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A Preliminary Study on Amateur French Show Jumper and Dressage Riders: Can Riders Accurately Recall the Duration and Content of Their Warm-Up Routines?

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Abstract

Effective warm-up (WU) prior to exercise can increase performance and decrease injury risk. Little is known about how riders design and implement WU routines in training and competition. A two-phase study aimed to understand show-jumper and dressage rider decision-making when selecting WU routines during flatwork sessions at home. An initial survey identified the rider's perception of warm-up use and decision-making. Then, ten riders competing at intermediate levels in dressage (DR) (n = 7: 39 warm-ups) and show-jumping (SJ) (n = 3: 22 warm-ups) videoed their horses' WU; duration, gaits, transitions, and specific movements were recorded by a single observer. A post-warm-up form was completed by riders (DR: 4; SJ: 2; total WU = 44) to assess riders' memory and perception of their warm-up and gain information on external temperature, and horse/rider age. Rider WU profiles were formulated and differences were assessed through a series of Friedmans and Kruskal-Wallis analyses. Riders warmed up for a total of 24 mins ± 7.1 mins (DR: 22 mins 3 secs ± 6 mins; SJ 27 mins, 29 secs ± 8 mins). Riders spent the most time in walk (DR: 48.3%; SJ: 56.4%). Riders (88%), who recorded > 3 WUs, WU sessions significantly differed over time ($p < 0.03$). Out of the 44 WU analyzed, riders accurately recalled 13.6% of the routines. No significant differences in total WU duration or total time spent warming up in walk were found between temperatures < 5°C and > 30°C even though riders said they adapted their warm-up to the weather during stage 1 of this study. Warm-ups at home seem to be rider and horse-dependent but are not discipline or climate-specific when preparing for a flatwork session at home.

Keywords

Warm-up; showjumping; dressage; competition; equestrian sport

1. Introduction

In order to compete successfully in equestrian sports, horse riders must prepare their horses physically and mentally for competition; this requires regular training sessions where the cardiovascular and musculoskeletal systems of the horse are put under physiological stress [1]. An exercise session should be composed of a preparatory warm-up phase (WU), a planned training phase consisting of targeted exercises, and followed by a cool-down phase to facilitate recovery [2]. These individual sessions should be components of a broader conditioning program designed to achieve the core principles of training: preparing the horse physiologically and psychologically for the work expected, developing required motor skills and conditioning the neuromuscular system to perform these skills, and promoting health to prevent injury and extend career longevity [3]. Exercise sessions should align to the stage and level of training of both horse and rider and should be assessed to monitor how well they are contributing to the attainment of planned short- and long-term performance goals, as well as enabling evidence-informed decision-making in the rider to enable them to meet their duty of care to safeguard equine health and welfare [4].

In horses, an efficient warm-up regime has been shown to increase the use of the aerobic metabolic pathway resulting in lower heart rates and respiratory rates during subsequent competition, diminishing glucose expenditure as well as reducing lactic acid accumulation in the muscles [1,5,6]. This approach delays the onset of fatigue providing a potential performance advantage over other horses competing, which may have not been warmed up adequately [7–11]. The rider will also contribute to the horse's physiologic load and should therefore complete their own warm-up to ensure they are suitably prepared to ride and to reduce loading on the horse but also to optimize the performance of the horse and rider combination [4,12]. Riders' knowledge, skills, experience, and emotional state can therefore influence how well the warm-up prepares the horse to perform at their best [4,13]. However, studies [14–17] recording warm-up length in dressage and showjumping (SJ) at competitions have riders' warm-up routines, both within and across disciplines vary substantially in practice. For example, the warm-up duration of show jumpers varies from 12-27 minutes [14] to 4-63 minutes [16]. The content and duration of the warm-up in competition also appear to depend on the experience level of the rider as well as on the competition level [15,17,18].

1.1. Warm-Up in Training

The training environment provides athletes with an opportunity to develop their fitness, skills, and partnership, including determining what constitutes a successful competition warm-up, as well as requiring effective warm-up approaches for the different exercise sessions within it, to promote performance and prevent injury. Chatel and Williams [2] reported that riders tended to reflect on warm-up routine content and duration in the competition environment with less consideration given to warm-ups in training. It is known from previous research that most injuries occur during training [19,20], therefore, individualizing each warm-up routine depending on the need of each horse (his age, pathology, and training methods) could potentially decrease injuries and therefore decrease the number of days lost in training [21]. Knowing that, field studies observing SJ and DR riders warm-up at home have not been achieved yet as most studies concentrate on the competition aspect.

1.2. Perception vs Reality

In sports, the difference between a winning or losing performance can be attributed to differences in the physiological or psychological status of athletes [22,23] as well as the efficacy of training regimens [24]. Time distortion has been reported in athletes participating in other sports, with differences between memory recall and actual training content increasing with intense exercise [25] and increased emotion [26]. This phenomenon has also been documented within athlete support networks, for example, football coaches were only able to accurately recall between 30% and 59.2% of critical events during a match after games [27,28]. While human athletes often have coaches during their warm-up and training sessions to assist and guide them in their decision-making and stress-management related to their sports, in equestrian sports, for many riders the majority of training sessions will be undertaken independently, with the rider acting as a coach for the horse and self-coaching themselves [2]. The unique nature of equestrian sport therefore, where the rider is responsible for and has to manage their own and direct their horse's performance is likely to reduce further the efficacy of memory recall. At competitions, riders are more likely to have support in place, which could facilitate the opportunity to engage with technology and record performances, but often the focus will be on the competitive test neglecting the warm-up period and precluding evaluation of how this key phase of the competition period has affected subsequent performance. Time perception can be affected by different factors including temperature, personal enjoyment and investment in the task to be realized, emotional state, stress, prospective or retrospective timing, familiar vs unknown location as well as age of the participant studied [29].

1.3. Effective Decision-Making

Effective decision-making requires the individual to utilize their working, prospective, and long-term memory centers in the brain [30]. Prospective memory is the ability to formulate plans and intentions, to retain them, and to execute them upon the occurrence of the appropriate cues [31]. Prospective memory should therefore be used by a rider when implementing a warm-up prior to a training session. In this study, as riders were aware they were required to recall their warm-up an event-based test was used. Working memory is required for the perception of time and has been associated with sports skills [32,33]. It uses a combination of short-term and long-term memory for storage and movement based on memory [34]. Working memory engages different parts of the brain to be stored and used for decision-making and does not always seem to be age-dependent. However, appears to be influenced by experience, with recalling of event-based working memory reported to be superior in inexperienced individuals compared to novice athletes [30,32–34]. Memory and time perception can also be affected by the emotional state of the rider. During a competition, the athletes are subjected to higher stress levels which could affect their decision-making [13,35]. Other factors can influence decision-making, at shows most riders have a coach on the ground to help them manage their time and have a fixed competition time that they must respect, giving them guidance for the timeline of their warm-up [2].

At home, the rider should be undergoing less stress as there is no competition arousal, however, the fact that most riders train alone and have no set time to follow, makes it difficult to understand how they perceive time while riding [2].

Equestrian sports are facing increased scrutiny from the public as equestrianism's social license to operate is questioned [36]. Horse owners and riders have a duty of care to manage their horses effectively including suitable preparation for exercise [4,36]. An increased knowledge and understanding of what constitutes an ideal equine warm-up, and what factors influence this, is currently lacking across horse sports. This information is needed to underpin evidence-informed practice and effective rider decision-making [2,4]. An important stage in this process is to understand current practice; therefore, this study aimed to evaluate the WU practices utilized by experienced dressage DR and SJ riders prior to flat work sessions in their home environment, to determine if extrinsic factors such as temperature affected WU content and duration. As well as determining if riders could recall the details of their horse's WU accurately. According to the results of previous studies in a competition environment,

it was also hypothesized that at home, dressage riders would warm up for a longer period than show-jumpers.

2. Materials and Methods

A two-stage study was designed to evaluate French riders' perception of equine warm-up via 1) an online survey, and 2) to assess their warm-up routine and recollection of their warm-up using video recording as well as a specific questionnaire. Ethical approval for the study was granted by the Hartpury University Ethics Committee (Ethics 2019-51 and Ethics No: 2019-58) for both the horses and riders, data protection was ensured and approved within this process.

2.1. Online Survey

The first stage of the study utilized data collected via an online survey of French riders' perception of their warm-up protocols and daily decision-making within these (for further details please refer to [2]. Part of the results collected in this survey have been published previously, however, the data used in this article were not analyzed in [2]. The study was designed as an online questionnaire (Survey Monkey®) with a total of 39 questions for DR riders and 41 questions for SJ riders. The first seven questions were common to both SJ and DR riders and were multiple choice questions related to the respondents' age, and nationality as well as their equestrian life and experiences. The survey was then divided into discipline-specific questions on warm-up routines. The dressage survey consisted of three open questions, ten Likert questions, and 26 multiple-choice questions. The survey for SJ riders consisted of three open questions, ten Likert questions, and 28 multiple-choice questions; the two additional questions for SJ riders related to using jumping within warm-up regimes. For this study, answers to questions 4, 18, 20, 27, 36, 38, 48, 50, 60, and 69 were analyzed to provide insight into non-ridden warm-up routines, and how rider perception of the influence of environmental conditions influenced decision-making related to warm-up. Please refer to [2] for a detailed overview of the method.

2.2. Warm-Up Routine, Perception vs Reality

A combined observational and phenomenological research design was utilized to gather video data of rider warm-up sessions and compare individual recall of the structure of these to the videos using self-completed training diaries. In this study, an event-based test was used to assess prospective and working memory in the recall of the warm-up routine.

Show jumpers and dressage riders were recruited via convenience sampling through social media to take part in the study. To be eligible to participate, riders had to be over 18 years old and be currently based and competing in DR or SJ in France (from Elementary up to Prix Saint George in dressage and from 90 to 120cm for SJ). Participating riders declared their horses to be sound and able to participate in the study. Horses were checked at least annually by a veterinarian. During the study, if a horse became lame, the videos recorded before the lameness occurred were used and the horse was then removed from the study; this was the case for one horse. If the horse had a known pathology, riders were asked to write it down and also write if their warm-up routine was changed due to the horse's prior pathology.

Riders were asked to record a video of the entirety of warm-up routines completed while training at home using either a smartphone or other device compatible with a computer between May 2020 and December 2020. Each rider was asked to record up to ten videos on different days over 8 months. To be eligible for the study, the recording device had to be placed in the same spot for the same rider to facilitate recording different warm-up routines. The video had to be of good quality and the horse rider dyad was not allowed to leave the video frame for more than 3 seconds if the recording device could not cover the entire riding arena. The riders were asked to record their usual warm-up routine for the training sessions about to take place. Within 12 hours of each training session being recorded, riders then had to complete a training session record form to recall the content and duration of activities within the warm-up recorded **(Table 1)**. On the form riders/participants were also asked to write what type of work session they were preparing for.

2.3. Data Collection and Analysis

Each rider recorded their own warm-up routine and uploaded the video to YouTube™ on a private listing. Training forms were completed and sent via email with the link to the video to be analyzed by a consistent observer (MC). Videos were played and paused every time a change of rein or gait/movement occurred. This allowed for analysis of variables studies as recorded in **Table 1**. The data collected were then entered anonymously on a Microsoft Excel Version 2019 (IBM, New York, USA) spreadsheet to enable rider warm-up profiles to be formulated.

Data met non-parametric assumptions and are reported as median ± IQR unless otherwise stated. A series of Friedman's analyses identified if significant differences occurred in individual riders' warm-up routines recorded by video and their perception of the same warm-up recorded via memory recall. Where significant differences existed, post-hoc Wilcoxon analyses identified where differences occurred between individual training sessions. For each rider, further Wilcoxon analyses examined if video-recorded warm-up content differed in perception or recalled content across all warm-up sessions recorded. For each warm-up routine, Wilcoxon analyses also compared warm-up duration and components to air temperature. Kruskal-Wallis analyses identified if differences occurred in warm-up duration and content, between riders and across the disciplines represented. Where significant differences existed, post-hoc Mann Whitney U analyses identified where differences occurred. Significance was set at $p < 0.05$; all analyses were conducted using SPSS Version 26 (IBM, London, UK).

Table 1: Variables recorded in training session form.

Rider name	Assigned unique identifier code
Horse name	Assigned unique identifier code
Date	Date of the training session
Weather	Raining, foggy, sunny, humid, windy
Environmental temperature	Air temperature recorded in Celsius degrees
Total warm-up time	Total seconds warming up; measured from mounting to when rider self-selected their flatwork warm-up was complete
Time spent in each gait	Cumulative seconds spent in halt, rein-back, walk, trot, and canter during the rider-defined warm-up period
Time spent on each rein	Cumulative seconds spent in walk, trot, and canter during the rider-defined warm-up period for the left and right rein, respectively
Time spent in different head carriage	Cumulative seconds spent in walk, trot, and canter during the rider-defined warm-up period where the horse's head is either below his chest level or above it (as seen in **Figure 1**)
Time spent in lateral work	Cumulative seconds spent engaging in specific lateral work movements (e.g. shoulder-in, half-pass) during the rider-defined warm-up period

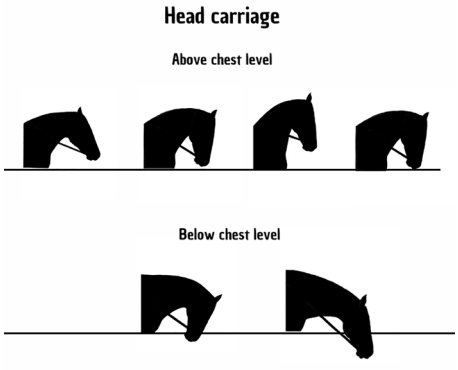

Figure 1: Head carriage classification.

3. Results

3.1. Stage 1: Survey

A total of 257 riders completed the survey and the majority (60%, n = 155) warmed up independently or with support from a coach or another rider (38,9%, n = 100). The survey found no significant differences between warm-up regimes between DR and SJ riders [2]. Respondents were asked to outline any non-ridden warm-up regimes followed, 70.1% (n = 125) stated they hand-walked their horses as a warm-up before riding, 61.8% (n = 54) manually massaged their horses and 16.5% (n = 24) used a massage pad on their horses before riding. Respondents were also asked what head carriage they used at the beginning of the warm-up during the walking phase, 93.8% (n = 160) responded they walked their horses either on a loose rein or in a low and round frame. The majority of DR (71%, n = 62) and SJ riders (70%, n = 64) included lateral work in the warm-up.

