [3,5].

Fractal analysis is a method used to describe complex structures and can be used to study biological systems, such as bone trabecular tissue, considered a natural fractal [2,6]. Fractal analysis is expressed as a fractal dimension (FD) and reflects the degree of complexity of an object, being correlated with the mechanical properties of the bone [7,8]. Lacunarity reflects the heterogeneity of an object, indicating the distribution of gaps in space [8]. The bone area fraction, called BA/TA (or BV/TV for three-dimensional images), is correlated with bone density and indicates the fraction of bone tissue in a two-dimensional image, consisting of a potential determinant of stiffness, elasticity, and trabecular strength [9–11]. Therefore, FD, lacunarity, and BA/TA are used for morphological descriptions of trabecular architecture and are potential indicators of bone quality [1,8].

The quantitative assessment of bone microarchitecture can be performed using two-dimensional digital images, such as radiographic examinations, or three-dimensional images, such as images obtained by computed tomography [12]. The *ImageJ* software is the most used in the medical field for the scientific evaluation of bone tissue images, as it is free, modern, simple to use, and easy to download [13].

Fractal analysis is useful in the clinical and scientific evaluation of horse bone tissue, but it is little studied and used in the species. Similarly, it is not yet known exactly how the trabecular bone architecture may vary in humans according to factors such as sex, age, anatomical region, and body mass, in addition to other (epi)genetic factors. Moreover, there is no methodological standardization for calculating the fractal dimension, which results in high variability of technique and results in studies that use fractal analysis.

This study aimed to measure the values of FD, lacunarity, and BA/TA of the trabecular bone of the proximal phalanx of male and female Pure Blood Lusitano and Brazilian Sport Horse and evaluate the agreement between two plugins for measuring the fractal dimension values. We hypothesize that the radiographic texture of the trabecular bone of the proximal phalanx of horses is different between breeds and sexes and that there is no agreement between the different plugins for measuring the fractal dimension.

2. Materials and Methods

2.1. Data Collection

The study evaluated 15 radiographic examinations of the left metacarpophalangeal joint of 15 female Pure Blood Lusitano (PSL) horses aged between 3 and 6 years old and weighing between 450 and 530 kg, and 50 radiographic examinations of the metacarpophalangeal joint of both forelimbs of 25 male (12/25) and female (13/25) Brazilian Sport Horses (BH) aged between 3 and 4 years old and weighing between 450 and 700 kg. The radiographic examinations were obtained from two studies previously carried out and approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo, under protocols numbers 4119210917 and 8840030417 [14,15]. The horses were evaluated through physical examination of the locomotor system and radiographic examination (lateromedial, dorsopalmar, dorsolateral-palmaromedial oblique, dorsomedial-palmarolateral oblique, and flexed lateromedial projections), showing to be clinically healthy and not presenting lameness or radiographic or ultrasound changes of the metacarpophalangeal joint.

2.2. Definition of Regions of Interest

Radiographic images in JPEG format were processed and analyzed using the *Fiji* image processing package of the *ImageJ* software (National Institutes of Health, USA). Regions of interest (ROIs) of 50 × 50 pixels from the dorsopalmar projection of the metacarpophalangeal joint were defined in the proximal epiphysis of the proximal phalanx, manually selected on the trabecular bone, distal to the subchondral region, medial and lateral to the sagittal groove. ROIs were positioned 30 pixels away from the joint line, centered on a line that ran from the sagittal groove to the lateral/medial end, to standardize their selection. ROIs were cropped, converted to 8 bits, duplicated, and binarized **(Figure 1)**.

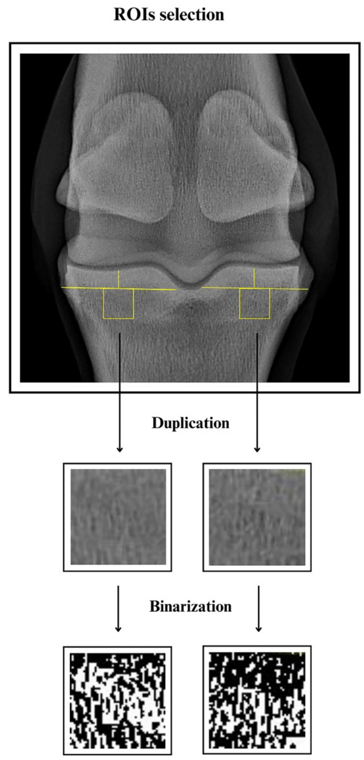

Figure 1: Demonstration of the definition and pre-processing of regions of interest (ROIs).

2.3. Fractal Dimension, Lacunarity, and Bone Area Fraction

Fractal dimension, using the box-counting method, and bone area fraction (BA/TA) of the binary images were measured using the *BoneJ* plugin, using the "Fractal Dimension" and "Area/Volume Fraction" tools, respectively. Fractal dimension and lacunarity were measured by the *FracLac* plugin using grayscale images ("Gray 1: Differential" image type), two scan positions and the differential box-counting method, which is the most suitable method for assessing the texture of objects [8,16–18]. All measurements were carried out by a single previously trained observer.

2.4. Statistical Analysis

Statistical analyses were performed using the Jamovi program (version 2.3.21). The analyzed variables were presented as a single population, being described with the mean, median, standard deviation, minimum, maximum, and 95% confidence interval. Box plots were used to present data separated by sex and breed. The normality of residuals and homogeneity of residuals of variances were analyzed using the Shapiro-Wilk and Levene tests, respectively. Q-Q plots were used to evaluate the data distribution. The Mann-Whitney U test was used to compare within the sex and breed categories. The Bland-Altman test [19] from the *blandr* package [20] was used to evaluate the agreement between the two methods for measuring FD. The one-sample T-test was used to test the hypothesis of similarity to zero of the differences relative to the means of the method data. Values of $p < 0.05$ were considered significant and $p < 0.1$ were considered a trend.

3. Results

3.1. Differences in Trabecular Bone Structure between Breeds

No significant difference was observed between the mean values of FD (calculated using the *BoneJ* plugin), lacunarity, and BA/TA of the medial and lateral ROIs for Brazilian Sport Horse and Pure Blood Lusitano horses ($p > 0.1$) **(Figure 2)**. The descriptive analysis of the data is available in **Supplementary Table 1**.

3.2. Differences in Trabecular Bone Structure between Sexes

A trend for a difference relative to lower mean fractal dimension (*BoneJ*) values was observed in the lateral ROI for male horses ($p = 0.06$). The other variables showed no significant differences between sexes ($p > 0.1$) **(Figure 3)**. The descriptive analysis of the data is available in **Supplementary Table 2**.

3.3. Comparison of Plugins for Measuring Fractal Dimension Values

No agreement was observed between the two plugins for measuring fractal dimension values ($p < 0.01$). The *FracLac* plugin tended to calculate higher values when compared to *BoneJ* (**Figure 4** and **Figure 5**). The mean FD values obtained using *FracLac* were 1.69 ± 0.07 and 1.66 ± 0.08 for the medial and lateral ROIs, respectively. The mean values obtained using *BoneJ* were 1.55 ± 0.04 for the medial ROI and 1.54 ± 0.05 for the lateral ROI **(Table 1)**.

4. Discussion

This study evaluated the fractal dimension, lacunarity, and bone area fraction of horses of different breeds and sexes, as well as the agreement between two plugins for measuring the fractal dimension. The results indicated no difference in the complexity, heterogeneity, and density of trabecular bone between Brazilian Sport Horse and Pure Blood Lusitano horses. Different breeds are subjected to different breeding systems, functions, and training programs. Consequently, horses of different breeds are subjected to different frequencies, magnitudes, and directions of mechanical stress, which cause tension in the bone and remodeling of the external and internal bone structure [3].

The close relationship between bone architecture and mechanical load causes exercise to alter bone density and the orientation of the trabeculae, and studies have suggested that this is a protective mechanism and a normal physiological condition for horses in training [21–28]. Therefore, no difference in the bone trabecular microstructure of horses of different breeds observed in our study can be explained by the similarity in the intensity and frequency of exercises to which these animals were subjected, as Pure Blood Lusitano mares were kept in stables and Brazilian Sport Horses were at the beginning of the training program for show jumping. There is still a lot that we do not understand about the influence of genetics and development on trabecular architecture, but perhaps changes in bone microstructure may be more closely related to the sport modality and training routine of horses than to the breed.

Our results demonstrated no difference in bone microstructure related to sex, except for the fractal dimension of the lateral ROI, which had a trend to differ between sexes ($p = 0.06$), with lower mean values observed in male horses. Bone growth is mediated by hormones at local and systemic levels, and the opposing action of sex steroids in humans is known to be responsible for sexual dimorphism in the skeleton, but the mechanisms underlying sexual dimorphism in cortical and trabecular bone are not well understood [3,4,29]. The main target of testosterone is cortical bone, while estrogens act essentially on trabecular bone and are powerful regulators of bone maturation and structure [30,31]. Circulating estrogen concentrations fluctuate significantly throughout the estrous cycle, as do concentrations of bone formation and resorption markers, which may be associated with altered bone remodeling in mares [29]. Prado Filho and Sterman [32] evaluated bone mineral density in male and female racing foals at the beginning of their athletic activity and found no significant difference between the sexes, which corroborates the findings of this study. On the other hand, Jackson *et al.* (2003) observed sex-associated differences in bone remodeling in racing horses, with males showing higher serum concentrations of biochemical markers of bone formation and resorption than females [33]. Further studies are needed to establish more precisely the influence of sex and sex steroids on horse bone microstructure and its clinical relevance.

Table 1: Descriptive analysis of fractal dimension (FD), lacunarity, and bone area fraction (BA/TA) values.

	Mean	Lower limit	Upper limit	Median	Standard deviation	Minimum	Maximum
Medial BA/TA	0.53	0.5	0.55	0.56	0.11	0.29	0.71
Medial FD (*BoneJ*)	1.55	1.54	1.56	1.55	0.03	1.43	1.61
Medial FD (*FracLac*)	1.69	1.67	1.71	1.7	0.07	1.35	1.76
Medial lacunarity	0.02	0.01	0.02	0.01	0.02	1.00e-4	0.07
Lateral BA/TA	0.49	0.46	0.52	0.47	0.12	0.3	0.74
Lateral FD (*BoneJ*)	1.54	1.52	1.55	1.54	0.05	1.34	1.61
Lateral FD (*FracLac*)	1.65	1.64	1.67	1.66	0.08	1.33	1.76
Lateral lacunarity	0.02	0.02	0.03	0.01	0.02	9.00e-4	0.12

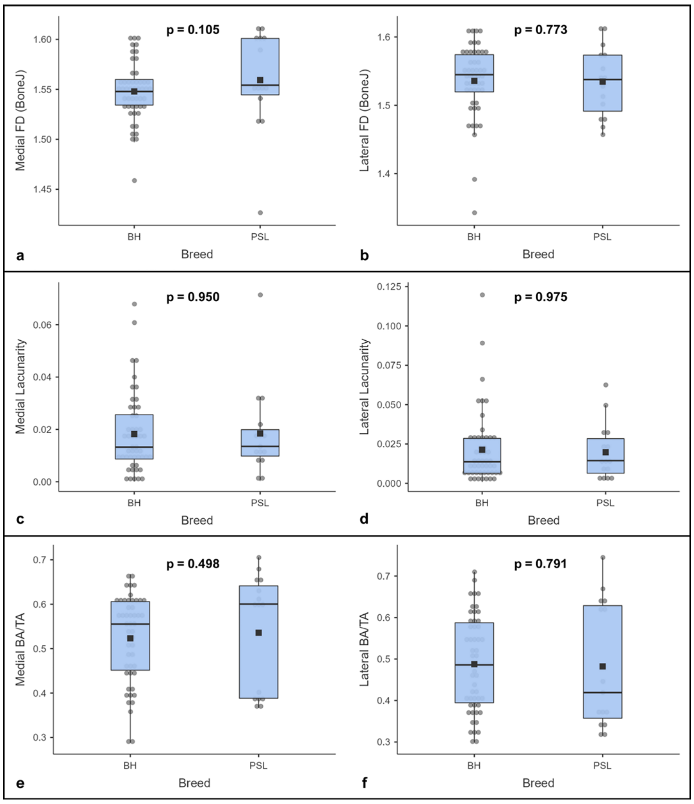

Figure 2: Box plots showing data on fractal dimension (a and b), lacunarity (c and b), and bone area fraction (e and f) of the medial and lateral ROIs for horses of BH and PSL breeds. FD = fractal dimension; BA/TA = bone area fraction; BH = Brazilian Sport Horse; PSL = Pure Blood Lusitano.

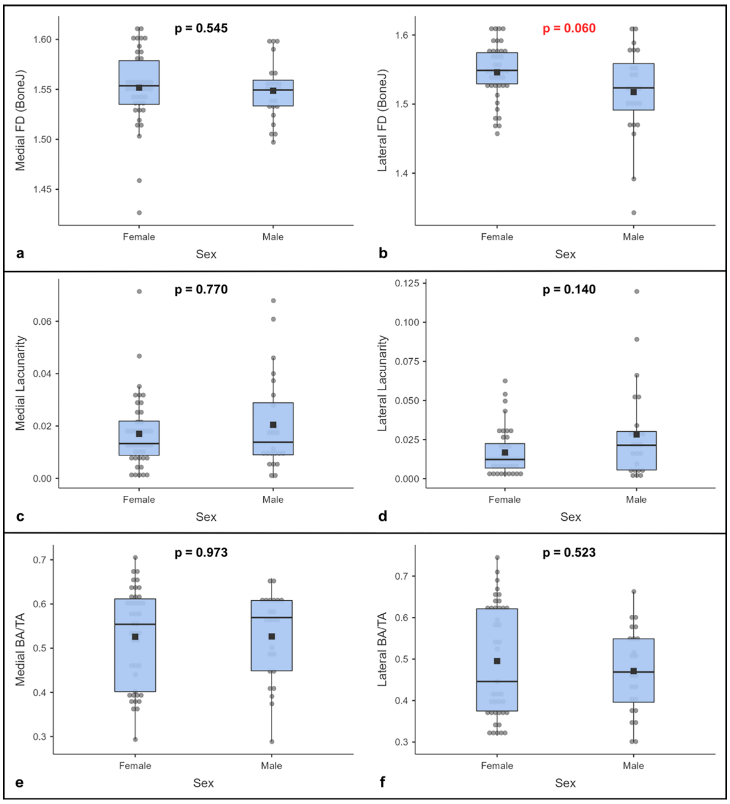

Figure 3: Box plots showing data on fractal dimension (a and b), lacunarity (c and b), and bone area fraction (e and f) of the medial and lateral ROIs for male and female horses. FD = fractal dimension; BA/TA = bone area fraction.