Respondents were also asked how their warm-up practice and decision-making varied with time of year and in response to environmental conditions; 60.1% (n = 107) agreed warm-up duration should vary depending on the time of the year. Respondents (24%, n = 22) self-reported that during winter, the warm-up should be longer as it takes longer to warm up horses' muscles. The original survey was used to inform stage 2 design (refer to [2] for additional survey results).

3.2. Stage 2: Warm-Up Routines, Perception vs. Reality

For the warm-up observation, ten female riders took part in the study (n = 7 DR; n = 3 show jumpers); however, only six of these (n = 4 dressage riders; n = 2 show jumpers) returned their training forms, aged 22 to 51yo age mean 33 ± 9.7 (mean: DR: 32yo; SJ: 35yo). Therefore, a total of 61 warm-up video routines were analyzed (n = 39 dressage; n = 22 show jumpers) and a total of 44 forms were completed (n = 21 dressage; n = 23 show jumpers). The total warm-up duration for each rider is described in **Table 2**. The age of the horses used in the study ranged from 8 to 19yo, mean of 12yo ± 3.1 (DR 12yo; SJ 11yo).

3.3. Video Analysis of Warm-Ups

Warm-up duration in the arena for all riders ranged from 8:04 minutes to 42:07 minutes. All riders warmed up in an outdoor arena, except for rider 2 who warmed up in an indoor arena. None of the riders hand-walked their horses as part of the warm-up regime; one rider (10%) used a massage pad on her horse prior to ridden warm-up. WU duration across all horse and rider combinations was 23 ± 8 mins (mean 24 ± 7 mins). Dressage riders warmed up for 22 ± 10 mins (mean 22 ± 6 mins), while showjumpers warmed up for 27 ± 10 mins (mean 27.5 ± 8 mins). Dressage riders spent ~2% longer on the left rein, while SJ riders spent ~2% longer on the right rein when warming up.

Riders spent the most time in walk (average: DR: 10 ± 4 mins; SJ: 15 ± 7 mins) and trot (DR: 7 ± 4; SJ: 8 ± 2 mins), and the least time in canter (DR: 4 ± 3 mins; SJ: 4 ± 2 mins). During the first walk phase (before trotting or cantering) 80% of the riders (n = 8) walked their horses with a low head carriage (head below chest level). The low head carriage in the first walk phase was used in 67% of the warm-up routines of dressage riders (n = 26) and in 95% of the show jumpers (n = 20).

All riders used halt (84 ± 7 secs) and rein-backs (32 ± 6 secs) when warming up. Dressage riders performed on average 26 transitions per warm-up routine, ~44% more than show jumpers who performed on average 18 transitions per warm-up routine. Lateral work was used by 100% of the DR and only by 30% of SJ riders during their WU. Overall, lateral work was used in 72% of dressage warm-up routines (28/39) and accumulated an average time of 1:30 min (±1), a median of 1 minute. Lateral work was used in 14% of SJ warm-up routines (3/22) and accumulated an average time of 12 seconds (±36), a median of 0.

When the warm-up routines of individual riders were compared across the period recorded, no significant differences were found for either the gaits used, warm-up duration, or the time spent on each rein during the warm-up. A similar pattern was observed when comparing intra- and inter-discipline warm-up routines across riders; with no significant differences found between riders' warm-up routines.

Table 2: Total warm-up (WU) duration range and mean (mins).

	Rider 1	Rider 2	Rider 3	Rider 4	Rider 5	Rider 6	Rider 7	Rider 8	Rider 9	Rider 10
WU length range (mins)	20–32	13–32	21–31	22–42	21–23	12–31	20	20–32	12–21	8–28
WU length (median)	23	24	26	31	22	18	20	24	20	22
WU length Mean (mins)	25	23	27	31	22	21	20	25	18	19

3.4. Rider Recall of Warm-Up Routines

Riders consistently demonstrated poor recall of the warm-up content, either over-estimating or under-estimating the total time spent warming up and the time spent in each gait **(Table 3)**. Out of the 44 warm-ups analyzed, riders recalled total WU time accurately in only 13.6% (n = 6) of the recorded routines. A total of 13 WU routines were under-estimated and 25 WU routines were overestimated. Time spent in walk, trot, and canter was correctly assessed in 11.4%, 13.6%, and 18.2% respectively.

Total warm-up routine time was under-estimated by up to 10 mins and over-estimated by up to 13 mins. Time spent in walk, trot, and canter were under-estimated by up to 13, 4, and 6 mins, respectively, and over-estimated at most by 12 mins for the walk and 10 mins for the trot and canter.

There was a trend for riders to over-estimate the time spent for the total warm-up, and time spent in walk, trot, or canter, rather than under-estimate the time spent warming up (**Table 4** and **Table 5**); however, the range of time over-estimation was smaller than under-estimation for all variables. The rider's age had no significant impact on warm-up recall.

Riders reported environmental temperatures ranging between 3°C and 39°C for the study period. The number of routines for each temperature variation is presented in **Table 6**. No significant differences in total warm-up duration or the total time spent warming up in walk were found between sessions in temperatures below 5°C and temperatures above 30°C.

Table 3: Duration of total warm-up, walk, trot, and canter in videos versus rider's perception; WU: warm-up.

Rider	WU	Total WU duration (mins)	Total WU duration (mins)	Diff in mins	Time in walk (mins)	Time in walk (mins)	Diff in mins	Time in trot (mins)	Time in trot (mins)	Diff in mins	Time in canter (mins)	Time in canter (mins)	Diff in mins
	Session	Actual	Perception		Actual	Perception		Actual	Perception		Actual	Perception	
1	1	21	20	-1	14	15	1	0	1	1	5	4	-1
	2	23	30	7	13	20	7	5	4	-1	12	6	-6
	3	29	30	1	13	20	7	4	4	0	5	6	1
	4	32	30	-2	10	20	10	4	2	-2	11	8	-3
	5	20	20	0	14	16	2	2	0	-2	2	4	2
2	1	21	20	-1	13	10	-3	7	10	3	0	0	0
	2	13	20	7	13	7	-6	5	10	5	1	3	2
	3	27	35	8	13	22	9	8	12	4	5	5	0
	4	32	27	-5	16	20	4	12	10	-2	2	2	0
	5	26	20	-6	26	26	0	8	10	2	2	3	1
	6	24	25	1	10	15	-5	8	10	2	2	5	3
	7	28	30	2	18	5	-13	7	10	3	0	5	5
	8	16	15	-1	6	19	-13	10	10	0	2	1	-1
3	1	22	30	8	9	6	-3	9	9	0	4	4	0
	2	31	30	-1	14	10	-4	8	8	0	8	8	0
	3	30	30	0	14	14	0	10	15	5	6	12	6
	4	28	30	2	15	8	-7	7	14	7	6	15	9
	5	23	30	7	20	14	-6	12	16	4	7	10	3
	6	23	30	7	11	20	9	8	16	8	4	12	8
	7	21	30	9	11	20	9	6	14	8	4	12	8
	8	26	30	4	17	20	4	7	14	7	2	12	10
	9	27	30	3	11	14	3	10	16	6	6	10	4

Rider	WU	Total WU duration (mins)	Total WU duration (mins)	Diff in mins	Time in walk (mins)	Time in walk (mins)	Diff in mins	Time in trot (mins)	Time in trot (mins)	Diff in mins	Time in canter (mins)	Time in canter (mins)	Diff in mins
	Session	Actual	Perception		Actual	Perception		Actual	Perception		Actual	Perception	
4	1	31	35	4	25	25	0	5	10	5	2	5	3
	2	39	45	6	28	29	1	8	12	4	3	5	2
	3	36	35	-1	25	22	-3	7	8	1	3	3	0
	4	22	25	3	14	18	4	5	8	3	2	2	0
	5	26	25	-1	14	12	-2	7	10	3	5	6	1
	6	33	30	-3	20	17	-3	7	6	-1	5	5	0
	7	42	45	3	25	29	4	10	10	1	4	6	2
	8	31	30	-1	20	17	-3	7	8	1	4	6	2
	9	20	30	10	11	16	5	6	8	2	3	6	3
5	1	21	33	12	11	10	-1	4	5	1	3	4	1
	2	22	35	13	8	20	12	9	5	-4	5	4	-1
	3	23	27	4	9	20	11	9	5	-4	5	4	-1
6	1	17	20	3	8	16	8	2	10	8	7	4	-3
	2	31	30	-1	13	12	-1	13	12	-1	5	6	2
	3	12	12	0	3	6	3	5	4	-1	3	2	-1
	4	19	19	0	9	9	0	5	5	0	3	5	2
	5	12	13	1	8	9	1	4	5	1	0	0	0
	6	27	27	0	8	10	2	13	13	0	4	6	2
	7	15	15	0	8	8	0	3	5	2	3	4	1
	8	25	26	1	14	9	-5	5	7	2	7	9	2
	9	29	19	-10	11	8	-3	10	7	-3	7	4	-3
	10	18	30	12	8	12	4	5	15	10	4	5	1

Table 4: Time overestimated by the riders for total warm-up duration, walk, trot, and canter; WU: warm-up.

Overestimation	Number of routines overestimated (out of 44)	% of routine overestimated	Median %
Total WU duration	25	56.8	14
Walk	24	54.5	30
Trot	28	63.6	32
Canter	26	59.1	28

Table 5: Time underestimated by the riders for total warm-up duration, walk, trot, and canter; WU: warm-up.

Underestimation	Number of routines underestimated (out of 44)	% of routine underestimated	Median (%)
Total WU duration	13	29.5	4
Walk	15	34.1	24
Trot	10	22.7	29
Canter	9	20.5	44

Table 6: Number of WU routines and temperatures.

Temperature in °C	Number of WU routines	Number of routine indoor	Number of routine outdoor
0–5	2	0	2
6–10	6	0	6
11–15	8	0	8
16–20	5	1	4
21–25	4	1	3
26–30	11	2	9
31–35	7	3	4
36–40	1	1	0

4. Discussion

4.1. Reality versus Perception – What Do Riders Recall of Their Warm-Up and How do they Assess Time?

Even though the number of riders was a limiting factor for phase two of this study, none of the riders could recall accurately their warm-up sessions in terms of duration or time spent in different gaits and activities each time. Interestingly, there was no clear trend for riders to under- or over-estimate the length of the warm-up completed but overestimation of the time seems. One rider could underestimate one training session

and overestimate the next one. There were however more WU routines overestimated than underestimated. While total warm-up duration does not seem to impact performance on the day in SJ, the warm-up intensity does impact performance [37,38]. If riders perform a more intense warm-up than they intend to, this could decrease their performance and increase injury risk by increasing repetitive stress on the horse's musculoskeletal structure [39]. Similarly, a shorter warm-up could lead to an increased injury risk as optimal core and muscle temperature might not be reached. Not knowing accurately how much time is spent in each gait could also be an issue for warming up specific muscles required for skilled movements such as jumping or lateral work. According to the survey, 71% of DR and 70% of SJ riders said they included lateral work in the warm-up, however, stage 2 of this study showed that 100% of the DR used lateral work and only 30% of SJ riders used lateral work during their WU. In humans, discipline-specific warm-up routines have been shown to be more efficient in reducing injury risk and increasing performance for athletes [40,41]. It is also important to note that human athletes can assess their pain level and adapt their warm-up and training accordingly, however, horses cannot verbally articulate how they are feeling and as a prey species, may pain guard making detection of pain and sub-clinical injuries more difficult to assess [42]. Riders have a duty of care to safeguard the welfare of horses in their care including ensuring they are adequately prepared for the demands humans place upon them [4]. Riders should therefore ensure they have sufficient knowledge and understanding of how to formulate warm-up routines that prepare their horses for the activities and demands of work expected of them but are also capable of recognizing behavioral and performance cues that could indicate distress, pain, or injury in their horse.

Inaccurate recall of prior activities is widely recorded in sports [25,43–45]. Many factors are associated with this including increased fatigue [43], higher exercise intensities [44,45], lower levels of training and experience [25], and heightened emotional states: stress, anxiety, or arousal [43]. From a rider's perspective, the potential impact of psychological state or fatigue on their own and their horses' performance should also be considered, especially in competitive environments to enable riders to plan ahead and perhaps adopt a more regimented warm-up. While this information was not recorded in the riders here, further research is warranted to determine if any underlying rider factors could be associated with the variability of under- and over-estimating both the duration and content of warm-ups observed here. The emotional state of the rider can also change within a riding session depending on horse behavior and the rider's emotional reaction to the environment [46] and fatigue may occur across a competition. Riders should be cognizant that based on these results, the likelihood of not accurately recalling warm-up is high, resulting in decision-making potentially being compromised or at best based on inaccurate appraisal. As such we would recommend the use of planned and documented exercise and training regimes aligned with core goals to optimize performance in the horse and rider [47].

4.2. Prospective and Working Memory

Time perception depends on prospective and working memory. In this study, participants were aged 22 to 51yo (mean 33yo). While aging can have an effect on long-term and working memory, no correlation was found in this study between the age of the participants and the accuracy of warm-up recollection [34]. Another parameter that can affect working memory and recall of activities is learning disorders, which were not assessed here [48].

Participants were asked to fill out the form recalling their warm-up within 12 hours post-warm-up; this timescale will rely on long-term memory recall [31]. Long-term memory can be disturbed by daily events as well as the emotional state and fatigue of the participant at the moment of memory recall [49]. Score recall in eventers was found to differ between riders depending on their final score [50]. This study did not concentrate on competition however it could be assumed that how the training session went could also affect the recall of the warm-up.

4.3. Warm-Up Duration

Warming-up is an essential component of the work routine of the equine athlete to increase performance and decrease injury risk [39,51]. Human studies have shown that core temperature starts to increase 3-5 mins after the onset of exercise, before reaching a plateau 15-20 mins later [40]. Increasing core temperature is an important factor of warm-up as it increases muscle blood flow and oxygen uptake, as well as optimizing metabolic reactions [40]. For the human athlete, a 1°C increase in muscle temperature has been reported to lead to an improved performance from 2 to 5% during short-duration exercise [52]. Our results found warm-up routine duration and content varied between riders and between warm-up routines for the same horse and rider combinations independently of the type of training session planned. This suggests the warm-up routines assessed in this study were rider and horse dependent, and were selected on a day-to-day basis, as the content of individual warm-up regimes was not consistent or linked to obvious extrinsic or intrinsic variables.