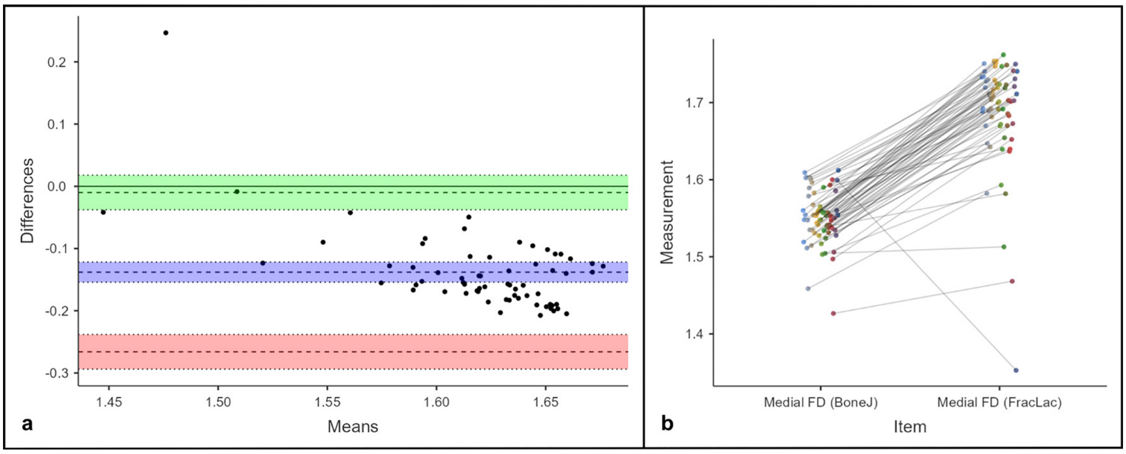

Figure 4: Bland Altman plot (a) and data reliability plot (b) representing the differences between the mean fractal dimension (FD) values of the medial ROI measured by two plugins.

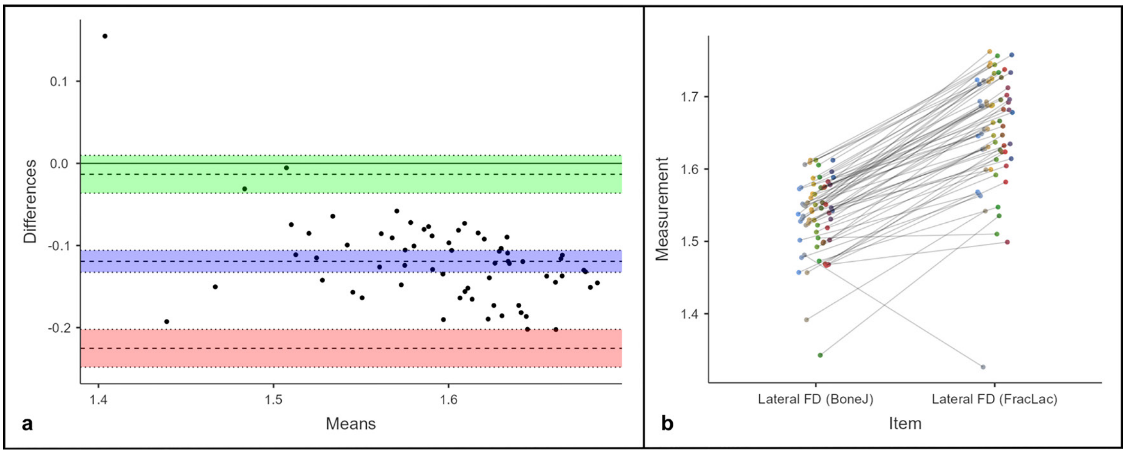

Figure 5: Bland Altman plot (a) and data reliability plot (b) representing the differences between the mean fractal dimension (FD) values of the lateral ROI measured by two plugins.

The values found in our study suggest that the trabecular bone of the proximal phalanx of healthy adult horses presents high complexity, density, and homogeneity, represented by high values of FD and BA/TA and low values of lacunarity. The body of the femur in a study evaluating human trabecular bone presented mean values of 1.2 ± 0.06 for FD and 11.05 ± 4.38 for BA/TA [34]. The subchondral trabecular bone of the femoral head in individuals with severe hip osteoarthritis presented lower FD and higher BV/TV values (1.05 ± 0.02 and 36.97 ± 10.39, respectively) than control individuals (1.10 ± 0.05 and 28.09 ± 0.06) [2]. Women with osteoporotic fractures have lower FD of the cortical bone of the femoral neck (2.11 ± 0.01) than women with hip osteoarthritis (2.57 ± 0.01) [8]. A systematic review that evaluated the use of fractal analysis in dental images found FD values that ranged from 0.78 to 2.79 for panoramic radiographs, 0.78 to 1.84 for periapical radiographs, and 0.91 to 2.4 for cone-beam computed tomography [12]. Yaşar and Akgünlü [35] evaluated dental radiographs of edentulous and dentate regions and observed that the edentulous regions had higher FD values (1.65 ± 0.08) and lower lacunarity values (0.34 ± 0.05) than dentate regions (1.36 ± 0.12 and 0.41 ± 0.06, respectively), possibly due to regional anatomical differences and occlusal loads. Foals presented BA/TA of 26.7 ± 4.6 for the trabecular bone of the parasagittal groove and 31.0 ± 4.4 for the condyle of the third metacarpal bone, while adult horses (>6 years) presented values of 56.6 ± 1.7 and 62.9 ± 5.4, respectively, demonstrating an increase in bone fraction with age and training [21]. These studies showed that the values of FD, lacunarity, and BA/TA (analogous to BV/TV) varied according to the methodology, such as the imaging modality (2D or 3D), image processing, ROI positioning (cortical, subchondral, or trabecular bone), and anatomical region, and the characteristics of the evaluated individuals, such as age, level of sporting activity, and clinical changes.

In order to perform fractal analysis, image pre-processing is necessary to standardize images and reduce artifacts by removing large-scale variations in image brightness, which can be caused, for example, by overlapping soft tissues. Many authors follow the methodology detailed by White and Rudolph [36], where a sequence of steps is carried out to assess trabecular bone, using *ImageJ* software [12,36]. The *BoneJ* and *FracLac* plugins automate image pre-processing, reducing the number of steps required for standardization, making it easier and faster. The several methods for calculating FD often result in obtaining different dimensions for the same object [37]. Ensuring standardization in the methodology of studies that analyze biological images is essential to guarantee reproducibility in research and a more accurate comparison between studies, as well as minimizing inconsistencies related to the object of study and facilitating applicability in clinical routine.

Our study found a non-equivalence between FD measurements performed by the *BoneJ* and *FracLac* plugins, using the box-counting method, which is considered easily accessible and is the most frequently used for fractal analysis [12]. In this method, grids of decreasing size are scanned over a binary image, and the number of boxes containing at least one foreground pixel is counted. As the box size decreases, the proportion of boxes with foreground pixels increases in a fractal structure. In short, the complexity of a structure is assessed by the change in detail with scale. While *BoneJ* measures pixels in a binary image, which can have one of two possible values (background or foreground/black or white), *FracLac* uses the differential box-counting method to measure pixels in grayscale images, which can have one of many possible values. Thus, *FracLac* finds the FD and lacunarity in grayscale images by counting the average pixel intensity per box, based on the relationship between the change in average intensity and the change in grid caliber [38]. We recommend standardizing FD measurements using the *ImageJ* software, as it is more widely used and better-established in the literature, and *BoneJ*, as it is a well-established plugin that brings together other important tools for assessing bone histomorphometry, such as trabecular thickness, trabecular separation, trabecular number, bone volume fraction, bone surface fraction, osteoclast activity, and degree of anisotropy, which allows quantitative evaluation of bone microarchitecture, bone formation, and bone remodeling [39–41].

Fractal analysis has been used to evaluate bone tissue in a wide variety of studies, such as bone functional adaptation [3,4,9,21,23,26,42,43], bone quality [10], osteopenia [44], osteoporosis [8,45–48], osteoarthritis [1,2,17], rheumatoid arthritis [49], osteointegration [50,51], bone failure analysis [16], and fibrous dysplasia [52]. Therefore, fractal analysis uses resources accessible to veterinarians, such as radiographs, to provide information with potential clinical application, diagnostic and prognostic value, which can help in the understanding of clinical cases, decision-making, therapeutic planning and evaluation of the evolution of treatment, as well as enabling early evaluation of microscopic alterations that precede late clinical manifestations.

We believe that our research can contribute to the emergence of new studies in the area of equine orthopedics that use fractal analysis for bone assessment in the species, such as for the assessment of bone quality, changes in the trabecular structure related to physiological and pathological conditions, clinical treatments and surgical procedures, standardization of research groups, assessment of bone adaptation to exercise, and prediction of fracture risk, among others. Our study evaluated the trabecular bone of the proximal phalanx of horses without clinical, radiographic or ultrasound alterations in this anatomical region, and the data obtained can be used for comparison with other individuals, provided that the same methodology is applied.

The use of radiographic images to evaluate the trabecular bone is among the limitations of this study. Despite the advantage of non-invasiveness and the high availability of retrospective data, radiographs are two-dimensional images that represent a three-dimensional trabecular structure and, therefore, are not accurate representations of the trabecular structure due to limitations imposed by this imaging modality, such as the resolution [3,6]. As the amount of information obtained is greater as image quality increases, studies suggest that 3D images are more suitable for fractal characterization of trabecular bone [7,53]. However, unfortunately, equipment for obtaining 3D images, such as computed tomography and magnetic resonance imaging, is still not widely available in the routine of equine veterinarians, which is why we used radiographic images as the object of study.

In conclusion, breed and sex have no significant influence on the values of fractal dimension, BA/TA, and lacunarity of the trabecular bone of the proximal phalanx of horses. The exercise routine to which the horse is subjected is assumed to have a higher influence on the trabecular bone structure than the breed. New studies evaluating other breeds, age groups, horses from different sports, and 3D images can be considered. Fractal dimension quantification using the *BoneJ* and *FracLac* plugins of the *ImageJ* software are not equivalent. Standardizing the measurement of fractal dimension values using the *BoneJ* plugin and detailing the methodology used in future studies are recommended, as these procedures have an important influence on the values of FD, lacunarity, and BA/TA.

Supplementary Materials

The tables of descriptive analysis of FD, lacunarity, and BA/TA values separated by breed and sex are available as **Supplementary Materials**.

Authors' Contributions

Literature review: L.O.P.; Methodology: A.F.S., D.R.A.S., and L.O.P.; Data collection: L.O.P.; Statistical analysis: A.F.S.; Writing: L.O.P.; Review: A.F.S., A.L.M.Y., D.R.A.S., and A.L.V.Z. All authors read and approved the final version of the manuscript.

Data Availability

Data supporting the conclusions of this study are available upon request from the corresponding author.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Ethical approval was not required to conduct this study as retrospective data was used.

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A Preliminary Study on Feeding Straw to Horses and Its Effects on Equine Chewing and Consumption Rates

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Abstract

Straw as a forage source has been investigated with regards to managing obese horses. However, its effect on chewing rate (CHR) and consumption rate (CR) lacks convincing evidence to encourage its inclusion. In this 2×7-day crossover study, the CHR (chews/5 mins) and CR (kg/hour) of eight horses were analyzed for differences in response to two diets: 50% oat straw, 50% haylage (S) and 100% haylage (CON). On Day 1, CR with S was lower compared to CON ($p > 0.05$), and by Day 7, this decrease was statistically significant ($p = 0.018$). CHR was lower on S on Day 1 than CON but higher on Day 7 ($p > 0.05$). The results suggest that oat straw provided at 50% of the forage ration slows consumption and alters chewing behavior in favor of managing obese horses. Adding oat straw to the forage diet can potentially improve the welfare of horses with low-energy requirements.

Keywords

Obesity; chewing; straw; satiety; forage; consumption

1. Introduction

Equine obesity has been a significant and challenging welfare concern for decades [1]. Restricting daily dry matter (DM) intake (DMI) is the most common method to induce weight loss [2], but this may compromise the horse's well-being/welfare [3,4]. Owners often find it difficult to adhere to strict management regimes and find it hard to identify and locate lower-energy grass-based forages [5]. Additionally, a horse's need to chew [6] and inability to self-regulate forage intake [7] make obesity prevention increasingly difficult to sustain.

It is recommended that horses perform natural feeding behavior for at least eight hours daily [8], spread across several meals to ensure fasting periods do not exceed four hours [9]. When periods without forage exceed six hours, it poses a significant risk to the development of EGUS [10]. Therefore, the forage requirement is strictly advised to reach a minimum DMI of 1.5% body weight (BW) per day to aid continuous foraging and reduce the risk of gastrointestinal disease and behavioral issues [8]. However, it can become difficult to balance continuous forage provision with energy content, even at this level of intake, as it can often exceed some horses' energy requirements. Therefore, slow feeders such as haynets and grazing muzzles are used to slow consumption and increase foraging opportunity [11,12]. Nevertheless, these may still have undesirable consequences such as musculoskeletal injury, refusal to eat, frustration due to unnatural feeding mechanisms, and increased poll pressure [6,13]. This is encouraging new research to find alternative methods that allow owners to feed more forage (i.e., 1.5-2% of BW in DM per day) to increase chewing opportunity and feeding frequency, without increasing the rate of intake or the risk of obesity.

Recent investigations indicate that the inclusion of straw as a partial hay or haylage replacement could promote equine welfare due to its high-fiber, low-calorie composition. The high neutral detergent fiber (NDF) content in straw reduces digestibility and palatability, thus may also decrease CR and CHR [14], resulting in prolonged feeding and reduced daily calorie consumption. Prolonged feeding increases chewing opportunity, which is believed to increase salivary secretion to buffer stomach acid [15] and promote satiation [16]. This may help horses feed on the same ration over a longer period, without requiring frequent top-ups, thus reducing daily calorie intake. Extended feeding periods by slowing consumption have been linked to slower passage rates and greater nutrient digestibility on straw diets [17]. Straw may also reduce insulinaemic responses, as was shown in the study by Jansson *et al.* [16] on a 50% straw and 50% haylage forage ration. However, it should be noted that replacing part of the forage diet with straw must consider additional vitamin and mineral supplementation as it is less nutritious than hay and haylage.

Presently, there is some reluctance among horse owners to include straw in rations due to a previous study associating straw with an increased risk of gastric ulcers [10]. The increased risk was associated with straw being the sole or predominant forage source, but this detail is missed by many. Conversely, recent studies found that 50% of straw-based rations did not cause gastric ulcers [16], promoted weight loss, and increased consumption time [16,18]. From these findings, Jansson *et al.* [16] proposed it is safe to feed straw, restricted to a maximum inclusion rate of 50% (as a percentage of the total daily forage ration).

There are also concerns about the risk of colic when horses are fed straw, predominantly in those with poor dentition or due to straw's poor hygiene quality. Poor hygiene quality is believed to increase digestive demand on the large intestine; however, it has only been anecdotally mentioned as a risk factor for feeding straw, with few studies evaluating this directly [19–21]. Additionally, the coarse texture of straw making it difficult to break down for horses with poor dentition has deemed it an unsuitable food source for many horses; Hammar [22] and Ralston *et al.* [23] have shown associations between poor dentition and poor digestibility, and consequently colic, but this has not been studied using straw forage. Thus far, most concerns regarding colic when feeding straw forage are assumptions based on situations where the forage was either introduced too quickly or the diet consisted of more than 50% straw. Studies [17] and [24], and more recently, [18] and [16] did not report incidences of colic during or after their studies, despite feeding straw diets up to 50% inclusion. In these studies, the inclusion rate was restricted to 50%, the forage was introduced slowly, and horses with dentition issues were excluded.

Existing research on straw forage is still limited, and inconsistencies in methods and variables have resulted in confusing and conflicting outcomes and communications about the safety and efficacy of feeding straw. Further investigation into how straw affects feeding behavior will provide greater insight into its suitability as a forage source for equines. The aim of the study was to evaluate how replacing 50% of the haylage ration with oat straw affects CHR and CR. It was hypothesized that the inclusion of straw would decrease CHR and increase the time spent foraging, thereby reducing CR.

2. Materials and Methods

2.1. Ethical Approval

To comply with the ARU Writtle ethical guidelines, an animal welfare monitoring protocol was established. Any horse used for the study was not to be subjected to pain and must be free of illness or pain (including colic and gastric ulcers) and up to date with dental examinations before commencing the investigation. Any horse refusing to eat during the study or showing sudden changes in behavior or stress had to be removed. All horses had to remain on the same daily routine to avoid additional stress factors. The ethical approval was granted under the number 1426.

2.2. Animals, Diets, and Experimental Design

Eight horses (age 14.88 ± 6.24 years old; weight 602.75 ± 53.49 kg; height 161.28 ± 8.31 cm) free from gastric ulcers, dental issues, colic, and other gastrointestinal diseases for the last 12 months were used for the study. The sample size was calculated using a formula (E = number of horses × number of groups - 2) to determine the maximum and minimum number of horses that could be recruited to ensure the study remains ethical. Eight horses were considered an appropriate sample size.

Prior to the study, all horses had been at the current premises for at least one year. All horses were only accustomed to a haylage diet. They had not been fed or bedded on straw before the study. Horses were housed in their usual single 3m × 3m stalls with rubber matting and shavings bedding overnight (17:00-11:00). Management routines remained unchanged from routines before the study period, and horses received daily group turnout for three to six hours (11:00-17:00) in bark paddocks. Water was provided ad libitum (AL). No additional feed or forage was available during turnout. The study diets were provided at the horses' usual feeding times, twice daily at 8:30 and 16:30 from haynets (4.5 cm openings), and once data collection was completed, they still had access to the remaining ration left in the haynet or were topped up if needed to fulfill their daily DM requirements. All horses were habituated to eating from the haynets used in the study. Due to the nature of a university yard setting, all attempts were made to ensure feeding times and data collection remained during hours when environmental disturbances were minimal. The study was performed as a 2×7-day crossover study with an initial acclimation period of two days **(Figure 1)** consisting of 75% haylage and 25% oat straw, to facilitate a smooth introduction of the study diet [16]. The choice of straw was informed by typical practice and availability. In the UK, oat straw is more widely available and more frequently used in horse feeds, like chaffs, in comparison to barley or wheat straw. It is also considered easier to handle and more digestible for horses, potentially making it a more suitable forage substitute. Horses were randomly divided into Group A (n = 4 horses) and Group B (n = 4 horses) using a computer-based random order generator. Each group received both study diets in different periods with each individual horse serving as the experimental unit. Diets fulfilled an average daily DMI of 1.71% (S) and 1.35% (CON) BW. The difference in DMI was due to the higher DM content in the oat straw and lower DM content in the haylage. As horses were required to be fed the same total daily forage quantities in the study diets that they received prior to the study, the rations could not be adjusted to be iso-caloric. The haylage and straw used during the study underwent wet chemistry and NIRS analysis [25] to determine their composition **(Table 1)**, as done in previous studies [16–18,24,26]. When preparing the forage rations, each haynet containing S was weighed regularly using a portable scale to ensure thorough mixing and an equal divide of the straw and haylage proportions of the ration.

2.3. Measurements

On Day 1 and Day 7 of each period, the BW, CHR, and CR were measured. BW was measured using a Horseweigh weighbridge before feeding at 7:30 at the beginning and end of P1 and the end of P2. The author (NM) was responsible and aware of the group allocation and conduct of the experiment and the data analysis, and therefore could not be blinded to the treatments.

2.4. Chewing Rate

Chewing was measured by visual observation of horses eating according to established methods previously described [6,27,28]. The methods described in these studies all consisted of manually counting chews for 1-to-10-minute intervals. Mueller *et al.* [27] also measured chewing rate at the start and end of a one-hour eating bout, which was not done in the other two studies. An attempt was made to replicate this in the present study by randomizing the order of counting chews.

In the present study, four horses were filmed for five minutes each between 8:30 and 9:30. The remaining four horses were filmed between 9:30 and 10:30. This was done to allow horses to become accustomed to the forage rations as it was anticipated that chewing frequency could be greater when forage was first presented after the overnight period where a period without forage is likely to have occurred. The order of filming horses eating the forage was randomized using a computer-based random order generator, with each horse being filmed at a different time for each data collection day. Filming took place outside of the stables to reduce disturbance.

CHR (chews/min) was counted while watching the replay of the videos for each individual horse using a Hand Tally Digital Click Counter (FEBSNOW). One chew was counted as either one leftward or rightward circular jaw movement, depending on individual chew laterality [29]. One chew corresponded to one click on the counter. Jaw movements associated with searching for forage, drinking, crib biting, and turning haynets around were not counted as chews [30]. When horses showed such behaviors for more than two seconds, the video recording was stopped and resumed when the horse returned to eating. Chews were counted for one minute and then the counter was reset before resuming counting at two minutes for another minute. This was repeated for a total of five minutes for each horse. Each minute of chewing was counted three times, and an average was taken to reduce human error during counting.

2.5. Consumption Rate

Consumption was measured using techniques described by Ellis *et al.* [6] and Glunk *et al.* [11]. Both studies removed the haynets from the stables before weighing, which was replicated in the present study. However, unlike the aforementioned studies, it was not feasible to repeat this process in the evening or measure CR for longer than two hours in the present study, but the horses were still fed the study diet rations throughout the night.

In the present study, all haynets were weighed before administering to the horses. As soon as all horses received their forage rations at 8:30, a one-hour timer was started. The CR was measured by removing all haynets from stables after one hour (at 9:30 exactly) to be weighed. This prevented some horses from receiving additional time to consume their forage. Before weighing the forage rations, the scale was calibrated using an object of known weight (1kg Equiblox Hi-Fiber block) to ensure accurate weighing of the forage. After recording weights, the haynets were put back in stables, another one-hour timer was started, and the process was repeated for the second hour.

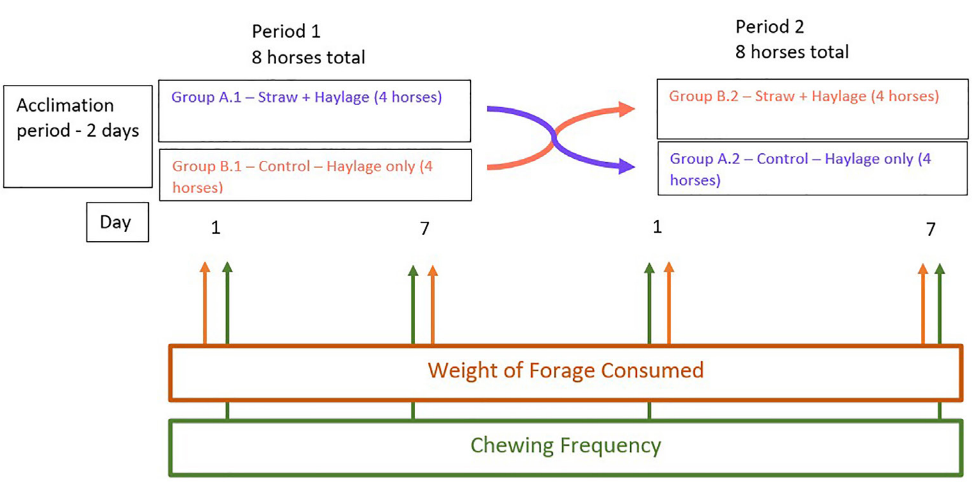

Figure 1: Illustration of the crossover study design (four horses in Groups A and B, respectively, that shifted diets from Period 1 (P1) to Period 2 (P2)). Days for data collection in each period are indicated by arrows and numbers. Periods followed each other with no washout period.

Table 1: Dry Matter (DM), energy, and nutrient content of the haylage and oat straw forages used in the study diet.

Dietary component	Haylage	Oat straw
Dry matter (%)	59.20	90.60
Crude protein (%)	13.20	3.60
Water-soluble carbohydrate (%)	5.10	2.18
Starch (%)	-	1.2
Neutral detergent fiber (g/kg)	56.60	59.60
Acid detergent fiber (g/kg)	33.70	34.70
Digestible energy (MJ/kg)	9.3	9.4
Metabolizable energy (MJ/kg)	8.1	6.5

2.6. Statistical Analysis

Data was first transferred to Microsoft Excel to produce descriptive statistics and subsequently analyzed by IBM SPSS Statistics (version 28, 2021) following data collection. Feed intake measurements included total chews in five minutes and consumption (weight in kg) of forage consumed per hour, both of which were analyzed as rates. All horses in Group A and Group B were subjected to both diets and the aim was to compare the diets. The data was split into Day 1 and Day 7, representing the first day and last day of administering the diet. For Day 1, CHR was calculated by averaging the number of chews/5 mins for each horse in Period 1, Group B and Period 2, Group A (for CON diet) and Period 2, Group B and Period 1, Group A (for S diet). The same was repeated using a separate dataset for Day 7. This process was repeated for CR. Period 1 and Period 2 only represented the duration of diet administration and illustrated the switching of diets between Group A and Group B.

The horses were included in the study if they continued to consume their forage for the total duration of the study. If horses showed negative health signs such as colic symptoms or refusal to eat, they were removed from the study and their data was excluded from analysis. As all eight horses did not show these behaviors, they were included in the analysis. A normality test (Shapiro-Wilk) was run to determine whether the data was parametrically or non-parametrically distributed. Parametrically distributed data was analyzed using a paired samples t-test. Non-parametrically distributed data was analyzed using Wilcoxon's Signed Rank test. A Pearson's Correlation test was run to determine the correlation coefficient between CR and CHR due to parametrically distributed data. For this test, chewing was extrapolated to a rate of chews/hour for a correct comparison. Statistical significance was assumed when $p < 0.05$.

3. Results

Data are represented as mean ± standard deviation unless otherwise stated. There were no outliers in any of the data, as assessed by visual inspection of boxplots. None of the horses were excluded from the data analysis. Shapiro-Wilk normality tests were used for all variables.

3.1. Chewing Rate

On Day 1, horses on CON had a higher chewing rate (317.25 ± 27.65 chews/5 mins; range: 285–353) than horses on S (308.63 ± 20.80 chews/5 mins; range: 274–341). This increase in CHR was not statistically significant, $t(7) = -0.868$, $p = 0.414$. On Day 7, horses on S had a higher CHR (322.38 ± 33.39 chews/5 mins; range: 264–367) than horses on CON (317.25 ± 31.78 chews/5 mins; range: 287–381), but this was not statistically significant, $t(7) = 0.282$, $p = 0.786$ **(Figure 2)**.

3.2. Consumption Rate

On Day 1, horses on CON consumed their forage ration faster (1.72 ± 0.59 kg/hour; range: 0.50–2.50) than horses on S (1.53 ± 0.45 kg/hour; range: 0.75–2.25); although, statistically this was only a trend, $t(7) = -0.917$, $p = 0.390$. On Day 7, CON elicited a statistically significant median increase in CR (Median = 1.65 kg/hour; range: 1–3) compared to S (Median = 1.00 kg/hour; range: 0.50–1.50), $z = 2.371$, $p = 0.018$. For comparison to Day 1, CR was plotted on the graph as a mean instead of a median **(Figure 3)**.

4. Discussion

4.1. Chewing Rate

This is the first study, to the authors' knowledge, to examine CHR on a 50% straw forage diet. The results indicate that CHR may be affected by the inclusion of oat straw due to the numerical differences between Day 1 and Day 7 on S, while CHR on CON remained constant. There are a few possible explanations for these findings, one being the novelty of the straw forage. Horses avoid unpalatable, indigestible, or poisonous plants and rely on olfaction to explore new scents and select suitable plants before ingestion **[31]**. Stachurska *et al.* **[32]** found that novel feeds were smelled for longer and more frequently rejected by horses. In the present study, oat straw probably had a novel odor as horses were not accustomed to eating it before the study. This may explain why horses were observed to spend less time chewing and more time searching for the more palatable haylage. Van den Berg and Hinch **[33]** suggested it takes three to four days to reduce variability in a horse's reaction to novel feeds. This could explain why CHR on S increased on Day 7, as oat straw was no longer a novelty.

Six horses were also observed to engage in more exploratory behaviors when straw was fed. These included flipping the haynet around, taking more pauses between feeding, and increasing periods of smelling the ration. They were performed intermittently, and no chewing occurred during this time. This may have been performed in search of the more palatable haylage, as a form of mental stimulation, or due to frustration. Several studies suggested that straw can provide mental stimulation as increased exploratory behaviors can indicate a motivation to search for alternative resources **[34–36]**. This may also be interpreted as frustration because of a reduction in diet palatability. However, this is less likely as the horses did not exhibit other behaviors indicative of frustration (i.e., muscle tension, aggression, displacement, stereotypies) **[37]**. Even if an increase in exploratory behaviors, rather than the composition of the straw itself, is the main cause of the decreased CHR, this effect is still beneficial as it slows intake and increases mental stimulation by allowing the horse to sort through the forage, mimicking what they do in the wild or in pasture. This indicates straw can elicit beneficial effects beyond just feeding and may be a source of enrichment.

Hunger level is another factor that could have promoted a higher CHR on S on Day 7. CHR was determined from the first five minutes of forage consumption in the morning when horses are likely to be hungrier. Other studies agree with this hypothesis, stating that increased hunger is one plausible reason for a higher CHR [28,38].

Due to the short-term study, an increase in search behaviors and hunger levels on S are the most plausible explanations for the lower CHR on Day 1 and higher CHR on Day 7. The existing literature suggests that CHR decreases with increasing NDF content as a greater masticatory effort is required to break down food particles [28,38,39], although the present study could not establish whether NDF content had an influence on CHR. Accordingly, further exploration of chewing in relation to straw composition is essential. While it is hypothesized that a decreased CHR is desirable to prolong feeding and increase saliva production, thus reducing the risk of EGUS, there is no concrete evidence of such benefits occurring in response to chewing, thus warranting additional investigation. However, the standardization of the units of measurement for CHR is fundamental for fair comparisons between different studies and to improve understanding of how different forages and feeding methods impact CHR. Chews/min or chews/5 mins might be the most realistic unit to report rates if the aim is to facilitate easy implementation of measuring feeding behavior into the horse owner's routine.

4.2. Consumption Rate

The findings on CR are arguably the most important as they suggest S had the greatest effect on CR and that statistically significant differences could be observed within a week. The lower CR on S could be due to the lower palatability and digestibility of oat straw, increased gut fill, and more demanding mastication. This may have created a sensation of fullness, thus reducing their appetite and making it difficult for horses to consume their forage rapidly. These results and hypotheses are consistent with several investigations on straw-based diets [16,18,24,26,40] although the study's short duration means other factors that influence CR may have had a more pronounced effect than oat straw composition.

An increase in search behaviors in response to a novel forage, as mentioned previously, reduces the time spent consuming forage, which may partially explain the lower CR [32]. However, such behaviors relating to novelty are likely to have ceased after 30 minutes so would have had less of an impact, especially by Day 7 as the straw was no longer novel. Nevertheless, horses may still sort forage based on palatability.