Anecdotally, equestrian lay literature suggests a low-intensity warm-up of between 10 and 20 minutes is warranted to prepare the horse for more intense exercise. The average warm-up duration in this study was 24 ± 7 mins, which equates to the warm-up duration (18-25 mins) advocated to increase core temperature in preparation for exercise in the human athlete. Few studies have evaluated the thermodynamics of equine warm-up. One of the first studies conducted on equine core temperature during exercise found that walking for 6 mins, followed by a 3-4 mins break, and then trotting for 11 mins had no significant changes on equine core temperature. When 14 minutes of cantering was added, the core temperature increased significantly [53]. More recently, Janczarek *et al.* [39] reported increasing external body temperature measured by thermography for very short-extended, and long-lasting warm-ups, although the duration of each of these was not stated. The duration of warm-up reported here is also slightly longer than previously reported in the assessment of WU at competitions [14–16,18]. This could be due to riders spending longer warming up at home than at shows, where increased flexibility in warm-up duration and content are more accessible. For the purpose of this study, riders were asked to only record their warm-up, they therefore had to differentiate between the warm-up phase and the actual training phase.

During competition differentiating both phases might be easier as the real exercise occurs in the show ring.

4.4. Warm-Up Content

In phase 2 of this study, none of the riders hand-walked their horses prior to their ridden warm-up which contradicts the survey results where 70.1% of the riders stated they hand-walked their horses before riding. Understanding, if hand walking is a useful stage of warm-up in order to prepare the horse's back without a rider, is needed.

DR warmed up on average 22 ± 6 mins while SJ warmed up on average 27.5 ± 8 mins. Out of the total time spent on warm-up, riders spent on average 10 ± 4 mins and 15 ± 7 mins in walking for DR and SJ, respectively. Therefore, approximately half of their warm-up is spent in a low energy-consuming gait, which does not raise core temperature significantly but will increase HR and respiratory frequency **[39,53]**. However, walking has its utility, during the walk the ground reaction force (GRF) of the forelimb fetlock joint increases to 20.6 N Kg^{-1}, which allows for the flexor tendons to warm up gradually **[54]**. In trot, the GRF is increased to 40.6 N Kg^{-1} and is further increased to 45.9 N Kg^{-1} in canter **[54]**. While cantering is the most efficient gait to increase core temperature and heart rate to maximal values, it also increases forces on the forelimb tendons and distal joints. The repetitive stress and high concussion forces can potentially cause damage to the distal limbs **[55–57]**.

A warm-up should also increase an athlete's heart rate to prepare the cardiovascular system for the demands of exercise. In human athletes, warm-up aims to increase the heart rate to 40–80% of HR max in preparation for the ongoing demands of exercise, increasing to up to 90% of HR max for explosive sports **[40]**. Barrey and Valette **[58]** reported a mean heart rate value of 166bpm (87.4% of HR_{max}) for show jumpers during a competition warm-up, despite all horses completing very different warm-up routines in terms of duration and number of jump attempts. Lower heart rates have been reported in dressage horses; Williams **[37]** reported average heart rates of 91bpm (63% and 62% of HR_{max} respectively for elementary and medium level) during warm-up for horses warming-up for elementary and medium level Dressage tests. Further work to consider the dynamic relationship between the duration, intensity, and type of activities performed within the warm-up is required to fully elucidate what constitutes an appropriate warm-up regime for different disciplines, levels of competition, and individual horses.

Another important factor to take into account is muscle warm-up. While both SJ and DR self-report that they know their horses are ready to work when they feel supple, each muscle will work differently depending on the gait and arena footing used **[21,59,60]**. Few studies compare muscular activation in all three gaits (walk, trot, and canter), but it is important to consider which muscles will be needed to facilitate the activities required by the horse during exercise to ensure these are prepared adequately in the warm-up. This approach is commonplace in human sports; for example, in basketball athletes, where the ankle joint is the main site of injuries, the use of a specific warm-up targeting ankle muscles three times a week, increases ankle range of motion and stability, decreasing injury **[61]**. In horses, muscle activity during work is poorly studied but muscle activation will vary in action across the gaits; gait velocity and in/decline will also modify muscle activation **[62–66]**. Understanding equine muscle activation with consideration of the environmental conditions and requirements of exercise is therefore important for riders to understand what muscles should be warmed up for different equestrian disciplines in order to promote optimal performance and decrease injury risk.

4.5. Show Jumping versus Dressage

Both SJ and DR riders utilized the walk with the horse's head and neck long and low, as the main warm-up gait at home. A similar approach was reported by Murray *et al.* **[15]** in the competition environment; however, Chatel *et al.* **[14]** found show jumpers self-reported they used the trot as their main warm-up gait. In the competition environment, it has been observed that show jumpers jump during their warm-up, and dressage riders practice dressage movements before their test **[15]**. However, this was not observed in this study as warm-up occurred at home. During training, show jumpers do not jump throughout every training session, therefore if they planned to have a flat work session or a dressage session to supple up their horses, jumping would not be necessary. The same can be observed for dressage riders at home, if the rider planned to have a stretching session, then practicing dressage movements during the warm-up would not be necessary. In the SJ horse, it has been observed that varying the type of work and surface could decrease injury risk, it is therefore important that both show jumpers and dressage riders vary their type of work and adapt their warm-up accordingly to the exercise planned for individual sessions **[19]**.

4.6. Environmental Conditions and Their Influence on Warm-Up

Within the initial stage of this study, 60.1% (n = 107) of respondents stated warm-up duration should vary depending on the time of the year; with 24% (n = 22) self-reporting that during winter, the warm-up should be longer as it takes longer to warm up horses' muscles. However, this proved to not be the case in practice for the riders recording warm-ups, with no significant differences in duration or gait used between warm-up routines reported at 3°C or 39°C. In human athletes it has been proposed that while warming up in a hot environment (30°C and above), muscle temperature should remain just above the resting temperature baseline as nerve transmission is impaired in higher temperatures and endurance capacity is also decreased **[67]**. Hot and/or humid conditions should be considered a core component of decision-making when determining warm-up routines and exercise levels for the equine athlete as muscle hyperthermia can lead to acidosis, decrease performance, and increase the risk of musculoskeletal pathologies, as well as contribute to potential heat thermodynamic compromise **[68]**. Therefore, in a hot environment, a shorter warm-up might be more beneficial for the cardiovascular health of the horse but also performance and welfare. Knowing that heat has an impact on the "cognitive load" of human athletes, it has been observed that increased core temperature has a negative effect on time perception **[67,69]**; further consideration if a similar impact occurs in riders is warranted.

5. Conclusion

This study has found that warm-up routines undertaken at home during training seem to be rider and horse-dependent

but are not discipline or climate-specific. On average, both dressage and show jumpers tend to warm up equally on both reins at home and use the walk as the main warm-up gait. Riders did not adapt their warm-up routines in response to external climate conditions. High levels of inaccurate recall of the duration and content of warm-up routines were also recorded, suggesting riders as athletes experience high levels of time distortion, which could impact decision-making, performance, and ultimately the welfare of the horse. Riders should take into consideration the individual horse, the demand of their discipline, and the external climate when planning and implementing their warm-up routines to safeguard equine performance and welfare. Equine warm-up remains an under-studied and poorly understood subject despite the warm-up underpinning performance and equine welfare by decreasing injury risks. Further research is warranted to evaluate how warm-up routines prepare the horse and rider as individual athletes, and as a combination, for the psychological and physiological demands of exercise and competition.

Authors' Contributions

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

Data Availability

Data are unavailable due to privacy restrictions.

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The authors declare no conflicts of interest.

Ethical Approval

This project received ethical approval from the Hartpury University Ethics Committee (Ethics 2019-51 and Ethics No: 2019-58))

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Taking the Bitter with the Sweet - A Preliminary Study of the Short-Term Response of Horses to Various Tastants in Solutions

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Abstract

Horses can distinguish sweet, salty, sour, and bitter tastes, but little is known about their preferences for various tastants. Understanding horse taste preferences can aid in increasing water intake by adding a preferred tastant or by masking an unpleasant taste to encourage administration of medications, for example. The quantity of water intake by horses was examined over five separate trials involving a two-choice preference test between tap water and water containing varying concentrations of sucrose (0-50g/100ml), citric acid (0-2.43mg/100ml), quinine (0-30mg/100ml) or a mix of sucrose (10mg/100ml)/citric acid (1.31mg/100ml) and sucrose (10mg/100ml)/quinine (20mg/100ml). Horses (n = 5) showed a weak preference for sweetened water up to 10mg/100ml ($p < .001$), with a rejection at higher concentrations. Horses rejected all concentrations of both sour (n = 12 horses; $p < .001$) and bitter (n = 6 horses; $p < .001$) solutions. In the mixed tastant trials, sucrose mixed with citric acid was only weakly rejected compared to the sucrose solution alone, which was moderately rejected (n = 5 horses; $p < .001$). Similarly, mixed sucrose/quinine solution intake increased over the quinine solution alone (n = 9 horses; $p < .001$). There was a large variation among individual horses within each trial, with some horses strongly rejecting sucrose solutions and others strongly preferring citric acid solutions. No horse indicated a preference for bitter solution in any trial. Age ($p < .001$), breed ($p < .001$), and exercise ($p = .004$) all influenced total fluid intake in the sour trial, not dependent on treatment ($p = .063$). These preliminary results show that some horses appear to prefer sweet and a preferred tastant can mask a less preferred tastant.

Keywords

Taste perception; preference test; water intake; sour taste

1. Introduction

Taste perception requires the integration of olfactory and gustatory (taste) stimuli to identify five basic tastes: sweet, salty, sour, bitter, and savory (umami). Horses are known to discriminate sweet, salty, sour, and bitter tastes although umami has not been tested [1]. Taste receptors are modified epithelial cells densely packed into groups called taste buds that are found mainly on the tongue [2] with each receptor coding for only one taste. Sweet and bitter tastes involve G-protein receptors [1] while sour and salty tastes activate ion channels and act upon both Na+ and H+ receptors [3,4].

Taste buds advise the animal about the substance in the mouth [5] by sending information to the brain thereby gathering information from the environment to determine what is edible [6]. Sweet and umami tastes signal the energy density of a food

source and animals will eat to meet caloric requirements [7]. Bitter and sour are both aversive tastes that inform the animal of spoiled or toxic food to be avoided [8]. Sour taste may be buffered by constituents in an individual's saliva [9]. The desire for salty taste may be affected by the balance between cellular water concentration and intracellular sodium concentration [10]. Over time, animals develop behavioral mechanisms to allow recognition of foods based on nutritional properties and post-ingestive consequences [11].

Although the equine nutrition and pharmaceutical industries are large, little previous work has been done on the taste sensitivities of horses. The most comprehensive work was carried out by Randall *et al.* in 1978 who reported on the discrimination of sweet, salty, sour, and bitter solutions in immature horses. The tested foals preferred a sucrose solution over tap water at concentrations ranging from 1.25 to 10 % sucrose. Above and below this concentration, they were indifferent. For salty, sour, and bitter solutions, the foals showed no preference until a concentration respectively of 0.63% NaCl, 0.16% acetic acid, and 20% quinine. Above those concentrations, all solutions were rejected. In similar experiments, goats and cattle preferred sucrose solutions; however, sheep [12] rejected the sucrose solutions, and chickens [13] and geese [14] were indifferent. Foals [15] appeared slightly more tolerant of higher concentrations across all four tastants than goats, sheep, or cattle [12,16–18]. A more recent study supported Randall *et al.*'s [15] original findings, indicating that the addition of sweet feed to the drinking water of hospitalized horses increased overall fluid intake [19].

Water consumption by horses is one of the key factors in their sporting performance. Endurance riding, for example, is undoubtedly one of the equestrian disciplines where hydration is essential as dehydration can quickly lead to a drop in performance and even more seriously to various pathologies such as colic or myositis [20]. Horses can become rapidly dehydrated due to their hypertonic sweat [20]. Rehydration can be accomplished more efficiently by providing electrolyte solutions rather than by oral pastes, and solutions containing dextrose facilitate sodium absorption and provide energy for ATP production [20]. As it is best to provide electrolytes in water, adding a preferred tastant could increase water intake. Preferred tastants can also be used to increase the palatability of various products. For example, medications often possess a bitter taste, and masking the bitterness with a taste that the horse particularly likes can facilitate administration and avoid rejection of the drug [20].

Improving the palatability of feedstuffs may increase the initiation of consumption and the total quantity consumed [21] but it requires understanding the taste preferences of the species. Horses are known as picky eaters and prefer to forage on a wide variety of feedstuffs [22] despite having little apparent cognition of post-ingestive feedback to avoid certain foods [23]. It could be that horses have individual taste preferences that supersede nutritional intelligence, meaning that they select what to ingest based on their preference rather than whether that substance is good for them. Researchers can measure preferences by presenting animals with a choice between different tastants. Adding a tastant to water instead of feed allows the direct determination of the effect of the tastant rather than the effects of ingestion and nutritional content associated with the food [24]. Taste and smell are inextricably linked, and little research has been done on the reception and sensitivity of horse taste and smell as compared to other mammals (but see [25]).

Animal behaviors in relation to taste are better described as evidence of preference, aversion, or indifference than as a neurobiological response [26]. To determine preference, aversion, and non-discrimination in two-choice preference tests the mean percent of treatment consumed is theoretically assumed to be 50% of total intake for control or non-discriminate tastes. Using a 95% confidence interval, consumption between 40-60% determines non-discrimination. A consumption level below 40% indicates aversion, with < 20% being a strong aversion. Likewise, above 60% is a preferred substance, with >80% being a strongly preferred substance [16]. This method of assuming preference and aversion to different tastants in solution has been widely used [12–18,27,28].

The purpose of this preliminary study was to determine preference or aversion of horses to sweet, sour, and bitter tastes or mixtures of these. It was hypothesized that horses would show a preference for sweet solutions but a rejection of bitter and sour solutions compared to untreated tap water. Based on these assumptions, it was further hypothesized that the provision of a bitter or sour solution mixed with sweet would increase the horse's acceptance.