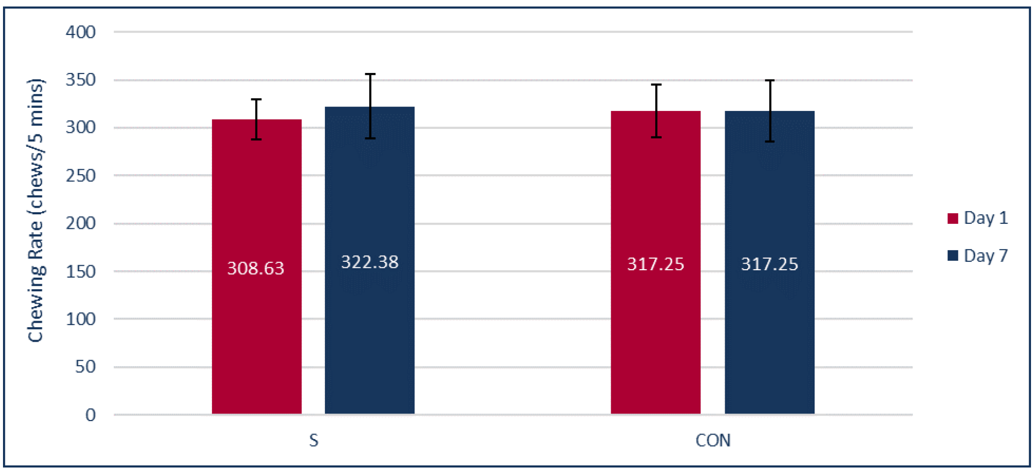

Figure 2: Mean CHR (chews/5 mins) on the S and CON diets on Day 1 and Day 7 (n = 8). Error bars show the standard deviation from the mean (p-value > 0.05).

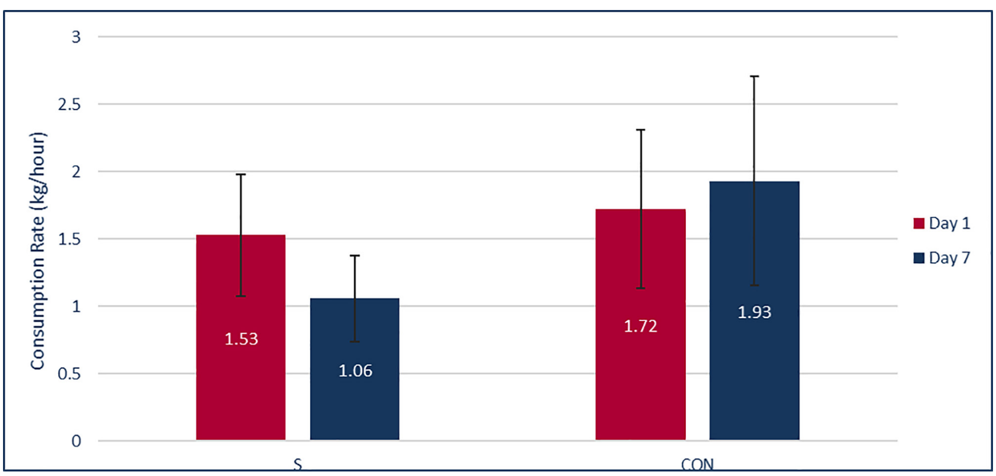

Figure 3: Mean CR (weight (kg) of forage consumed/hour) on the S and CON diets on Day 1 and Day 7 (n = 8). Error bars show the standard deviation from the mean (p-value < 0.05).

Alternatively, the S diet may have been retained inside the GIT for longer, thus decreasing ghrelin secretion, and subsequently, hunger levels, which may have increased satiety **[41]**. This decreased passage rate may have triggered a feedback loop that reduced CR in response to higher satiety levels **[42]**. Although hormonal responses to the diets are beyond the scope of the present study, there is evidence in previous research that supports this; Jansson *et al*. **[16]** found a higher serotonin production and a greater number of pauses between feeding bouts, which corresponded to a prolonged intake on the straw diet. They suggested this occurred in response to increased satiety with straw.

It is important to note that the use of haynets was a confounding factor. Haynets were used to prevent horses from separating haylage and straw, which made it difficult to isolate the effect of haynets from straw inclusion on CR. Jansson *et al*. **[16]** found that the straw diet resulted in an 80% increase in feeding time, which is comparable to feeding from haynets with small openings (3.2 cm) **[11]**. However, the haynets used in their study had 3.5 cm openings, which is smaller than the medium-sized openings (4.4 cm) in **[11]**. Therefore, the 80% increase in feeding time might not be solely due to straw inclusion. The haynets used in the present study had 4.5 cm openings so would have had a lower impact, meaning the CR obtained is more reflective of oat straw inclusion. However, more recently, Bordin *et al*. **[43]** highlighted that haynet position influences the posture and mandibular angle, which may have influenced feeding behavior differently than if the diets were fed on the floor. Thus, the size of the haynet alone is not the only influence that could have affected results, making it necessary to repeat the study while feeding on the floor to compare it against a horse's natural feeding position.

5. Limitations

This study has some limitations, and thus, the data presented should be considered a pilot study. The short study duration made it difficult to determine whether BW changes were reflective of a diet effect, hence why it was not a focus of the present study. A longer study duration would allow a longer adaptation to both diets to determine how BW, CHR, and CR change as horses become more accustomed to the study diets over time.

The difference of DM provided to horses is to be noted as a limitation as diets were not iso-caloric. However, as it was not in the authors' control to change diet quantity, it would be necessary to repeat this study with iso-caloric diets.

It was not feasible to conduct a longer-term study, and thus a longer acclimation and washout period had to be eliminated from the study design. While it is evident that this could have influenced results as carryover effects were not eliminated, washout periods and longer acclimation periods were also missing in similar studies **[16,18]**, indicating that more research ruling out these limitations is needed.

Measuring chewing by counting may have resulted in some human error. Initial proposals to use a rumination collar (RumiWatch) were not feasible but would have reduced human error and allowed a more extensive collection of chewing data. Establishing baseline CHR in individual horses would have been valuable to improve the interpretation of results. Notwithstanding these limitations, this study provided insight into variables that can be affected by different diets and how the inclusion of straw presents a debatable, but optimistic approach to equine obesity management.

6. Future Directions

As research continually pursues new ways to address the ongoing increase in equine obesity, the manipulation of feeding behavior by replacing half of the forage ration with straw is potentially an important focus for developing weight management strategies. Therefore, future research could benefit from using longitudinal studies and larger sample groups consisting of obese horses when investigating the effect of adding straw to the forage diet. This may reduce the influence of short-term responses, such as hunger levels and search behaviors, and therefore, more accurately describe the effect of straw on CHR and CR as a sustainable, long-term forage option. Including obese horses is necessary to evaluate the influence of straw on the target equine population. To facilitate measuring CHR more easily for longer periods and with greater accuracy, a rumination collar could be used. Moreover, as bite rate may explain the relationship between CHR and CR, it would be valuable to incorporate this into future research.

Standardizing the reporting of chewing rates in research is vital to improve our understanding of how CHR affects consumption. Such information can then be used to directly investigate the relationship between CHR, CR, and serotonin production as well as the relationship between chewing, saliva production, and the prevalence of EGUS. This can subsequently inform guidelines that assist in the formulation of suitable diets to increase satiation and manage obesity and ulcer-prone horses, therefore ultimately enhancing the welfare of a range of horses.

7. Conclusion

This study aimed to measure the impact of substituting 50% of the grass forage diet with oat straw on chewing rate and consumption rate. As emerging research suggested that oat straw may provide psychological and physiological benefits to horses, this study sought to investigate whether straw could help increase chewing and slow intake rates. This could reduce the length of time a horse is left without forage and make it a suitable partial forage replacement for horses on low-energy diets. Findings revealed that the inclusion of oat straw decreased the consumption rate more than the haylage-only diet. Its effect on chewing rate did not establish a clear trend but more evident changes occurred on the straw diet in comparison to the haylage-only diet. The findings agree with previous literature, highlighting the potential welfare benefits straw may provide to horses with lower-energy requirements, while also challenging previously unsubstantiated assumptions of its unsuitability.

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Authors' Contributions

N.L.M.: Methodology and analysis, K.W.: Conceptualization and manuscript development, B.A.W.: Conceptualization and manuscript development.

Data Availability

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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The research has not received any funding.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Approval

The authors confirm that the ethical policies of the journal, as noted on the journal's guidelines page, have been adhered to and the research received approval from the ARU Writtle ethical review committee (ethical approval number: 1426). The authors confirm they have followed EU standards for the protection of animals used for scientific purposes and feed legislation as well as the guidelines in the Declaration of Helsinki.

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Unilateral-Dominant Lameness Induces Changes in Breakover Duration Symmetry in Equine Walk

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Abstract

Lameness is widely regarded as the most prevalent problem affecting equines globally. Much is understood about the adjustment of upper body posture to reduce loading in an affected limb. However, the relationship between lameness and breakover duration, when the distal limb experiences high tensile stresses, remains an underinvestigated area. Thus, this study aimed to investigate breakover duration at walk in a cohort of horses, quantifying the effect of fore- and hindlimb lameness. It was hypothesized that lameness would induce an asymmetry between breakover durations of affected contralateral limb pairs. Breakover durations of sixteen horses (five sound and eleven lame, as presented by owners) were measured using data collected by hoof-mounted gyroscopes. Breakover durations of the limbs of contralateral pairs were compared, and paired Student's t-tests were used to determine whether differences were significant ($p < 0.01$). A high degree of symmetry was seen in breakover durations of sound horses, with a mean (SD) duration of 168(19)ms and a negligible mean absolute difference (6ms, $p = 0.07$). In lame horses, breakover durations of lame limbs (167(22)ms) were longer than those of contralateral limbs (146(23)ms, $p < 0.001$); and breakover durations of the ipsilateral (160(26)ms) and diagonal (162(24)ms) limbs were equivalent and comparable to those of sound limb pairs. These results indicate that where there is lameness present in a contralateral limb pair, there will be a breakdown in the symmetry of breakover duration, with the most severely affected limb having a significantly longer breakover duration than the contralateral. This pattern should be investigated in the future as a marker to indicate lameness.

Keywords

Breakover; wearable technology; lameness classification; gait analysis; biomechanics; IMU

1. Introduction

In a stride cycle, limb motion consists of swing and stance phases, the latter describing the period from first hoof-ground contact (*hoof-on*) to the instant the toe is lifted (*hoof-off*). The stance phase can further be broken down into instants of primary and secondary impacts [1], during which the hoof is loaded, experiencing ground reaction forces (GRFs) initially applied at a point dorsal to the center of rotation of the distal limb, which creates the extending moment of the digital interphalangeal joint [2]. This is opposed by a flexing moment applied by increasing tension in the deep digital flexor tendon (DDFT) and other soft tissues. When the flexing moment overcomes extending, the heel is lifted from the ground (*onset of breakover*), and the hoof rotates around the toe until it is lifted totally (*hoof-off*) [3]. This terminal part of the stance phase is known as *breakover* [3].

In the literature, there is slight disagreement about the breakover duration at walk as a percentage of stance duration, with one group reporting 10% [4,5] for the forelimbs, in contrast to others who published values of 14.1% [6] and 17(5)% [7]. For the hindlimbs, 15.4% [8] and 13(4)% [7] have been reported.

Most previous studies of breakover duration focused on the effects of different farriery methods [9–11] and speed and surface conditions [12]. These aimed to assess the effectiveness of farriery techniques to improve musculoskeletal health by influencing breakover mechanics and to evaluate the risk of injury posed by different exercise surfaces and speeds.

In the lame horse, movement adaptations alleviate pain in an affected limb by redistributing loading to other limbs [13], resulting in significantly smaller vertical GRFs in lame limbs compared to compensating limbs [14–16]. Clayton *et al.* [17] mooted a causal link between breakover duration and the reduction in vertical GRFs observed during lameness. In lame horses trotting over a force plate, they reported that the center of pressure in the lame limb began to move rapidly in the dorsal direction at a relatively early stage of the stance duration resulting in an early onset of breakover and, thus, prolonged breakover duration. They suggested that the lower vertical GRFs experienced by the lame limb were responsible for the prolonged breakover duration as the extending moment they induced would be more easily overcome by the opposing moment created by tension in the soft tissues.

Since this suggestion, only a handful of studies have reported on the relationship between lameness and breakover duration. Small differences have been found at walk between breakover durations of an affected limb at baseline readings and after induction of unilateral forelimb lameness [18]. No significant differences were reported between the breakover durations of treated and contralateral forelimbs, suggesting lameness did not affect the left/right symmetry of breakover durations. Studies of trot found the breakover duration of the affected limb was significantly longer than that of the contralateral in cases of severe forelimb lameness caused by a non-articular shoulder fracture [19], chronic sesamoiditis of the fetlock joint [20] and fracture of the third carpal bone [21]. To the best of the author's knowledge, no previous investigations specifically explored the effect of hindlimb lameness on breakover duration.

The aim of this research was, hence, to investigate breakover duration in a cohort of horses, quantifying the effect of fore and hindlimb lameness. It was hypothesized that lameness would influence breakover duration, inducing a longer breakover in the most severely affected limb compared to the contralateral limb.

2. Materials and Methods

2.1. Horses

Sixteen riding horses (eight geldings; eight mares) of various breeds and uses, including sports and leisure, with a mean (SD) height of 164(9) cm and age of 13(5) years were included **(Table 1)**. Five were presented as sound by the owners (Horses 1-5). These horses had not been referred to a veterinarian with any lameness concerns for at least the past 5 years and had not shown any changes in their performance that might indicate a developing issue.

Eleven horses were also recruited that presented with unilateral or unilateral-dominant lameness (where one limb of the pair was markedly more affected than the other), but no perfectly bilateral lameness at the time of data collection. Among these, three suffered from forelimb lameness (Horses 6-8) and seven from hindlimb lameness (Horses 9-15), while one (Horse 16) had lameness predominating in one forelimb and the diagonal hindlimb. The horses were not assessed by a clinician specifically for the study, but each of them was classified as sound or lame based on the history provided by their owner, provided that they had been assessed by their own veterinarian in the two weeks prior to data collection. The findings of these assessments are recorded in **Supplementary Table 1**. Given that no study-specific veterinary assessment was performed, lameness grades were not obtained

The lack of published data in the literature meant that sample size calculations were not possible during the study design stages, but effect sizes are reported in the results to account for the limited sample size.

2.2. Data Collection and Measuring Protocol

Inertial measurement units (IMUs; Shimmer3 IMU, Shimmer Sensing, Dublin; **Figure 1**) containing tri-axial gyroscopes (range ±2000 deg/s; sampling frequency 200 Hz) were firmly attached to the lateral aspect of the four hooves using sticky-back hook-and-loop fastenings (VELCRO® Brand, Manchester, New Hampshire). Horses were walked in-hand at self-selected speeds along a flat 30m asphalt track, with the central 20m being used for data processing. Three passes were recorded per horse. Trials with significant disturbances (such as the horse trotting or halting) were repeated. The methods were reviewed and approved by The University of Sheffield, Ethics Department (Reference Number 033398), and owners gave informed consent for their animals' involvement.

2.3. Data Analysis

2.3.1. Calculating Temporal Parameters

The hoof-on, -off, and onset of breakover were detected from the angular velocities **(Figure 2)** using previously validated methods [7,22,23]. Briefly, the resultant of angular velocity (ω_R) was calculated and filtered using a second-order Butterworth filter with a cutoff frequency of 40Hz. For each stride, the flat portion (stance phase) was identified, and the instant the signal began to rise, at the end of this, was taken to be the onset of breakover (b_{ov}). The highest subsequent peak was taken as hoof-off (h_{off}), and the last peak before the stance phase as hoof-on (h_{on}).