2. Materials and Methods

2.1. Research Protocol

A total of 37 horses participated in five different trials. Each trial was performed independently with no horse participating in more than one trial. The number and details of horses participating in each trial are indicated below. All of the trials with the exception of the bitter trial were held at the same facility. All horses were housed in individual box stalls bedded with wood shavings and received group turnout daily. All horses were lesson horses participating in weekly beginner and intermediate English riding lessons with the exception of the sour trial. The horses participating in this trial were draft horses and Caspians some of whom were in light training (English riding) and some who were not ridden at all. All horses were fed hay and concentrates according to their needs. All horses had been present in the facility for at least three weeks prior to any testing to acclimate them to the local water.

2.2. General Procedure

Horses were exposed to a two-choice preference test during the time they were in their stalls. The order of treatments was randomized across horses with each horse receiving each treatment for four or five days with at least two days washout in between treatments. Each horse was presented with two identical 19L black water buckets that were scrubbed and rinsed clean of any debris each day prior to filling with water. One bucket contained the treatment solution and the other bucket contained the untreated tap water. Buckets were hung on the front wall of the stall and the position of each bucket was alternated each day to account for any side effects. Buckets were observed multiple times throughout the day and weighed (Matzuo hanging digital fishing scale, Morehead City, NC, USA) and refilled if they contained less than two-thirds the volume to ensure a choice was available at all times. All buckets were weighed and refilled at 9 pm and

again at 6 am to make certain free choice water was available throughout the night. Daily values of the volume of tap water and treatment solutions consumed were calculated and expressed as a percent of the total amount of water consumed. Ambient temperature and water temperature were recorded at each weighing time.

2.3. Trial 1: Sweet Tastant

Five horses (1 mare, 4 geldings) of various breeds participated in this trial ranging in age from 8-17 years. Each horse was ridden for 5-10 h/week in light training consisting of intermediate-level English riding lessons. Six different concentrations of sucrose (Lantic and Rogers, Toronto, ON, Canada) were tested (0, 1, 5, 10, 20, 50 g/100mL) based on the results from [15]. The trial lasted six weeks with each horse receiving each treatment for five consecutive days with two washout days in between.

2.4. Trial 2: Sour Tastant

Twelve horses (1 mare, 2 stallions, 9 geldings) participated in this trial ranging in age from 2-18 years. Three of the horses were Caspians and the remainder were draft or draft crosses. Exercise in the form of riding ranged from 0-5h/week of light training consisting of English-style flat work to maintain fitness. Citric acid (Rougier, Mirabel, QC, Canada) was added to the water at 0, 0.49, 1.31, and 2.43 mg/100ml to obtain a pH of 7.6, 5.2, 3.6, and 3.0 respectively. These solutions corresponded to neutral, detectable, weakly sour, and moderately sour [9]. The trial lasted four weeks with each horse receiving each treatment for four consecutive days with two days wash out in between. A treatment solution sample was taken each time a new solution was added to the bucket for pH measurements (Fuzion CL-500 pH meter, Fisher Scientific, Mississauga, ON).

2.5. Trial 3: Bitter Tastant

Six Thoroughbred geldings between 3-10 years participated in this trial. All horses were worked under saddle 5-10h/week in light training consisting of English-style riding as determined by their owners. Four different concentrations of quinine monohydrochloride dihydrate (Sigma-Aldrich, Oakville, ON, Canada) were tested at 0, 10, 20, and 30 mg/100ml based on the results from [15]. The trial lasted four weeks with each horse receiving each treatment for four consecutive days with three days wash out in between.

2.6. Trial 4: Mixed Sweet plus Bitter Tastants

Nine horses (2 mares, 7 geldings) of various breeds ranging in age from 5-13 years participated in this trial. All horses were worked under saddle between 3-6h/week in light training consisting of intermediate-level English riding lessons. Four treatments were tested based on the results of the previous experiments: tap water, a bitter solution with quinine monohydrochloride dehydrate (20 mg/100ml; Sigma-Aldrich, Oakville, ON, Canada), a sweet solution with sucrose (10g/100ml; Lantic and Rogers, Toronto, ON, Canada), and a mixed solution of quinine monohydrochloride dehydrate and sucrose at a concentration of 20mg/100ml and 10g/100ml respectively. The trial lasted four weeks with each horse receiving each treatment for five consecutive days followed by two washout days in between.

2.7. Trial 5: Mixed Sweet plus Sour Tastants

Five horses (3 mares, 2 geldings) of various breeds ranging in age from 5-13 years participated in this trial. All horses were worked under saddle between 3-6h/week in light training consisting of intermediate-level English riding lessons. Four treatments were tested: tap water, a sour solution with citric acid (1.31 mg/100ml; Rougier, Mirabel, QC, Canada), a sweet solution with sucrose (10g/100ml; Lantic and Rogers, Toronto, ON, Canada), and a mixed solution of citric acid and sucrose at a concentration of 1.31mg/100ml and 10g/100ml respectively. The trial lasted four weeks with each horse receiving each treatment for five consecutive days followed by two washout days in between. A treatment solution sample was taken each time a new solution was added to the bucket for pH measurements (Fuzion CL-500 pH meter, Fisher Scientific, Mississauga, ON).

2.8. Data Analysis

A general linear mixed model with repeated measures was used to analyze the effect of the percent of treatment solution consumed by the horses using SPSS (v28.0.1.1, IBM Statistics, Armonk, NY, USA). Each trial was analyzed separately. The model included week, day, temperature, treatment bucket side and their interactions as fixed factors and horse as the random factor to determine their effects on the amount of treatment water consumed and the total amount of water consumed. Horse age, breed, exercise, and solution pH were included as fixed factors in Trial 2 (sour tastant), and exercise was included as a fixed factor in both mixed tastant trials (Trials 4 and 5). Estimated marginal means determined differences among levels of significant factors. The volume of solutions consumed by the horses is presented in terms of discrimination zones as reported by Randall *et al.* [15] (**Figure 1**).

3. Results

3.1. Trial 1: Sweet Tastant

Horses displayed a weak preference for sucrose solution at concentrations of 1, 5, and 10 g/100ml and moderate to strong rejection at concentrations of 20 and 50 g/100ml respectively compared to tap water ($F(4,125) = 26.045$, $p < .001$; **Figure 2**).

There was no effect of week ($F(4,125) = 0.611$, $p = .656$), day ($F(4,125) = 1.920$, $p = .111$), the position of the buckets ($F(1,125) = 0.064$, $p = .800$) or the ambient temperature ($F(1,124) = 0.092$, $p = .792$) on the percent consumption of sucrose solution by the horses. Horses did not differ in their individual intake of treatment solutions ($F(4,132) = 0.159$, $p = .959$).

The total amount of fluid consumed (tap water plus sucrose solution) was not influenced by treatment ($F(4,130) = 1.163$, $p = .330$). However both week ($F(4,135) = 4.135$, $p = .006$) and day ($F(4,135) = 12.875$, $p < .001$) did influence the total amount of fluid consumed, with less total fluid consumed in the second week (average 28.6 ± 10.22 kg/d) compared to the fifth week (average 37.2 ± 11.68 kg/d), and less water consumed on Day 1 (average 23.9 ± 9.42 kg/d) than the other four days (average 34.3 ± 9.16 kg/d).

Figure 1: Preference, rejection, and non-discrimination zones in two-choice preference tests where the percent of solution consumed is theoretically 50% of total intake. Using a 95% confidence interval, consumption between 40 and 60% determines non-discrimination. Consumption below 40% indicates rejection (dark grey area) and above 60% is a preference (light grey area). Adapted from Figure 1 in **[15]**.

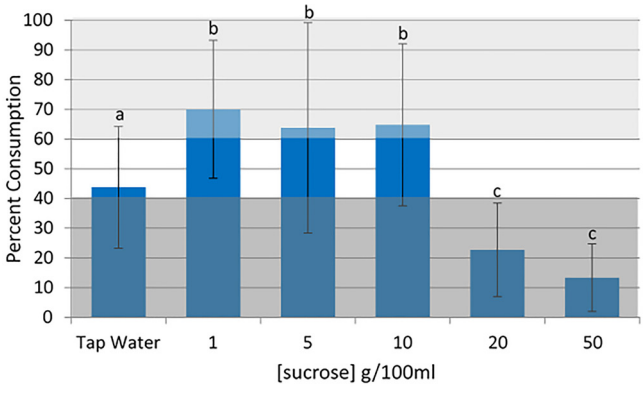

Figure 2: Mean percent (±SD) of water sweetened with sucrose at varying concentrations consumed by horses (n = 5). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) and above 60% is a preference (light grey area) according to **[15]**. a, b, c differ $p < .001$.

3.2. Trial 2: Sour Tastant

Due to variations in the daily pH of tap water, each treatment had a pH that varied over the course of the trial **(Table 1)** however no treatments overlapped in the pH readings.

Table 1: pH measurements of water with various concentrations of citric acid added in a two-choice preference test to determine taste preferences of horses (n = 12). pH measurements were taken multiple times per day and averaged over the trial.

Treatment (citric acid in water mg/100ml)	Mean pH ± SD	Minimum pH	Maximum pH
0 (Tap Water)	7.55 ± 0.116	7.28	7.89
0.49	5.17 ± 0.34	4.69	6.57
1.31	3.62 ± 0.11	3.38	3.96
2.43	3.02 ± 0.06	2.87	3.13

Horses consumed more tap water than any other treatment ($F(3,130) = 15.114$, $p < .001$; **Figure 3**). Horses displayed a weak rejection of tap water, a moderate rejection of the lowest concentration, and a strong rejection of the two highest concentrations of citric acid.

Horses consumed more citric acid solution during the first (average 30.8%) and second (average 34.8%) weeks compared to the third (average 21.7%) and fourth (average 16.2%) weeks ($F(3,130) = 10.159$, $p < .001$). There was no interaction of treatment by week ($F(9,49) = 1.714$, $p = .111$).

Ambient temperature did influence the percent citric acid solution consumed ($F(1,130) = 11.953$, $p < .001$) with no clear pattern (i.e. higher temperature did not correlate to higher intake). Ambient temperature within the barn ranged from 18-33 °C and water temperature ranged from 11-29 °C over the course of the trial. There was no effect of day ($F(3,133) = .788$, $p = .508$), position of the buckets ($F(1,146) = 3.781$, $p = .054$), horse age ($F(1,133) = 1.095$, $p = .297$), exercise ($F(1,133) = 0.036$, $p = .850$) or breed ($F(1,133) = 0.892$, $p = .347$) on the percent citric acid solution consumption.

Horses consumed less total fluid (tap water plus treatment solution) in the first (average 11.3 kg/d) and second (average 12.3 kg/d) weeks compared to the third (average 16.1 kg/d) and fourth (average 17.6 kg/d) weeks ($F(3,142) = 26.712$, $p < .001$). The total amount of water consumed was not influenced by treatment ($F(3,142) = 2.488$, $p = .063$). Breed ($F(1,142) = 22.312$, $p < .001$) influenced the total amount of water consumed with draft horses (average 17.4 ± 8.60 kg/d) drinking more than Caspian horses (average 5.2 ± 3.27 kg/d). Horses less than 8 years old (average 15.5 ± 9.25 kg/d) consumed more total water than horses 8 years and older (average 12.7 ± 9.07 kg/d; $F(1,142) = 18.938$, $p < .001$). Horses that worked 3-5 hours per day consumed more (average 18.2 ± 6.87 kg/d) than horses who worked one or less hours per day (average 9.15 ± 6.57 kg/d; $F(1,142) = 8.583$, $p = .004$).

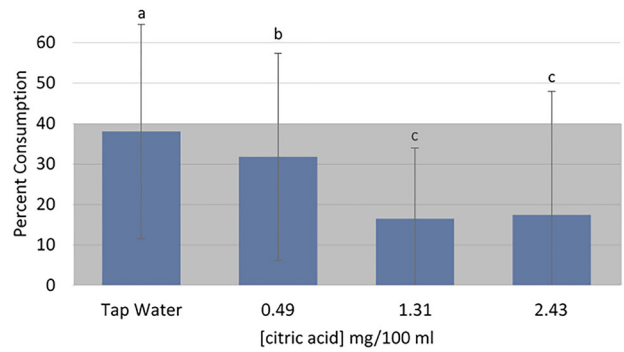

Figure 3: Mean percent (±SD) of water treated with citric acid at varying concentrations consumed by horses (n = 12). The pH of tap water was 7.6, with the pH of the citric acid treatments corresponding to 5.2, 3.6, and 3.0, respectively. Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b, c differ $p < .001$.

3.3. Trial 3: Bitter Tastant

Horses consumed more tap water and less 10 and 30 mg/100ml quinine solution while the 20 mg/100ml solution was intermediate ($F(3,70) = 11.604$, $p < .001$; **Figure 4**). There was a weak rejection of the 20 mg/100ml solution, a moderate rejection of the 10 mg/100ml solution and a strong rejection of the 30 mg/100ml solution.

Horses consumed more quinine solution during week 3 (average 43.6%) compared to week 4 (average 21.1%; $F(3,70) = 3.681$, $p = .016$). There was a treatment by week interaction ($F(7,72) = 14.903$, $p < .001$). There was no effect of day ($F(3,70) = 0.405$, $p = .750$), horse ($F(5,70) = .937$, $p = .463$), position of the buckets ($F(1,77) = 3.588$, $p = .328$) or temperature ($F(1,70) = 0.001$, $p = .976$) on quinine solution consumed. The temperature inside the barn during the trial remained above 0 °C but was not greater than 10 °C.

Treatment had no effect on the total quantity of fluid (tap water plus treatment solution) consumed by the horses ($F(3,80) = 1.985$, $p = .123$). There was an effect of week ($F(3,80) = 8.679$, $p < .001$) with horses drinking less total water during the first week (average 14.7 ± 4.98 kg/d) compared to the other three weeks (average 20.8 ± 5.69 kg/d).

3.4. Trial 4: Mixed Sweet plus Bitter Tastants

Horses showed a strong rejection of the bitter solution compared to all other treatments ($F(3,149) = 30.325$, $p < .001$). The percent intake of tap water, sweet, and mixed solutions were similar **(Figure 5)**.

There was no effect of week ($F(3,149) = 2.468$, $p = .064$), day ($F(4,149) = 0.673$, $p = .612$), horse ($F(8,149) = 1.047$, $p = .404$), exercise ($F(1,149) = 1.018$, $p = .315$), position of the buckets ($F(1,149) = 2.230$, $p = .137$) or ambient temperature ($F(1,149) = 1.489$, $p = .224$) on the percent of treatment solution consumed by the horses. The temperature inside the barn during the trial remained above 3 °C and was not greater than 15 °C.

Horses drank less total fluid (tap water plus treatment solution) when provided with bitter treatment (average 31.9 ± 8.94 kg/d) compared to the sweet treatment (average 36.7 ± 11.63 kg/d; $F(3,165) = 4.922$, $p = .003$).

3.5. Trial 5: Mixed Sweet plus Sour Tastants

Horses consumed more tap water compared with all other treatments. The quantity consumed for sweet, sour, and mixed solutions was similar ($F(3,63) = 11.410$, $p < .001$; **Figure 6**) with sweet water being moderately rejected and the sour and mixed solutions weakly rejected.

Horses consumed more treatment water during week 2 (45.4%) compared to week 1 (28.4%) with the other weeks not differing ($F(3,63) = 7.970$, $p < .001$). There was a treatment by week interaction ($F(7,64) = 2.932$, $p = .01$). There was no effect of day ($F(3,63) = 1.350$, $p = .266$), horse ($F(4,60) = .831$, $p = .511$), position of the buckets ($F(1,63) = 1.373$, $p = .246$), exercise ($F(1,63) = 1.467$, $p = .230$), treatment pH ($F(1,63) = 0.008$, $p = .930$) or temperature ($F(1,63) = 2.597$, $p = .112$) on treatment water consumed. The temperature inside the barn during the trial remained above 5 °C but was not greater than 16 °C.

The total amount of fluid consumed (tap water plus treatment solution) was not influenced by treatment ($F(3,56) = 2.303$, $p = .087$).

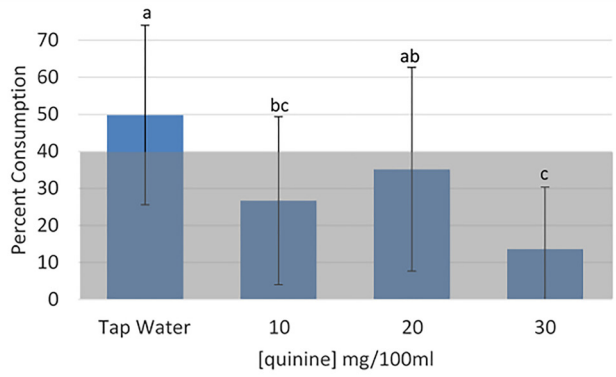

Figure 4: Mean percent (±SD) of water treated with quinine at 0 (tap water), 10, 20, and 30 mg/100ml consumed by horses (n = 6). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b differ $p < .001$.

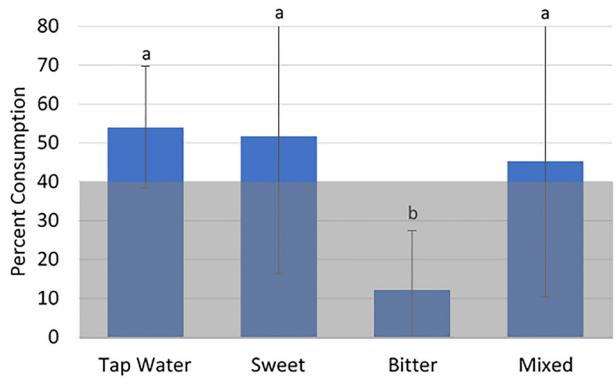

Figure 5: Mean percent (±SD) of water consumed by horses (n = 9) consisting of tap water, sweet solution (sucrose concentration of 10g/100ml), bitter solution (quinine concentration of 20mg/100ml), and mixed solution (containing both sucrose (10 g/100ml) and quinine (10 mg/100ml)). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b differ $p < .001$.

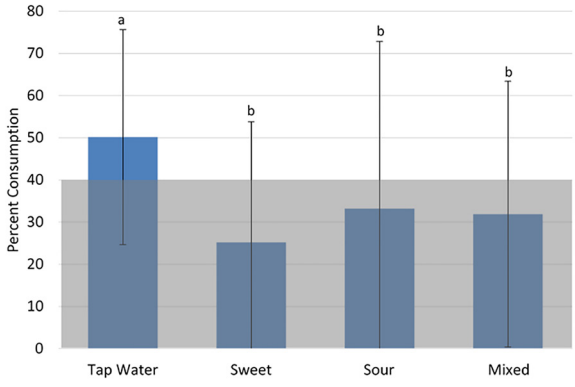

Figure 6: Mean percent (±SD) of water consumed by horses (n = 5) consisting of tap water, sweet solution (sucrose concentration of 10g/100ml), sour solution (citric acid concentration of 1.31 mg/100ml), and mixed solution (containing both sucrose (10 g/100ml) and citric acid (1.31 mg/100ml)). Treatments were presented as a two-choice preference test with tap water in one bucket and treatment solution in the other bucket. The bucket location was switched daily. Consumption below 40% indicates rejection (dark grey area) according to Randall *et al.* **[15]**. a, b differ $p < .001$.

4. Discussion

In a two-choice preference test, horses showed distinct preferences for various tastants provided to them in solution indicating that they are able to discern sweet, sour, and bitter tastes. Similar to previous research **[15]** the results of our various trials show that horses, when given a choice between tap water and sweet solution, displayed a weak preference for sweet, and when given a choice between tap water and sour or bitter solutions, showed a rejection of sour and bitter tastants. We also showed that a less preferred taste can be masked by a more preferred taste which is important when having to administer certain substances like medications.

These results should be interpreted with caution since all trials in this study were carried out independently and involved small numbers of horses. Only a sufficient number of horses participated in the sour trial to be able to analyze age, breed, and exercise factors on total water intake. Additionally, preference tests can only measure an animal's preference of one choice in comparison to another, which may not be indicative of the animal's overall preference **[29]**. One drawback of this forced choice test is that the consumer does not have the ability to state "no preference." Thus while it is assumed that a 50% consumption of one option indicates no preference, it can just as easily mean that half of the consumers preferred option A and the other half preferred option B **[30]**. However, our results are not dissimilar to other published studies.

The addition of sweet to various feedstuffs made available to horses is not uncommon. Perhaps it is because humans find sweet flavors so attractive **[31]** that perpetuates the idea that non-human animals should also prefer sweet. Indeed, sweeteners are routinely added to piglet diets to encourage feed intake upon weaning **[32]**. Limited research in this area shows that horses do have some preference for sweet **[15,19,33]** but perhaps this is overrated. Our results in the sweet trial, similar to **[15]**, show only a weak preference for sweet when tested alone, and in our mixed tastant trials, horses showed no preference or even a moderate rejection of sweet solutions. Likewise, when testing taste additives to feedstuffs, results from other studies showed horses preferred salt and sour apple pellets over sweet apple or sugar beet pellets **[34]** and preferred higher protein content versus sweetener **[25]**.

Our results showed all concentrations of sour solutions provided to the horses were rejected whereas Randall *et al.* **[15]** reported no discrimination of sour solutions in weanlings until a pH of 3.1. This difference could be due to the fact that Randall *et al.* **[15]** used acetic acid in their solutions while we used citric acid. Although response to sour taste is pH dependent, the pH of a solution is not necessarily proportional to the magnitude of sourness **[35]**. Citric acid ($C_3H_5O(COOH)_3$) has a higher pH and greater solubility than acetic acid (CH_3COOH) and is the most widely used organic acid in the food industry due to its appealing effects on taste **[36]**.

Horses in our study showed a rejection of all bitter solutions whereas Randall *et al.* **[15]** showed no discrimination at the lower concentrations of bitter. This could be a result of acclimatization since the concentration of bitter solute was doubled every other day in Randall *et al.*'s **[15]** protocol whereas in our study the horses were presented with the randomized concentration of bitter solution; some horses received a higher concentration for the first week followed by a lower concentration and vice versa. Rejection could also have been a result of neophobia, although we observed no change in water intake over the four days of the bitter trial. Other researchers have demonstrated neophobia to foodstuffs resolving after two days **[25]**.

Bitter substances often contain alkaloids which can be toxic when ingested, thus the ability to perceive bitter taste is essential to avoid poisoning **[37]**. This preservation strategy becomes problematic when it is necessary to administer, for example, medications **[21]**. Understanding taste preferences can aid in encouraging ingestion of required feedstuffs or solutions. Despite horses rejecting bitter solutions, our results in the mixed trial showed that when the bitter taste was masked with a sweet taste, horses no longer rejected the solution. Although our results with the mixed sweet and sour tastants were unexpected, the same outcome was achieved – the addition of a more preferred tastant to a less preferred tastant increased the tolerance for the less preferred tastant.

Individual taste preferences were evident in all our trials as indicated by the large standard deviations in all the graphs. While no treatment was completely rejected in any trial, certain horses demonstrated specific likes or dislikes to the various tastants. In the sweet trial, there was a high variation among the horses ranging from strong preference to strong rejection. In the sour trial, some horses showed a strong preference for sour solutions. However, no horse showed a preference for any bitter solution in the bitter trial. High variability was noted previously both among and within individual horses in a two-choice preference test for water treated with increasing salt concentrations **[27]**. Individual horse variation led to inconclusive results when Murphy *et al.* **[38]** tested a variety of flavored solutions in Thoroughbred horses. Similarly, van den Berg *et al.* **[39]** reported a large individual variation in the acceptance of novel foodstuffs. Our results also showed a variation in solution intake across

days or weeks of the trials which could be influenced by many independent factors that could not be controlled such as ambient temperature, humidity, forage type, salt availability, breed, age, and exercise. Future research should endeavor to use a more controlled methodology with larger numbers of animals who participate in testing all the treatments.

Ambient temperature did affect intake of sour solution in our study but no clear pattern emerged. This trial was the only trial conducted during the summer months when the ambient temperature was generally quite hot. Previous studies reported ponies drinking more when warm water was provided during the winter months compared to cold water [40] but during the hot summer months, ponies did not alter their water intake regardless of whether they were provided with cold or warm water [41].

An important aspect of understanding taste preferences in horses is in regard to monitoring water intake. Horses who travel to different locations may reject a local water source but the addition of a tastant to the water could override that. Mars *et al.* [42] found that mares drank more apple-flavored water at an unfamiliar location compared to clover-flavored water. Water intake is highly important in horses competing in high-performance disciplines such as three-day eventing who can quickly lose electrolytes and experience dehydration through exertion [20,43]. Both problems can be ameliorated by offering electrolytes in solution for the horse to ingest, and the volume of voluntary intake can be increased with the addition of a preferred taste [20,43].

The total amount of fluid ingested daily by the horses across all our trials was not affected by treatment with the exception of the bitter tastant during the mixed trial. In this instance, horses drank less total water when presented with bitter solution compared to the sweet or mixed solutions. Rats were also reported to diminish daily fluid intake when presented with quinine-adulterated water [44]. Horses are noted to have a higher sensitivity to bitter [2] which may aid them in avoiding toxic plants such as artemisia [45]. The presence of the bitter tastant in the water in our trial may have resulted in a learned response to avoid water as much as possible [2].

Age, breed, and exercise all affected the total amount of fluid ingested by the horses in our sour trial. Older horses ingested less total fluid than younger horses which has previously been noted [40]. The breed effect is not surprising as Caspian Horses, who weigh an average of 270kg, are significantly smaller than draft horses, with an average weight of 800kg (**https://equi-analytical.com/resources/typical-body-weights/),** thus fluid intake would be significantly less for the Caspian horses. Breed differences have also been noted in taste preferences, with cold-blooded horses preferring salty feeds and Arabians (hot-blooded) preferring sour [34]. However, our results presented here did not show any breed effect in the consumption of sour solutions. This may indicate that aversion to sour is a factor of evolution, as suggested by Kyriazakis [11]. Horses in our sour trial who received more daily exercise ingested more total fluid than those who were idle. This would be expected as moderate exercise in temperate weather can result in a water loss of 25ml/m^2/min [43] which would need to be regained through drinking.

It should be stressed that these trials were all short-term, where horses were exposed to various tastants for only four or five consecutive days. It could be that longer-term exposure would lead to recognition of post-ingestive consequences [11] or an adaptation to the presence of a particular tastant, resulting in more obvious preferences. Nevertheless, these results shed some light on the taste preference of horses for sweet, sour, bitter, and mixed solutions.

5. Conclusions

It is known that horses can distinguish between sweet, salty, sour, and bitter. The results of this study show that some horses have only a weak preference for sweet solutions and some reject sour and bitter solutions. Importantly, a less preferred taste can be masked by a more preferred taste to increase consumption. This is helpful when it is necessary to administer unpalatable substances to horses such as medications. This information adds to the scarce research on this topic to date.

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Authors' Contributions

K.M., M.V., J.D, J.C., and E.D. conceived the idea and planned the study. M.V., J.D, J.C., E.D., and L.F. collected and curated the data. K.M., M.V., J.D, J.C., E.D., and L.F. analyzed the data and contributed to the interpretation of the results. K.M. took the lead in writing the manuscript and M.V., J.D, J.C., E.D., and L.F. contributed to the final version. K.M. supervised the project.

Data Availability

Data supporting the findings of the study can be supplied upon request to the corresponding author.

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No funding was received for this research.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

All procedures were approved by the Institutional Animal Care Committee in concordance with the Canadian Council for Animal Care guidelines for the use of animals in research.

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Are Current Efforts to Prevent Grooms from Leaving the Industry Effective? An Analysis Based on Principles of Behavior Change

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Abstract

Grooms play a vital role in the equestrian sector, providing horses with expert care. Yet the grooming industry is finding itself amid continued outflow of experienced staff due to poor working conditions, insufficient remuneration, and lack of respect towards the profession. In 2022, four high-profile initiatives, the formation of the International Grooming Association (IGA), the 2022 FEI ECCO World Championships, the #ChampionsAsOne initiative, and the Cavalor Best Groom Award aimed to galvanize the grooming industry. Drawing on the COM-B model and Behaviour Change Wheel as a theoretical framework and using a four-part online questionnaire, the current study aimed to determine whether these initiatives were perceived by grooms (N = 1389) as addressing prevalent barriers that currently prevent them from staying in the industry. Statistical analysis using chi-square tests, Kruskal-Wallis and Mann-Whitney U tests revealed that the initiatives were not perceived as effective. 58.5% of the grooms indicated that the initiatives had no impact on them personally, arguably because the initiatives failed to address those issues perceived as most pressing, namely a high mental and physical workload (physical opportunity) and insufficient remuneration and time off (physical capability). Grooms considered employers, followed by the FEI and the IGA as the most important stakeholders to work towards sustainable change in the grooming industry.