Temporal stride parameters were calculated for each stride cycle of each limb. Stride durations (T_{stride}, ms) were calculated as the time from one hoof-on to the next (Eq. 1).

$$T_{stride} = h_{on_{n+1}} - h_{on_n} \quad (1)$$

Where T_{stride} is the stride duration, h_{on_n} the instant of one hoof-on, and $h_{on_{n+1}}$ that of the next.

Stance durations (T_{stance}, ms) were calculated as the time from hoof-on to the subsequent hoof-off (Eq. 2).

$$T_{stance} = h_{off} - h_{on} \quad (2)$$

Where T_{stance} is the stance duration and h_{off} is the instant of hoof-off.

Breakover durations (T_{BO}, ms) were calculated as the time from the onset of breakover to hoof-off (Eq. 3). Breakover durations were also calculated as a percentage of the stride duration.

$$T_{BO} = h_{off} - b_{ov} \quad (3)$$

Where T_{BO} is the breakover duration, and b_{ov} is the instant of the onset of breakover.

Table 1: Details of the cohort. Horses 1 and 16 were barefoot, while Horse 4 was shod only in front. All other horses were fully shod.

Horse ID	Age (years)	Height (cm)	Lameness state
1-5	13(6)	162(9)	Sound
6-8	16(4)	163(14)	Forelimb lame
9-15	14(5)	164(5)	Hindlimb lame
16	14	168	Left fore & right hindlimb lame
Mean (SD)	13(5)	164(9)	-

Figure 1: Shimmer IMU attached to the lateral aspect of the hoof wall using sticky-back hook-and-loop fastenings.

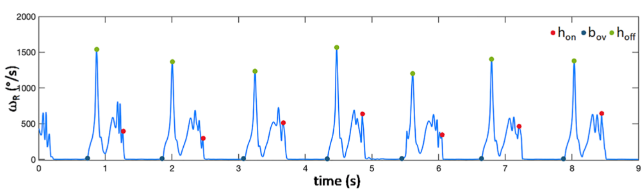

Figure 2: Example of hoof-on (h_{on}, red dots), -off (h_{off}, green dots), and onset of breakover (b_{ov}, indigo dots) detected from resultant of angular velocity (ω_R); seven consecutive stride cycles for one limb are presented.

To investigate stride, stance, and breakover durations, data were split into groups of sound, lame, and opposite limb pairs **(Table 2)**, not including data from Horse 16 (which presented both fore and hindlimb lame). The between-limb differences (those between the sound and contralateral limbs of a sound limb pair, or lame and contralateral limbs of a lame pair) were tested for significance using statistical methods. The breakover durations (ms) of sound limb pairs were found to be not normally distributed by visual inspection of the QQ plots and Shapiro-Wilks test (p = 0.004). Therefore, differences between breakover durations of these and the contralateral limbs of sound pairs were tested for significance using a Wilcoxon Signed Rank test. All other datasets proved normal, and significance of between-limb differences was tested using paired Student's t-tests.

2.3.2. Comparing Contralateral Breakover Durations

For each horse, the mean difference ($\overline{\Delta T_{BO}}$, ms, Eq 4) between the breakover durations of the right and left limb of each contralateral limb pair was calculated over the total number of strides.

$$\overline{\Delta T_{BO}} = \frac{1}{n}\sum_{i=1}^{n}\left(T_{BO_R} - T_{BO_L}\right)_i \quad (4)$$

Where $\overline{\Delta T_{BO}}$ is the mean difference between breakover durations of the right (T_{BO_R}) and left (T_{BO_L}) limb of each contralateral limb pair measured over n strides.

The sign of $\overline{\Delta T_{BO}}$ indicated whether the breakover duration of the right (positive) or left (negative) limb of the pair was longer.

Absolute values of $\overline{\Delta T_{BO}}$ for groups of sound, lame, and opposite limb pairs were found to be normally distributed (by visual inspection of QQ plots and Shapiro-Wilks test; $p \geq 0.03$ in all cases). Therefore, absolute values of $\overline{\Delta T_{BO}}$ for the limb pair groups were compared, and differences tested for significance using unpaired Student's t-tests (sound vs. lame, and sound vs. opposite limb pairs) and a paired Student's t-test (lame vs. opposite limb pairs).

Finally, statistical methods were used to test the null hypothesis that, for each individual horse, the mean breakover duration of the left and right limbs of each contralateral limb pair was not significantly different. Shapiro-Wilks test for normality and visual inspection of the QQ plots indicated datasets were normally distributed. Therefore, for each horse, paired Student's t-tests were used to detect statistically significant differences between breakover durations of the left and right limbs of the fore and hindlimb pairs, with $p < 0.01$ indicating significance. The effectiveness of the methods to classify lame horses, detecting lame limb pairs, and identifying the most severely affected limb, were tested on the cohort. All data and statistical analyses were carried out using custom scripts written in Matlab (version 2024Ra).

3. Results and Discussion

A total of 700 walk strides were analyzed, with an average of 41(10) strides per horse.

3.1. Effect of Lameness on Temporal Stride Parameters

There was no difference between mean stride durations of sound and lame horses, and the distributions were similar as both groups consisted of a varied range of horse heights and types **(Figure 3a)**. For sound limb pairs, stance durations were symmetric with no difference between those of the sound and contralateral limbs (**Figure 3b**; p = 0.4, effect size = -0.197), in agreement with the literature **[18,24]**. Similarly, no differences were observed between the stance durations of the lame and contralateral limbs of lame limb pairs (p = 0.96, effect size = 0.009), indicating that the prevalence of lameness in one limb of the contralateral pair did not affect stance duration symmetry. Moreover, the mean stance durations of the lame group (801(45)ms) were comparable to those of the sound (795(29)ms). Thus, results suggest lameness does not affect the symmetry of the stance durations at walk, in agreement with previous literature **[24]**.

For sound limb pairs, recorded breakover durations were slightly longer (21(2)% of stance duration) than previously reported **[4–6,8]**, which may be due to cohort morphology. In literature, four French Trotters of height 158(4)cm recorded a mean breakover duration of 10% **[4,5]** of the stance duration, while five horses of various breeds and heights 143-156 cm recorded breakover duration of 15% [6]. In the present study, horses were also of various breeds, with a larger range of heights (147-178 cm) and taller mean height (164 (9)cm) than in previous reports which may explain why the average breakover durations recorded here were longer. The disagreements in breakover duration, here and in the literature, warrant further investigation.

Breakover durations of the two limbs of sound limb pairs showed negligible differences (**Figure 3c** and **3d**; $p = 0.07$, effect size = -0.3), reflecting the symmetrical nature of healthy walk. One publication [18] reported a significant difference between the breakover durations of sound and contralateral limbs of sound forelimb pairs. However, the magnitude of that difference (4ms) was negligible. Thus, it can be concluded that there should be left/right symmetry of breakover durations in fore and hind contralateral limb pairs at walk, in a sound horse.

A breakdown in the symmetry of breakover durations emerged for lame limb pairs. The mean breakover duration of the lame limb (**Figure 3c**, solid red box; 167(22)ms) was comparable to that of sound limb pairs (168(19)ms), with a 2ms difference. In contrast, the breakover duration of limbs contralateral to lame limbs (**Figure 3c**, empty red box) was 14% shorter (146(23) ms, $p < 0.0001$, effect size = 0.9). These results also hold when breakover is considered normalized to the percentage of stride duration **(Figure 3d)**, with a mean of 13 (2)% for lame limbs and significantly ($p < 0.0001$) lower mean of 11 (2)% for those contralateral to them. The results support the hypothesis that lameness induces a longer breakover duration in the lame limb compared to the contralateral limb.

Previously, breakover duration was reported to increase with the induction of Grades 1 (+2ms), 2 (+3ms), and 3 (+1ms) lameness, compared to baseline values [18], at walk. These values are significantly smaller than the differences found in the current study, and no differences were reported between breakover durations of the lame limb and that contralateral to it. There are several reasons why our results may differ. Firstly, the earlier study recorded data over a surface covered by a 9.3mm thick, rubberized mat. This may have acted as a cushion, attenuating some of the impact of the hoof-surface collision, and thus relieving discomfort due to shockwaves [25] traveling up the painful limb and reducing the need for the horse to adopt such a pronounced compensatory movement as those horses in the current study, where data was collected on a hard surface. The lameness models used may have also had an effect. Moorman *et al*. [18] used a method of sole pressure to induce unilateral lameness in six sound horses. While this method has been widely used to stimulate a reversible and controllable lameness [14,24,26], we suggest that the compensatory mechanisms it induces may not comprehensively represent those adopted by horses suffering spontaneous lameness (as in the current study; **Supplementary Table 1**) the causes of which can be many varied and complex.

A prolonged breakover has been associated with a longer toe length and thus an increase in the risk of developing specific pathologies (such as navicular disease [27] or tendon injury [3,7,9]) as a result of increased tensile stresses in the DDFT and impar ligament, and related increased compression of the navicular bursa and navicular bone [3]. However, the diverse range of lameness causes represented in this cohort **(Supplementary Table 1)** suggests that not only can prolonged breakover predispose an animal to injury or disease, but it may also develop as a result of underlying pathologies. These results support the proposal of Clayton *et al*. [28] that the lower GRFs seen in lame limbs might allow the earlier onset of breakover in the affected limb and, hence, a longer breakover duration. Thus, we suggest breakdowns in the left/right symmetry of breakover duration may develop as a coping strategy for accommodating lameness.

The result is perhaps surprising as persistent lameness is often believed to lead to increased hoof angles and a more upright dorsal hoof wall [29] which would tend to shorten breakover duration. However, in literature, only a small and statistically insignificant difference in hoof angle has been reported to support this claim (53 (3)° for lame limb compared to 52 (4)° for non-lame, $p = 0.4$ [30]). Furthermore, other studies have found decreased hoof angles (characterized by long toes and low heels) to correlate with both fore [31] and hindlimb [32,33] lameness, and poor performance [34]. In these studies, breakover durations were not reported but, had they been, they may have been found to be prolonged as a result of the long toe. The findings of these and other publications, along with the results of the current study indicate that further kinematic studies are needed to understand whether the relationship between hoof angle and lameness is a *cause* or *effect* relationship [33], with it being unknown whether low hoof angles precede the onset of lameness or vice versa [32].

Hoof imbalance, particularly of the hindlimbs, has been a consistent clinical finding in horses with back soreness [35,36]. While hoof balance was not assessed in the present study, this previous finding could support a hypothesis that the effect of axial-related lameness (Horses 11-13) on breakover duration might be connected to hoof imbalance. Again, this raises the question of whether the association between hoof morphology and lameness is cause or effect, with Melo *et al*. suggesting that hoof imbalance predisposes horses to musculoskeletal pathologies [36]. Further studies would be needed to answer these questions.

Table 2: Definition of groups into which limb pairs were sorted for analysis. The name of the group, definition, and examples of which limbs would be assigned to each are provided. LF = left forelimb; RF = right forelimb; LH = left hindlimb; RH = right hindlimb.

Group	Description	Example
Sound	Both contralateral limb pairs (fore and hind) of each sound horse, with limbs dubbed *sound* and *contralateral*.	The forelimb- LF (sound) and RF (contralateral)- and hindlimb pair- LH (sound) and RH (contralateral)- of a sound horse.
Lame	The *lame* and *contralateral* (with respect to lame limb) limbs of lame horses.	LF lame horse: LF (lame) and RF (contralateral). RH lame horse: RH (lame) and LH (contralateral).
Opposite	The *ipsilateral* and *diagonal* (with respect to lame limb) limbs of lame horses.	LF lame horse: LH (ipsilateral) and RH (diagonal). RH lame horse: RF (ipsilateral) and LF (diagonal).

Standard deviations of breakover duration were large for all groups, ranging from 17ms to 22ms, reflecting the highly varied nature of both sound and lame cohorts. As the phenomenon is dependent on individual hoof shape [3] as well as overall stride duration and, thus, morphology of the horse, breakover duration could vary substantially between subjects. Hence, actual mean values of breakover duration might prove to be horse-specific. Nonetheless, the pattern of symmetry in breakover duration of sound limb pairs and asymmetry in lame limb pairs is expected to be maintained. Further studies using varied cohorts are needed to verify this.

The pairs of outliers identified in **Figure 3c** were attributable to the forelimbs of sound Horse 4 and lame Horse 8, which were also the tallest horses studied (178cm and 174cm, respectively). These horses also recorded the longest breakover when normalized to total stride duration **(Figure 3d)**. The results could be due to the horses' heights. Despite appearing as outliers, both horses follow the pattern of their respective groups—Horse 4's forelimbs have similar breakover durations, and Horse 8's lame forelimb demonstrates a longer breakover duration compared to the contralateral limb.

The small absolute mean difference between the breakover duration of the two limbs of sound limb pairs (**Figure 4**, $|\overline{\Delta T_{BO}}|$=6(5)ms, n = 10), seems to confirm the well-reported fact that horses demonstrate some natural asymmetry due to sidedness [37–39]. As a direct practical application of these results, $|\overline{\Delta T_{BO}}|$ of sound limb pairs could be used to establish a threshold of allowable difference to distinguish between natural sidedness and lameness. However, a larger cohort of sound horses would be needed to ensure the robustness and generalizability of these results.

Compared to the sound group, $|\overline{\Delta T_{BO}}|$ obtained for the lame limb pairs (**Figure 4**, 21(5)ms, n = 10) was more than three times greater ($p < 0.001$), indicating a much higher degree of asymmetry, with power calculations revealing an observed power of over 99% ($\alpha = 0.01$). Thus, results suggest there exists a real significant difference between $|\overline{\Delta T_{BO}}|$ values recorded from sound limb pairs compared to lame. $|\overline{\Delta T_{BO}}|$ for opposite limb pairs (7(5)ms) was equivalent to the value obtained for sound pairs ($p = 0.7$) and 67% smaller ($p < 0.001$) than that obtained for lame pairs. The degree of symmetry of opposite limb pairs being comparable to that of sound indicates they did not demonstrate a compensatory effect due to lameness. In studies of upper body movement symmetry, compensatory lameness mechanisms are widely reported, and methods of lameness quantification which use upper body parameters can be complicated [40]. If they continue to prove robust to the effects of compensatory lameness, methods of breakover analysis will surely be a useful addition to the currently used upper body symmetry analyses as a means of distinguishing true lameness from compensatory.

The outliers identified in lame and opposite limb pair groups **(Figure 4)** were the results of Horse 9. Although the magnitude of $|\overline{\Delta T_{BO}}|$ of the opposite limb pair (20ms, Horse 9_F) was higher than those recorded for other horses in the group, it was still substantially (39%) smaller than that recorded for the corresponding lame limb pair (33ms, Horse 9_H). Hence, despite appearing as an outlier, the behavior of Horse 9 fitted the cohort pattern. These results indicate that a pattern in breakover duration was observable for the sound and lame cohorts, with sound limb pairs and those opposite to a lame pair displaying a high degree of symmetry but a breakdown of this symmetry being seen in lame limb pairs. The results confirm the study hypothesis that longer breakover durations, compared to the contralateral, are a feature characteristic of lame limbs. Furthermore, **Figure 4** indicates that the result is the same and holds for both horses suffering axial- and appendicular-related lameness, as there is no distinction between the behavior of each group. Therefore, comparing concurrently recorded breakover durations of the left and right limbs of contralateral pairs may prove a valuable addition to methods to detect and monitor unilateral-dominant lameness attributable to both axial- and appendicular-pathologies.