Keywords

Grooming industry; human behavior; staffing crisis; equestrianism; sustainability

1. Introduction

Grooms are considered the backbone of the equestrian industry [1]. They are responsible for the daily care and management of horses, their health and welfare, as well as the running and general maintenance of the yard, be that at an equine veterinary clinic, a riding school, a stud farm, or the training and competition yard of a medal-winning rider. However, over the past few years, an increasing number of grooms have decided to leave the industry [2]. Challenging working conditions, including long hours of physically and mentally demanding work, resulting health issues, little recognition or respect from employers combined with below-minimum wages, and a poor work-life balance are being cited as the underlying reasons for giving up their life's work [3–5]. Grooms who have been doing the job for decades consider their profession a "dying breed" and demand that "something needs to get done to get people back to the industry and feeling like it is a long-term career outlet" [4]. Showjumping Olympic Gold Medalist Ludger Beerbaum has been publicly quoted as saying that finding committed staff is "something we face in our [equestrian] industry as well," [4] while the director of the Dutch Federation for Riding Schools (FNRS) Haike Blaauw acknowledged that staffing is a "challenging area in many industries at the moment" [6].

In 2022, in response to the growing concerns regarding the grooming industry, the governing body for the equestrian sport, the Fédération Equestre Internationale (FEI), newly initiated or supported four high-profile initiatives aimed at boosting the image of grooms and acknowledging the importance of their role in equestrian sports.

1.1. Formation of the IGA

At the FEI's request, the International Grooms Association (IGA) was formed on 26th April 2022, formally representing all professional grooms within the equestrian sports community. The IGA aims to achieve greater representation and career recognition for grooms as well as to improve their working conditions [7].

1.2. #ChampionsAsOne

Prior to the 2022 ECCO FEI World Championships, the FEI launched the initiative #ChampionsAsOne that "pays homage to each person's unique and valuable contribution and also allows them to be a part of the celebration," [8]. Essentially, the essence of the initiative is to demonstrate that it takes more than just the rider and the horse to achieve top results in the sport, but that it is the grooms and other individuals working behind the scenes who have an equally important role to play.

1.3. 2022 ECCO FEI World Championships

Moreover, the 2022 ECCO FEI World Championships in Herning made a concerted and well-publicized effort to improve amenities for the grooms and to foster public recognition of grooms as vital partners to any horse-rider team [9]. In Herning, grooms had their own dedicated lounge with proper catering and an on-site accommodation opportunity and they were clearly included in the TV graphics which introduced each horse-rider combination [10].

1.4. FEI Cavalor Best Groom Award

Each year, the FEI announces the winner of the FEI Cavalor Best Groom award. Presented at the FEI Awards Gala, grooms can be nominated for this award by their athletes, colleagues, and others within the sport. The shortlist is drawn up by a judging panel and the winner is chosen from the said list based on 50% public votes and 50% by an FEI panel [11].

As a result of these initiatives, grooms have been experiencing increased media coverage as well as having been asked to attend prize-giving ceremonies and being given hot meals at shows or "goody bags" to take home [12]. Yet, while such public acknowledgment of grooms may be considered more than welcome, it is important to investigate whether these initiatives improved, let alone solved some of the more fundamental reasons why grooms decided to leave the industry in the first place.

So far, no concerted effort has been made to examine the grooming crisis within the context of human behavior change. Research has demonstrated that behavior is influenced by a combination of internal and external factors [13,14]. To carry out any type of behavior, an individual must have the relevant knowledge and skill set, be motivated to do so as well as have access to the necessary physical resources, and enjoy social support from their family, peers, and larger public [15]. The COM-B model (see **Figure 1**), developed by Michie *et al.* [15] captures these ideas and, together with the Behavior Change Wheel [15,16] (see **Figure 2**) serves as a framework for understanding behavior as well as establishing grounds for behavior change [15]. The COM-B model implies that regardless of whether the behavior is intentional or not, it depends on three behavioral sources, and their interaction: capability, motivation, and opportunity [15]. Each behavioral source is further divided into two sub-categories. Capability may be divided into "capability psychological", which denotes the levels of knowledge or insight required to perform a behavior, and "capability physical", which describes the required skills, strength, or stamina. Opportunity focuses on both the "physical" aspects, i.e. time, money, and resources, as well as the "social" support provided by others, such as friends, family, employers, etc. Lastly, motivation may be divided into "automatic" motivation, affected by habit, desire, or emotional reactions, or "reflective", referring to conscious thought processes, such as planning or goal setting [15,16]. When trying to elicit behavior change, it is important to determine whether any (or all) of these six behavioral sources might present a barrier to the desired behavior being performed. Does an individual have the necessary knowledge or skills to perform a behavior? Are the physical resources and/or social support appropriate? Is the individual motivated to perform the behavior?

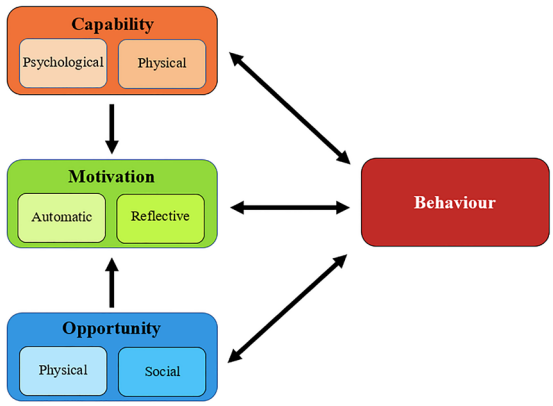

Figure 1: The COM-B model, showing the three sources of behavior and their sub-components. Reproduced with permission from [16].

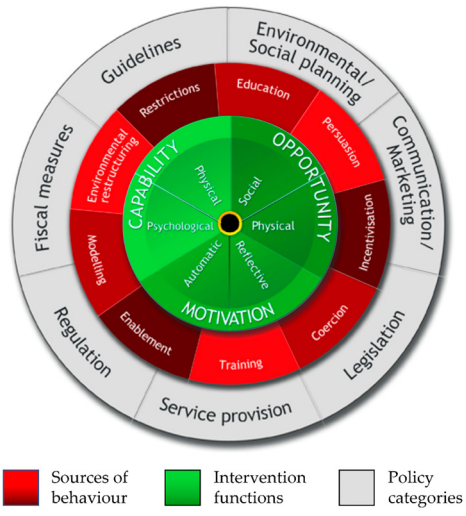

Figure 2: The original Behavior Change Wheel, showing the three sources of behavior, the nine intervention functions, and the seven policy categories. Reproduced with permission from [16].

In order to initiate behavior change, Michie *et al.* [15,16] furthermore describe nine intervention functions, e.g. education, training, persuasion, incentivization, coercion, restriction, environmental restructuring, modeling, and enablement. Each intervention function addresses specific COM-B behavioral sources, which in turn, affects behavior change [16,17] (see **Table 1**). It follows that any sustainable intervention strategy aimed at changing the underlying behavior of individuals or organizations should first identify existing barriers that prevent grooms from staying in the industry [15,17].

While there are very few scientific studies investigating the current state of the grooming industry, much media attention has been paid to why grooms might be leaving the industry, or rather, in the language of behavior change, what may be preventing grooms from staying in the industry [4,18,19]. These reasons or 'barriers' may be categorized according to the COM-B model as follows:

Capability physical: As horses are animals who do not take "days off" and need care 7 days a week, grooms regularly work longer than the average 8-hour work day [4,20]. This is taxing both physically and mentally, limiting the possibility of proper rest, and increasing the risk for mental and physical illness, and an increased risk of injury as highlighted in studies focusing on the UK horse racing industry [21,22].

Capability psychological: Caring for horses requires extensive theoretical knowledge of horse care and relevant rules/regulations [23]. In high-level sports, such rules and regulations can be complex and demanding as grooms have to be aware of general guidelines as well as discipline-specific regulations and equine medication rules dictated by the FEI [9].

Opportunity physical: A study [5] done on employment satisfaction among Dutch primary equestrian sector employees showed that employees are not only unhappy with current working conditions, but they also consider monetary remuneration to be insufficient while the opportunity to take time off is limited [21].

Opportunity social: Grooms rarely receive the support and understanding from the sector they would like. Their concerns are often not being listened to and attended to [24].

Motivation automatic: A groom's job is often not respected by employers, the public, etc. which negatively affects a person's self-worth.

Motivation reflective: The profession lacks possibilities for career progression [21] – grooms are often limited to move only laterally within the profession (home groom, show groom, stable manager).

In line with the theoretical foundations of the Behavior Change Wheel; any intervention strategy that hopes to evoke sustainable change in the grooming industry should be directed at what the target group – grooms – experience as the most salient barriers [15–17]. While the 2022 initiatives were arguably devised to address some of the underlying aspects of the current grooming crisis, it remains unclear whether the grooms themselves consider these initiatives effective in targeting the barriers they consider most important. Drawing on the Behavior Change Wheel as a theoretical framework, the current study therefore aims to investigate whether the initiatives effectively addressed the perceived barriers that prevent professional grooms from staying in the industry.

2. Materials and Methods

2.1. Study Design

A descriptive study design was used to gain an in-depth understanding of how professional grooms perceived the four biggest initiatives directed at them in 2022. An online questionnaire was used to collect data from as many grooms as possible. The study received ethical approval from the Hanze Hogeschool Ethics Advisory Committee (dossier number: heac.2023.006) and the study followed the guidelines of the Declaration of Helsinki.

The survey was hosted on the Microsoft Forms platform and was open for participation from 1st February 2023 until 26th February 2023 prior to which three grooms participated in pilot testing. The survey was aimed at current and former professional grooms from all over the world, aged 18 and over. Participation was voluntary and anonymous with an option to withdraw at any time before final submission of answers. The survey was distributed through various social media channels. Link to the survey was sent to both via private messages as well as to relevant parties and media channels such as the university, World of Showjumping, Horse & Hound, Equnews.com, and Equnews.be to facilitate snowball sampling. The questionnaire was available only in the English language. All submitted data were kept strictly confidential and processed anonymously. A representative sample with a confidence level of 95%, a population proportion of 50%, and a population size of 5000 was calculated to be 357 participants.

2.2. Survey Design

A four-part, 31-question online survey was developed using Microsoft Forms (see **Supplementary Materials**). Questions used both multiple-choice and open free text format. The survey took approximately 10 minutes to complete and included different routing options to improve ease of use. In the first section (1 question), prior to getting access to the rest of the survey, grooms were informed of the aims of the study after which they were asked to give explicit consent to participating in the survey. The second section (8 questions) focused on general demographics and work position-related information. The third section (6 questions) concentrated on initiatives directed at grooms in 2022. Those initiatives were: the formation of the IGA, the 2022 ECCO FEI World Championships, the #ChampionsAsOne initiative, and the FEI Cavalor Best Groom Award.

The COM-B model [15] provided the theoretical framework for the questions aimed at establishing the effect that the initiatives had on behavioral barriers. Participating grooms were presented with a short description of the four initiatives and asked whether they were familiar with any or all of them. Subsequent questions in the same section focused on determining which of the initiatives was perceived as having the greatest impact on the industry as a whole and at a personal level. Respondents were also given the option of selecting "none" if they believed that none of the initiatives had any impact.

Table 1: Mapping of different Behavior Change Wheel intervention functions onto specific COM-B sources of behavior [16,17]. Shaded areas indicate a relation between intervention function and COM-B source of behavior.

Behavior Change Wheel Intervention functions	COM-B sources of behavior					
	Capability		Opportunity		Motivation	
	Psychological	Physical	Physical	Social	Automatic	Reflective
Education Increasing knowledge or understanding	■					■
Training Imparting skills	■	■	■		■	
Persuasion Using communication to induce positive or negative feelings or stimulate action					■	■
Incentivization Creating an expectation of reward					■	■
Coercion Creating an expectation of punishment or cost					■	■
Restriction Using rules to: i) increase the opportunity to engage in the target behavior or ii) increase the target behavior by reducing the opportunity to engage in competing behavior			■	■		
Environmental restructuring Changing the physical or social context			■	■	■	
Modeling Providing an example that people can imitate or to which they may aspire				■	■	
Enablement Increasing means/reducing barriers to increase capability or opportunity	■	■	■	■	■	

The fourth part of the questionnaire (16 questions) was directed at grooms who chose a personally impactful initiative in the third section. Participants were asked about the impact that their chosen initiative had on key areas associated with their work, drawing on the COM-B sources of behavior, capability psychological, capability physical, opportunity physical, opportunity social, motivation reflective, and motivation automatic. For example, in order to investigate whether an initiative impacted a groom's automatic motivation, participants were asked whether the initiative affected their feelings about their profession. To keep response options as simple as possible, answers were constructed on a three-point Likert scale: "It had a positive impact", "It had no impact", or "It had a negative impact". For questions regarding psychological and physical capability, the wording was adjusted to reflect the fact that it is not possible to unlearn knowledge or skills. In those two cases, the possible answers were: "Yes, significantly", "Yes, a bit", or "It had no impact".

Finally, participants were asked to indicate which industry stakeholders were primarily responsible for affecting sustainable change for grooms. The listed stakeholders were as follows: the FEI (International Equestrian Federation), IGA (International Grooms Association), EEF (European Equestrian Federation), national federations, show organizers, employers, grooms themselves, and other.

2.3. Data Analysis

Once the survey was closed, all responses were downloaded from Microsoft Forms and transferred to IBM SPSS 28.0 for statistical analysis. To test for differences between grooms with various backgrounds, their familiarity with the different initiatives, and their perceived impact, Kruskal-Wallis, Mann-Whitney U, and Pearson chi-square tests were used. In order to determine differences in perceived impact between the initiatives and COM-B behavioral sources a Kruskal-Wallis test and subsequent post-hoc Mann-Whitney U tests were used. Statistical significance was set at $p < 0.05$. Whenever multiple comparisons were carried out, a Bonferroni correction was applied to the alpha value with $p < 0.05/N$.

Data storage was conducted according to the Research Data Management policy framework of the University of Applied Sciences Van Hall Larenstein. Data management will adhere to the principles of Open Science.

3. Results

3.1. General Overview

A total of 1397 participants filled in the survey. Eight surveys were only partially completed and thus removed from further analysis, resulting in a data set of 1389 complete responses.