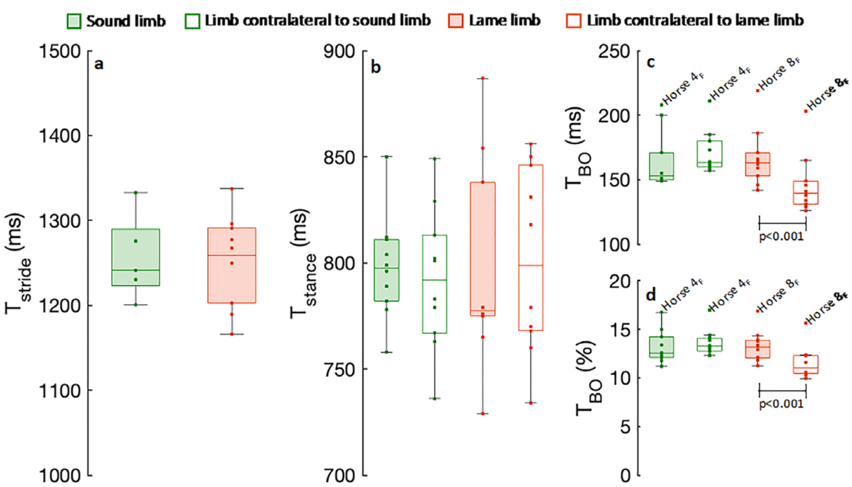

Figure 3: Stride (T_{stride}, a) and stance durations (T_{stance}, b) in ms, and breakover durations in ms (T_{BO}, c) and as percentage of stride duration (T_{BO}, d). Temporal parameters are shown for limb pairs of sound horses (sound and corresponding contralateral limbs) and lame limb pairs of fore and hindlimb lame horses (lame and corresponding contralateral limbs). Solid green boxes represent the sound limbs and empty green boxes represent those contralateral to them; solid red boxes represent lame limbs and empty red boxes represent those contralateral to them. Individual points are shown as dots, and outliers are labeled. Significant differences are indicated with *p*-value. These results do not include Horse 16.

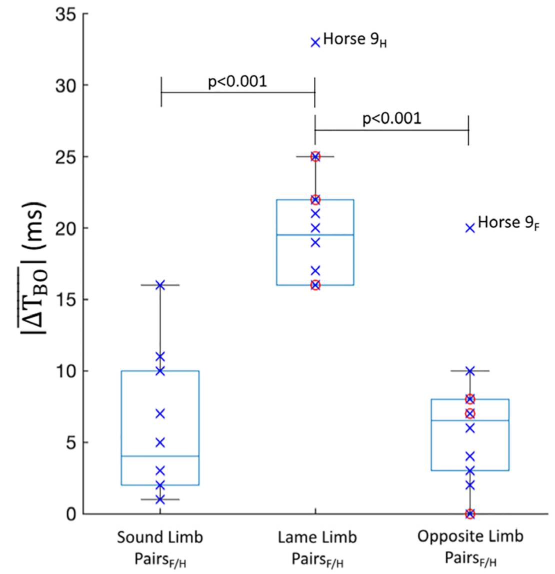

Figure 4: Absolute mean values of the difference in breakover durations of the limbs of fore (F) and hind (H) contralateral limb pairs ($|\overline{\Delta T_{BO}}|$, ms) for sound, lame, and opposite limb pairs. Significant differences are indicated with *p*-values, and outliers are labeled. For lame and opposite limb pairs, results of horses with axial-related lameness causes (Horses 11-13) are highlighted with red circles, compared to those with appendicular-related causes. These results do not include Horse 16.

3.2. Breakover Duration as a Tool for Lameness Detection

An example of how, with further validation studies, breakover data could be used to classify lameness in individual horses is presented in **Table 3**. The presence of a statistically significant difference ($p < 0.01$) between breakover durations of a contralateral limb pair, recorded over a given number of strides, would indicate lameness (bold values). Sensitivity analyses revealed that ten strides were sufficient to establish steady values of $\overline{\Delta T_{BO}}$, while thirty were required to obtain steady *p*-values from the paired Student's t-tests of lame limb pairs. The *p*-values for sound limb pairs did not converge to a steady value, regardless of the number of strides analyzed, as expected. Thus, it is advised that a minimum of thirty strides be recorded for the application of these methods.

No lameness was detected for the fore or hindlimbs of the five sound horses ($p \geq 0.03$). The method correctly classified all lame horses, with limb pairs where lameness was prevalent displaying statistically significant differences in breakover duration. Furthermore, the sign of $\overline{\Delta T_{BO}}$ correctly identified whether lameness predominated in the left (negative) or right (positive) limb in every case. For both fore and hindlimb lame horses, $\overline{\Delta T_{BO}}$ of the lame limb pair was substantially larger in magnitude than that of the opposite limb pair. Indeed, in all cases but one (Horse 9), absolute $\overline{\Delta T_{BO}}$ of the lame limb pair was at least 90% longer (range 16 to 33ms) than that of the opposite limb pair (range 0 to 10ms). This supports the suggestion that, with a larger cohort, threshold values of $\overline{\Delta T_{BO}}$ might be used in the future to classify lame and sound limb pairs.

Horse 16 was presented with severe lameness of the left fore and lameness of the right hindlimb. This was one of only three horses currently out of work due to lameness **(Supplementary Table 1)**. The severity of Horse 16's forelimb lameness appears to be reflected in the magnitude of $\overline{\Delta T_{BO}}$ (51ms), the highest recorded. Future studies could determine whether the magnitude of $\overline{\Delta T_{BO}}$ correlates with the severity of lameness. Horse 16's results also indicate that the methods might be used to assess horses with concurrent fore and hindlimb lameness, provided one limb of each contralateral pair is sufficiently more affected than the other to allow the detection of breakover asymmetry. Further studies on larger populations of horses, with complex multi-limb lameness, are of course needed to explore this hypothesis.

3.3. Limitations and Future Work

The most significant limitation of the work was that lameness states were not classified and graded by the same veterinarian at the time of data collection. While all lame horses had been assessed by a vet during the two weeks prior to data collection, initial classification depended on the history provided by the owner. Future studies should prioritize having a clinician involved for subjective lameness assessment using, for example, the AAEP scale. It may also be highly beneficial to use another system for lameness detection concurrently (for example one which analyses upper body movement) to compare results obtained by both systems.

Table 3: Mean (SD) values of the difference in breakover duration of right and left limbs ($\overline{\Delta T_{BO}}$, ms) for the fore and hindlimb pairs of all horses and p-value result of the paired Student's t-tests. Clinical observations indicate whether the horse was presented as sound (S) or having lameness predominating in the left (L) or right (R) fore (F) and/or hindlimb (H). Values in bold indicate where the difference was significant ($p < 0.01$). Horse 16, with lameness of both left fore (LF) and right hindlimb (RH), is presented in the bottom row.

Horse ID	Forelimbs (ms)		Hindlimbs (ms)		Clinical observations
	Mean(SD)	*p*-value	Mean(SD)	*p*-value	
1	7(18)	0.08	-3(22)	0.5	S
2	10(33)	0.1	-11(30)	0.03	S
3	1(27)	0.7	5(34)	0.4	S
4	2(30)	0.6	1(19)	0.8	S
5	-16(40)	0.03	3(29)	0.6	S
6	**21(34)**	**<0.001**	3(34)	0.4	RF
7	**20(18)**	**<0.001**	2(17)	0.3	RF
8	**16(33)**	**0.006**	-4(36)	0.5	RF
9	-20(38)	0.01	**-33(32)**	**<0.001**	LH
10	-7(22)	0.03	**16(14)**	**<0.001**	RH
11	-8(16)	0.02	**22(21)**	**<0.001**	RH
12	0(17)	0.8	**25(18)**	**<0.001**	RH
13	-7(15)	0.02	**16(26)**	**<0.001**	RH
14	-10(40)	0.3	**19(16)**	**<0.001**	RH
15	6(36)	0.3	**-17(24)**	**<0.001**	LH
16	**-51(23)**	**<0.001**	**13(12)**	**<0.001**	LF, RH

In a future study, hoof morphology ought to be recorded as trimming [41] and shoeing [10] are known to have a significant effect on the shape of the hooves, although the latter has been found to affect breakover duration only insignificantly. As the left and right hooves of contralateral pairs tend to be trimmed at the same time and undergo the same shoeing treatment, it is anticipated that the methods of breakover duration symmetry analysis will hold, regardless. Similarly, future studies might also include an assessment of hoof balance, given that hindlimb hoof imbalance appears as a consistent clinical finding in the presence of back soreness [35,36].

This cohort, although larger than many similar studies [18,42], was small. However, the results are extremely useful as they allowed sample size calculations to be conducted to inform future study design. Using $|\overline{\Delta T_{BO}}|$ values for sound (n = 5) and lame (n = 4) forelimb pairs revealed a cohort of twenty-one sound and seventeen forelimb lame horses would be needed to establish that there exists a real significant difference between the $|\overline{\Delta T_{BO}}|$ values of sound and lame forelimb pairs (power 90%, α = 0.01). The results of sound (n = 5) and lame (n = 8) hindlimb pairs indicated that eight sound and five hindlimb lame horses would be needed to prove a real difference between $|\overline{\Delta T_{BO}}|$ of sound and lame hindlimbs (power 90%, α = 0.01).

4. Conclusion

In cases of lameness, lame limbs were found to have a significantly longer breakover duration at walk than the contralateral limb of the pair. With further validation, this finding could form the basis of a valuable tool for the detection and assessment of lameness, requiring the horse to be assessed only at walk. The tool will be a beneficial addition to the current state-of-the-art methods based on upper body motion symmetry, particularly if the finding continues to prove robust to the effects of compensatory lameness.

Supplementary Materials

Morphological details of the cohort of sound and lame horses. The lameness histories of lame horses are given. Horses are organized into sound, forelimb lame, hindlimb lame, and the one horse (Horse 16) which had severe lameness in the left fore (LF) and right hind (RH).

Authors' Contribution

E.V.B: Conceptualization, formal analysis, investigation, writing, reviewing, and editing; C.M.: Conceptualization, reviewing, and editing.

Data Availability

The data underpinning this research is available at: https://figshare.com/s/dfec5cdac816ff46cfcd.

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Conflicts of Interest

The commercial funder Worldbase Ltd. is a manufacturing company, specializing in agricultural machinery, and currently has no products or services, existing or in design, related to the content of this research. Funders had no role in study design, data collection and analysis, decision to publish, or manuscript preparation.

Ethical Approval

The study was reviewed and approved by The University of Sheffield, Ethics Department (Reference Number 033398), and owners gave informed consent for their animal's involvement.

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Investigating Equestrians' Knowledge, Perceptions, and Experiences with Domestic Horse Oncology

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Abstract

Common equine cancers, such as sarcoids, melanomas, and squamous cell carcinomas, can lead to pain, discomfort, and decreased quality of life, especially if improperly treated. These tumors often affect vital areas like the skin, eyes, and internal organs, impairing mobility and function. The management of equine cancer requires careful consideration of treatment options, many of which may be invasive or costly, and often necessitate long-term care. While incidence rates of different types of cancer are difficult to establish, identifying high-risk individuals is needed as part of clinical decision-making protocol. This study examines equine oncology through a mixed-methods approach, incorporating a survey of equestrians (n = 287), case studies (n = 164), and social media analysis (243 social media posts). The results indicate that equine skin cancers, particularly sarcoids, melanomas, and squamous cell carcinomas, are the most commonly reported among owners regardless of horse age, breed, and sex. Treatment methods, often chosen based on anecdotal evidence, vary widely, with combination therapies perceived as more effective than individual treatments. The study also reveals a concerning trend of equine owners relying on social media for cancer diagnosis and treatment advice, often in lieu of veterinary consultation. This underscores the need for better educational resources and support systems for equine caretakers. The findings highlight the challenges in diagnosing and treating equine cancer, emphasizing the importance of early detection and a multidisciplinary approach to improve equine welfare.

Keywords

Equine cancer; equine welfare, sarcoids, equine oncology, owner knowledge

1. Introduction

The exact prevalence and incidence rates of cancer in horses are difficult to determine due to several factors, including underreporting, limited studies, and difficulties in diagnosis [1,2]. Sarcoids are the most commonly diagnosed equine tumor, with melanomas and squamous cell carcinomas (SCCs) also representing a substantial proportion of cancer cases in horses, with melanomas being especially common in grey horses [3–5]. Age is a major risk factor for cancer in horses, with older horses, particularly those aged 16 years or older, demonstrating higher incidence rates of tumors like lymphosarcomas and melanomas [6]. There is an increasing number of geriatric horses over 16 years of age globally and particularly in the UK [7]. The exact incidence rates vary by breed and geographic location, but as horses age, the likelihood of developing cancer increases significantly [8,9]. Breed does play a significant role in the increased risk of cancer development. Arabians are more prone to melanomas, while Thoroughbreds and their crosses are more susceptible to sarcoids [10]. However, equine cancer prevalence is still thought to be lower than in other species due to shorter life expectancy and less frequent diagnostic testing [11,12].

1.1. Common Types of Equine Cancers

Sarcoids are the most frequently diagnosed type of tumor in horses, typically appearing as skin masses [13,14]. They are generally non-metastatic but locally invasive and can recur after treatment [10,15]. Sarcoids come in several forms, including verrucous (wart-like), fibroblastic (fleshy), and nodular, affecting different parts of the horse's body [16]. These tumors are caused by the bovine papillomavirus (BPV) and often require various treatments, including surgical removal, topical chemotherapy, and immunotherapy [16,17]. Equine sarcoid tumors are more likely to be noticed, sampled, or excised and submitted to diagnostic laboratories, and therefore recorded in surveys as being accountable for 40% of equine cancers [18]. Melanomas are most commonly observed in grey horses, affecting up to 80% of this population by the age of 15 [8]. These tumors develop from melanocytes (pigment-producing cells) and tend to appear on the horse's perineum, tail, or head. Unlike sarcoids, melanomas can be both benign and malignant, with a risk of metastasis to internal organs over time [19]. Although surgical excision is the most common treatment, laser therapy and immunotherapy are also used to manage melanomas [20]. Squamous Cell Carcinomas (SCCs) affect the mucocutaneous junctions, such as the eyes, nose, and genitalia, and are associated with ultraviolet (UV) exposure, particularly in light-colored horses [21]. These tumors are malignant and can invade surrounding tissues, requiring early intervention [22,23]. Treatment options include surgery, cryotherapy, radiation therapy, and topical chemotherapy [24]. Although SCCs can be managed if caught early, they carry a higher risk of morbidity if left untreated. Lymphosarcomas are less common than the aforementioned cancers but are still noteworthy, particularly in older horses [25,26]. This cancer affects the lymphatic system and can present in various forms, including multicentric (affecting multiple organs) or cutaneous (restricted to the skin) [27]. Lymphosarcomas tend to be more aggressive and are often treated with systemic chemotherapy or corticosteroids [28]. The prognosis for horses with lymphosarcoma is generally poor, particularly for multicentric forms [29].