Participating grooms had a mean age of 34 (±11.47 years). Fifty-four percent (54%) were former grooms and 46% currently worked as professional grooms. Ninety-one percent (91%) of participants were female, 8% male, 0.4% non-binary and 0.5% preferred not to say. Participants from the United States were represented by 33.8% (N = 469) followed by the United Kingdom (12.2%), Germany (9.1%), Canada (8.6%), and the Netherlands (7.8%). Most grooms worked in the discipline of showjumping (74%), followed by dressage (9.6%) and other (different discipline or type of stable) with 9%. A total of 87.4% of participating grooms attended competitions (49.5% national and 50.5% international). 94.2% of those did that at least once a month.

3.2. Familiarity with and Impact of Initiatives Directed at Grooms in 2022

Out of all the participants, 23.9% of current or former grooms had heard of all four of the biggest initiatives directed at grooms in 2022. 52.3% of them had heard of some, but not all of them, and 23.8% were not familiar with any of the initiatives. Formation of the IGA was named to be the most impactful initiative for the grooming industry by 36.4% of the grooms (see **Figure 3**), followed by the 2022 ECCO FEI World Championships (27.9%). However, 20.1% of the grooms thought none of the initiatives had any real impact.

When asked about the personal impact of the initiatives (see **Figure 4**), the majority of participants chose none (58.5%). However, out of the four interventions, the 2022 ECCO FEI World Championships (16.7%) was considered marginally more impactful than the formation of IGA (16.4%).

3.2.1. Familiarity with the Initiatives

A Mann-Whitney U test found a significant difference in familiarity with the initiatives between current and former grooms (U = 198566, z = -6.04, $p < 0.001$). Current grooms (N = 638) reported greater familiarity with the initiatives compared to former grooms (N = 751) (mean: 1.88 ± 0.69 vs. 2.1 ± 0.67). A significant difference was also found between show grooms (N = 1213) and home grooms (N = 176), Also, show grooms (N = 1213) reported significantly greater familiarity with the initiatives than home grooms (N = 176) (U = 92397.5, z = -3.17, $p < 0.01$; mean: 1.98 ± 0.69 vs. 2.15 ± 0.12, respectively).

A Kruskal-Wallis test revealed a significant difference between grooms working in different disciplines ($\chi 2$ (3, N = 1389) = 64.59, $p < 0.001$). Post-hoc Mann-Whitey U tests with a Bonferroni-adjusted alpha value of 0.0125 showed significant differences between showjumping grooms (N = 1027) and other grooms (N = 133) (U = 42137.5, z = -7.87; $p < 0.001$; mean: 1.94 ± 0.48 vs. 2.44 ± 0.61); dressage/para-dressage grooms (N = 134) and other grooms (N = 133) (U = 6136.5, z = -4.884, $p < 0.001$; mean: 2.06 ± 0.63 vs. 2.44 ± 0.61) and eventing grooms (N = 95) and other grooms (N = 133) (U = 4006, z = -5.168; $p < 0.001$; mean: 1.97 ± 0.68 vs. 2.44 ± 0.61).

No significant differences were found between showjumping grooms, dressage/para-dressage grooms, or eventing grooms.

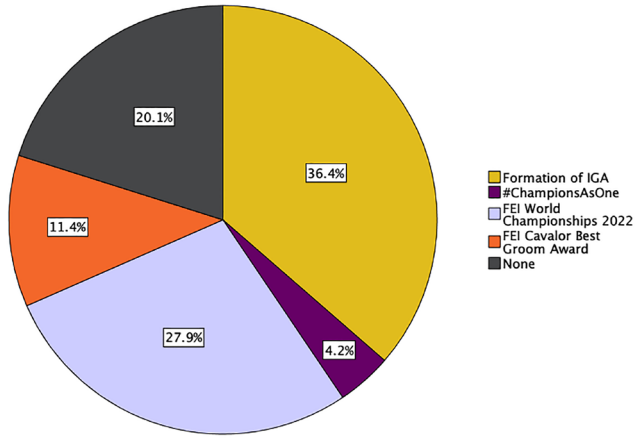

Figure 3: Most impactful initiatives for the grooming industry.

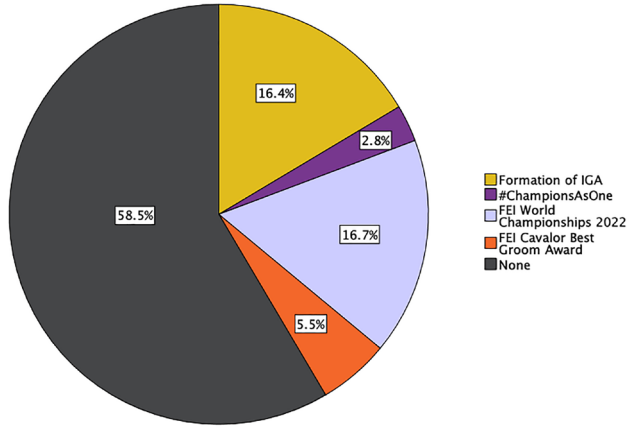

Figure 4: Most impactful initiatives for the grooms on a personal level.

3.3. Impact of the Initiatives on the Grooming Industry and Personally

Using the chi-square test, a significant difference was found between current and former grooms regarding which initiative they considered to have the most impact on the grooming industry ($\chi 2$ (4, N = 1389) = 20.912, $p < 0.001$). Former grooms considered the formation of IGA (34.3%) and 2022 ECCO FEI World Championships (24.6%) as less impactful compared to current grooms (38.6% and 31.3% respectively). FEI Cavalor Best Groom Award is seen as more impactful by former grooms (14.6% and 8.1% respectively). In terms of the personal impact of the initiatives, there is a significant difference between the current and former grooms ($\chi 2$ (4, N = 1389) = 35.919, $p < 0.001$). Former grooms see the formation of IGA (11.1%) and 2022 ECCO FEI World Championships (14.3%) as less impactful compared to current grooms (22% and 19.3% respectively).

A significant difference was found in the perceived impact of the initiatives when comparing show grooms to home grooms ($\chi 2$ (4, N = 1389) = 10.319, $p = 0.035$). Show grooms consider the formation of the IGA more impactful than home grooms (37,5% vs. 28,3% respectively), while home grooms consider the FEI Cavalor Best Groom award as more impactful compared to show grooms (16.5% and 10.7%, respectively). Regarding the personal impact of the interventions, no

significant difference was found between grooms going to shows and home grooms ($p = 0.127$).

Pearson's chi-square tests showed no significant differences between grooms from various disciplines, regarding the impact of the different initiatives on the grooming industry in general ($p = 0.183$) and on grooms personally ($p = 0.145$).

3.4. Initiatives and COM-B Factors

In total, 439 grooms found one of the initiatives to have had an impact on them personally. A Kruskal-Wallis test revealed that they significantly affected three COM-B factors (see **Table 2**). Psychological capability ($H (3, N = 439) = 18.479$, $p < 0.001$) and automatic motivation ($H (3, N = 439) = 9.860$, $p = 0.020$) were impacted in a positive direction. Physical opportunity ($H (3, N = 439) = 14.170$, $p = 0.003$) was the least impacted by any of the initiatives. The rest of the factors were not significantly impacted.

Post-hoc comparisons were conducted using the Mann-Whitney U Test with a Bonferroni-adjusted alpha level of .01 (0.05/5). By comparing the two most impactful initiatives which were the formation of IGA (N = 174) and 2022 ECCO FEI World Championships (N = 177), the formation of IGA had a significantly more positive impact on psychological capability ($U = 12047$, $z = -3.921$, $p < 0.001$) and physical opportunity ($U = 12996.5$, $z = -3.820$, $p < 0.001$).

3.5. Responsible Stakeholder for Leading the Change

Out of 1389 participants, 29% (N = 405) would like to see employers take the lead in developing a sustainable grooming industry (see **Figure 5**). The FEI was chosen by 23.5% of participants (N = 326), followed by the IGA (N = 215, 15.5%) and national federations with 10.7% (N = 148).

4. Discussion

The current study aimed to determine whether any of the four recent initiatives directed at grooms were perceived by grooms to address the barriers preventing them from staying in the industry. The Behavior Change Wheel was used as a theoretical framework in the analysis. Findings showed that participating grooms viewed the formation of the IGA and the 2022 ECCO FEI World Championships to be most impactful to the grooming sector as a whole. Arguably, these two initiatives were the most high-profile and promoted unity for a profession (the IGA) and within the realms of competitive sports (the 2022 ECCO FEI World Championships) **[7,10]**.

However, these results did not appear to translate to perceptions of effectiveness at the personal level. More than half of the grooms who indicated that they were aware of the campaigns (i.e. 58.5%) failed to see the relevance of the initiatives to themselves personally, likely because the initiatives failed to address the issues grooms perceive as the most salient barriers.

The 2022 initiatives appear to have failed to address barriers relating to physical capability. Effective interventions would have focused on reducing the mental and physical workload of grooms, thereby 'enabling' them to take better care of their health and physical fitness. The initiatives also failed to tackle barriers related to physical opportunity. Providing better remuneration or time off would likely have been much more effective seeing that these are concrete examples of the relevant intervention functions enablement or restructuring of the (working) environment. Lastly, the initiatives seem to have done little to clamp down on, or 'restrict' levels of disrespect by employers (rather than the general public), thus failing to attend to pervasive barriers relating to social opportunity. In fact, most of the barriers that were left unaddressed fall within the responsibility of employers. Tellingly, grooms named employers as the responsible stakeholders for leading the change in the grooming industry. These findings mirror those from previous studies investigating the effectiveness of public health interventions, where, in the case of the study, the responsible governmental stakeholders, refrained from implementing numerous appropriate intervention functions, and subsequent behavior change failed to manifest **[25,26]**.

Findings did demonstrate that the initiatives appear to have successfully addressed the behavioral sources of automatic motivation and psychological capability. The 2022 ECCO FEI World Championship, #ChampionsAsOnes, and the Cavalor Best Groom Award all focused on raising the image of the profession by evoking a positive emotional response, categorized by Michie *et al.* **[15,16]** 'persuasion', as well as creating expectations of reward, i.e. 'incentivization'. Both these intervention functions will affect levels of automatic motivation. What is more, the IGA, as an organization focused, at least in part, on educating grooms as well as helping them voice their concerns in the industry, may be perceived as increasing levels of knowledge, i.e. psychological capability.

Results also indicated that overall, 76% of grooms had heard about at least one initiative. However, levels of familiarity differed between grooms according to employment status and type of discipline. For example, former grooms were found to be less familiar with the initiatives. In all likelihood, this is due to them having retired from their profession prior to 2022. A study in 2023 on the acceptability of behavior change interventions reported that the success of interventions is tied to their relevance to a specific demographic group **[27]**. Therefore, former grooms may have not perceived the initiatives as particularly relevant to them and thus failed to familiarize themselves with their aims and activities.

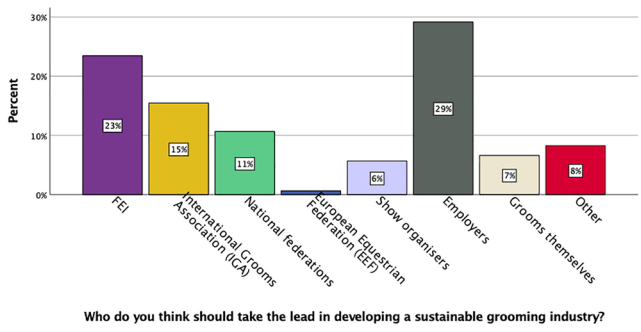

Figure 5: Responsible stakeholder to lead the change.

Table 2: The degree of impact of different initiatives on various COM-B factors (in %), with significant factors highlighted in bold.

	"Yes, significantly" [a]	"Yes, a bit" [a]	"It had a positive impact" [b]	"It had no impact" [a, b]	"It had a negative impact" [b]
Physical capability [a]	2.5%	29.4%	n/a	86.1%	n/a
Psychological capability [a]	7.7%	48.3%	n/a	44%	n/a
Automatic motivation [b]	n/a	n/a	67%	31%	2.1%
Reflective motivation [b]	n/a	n/a	41.7%	55.4%	3%
Social opportunity [b]	n/a	n/a	35.3%	61.5%	3.2%
Physical opportunity [b]	n/a	n/a	16.2%	81.8%	2.1%

[a] These response options only applied to the questions relating to the behavioral sources 'physical capability' and 'psychological capability'

[b] These response options only applied to the questions relating to the behavioral sources 'automatic motivation', 'reflective motivation', 'social opportunity', and 'physical opportunity'

Grooms involved in one of the Olympic disciplines (showjumping, dressage/para-dressage, eventing), were much more likely to have heard of one or more initiatives. For example, the 2022 ECCO FEI World Championships in Herning were held only for showjumping and dressage/para-dressage, meaning grooms working in these disciplines were the main group exposed to the initiative. Grooms not involved in the aforementioned disciplines are likely to be less aware of initiatives aimed primarily at the Olympic disciplines. Again, these findings echo the conclusions drawn by Novoradovskaya *et al.* **[27]**, that the success of an intervention strategy depends on its relevance to the target group. By failing to take the diversity of the grooming population into account, the initiatives may have ended up being less effective.

Additionally, compared to show grooms, home grooms perceived the initiatives to be less impactful. It could be argued that the formation of the IGA made less of an impact in the eyes of home grooms due to their lack of connection to the FEI. Since the FEI is best known for representing the international equestrian sports scene, home grooms are much less directly affiliated with the FEI. Secondly, it is important to note that 25% of the home grooms who took part in the survey are not involved with any of the Olympic disciplines. Since the initiatives were primarily publicized through FEI and affiliated media channels and at FEI events **[8,10,11,28]** the message and intended impact of the interventions might not have resonated with home grooms. Even though the formation of the IGA may arguably be relevant to all grooms, regardless of their specific tasks, the close connection of the IGA with the FEI may have led home grooms to assume that the work of the IGA is as irrelevant to them as other FEI activities. Such a phenomenon is a commonly acknowledged bias, the so-called 'halo effect', which leads individuals to base their assessment of a specific aspect of a person, product, or organization on the nature of previous assessments of that person, product or organization **[29]**. As such, the close association of current initiatives with the FEI may have inadvertently led to the alienation of certain groups of grooms and the subsequent perceived ineffectiveness of the initiatives. As has been highlighted by Henley *et al.* **[30]**, identifying the themes most relevant for a target group is essential to evoke sustainable behavior change in that group.