1.2. Diagnostic Tools for Equine Cancer

Early and accurate diagnosis is critical for managing equine cancer, but it is often challenging due to the variety of tumor presentations and limited access to advanced diagnostic tools in some areas [30]. Common methods include clinical examination, histopathology, cytology, imaging, and biopsy. Diagnosis typically begins with visual inspection of the affected area, as many equine cancers like sarcoids and melanomas present with distinctive external symptoms, though this alone is not definitive [9,12,20]. Histopathology, involving tissue sample analysis, is one of the most reliable methods for distinguishing between benign and malignant tumors [31], while cytology, though less invasive, may not provide as detailed a diagnosis. Imaging techniques such as ultrasound, X-rays, and MRI are valuable for assessing tumor extent and planning treatment. Additionally, molecular diagnostics like PCR can detect viral agents like BPV in sarcoids, and genetic profiling offers insights for personalized treatments.

Equine cancer is a multifaceted health issue, with sarcoids, melanomas, squamous cell carcinomas, and lymphosarcomas being the most common types observed in horses. Diagnosis is often complicated by the diversity of tumor presentations and the availability of diagnostic tools. While clinical examination, histopathology, cytology, and imaging remain the cornerstone of cancer diagnosis in horses, emerging molecular diagnostic techniques hold promise for more accurate and early detection [32,33]. Ultimately, increased awareness, research, and advancements in diagnostic methods are essential for improving the management and treatment of equine cancer, thus enhancing horse welfare and longevity [34].

The aim of the current study was to investigate the prevalence and characteristics of different equine cancers, the diagnostic and treatment methods employed by horse owners, and the role of social media in disseminating information and support regarding equine oncology between horse owners. Through a detailed survey and case study analysis, this research seeks to explore the experiences of equestrians globally in managing common equine cancers. There is currently a lack of data investigating the equestrian's knowledge, perceptions, and experiences with equine cancer on a multinational scale. By examining the relationships between variables such as horse breed, age, cancer type, and treatment success, this study aims to identify patterns and provide insights into improving cancer management and equine welfare outcomes. Additionally, the study investigates the influence of social media on diagnosis, treatment decisions, and community support, highlighting both the benefits and risks of using online platforms for equine health advice.

2. Materials and Methods

2.1. Initial Survey Design

An initial survey comprising 12 questions that could be completed in approximately eight minutes was constructed and piloted with ten adult equestrians of different nationalities. The survey included 2 binary, 6 open-ended, and 6 multiple-choice questions, and was distributed via Microsoft and Google Forms. The survey consisted of two sections. The first section included demographic questions: gender, nationality, horse ownership, horse-related experience, horse information such as sex, age, breed, exercise, feeding, and housing regime. The second section focused on participants' experience with equine cancer; "which of these equine cancers have you had experience with, please outline your experience with these cancers, what diagnostic methods were utilized to diagnose the equine cancer, what symptoms did the horse display, what was the prognosis and what treatment methods did you utilize?" Participants were also given the opportunity to share with researcher specific case studies that they wished to highlight regarding cancer diagnosis, treatment, and prognosis in equines.

Three Facebook social media pages dedicated to equine oncology were selected for analysis, all of which opted to remain anonymous for the purpose of this study. These pages were chosen because they had over 10,000 members and averaged more than 10 posts per day. All pages were in English to avoid misinterpretation due to translation. Criterion sampling was used to select Facebook posts meeting specific criteria, including a description of the cancer the horse had, either through an image or written description, details about the horse such as age, breed, and sex. The post also needed to contain information about the diagnosis of the cancer e.g., whether the cancer had been diagnosed by a veterinarian and what treatment options had been attempted and their outcomes. The posts then were categorized according to their content, what type of cancer the horse had, whether veterinary diagnosis was sought or not, and whether treatment had or had not been prescribed by a veterinarian.

2.2. Participant Recruitment

The target population consisted of English-speaking equestrians over 18 years old. Participation in the questionnaire and case study analysis was voluntary. For the social media analysis, administrators of pages granted permission for analysis. No personal data was gathered, and no distinguishing characteristics of participants were recorded. Ethical approval was granted by the UCNL Ethics Committee. To be eligible for participation, respondents had to meet the research criteria of being over 18 and have experience caring for an equine. By completing the questionnaire and submitting case studies, participants consented to their data being used for this study. The survey link was distributed online via personal social media and equestrian-related social media platforms e.g. UK Horse Owners. Social media pages were chosen that had over 1,000 members for questionnaire distribution and link sharing. Participants were required to have previously owned or been responsible for the health and welfare of a horse. The questionnaire was open from November 2022 to June 2023. Posts for the social media analysis were selected from social media pages that only allowed members over the age of 18. The social media posts that were used in the analysis were those posted between March 2023 and June 2023.

2.3. Data Collating

All questionnaire responses were downloaded from Forms into Microsoft Excel. A total of 287 participants completed the questionnaire, 6 of which were dismissed due to an incomplete response. This left 281 valid responses being analyzed. Following completion of the questionnaire, participants were invited to provide further information relating to their individual experiences with equine cancer. Furthermore, 164 participants provided additional case studies for analysis. The case study information had to include the horse's breed, sex, and age when the cancer was first diagnosed or identified, an image of the cancer at initial diagnosis, an image of the cancer post-treatment, and an approximate size of the tumor at various stages throughout the treatment period. A total of 243 social media posts were analyzed for the social media analysis, with comments from the posts also being categorized based on their content. Answer choices for questions were open-ended to allow for a full description of the events and experiences of participants related to equine cancer.

2.4. Statistical Analysis

Data was analyzed using IBM SPSS statistics software (version 24). Frequency analysis was undertaken for the demographic data of participants and the data relating to their horses. The analyzed data consisted of nominal/categorical data and binary data; therefore, a chi-squared test for association was used to analyze the data. A p-value < 0.05 was considered statistically significant. To meet the test assumptions, all cell frequencies involved in the analysis contained values greater than 5. Some answer categories were combined to provide sufficient data for analysis.

3. Results

A total of 287 participants completed the survey, with 51.2% of responses being received in the first week of distribution. After discarding incomplete surveys, results from n = 281 participants were analyzed, representing a margin of error of ±5% based on 15 million global English-speaking equestrians and a 95% confidence rate. It could be predicted that a repeat of data collection would be comparable if 95 out of 100 people were randomly selected to complete the same survey.

3.1. Participants Demographics

Sixteen nationalities were represented, with the majority of participants originating from the UK (67%, n = 188), the USA (7%, n = 20), and Ireland (7%, n = 20). The remaining 53 participants (19%) comprised nationalities such as Australian, Norwegian, French, South African, and Canadian. 96% of participants (n = 270) were female, and 4% were male (n = 11). Nationality, gender, horse information, and experiences with equine cancers are all summarized in **Table 1**.

3.2. Participants Experience with Equine Oncology – Questionnaire Results

Most participants declared they had experiences with their horses having sarcoids (93%, n = 261), 35% (n = 98) of participants had experiences with equine melanomas, 20% (n = 56) had experiences with Squamous Cell Carcinomas, and 13% (n = 37) of participants had experiences with subcutaneous skin tumors and Lymphosarcomas **(Figure 1)**.

9% (n = 25) of participants declared their horse to be below 4 years of age, 43% (n = 121) of participants declared their horse to be 4–10 years old, 22% (n = 62) declared their horse to be 10–16 years old, and 26% (n = 73) declared their horse to be over 16 years old **(Figure 2)**.

Table 1: Questionnaire respondents' demographics: nationality, gender, type of cancer experienced, age of horse, breed of horse, and sex of horse.

Question	Answer categories
Nationality	UK (67%, n = 188), USA (7%, n = 20), Ireland (7%, n = 20), Australia (6%, n = 17), Norway (5% n = 14), France (3%, n = 8), Canada (3%, n = 8), South Africa (2%, n = 6)
Gender	Female (96%, n = 270), Male (4%, n = 11)

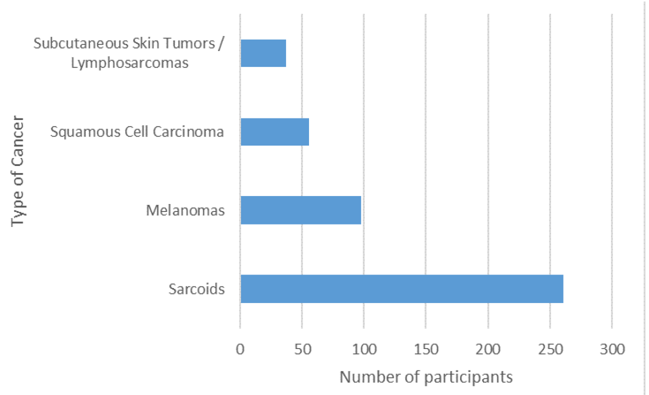

Figure 1: The types of cancer compared with the number of participants who have had experience with equine cancer; Sarcoids (93%, n = 261), Melanomas (35%, n = 98), Squamous Cell Carcinomas (20%, n = 56), and Lymphosarcomas (13%, n = 37).

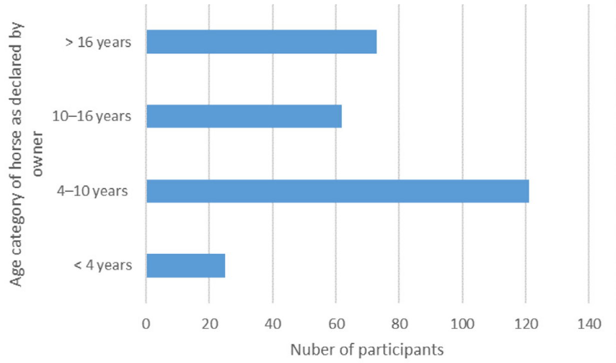

Figure 2: The age categories of horses as declared by participants of questionnaire; Below 4 years (9%, n = 25), 4–10 years of age (43%, n = 121), 10–16 years of age (22%, n = 62), and 16 years and above (26%, n = 73).

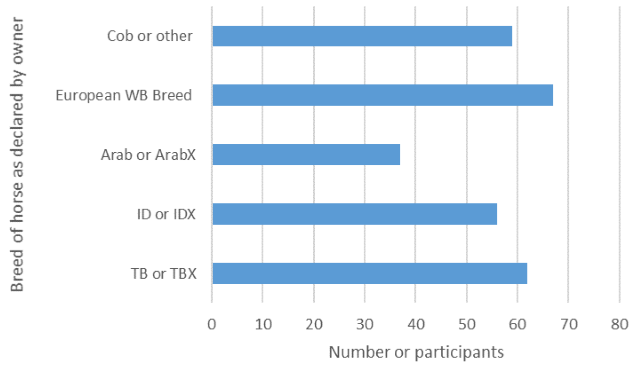

Figure 3: The breed of horses as declared by participants of questionnaire; TB or TBX (22%, n = 62), ID or IDX (20%, n = 56), Arab or ArabX (13%, n = 37), European WB Breed (24%, n = 67), and Cob or other (21%, n = 59).

75% (n = 211) of horses were declared as geldings when the cancer was diagnosed, 22% (n = 62) were declared as mares, and 3% (n = 8) were declared as stallions.

80% (n = 225) of participants stated that they had sought and received a confirmed veterinary diagnosis for the cancer present in their horse, while the remaining 20% (n = 56) had not received a confirmed veterinary diagnosis. Visual diagnosis of the cancer type was the most utilized method for diagnosing the cancer in horses, with 98% (n = 275) relying on visual inspection. All participants who had not sought veterinary diagnosis relied on their own visual inspection to diagnose the cancer in the horse. All participants mentioned visual inspection, and "evidence of a visible mass" was mentioned by all participants, regardless of whether self or veterinary diagnosis was utilized.

There was no statistically significant association between the breed of the horse and the type of cancer declared by the owner, regardless of whether a veterinary diagnosis was present ($p > 0.05$). However, horses whose owners sought veterinary diagnosis were analyzed alone, and the breed did demonstrate an association with the type of cancer declared by the owner ($X^2(9), = 10.78, p < 0.05$). The highest incidence rates were demonstrated in Arabs and Arab crosses associated with melanomas and TB and TB crosses associated with sarcoid presence **(Figure 3)**. There was no association between the sex of horses and the types of cancer present within the individuals ($X^2(6), = 1.25, p > 0.05$). There was a statistically significant association between the age of horses and the type of cancer they presented with ($X^2(9), = 13.56, p < 0.05$). Horses in the older category (16+ years old) demonstrated a greater incidence of Lymphosarcomas than the other age categories (n = 28).

There was a significant association between the type of cancer diagnosed and the treatment options explored by participants ($X^2(6), = 8.72, p < 0.05$). Different treatment methods were highlighted by participants for the different cancers, with several participants using multiple treatment methods in conjunction with one another **(Figure 1)**. Typically, the topical chemotherapy treatments used by participants were AW4 – LUDES and AW5 cream. The topical herbal creams typically included ingredients such as Thuja, Mistletoe, and Echinacea. Homemade topical treatments included ingredients such as flour, water, sugar, manuka honey, turmeric, and toothpaste **(Figure 4)**.

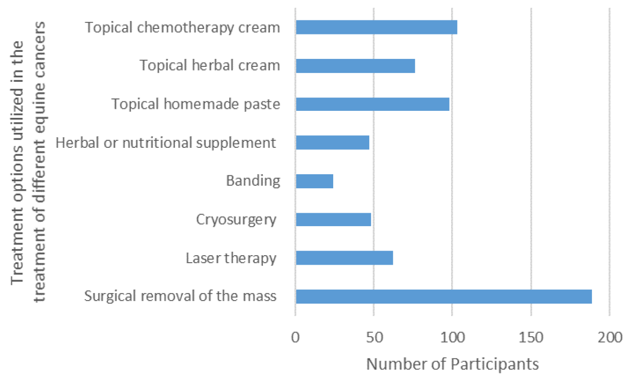

Figure 4: Treatment options utilized in the treatment of different equine cancers; Surgical removal of the mass (n = 189), Laser therapy (n = 62), Cryosurgery (n = 48), Topical chemotherapy cream (n = 103), Topical herbal cream (n = 76), Topical homemade paste (n = 98), Herbal or nutritional supplement (n = 47), and Banding (n = 24).

All respondents who had sought veterinary diagnosis, when asked about the prognosis provided by the veterinarian, declared that their horse would continue to have "a good quality of life." All commented on the management of the cancer being positive even if treatment did not eradicate; there was an emphasis on trying to maintain the horse's welfare. There was an association between the treatment method utilized and the perceived success of the treatment by the respondents ($p > 0.05$). Combination treatments were associated with a higher perceived success rate in the treatment of equine cancer compared to single treatment methods ($X^2(1), = 12.43, p < 0.05$). There was no association between the type of treatment utilized and the perceived success rate of the participants.

3.3. Participants Experience with Equine Oncology – Case Study results

A total of 164 participants provided additional information regarding specific case studies involving equines they are responsible for, including image evidence of the cancer and before and post-treatment images. Of the 164 participants who sent case studies, 38 had not sought a veterinary diagnosis, while the remaining 126 had a confirmed veterinary diagnosis for the present cancer. Additional analysis related to the previous questionnaire result analysis from the case studies included the location of the cancer as confirmed by image evidence, the size of the skin tumor as confirmed by the owner's description, and the success of treatment as provided by image evidence and participant commentary where applicable.

All case study horses presented with skin tumors in the images. Some had multiple sarcoids in a single image, resulting in 258 tumors being presented. However, in 18 of the case study horses, the photographs were not clear enough to categorize the tumor based on tissue morphology. The photographic evidence demonstrated a variety of tumors with different tissue morphology, including hairless circles flush to the skin, protruding wart-like growth with thickened nodules, smooth and firm protruding growth, and ulcerated protruding mass. Most of the tumors presented were smooth and firm protruding masses (n = 146), hairless circles (n = 56), protruding wart-like growth (n = 32), and ulcerated protruding mass (n = 24).

Of the 164 case study horses, the most common site of cancer development was the genitals and groin area, with 114 horses presenting with tumor development in this area. The remaining locations on the body where the cancer developed were the head and neck (n = 30), the legs (n = 18), and the main body mass (n = 2). Regarding tumor size, 11 horses (7%) presented with skin tumors smaller than 2 cm in diameter at their widest part, 82 horses (50%) presented with tumors between 2–5 cm in size, 69 horses (42%) presented with tumors 5–10 cm in size, and 2 horses (1%) presented with skin tumors greater than 10 cm **(Figure 5)**.

Of the 164 case studies presented, 96 provided additional photographic evidence of the skin tumor before treatment commencement and following treatment. Images of tumors before treatment were provided at different stages of development. Some individuals provided images from the date of detection, some from the date of veterinary diagnosis, and others from the date that treatment commenced. The images of the tumors after treatment were taken at various stages, ranging from 1 week after treatment to one year after treatment. Of the 96 participants who submitted the images, 90% (n = 86) presented images that they determined as successful. These participants made comments relating to tumor size decreasing, changes in tissue morphology, and horse behavior related to discomfort. The remaining 10% (n = 10) submitted images of tumors following treatment that they did not deem successful. These participants made comments relating to tumor size increasing or remaining the same, an increase in horse discomfort, and negatively perceived tissues morphology changes, such as bleeding.

3.4. Participants Experience with Equine Oncology – Social Media Analysis

A total of 243 social media posts were analyzed concerning their content relating to equine cancer: diagnosis, treatment, and prognosis. Social media analysis indicated a strong community support system, with many users sharing personal experiences and advice on managing equine cancer. Keywords frequently associated with equine oncology in social media posts included "treatment," "hope," "support," and "awareness."

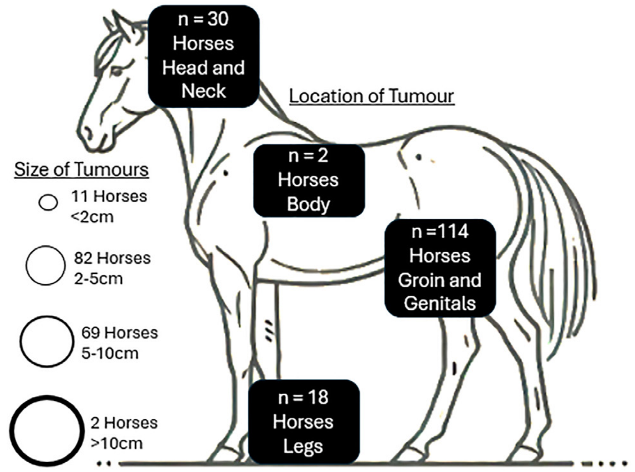

Figure 5: Location and size of tumors in case study horses (n =164) presented for case study analysis.

All posts were divided into three categories: those relating to seeking aid in the diagnosis of equine cancer (n = 60), those seeking advice on treatment options available for cancer following diagnosis (n = 147), and those raising awareness of treatment options previously utilized and the subsequent prognosis (n = 26).

60 posts were categorized due to the post asking the public/ social media members to aid in the diagnosis of suspected equine cancer presence. None of the respondents in the comments of these posts who gave advice regarding diagnosis, all analyzed within 24 hours of posting, declared previous veterinary experience as their reasoning behind their "diagnosis." Several comments advised the original poster to seek the advice of a veterinarian. This advice was present on 16 of the 60 categorized posts.

Participants reported using a variety of sources on social media to seek advice on the treatment of equine cancer. The most frequently cited sources included veterinary professionals and clinics (45%), Equestrian influencers and bloggers (35%), Equine health organizations (30%), and personal anecdotes and experiences from other equestrians (60%). Treatment success varied within those relating to prognosis, with 50% (n = 13) reporting positive outcomes, 30% (n = 8) reporting mixed results, and 20% (n = 5) reporting negative experiences. Common treatments mentioned included surgery, chemotherapy, and radiation therapy alongside alternative treatments such as topical cream application, alternative therapies, and herbal product application.

The analysis of social media posts showed a significant amount of engagement, with posts about treatment options particularly receiving an average of 67 likes and 12 comments. The sentiment analysis of these posts revealed a predominantly positive tone, with 70% (n = 862) of all comments (n = 1232) offering support and encouragement to the original poster. Common forms of positive support included content referring to emotional encouragement, practical advice, shared experiences, and recommendations for care, treatment, and veterinary support.

4. Discussion

This research investigated knowledge, perceptions, and experiences with domestic horse oncology. The study explored a comprehensive examination of the diagnosis and treatment options sought within equine oncology through dual data collection methods, which included participant questionnaires and analysis of social media posts. Incident rates for equine cancer have not been accurately established to a significant extent globally [35]. The current study's results included 16 nationalities being represented, providing a global perspective on the incidence rates of different cancers. For future research, emphasis could be placed on comparing incidence rates across different countries to determine whether this correlates with increased incident rates in specific cancers.

Knowles *et al*. [10] analyzed records from a database containing neoplastic equine histology submissions from 1982–2010, with 964 cases examined. They found that the majority of tumor types were sarcoids (24%), which aligns with the current study's finding that 93% of participants had experience with sarcoids. Knowles *et al*. [10] also identified submitted cases of gonadal stromal tumors or mast cell tumors (MCT) in their study, while no individuals in the current study mentioned their horses suffering from gonadal stromal tumors or mast cell tumors. This could have been due to the breeds of horses included in the current study or lack of owner and veterinarian knowledge regarding the symptoms associated with these tumors. Regarding breed, both the current study and Knowles *et al*. [10] found that Thoroughbred and Thoroughbred cross individuals had an increased risk of sarcoid development.

Knowles *et al*. [10] found that Cob/Cob crosses had an increased risk of SCC and MCTs, and Arab and Arab crosses were at a higher risk of developing Mast Cell Tumors. In comparison, within the current study, Arab/Arab crosses were statistically associated with melanoma development, and Cob/Cob crosses did not associate with any particular tumor development. Melanoma has been recognized to appear most frequently in specific equines, with figures showing 80% of these cases have appeared in grey horses, indicating genetic predisposition [36]. The current study did not ask for the color of the horses, although the case studies would have provided this for some of the individuals in which images were clear enough. In the future, further information from participants, including coat color, could be obtained to assess their association with specific tumor development. Knowles *et al*. [10] also found that mares were at a reduced risk of SCC, and the current study found that there was no association between the sex of horses and the types of tumors presented in the current study.

Hollis [37] discusses the evidence behind the most used treatments for equine sarcoids, ultimately concluding that no one treatment is universally successful, and there are many treatments with varying levels of scientific evaluation and reported success rates. This would relate to the current study's findings of varying treatment options being employed by horse owners for various tumor types. Previous studies have identified the success of surgery, chemotherapy, radiotherapy, and immunotherapy to treat sarcoid patients [16,38,39]; however, the difficulty lies with the varying success of cheaper, more accessible options employed by horse owners. Unfortunately, due to no treatment modality being proven to be the singular most effective when attempting to treat equine patients diagnosed with sarcoid tumors [38,40], this opens the door to less scientifically grounded methods of treatment and potentially even those that may impact equine welfare in a negative manner. In addition to this, limitations due to tumor size and the severity of tissue invasion may make the surgical removal of the tumor impossible in advanced cases [41]. These instances require the use of drugs such as chemotherapy but are often complicated because of inconsistent results and costs [41]. Pitman [42] states that due to a lack of research and reported incidence within equine cancer, limitations are apparent within the development of treatment options, further suggesting the challenges including cost and limited information for horse owners and carers to restrict the conduction of reliable studies to develop a greater level of understanding.

Most participants had a gelding (75%), and most case study horses (n = 114) presented with tumors in the groin and genital area. Penile SCCs are a common, potentially life-threatening neoplasm, thought to be commonly caused by Equine caballus papillomavirus (EcPV) [23]. Giuliano [43] declared squamous cell carcinoma (SCC) as the most prevalent tumor affecting the equine eye and equine genitals worldwide. For the current study, sarcoids were declared the most common tumor seen in participants' horses; however, this could be due to the Facebook groups participants were recruited from causing a bias in results. With SCCs, if the tumors are minor or recognized as carcinomas in situ, surgical excision of the SCC may be sufficient [44]. However, inadequate surgical excision has been reported to cause tumor recurrence, concluding this method of treatment success rate to not be the best practice [45]. A larger scale more focused study on genital tumors in male horses is needed to assess the incidence rates and to make any associations between age, breed and castration status.

While earlier studies have explored the varying processes to diagnose equine cancer with a need for veterinarian physical examination [9], they have not explicitly addressed the use of social media within an owner's remit of diagnosis. The results of this study indicated a strong correlation between the various veterinary treatment modalities, with combination therapies being perceived as more effective than individual ones. With owners' views being positive in relation to cancer management, focusing on maintaining the horse's welfare even if the treatment did not eradicate the cancer. Social media analysis highlighted owners seeking support from alternative sources as opposed to a veterinary diagnosis, indicating further support for equine ownership is necessary in relation to cancer.

Through the analysis of questionnaire results, our study suggests that participants use a variety of sources on social media to seek advice on the treatment of equine cancer. The results demonstrate that social media is a vital resource for equestrians regarding equine cancer, significantly enhancing knowledge and providing emotional support. However, the presence of misinformation highlights the necessity for improved information accuracy and expert involvement. Leveraging social media's strengths while addressing its weaknesses can further benefit the equestrian community in managing equine cancer. However, the obvious reliance of equine owners on the support of peers when investigating abnormalities within their equine suggests a lack of veterinarian investigation being sought due to the owner's need for emotional support or reassurance in these circumstances, which may result in misinformation being provided, and a delay in care being obtained. In relation to existing research **[32]**, our research voids the gap between veterinary diagnosis and seeking advice through social media, suggesting that 70% of post responses are positive and helpful, in turn leaving 30% of responses being unhelpful or inclusive of misinformation. In relation to the questionnaire, there was a significant association between the type of cancer diagnosed and the treatment options explored ($X^2(6)$, = 8.72, $p < 0.05$), which is supportive of equine owners seeking veterinary input into diagnosis. However, 38 of 164 participants who provided case studies had not sought veterinary diagnosis, instead relying on image evidence and support group input into their presumed diagnosis based upon appearance, location, and other owners' experience.

Recent observations suggest that there is extensive research and knowledge of common types of cancer and treatment options available; however, these are not always plausible for equine owners to access, which results in them seeking support online and undertaking self-treatment options as a primary action. However, as Durham **[32]** suggests, the goal of equine clinicians is early cancer detection, with a priority to treat the oncology patient and improve its quality of life. Our findings provide conclusive evidence that this phenomenon is associated with a need for greater support for the owner than is able to be provided by a veterinary professional, resulting in social media support being pursued, sometimes as the only source of diagnosis. Further research into the reasons behind this would be of benefit to aid in a greater support network with solely reliable input being provided. This would be in keeping with Smith *et al.* **[46]**, who discuss appropriate treatment methods and choices made by the owner being dependent on personal beliefs and often result in inexperienced and inadequate self-treatment options.

5. Limitations

This study employed an online survey and social media analysis to collect data on equine oncology experiences. However, it has several notable limitations which could affect the overall validity and reliability of its findings.

One significant limitation of this study is the reliance on self-reported data from participants. Although the survey includes questions on equine cancer diagnoses, symptoms, and treatments, these responses heavily rely on the participants' recollection and interpretation of their experiences. Memory biases and misinterpretations of events or symptoms could lead to inaccurate reporting. Moreover, self-diagnosis of cancer, particularly for respondents who did not seek veterinary advice, may introduce substantial errors in data accuracy. Participants who based their assessment on visual inspection might misclassify other health issues as cancer, which can distort the results.

Another limitation arises from the sample composition. Despite including participants from multiple nationalities, the majority (67%) are from the UK, with females comprising 96% of the participants. While this might reflect the demographics of equestrians, it limits the generalizability of the findings to a broader population of horse owners. This underrepresentation of males and other nationalities means the results may not accurately reflect the experiences or practices of the global equestrian community, particularly in regions with different veterinary practices or cancer awareness levels.

The use of social media platforms and equestrian-specific groups for recruiting participants and obtaining case studies introduces selection bias. Individuals active in such groups may already have a heightened interest or awareness in equine cancer, leading to an overrepresentation of highly engaged or informed participants. Additionally, the study may overlook the perspectives of horse owners who do not participate in these online communities, thus limiting the diversity of experiences and knowledge within the sample. This is particularly important when analyzing social media posts, as the content might be skewed towards individuals who are more vocal or actively seek advice and support on these platforms.

A significant portion of the survey and social media data relies on visual diagnosis or owner assumptions without veterinary confirmation, posing a challenge to the study's diagnostic reliability. Without consistent professional diagnosis across all cases, it is difficult to determine the true prevalence and type of equine cancers. This limitation introduces the possibility of misclassification, as what one participant identifies as sarcoids may be an entirely different condition. This affects the study's ability to draw accurate conclusions about the types and distribution of cancers in the equine population.

Although the survey included a mix of binary, multiple-choice, and open-ended questions, the structure may still limit the depth of responses. While open-ended questions allow for nuanced answers, they also make it challenging to ensure consistency in the data provided. Respondents may interpret questions differently, leading to variability in the quality and scope of the answers. Additionally, while the survey was piloted on a small group of participants, a broader pilot might have uncovered issues related to question phrasing or answer categorization that could enhance clarity and consistency in responses.

In summary, while the study provides useful insights into equine oncology from a large sample of participants, its reliance on self-reported data, non-random sampling, and lack of consistent professional diagnostic validation are key limitations that may affect the generalizability and accuracy of its conclusions.

6. Conclusion

In conclusion, the results of this study offer valuable insights into the experiences and knowledge of equestrians regarding equine cancer, highlighting significant trends in diagnosis, treatment, and prognosis. The participant demographic demonstrated strong representation from the UK, with females dominating the responses. Experiences with sarcoids were overwhelmingly common, with veterinary diagnosis sought by a majority of participants. Notably, breed and age were significantly associated with specific cancer types, particularly melanomas in Arabs and sarcoids in TB crosses, echoing findings from prior research on breed predispositions to equine cancer. The association between combination treatments and higher perceived success rates also aligns with literature advocating for multimodal approaches in cancer treatment. Social media emerged as an essential platform for community support and shared experiences, although the prevalence of misinformation underscores the need for expert-led guidance. These findings contribute to the growing body of literature on equine oncology, particularly in the context of breed-specific risks and the evolving role of social media in equestrian health care, as observed in prior studies on digital health communication and animal welfare.

Authors' Contribution

Conceptualization; G.B., methodology; G.B., formal analysis; G.B., writing original draft preparation; G.B. & J.W., writing review and editing; G.B. and J.W. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data supporting the findings of this study are available within the article.

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The research did not receive any funding.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

Data collection adhered to the DN Colleges Ethical Policy and followed the guidelines of the Declaration of Helsinki.

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