The minority of grooms who did choose a personally impactful initiative also settled on the formation of the IGA and the 2022 ECCO FEI World Championships. To reiterate, both initiatives focused strongly on automatic motivation (i.e. feeling about the job) and psychological capability (i.e. levels of knowledge). However, as research from Michie *et al.* **[15]** has shown automatic motivation and psychological capability are only pieces of the larger puzzle. Successful behavior change is a result of addressing all relevant behavioral sources and using appropriate intervention functions in the process **[13,31]**. Research within the nursing sector indicates that interventions which target all COM-B factors could help improve staffing shortages. Successful interventions include expanding the educational capacity and opportunity, expanding career options and improving compensation, regarding nurses as strategic assets and making positive changes in the work environment **[32]** as well as increasing funding for educational purposes, non-profits and organizations helping nurses **[33]**.

The findings of the current study demonstrate that, albeit undoubtedly well-intentioned, the effect of the four initiatives aimed at galvanizing the grooming sector is perceived to have been marginal at best. However, by drawing on the existing theoretical frameworks such as the COM-B model and the Behavior Change Wheel **[15,16]**, it has become possible to pinpoint why this might be: launching initiatives that target secondary issues, rather than attempting to tackle those barriers considered essential. As such, the current approach of using the Behavior Change Wheel shows considerable promise in assisting governing bodies when drawing up initiatives aimed at (behavior) change within the equine industry in general and the grooming profession in particular. Future research should focus on investigating how grooms themselves see the future of the industry, what changes they consider essential, and what might be needed to prevent fellow grooms from leaving their jobs.

The main limitations of the study include the high number of showjumping grooms taking part in the research. This was largely due to the fact that the main media outlets who spread the survey are highly active in covering news related to that specific discipline. However, showjumping is also by far the biggest equestrian discipline in the world so the high number of showjumping grooms might, in fact, be considered highly representative and may reflect the overall opinion of the grooming industry. Also, it needs to be borne in mind that the initiatives were launched in the spring and summer of 2022, while the current study was executed at the start of 2023. Seeing that the process of change is incredibly complex **[34]**

and time-consuming [35], it may be that the perception of the effectiveness of the initiatives may change as time goes on.

5. Conclusions

The main aim of the current study was to determine whether professional grooms consider the initiatives launched in 2022 by the FEI and stakeholders to be effective in addressing the current staffing crisis and the underlying issues that prevent them and their colleagues from staying in the industry. The answers from participating grooms indicate that last year's interventions have – so far – failed to address the issues perceived as the most pressing barriers to grooms staying in the industry. Current results showed that, in general, the initiatives investigated were perceived as unsuccessful in addressing barriers related to physical opportunity and physical capability. In addition, a majority of the grooms were not personally impacted by any of the initiatives.

Out of four presented initiatives, the formation of IGA and the 2022 ECCO FEI World Championships were considered to be the most impactful both on a personal level and to the industry at large. There was a significant difference in the behavior barriers that the initiatives addressed with psychological capability, automatic motivation, and physical opportunity being the behavioral sources most readily addressed. Employers, the FEI, and the IGA were considered to be three of the most important stakeholders responsible for initiating and managing relevant interventions to affect change in the grooming industry. Future interventions have to focus more on tackling the barriers of physical opportunity and physical capability which may be argued to be the most relevant in ensuring the longevity of a groom's career.

Supplementary Materials

A four-part, 31-question online survey was developed using Microsoft Forms®.

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Authors' Contributions

Conceptualization, S.L.O. and I.W.; methodology, S.L.O and I.W.; formal analysis, S.L.O; writing—original draft preparation, S.L.O.; writing—review and editing, I.W.; visualization, S.L.O. and I.W.; supervision, I.W.; All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data storage was conducted according to the Research Data Management policy framework of the University of Applied Sciences Van Hall Larenstein. Data management will adhere to the principles of Open Science and data is accessible on request.

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The study received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

The study received ethical approval from the Hanze Hogeschool Ethics Advisory Committee (dossier number: heac.2023.006) and the study followed the guidelines of the Declaration of Helsinki.

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The Impact of Online Educational Talks on Young Equestrians' Knowledge of Breast Health and Breast Issues

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Abstract

The breasts are a barrier to female participation in physical activity. Breast and bra issues are prevalent in female horse riders. Wearing a sports bra can minimize these issues, but many do not exclusively wear one for horse-riding. This study investigated the impact of live online breast educational talks on subsequent self-perception of knowledge and understanding. Two online surveys (GoogleForms™), one immediately pre-talk and one immediately post-talk, were created to assess perceived impact. The talks consisted of five sections: breast anatomy; types of sports bras; breast issues in relation to exercise; breast issues specifically related to horse riders and the importance of increasing awareness in the horse-riding community. Four educational talks were delivered to different groups of college and university students (n = 67) studying equine courses. Completed pre- and post-talk surveys (40 female, 2 male) were analyzed using a generalized linear model and post hoc Tukey tests. Comfort talking to others about breast health issues increased significantly after the talks, particularly for larger-breasted (≥ D cup) participants ($p = 0.032$). Knowledge of bra fit, breast support, and breast pain significantly increased ($p < 0.01$) post-talk, particularly in those who had not previously experienced breast pain whilst horse-riding ($p \leq 0.001$). The intervention was successful at increasing participant understanding and knowledge of breast health issues, although different educational tools such as access to online resources or in-person talks may prove beneficial to equestrians to further increase comfort in broaching breast health issues with peers and support networks in future.

Keywords

Horse rider; breast issues; stigma; education

1. Introduction

Participation in regular exercise improves both physical and mental wellbeing [1]. Regular physical activity drastically reduces the development of serious diseases, i.e. Type 2 diabetes, heart disease, and osteoarthritis, and reduces the risk of stress and depression [2]. Recently emphasis has been placed on increasing levels of physical activity, with the World Health Organization (WHO) recommending adults participate in at least 150 minutes of moderate intensity exercise per week [3]. Despite this, only 50-60% of adults meet aerobic exercise guidelines, lessening to 30% who meet aerobic and resistance training guidelines [4]. Increasing rates of obesity, a factor known to contribute to health problems, highlight the crucial need for more people to engage in regular exercise. However, Audickas [5] found that 63% of men in the United Kingdom (UK) participate in exercise, and only 58% of women. A popular sport for females is equestrian activities, with 74% of horse riders in the UK being female [6]. For many, it is also the only form of physical activity undertaken [7]. To increase female physical activity and participation in equestrianism, it is important to understand barriers to participation.

1.1. The Breast as a Barrier to Participation

The breast has been identified as a significant barrier to female participation in equestrian activity [8] and Burnett *et al.* [9] found that the breast was the fourth greatest barrier to participation in sport. Breast pain is a common barrier and often increases with exercise intensity [10,11]. Excessive breast motion during exercise is more prevalent in larger-breasted women (≥ D cup) which may prove problematic in equestrianism as Burbage and Cameron [12] found that over 50% of equestrian survey respondents were in this category. This increased breast movement not only causes pain, but also embarrassment [13], and may impact female equestrian participation.

The female breast is a malleable structure, readily deformed by external forces [14]. The breasts contain between 15-20 lobes, adipose tissue filling spaces between, interspersed with nerves and blood vessels [15]. The breast lies over the pectoralis major muscle but the breasts themselves contain no muscle; their main supporting structures are Cooper's Ligaments and covering skin, which if damaged are irreparable [16]. As little as 2cm of relative breast movement can cause pain, however, breasts can move up to 19cm vertically and 4cm in the medial-lateral and anterior-posterior planes during physical activity [13]. Research has shown that wearing suitable breast support can minimize the risk of excessive breast movement and alleviate associated breast pain (mastalgia) [11,17–19].

Activity levels in females have been shown to decline during adolescent years [9] with breast concerns contributing to unwillingness to exercise. Scurr *et al.* [20] found nearly half of over two thousand schoolgirls reported that their breasts contributed to their unwillingness to complete compulsory exercise, and yet, did not wear a sports bra. Horse-riding is popular among adolescent females, but their participation may decline if they experience breast issues when riding, suggesting that breast health and breast support education may have an important role to play in maintaining female equestrian participation.

1.2. Breast Issues in Female Horse Riders

Horse-riding is a moderate-intensity exercise inducing similar breast movements to running and jumping activities [12]. Burbage and Cameron [8] reported that 40% of equestrian survey respondents experienced breast pain with 21% reporting negative riding performance effects. Burbage and Cameron [12] found at least one breast-associated barrier reported by 25% of respondents. Despite breast pain, many women do not wear a sports bra for horse-riding [8]. Studies have shown that wearing appropriate breast support for horse-riding reduces breast movement and exercise-induced breast pain (EIBP) [18,19] and has rider performance implications. Given the positive effects of wearing a sports bra, it is surprising that uptake is low when horse-riding. However, not wearing a sports bra is not limited to female equestrians [21] and greater education on breast health and bra usage across a wide demographic of exercising females is warranted.

1.3. A Need for Breast Health Education

Many females suffer from breast-related issues including incorrect bra fit, excessive breast movement and pain, breast sag (ptosis), and embarrassment, all of which can negatively affect health and well-being. However, knowledge and awareness of these issues are low, especially in adolescent females compared to adult females [22]. McGhee *et al.* [23] identified a need to educate adolescent female athletes about bra knowledge and fit. McGhee and Steele [24] argue that a well-fitted sports bra is essential equipment for those wishing to participate in sports at any level. However, there is a lack of evidence-based guidelines providing information on breast health and bra fit and sporting females may be unwilling to discuss such issues with male coaching staff. Creating resources that male coaches can signpost their clients to, to find useful and correct information, may lessen these difficulties.

These resources are not available within the school curriculum [25] and Omrani *et al.* [22] found that educating adolescent girls improves their breast knowledge making them feel more informed and less embarrassed. For equestrian adolescents, horse-riding may be their only physical activity, and as activity levels decline during the post-adolescent years, improving breast and bra knowledge through specific equestrian-focussed educational interventions may help young females to feel better equipped, more comfortable when horse-riding and more likely to continue this activity into adult life.

The aim of this study was to assess the impact of equestrian-focused educational talks on the topic of female horse rider breast health and related issues on listener knowledge and awareness of the subject. It was hypothesized that talks would significantly increase listener knowledge about breast health and would increase listener comfort in discussing breast health issues with others.

2. Method

2.1. Study Design

Following institutional ethical approval from the Sparsholt Research and Ethics Group, a breast education intervention titled "Breast Health in the Female Equestrian" was offered to a range of equine students in the form of an online short lecture. Four groups of students, and their lecturers, studying equine courses at further education level and undergraduate level were recruited between March and April 2021. All participants were over 18 years of age and confirmed they were current equestrians. To assess the impact of this educational talk, two surveys were created using Google Forms™, completed immediately pre- and post-talk. These surveys were designed to take no more than 10 minutes to complete and included yes/no, multiple-choice, 5-point Likert scales, 10-point Numeric Rating Scales (NRS), and short answer questions.

2.1.1. Pre-talk Survey

The pre-talk survey had two versions, one for participants identifying as female and one for other participants (identifying as male or non-binary or who chose not to declare a self-identification). Part one of the pre-talk survey for those participants identifying as female collected demographic information on age, self-reported bra-band size, bra cup size, and the types of bras worn for horse-riding. Participants were asked to provide a word to allow pre- and post-talk surveys to be matched. Part two identified bra fitting issues (chafing, straps/underwire digging in, muscle ache, poor posture) and whether participants felt that their bra choices met their needs for horse-riding using a multiple-choice grid

and Likert scale. Part three explored breast pain in relation to horse-riding and whether they had spoken to anyone about it using yes/no, multiple-choice, Likert scales, and free text questions. The final part asked how comfortable participants felt talking about the topic of breast health issues to others and to rate their knowledge of bra-fit, breast support, and breast pain using a 10-point NRS (1- not comfortable, 10 - very comfortable). Pre-talk surveys for those participants identifying as male, non-binary, or prefer not to say had similar questions excluding questions relating to bra size, bra comfort, and experience of breast pain. Part one asked for age and their association with horse-riding. Part two identified breast-related issues they were aware of, and their comfort in talking to horse-riding associates about breast issues using a multiple-choice and a 10-point NRS question. The final part asked all participants if they would like access to resources about breast health issues in horse riders. Pre-talk surveys were completed immediately before the educational talks.

2.1.2. Post-talk Survey

Part one of the female post-talk survey asked if participants would now talk to someone about their breast pain if applicable and if yes, who they would talk to, using a multiple-choice question. Part two explored the area of how comfortable they now were talking to others about breast issues and if they would find online resources useful. Part three asked them to again rate their knowledge of bra fit, breast support, and breast pain and whether the information given in the talk had impacted their choice of bra for horse-riding using multiple-choice questions. The final part asked for participant views on how useful the talk had been using a 10-point NRS (1- not useful, 10 - very useful) and for any comments on areas they thought needed more research or should have been discussed in the talk using free text questions. Questions were formatted to be short and clear, leading questions were avoided, Likert scales were utilized and the overall time for completion of the questionnaire was kept short to limit potential bias. For participants identifying as male, non-binary, or prefer not to say post-talk surveys used Likert scales and yes/no questions to ask how comfortable they now felt about discussing breast issues with others, whether they would find online resources useful, and how useful the educational talk had been as for female participants. The post-talk surveys were completed immediately following the educational talks to limit any bias due to recall memory.

2.1.3. Educational Intervention

The educational intervention was presented to four groups of students, and their lecturers, studying equine courses at further education level (over 18 years of age) and undergraduate level where the course leader had agreed to time within timetabled sessions being used for the study and the students had not already experienced a breast health education talk within their syllabus. The lecture was delivered via Microsoft Teams™ and in total, including time for completing the surveys, took 40 minutes to adhere to existing timetabled sessions within the institution. Prior to data collection, the educational talk had been piloted with staff members to ensure it was evidence-based. The talk included five sections: section one explained the anatomy of the female breast; Section two described the common types of sports bras that are available on the market (encapsulation, compression, combination); Section three detailed breast issues in relation to exercise (bra fit, breast pain, performance, embarrassment); section four explained breast issues specifically related to horse riders; section five explained the importance of increasing awareness and knowledge of these issues in the horse-riding community and how individuals can know if their bra is a good fit. The talk finished with the opportunity for any questions to be asked.

In total, 67 pre-talk survey responses (64 females, 3 males), and 49 post-talk survey responses (47 females, 2 males) were downloaded from Google Forms™ into a Microsoft Excel™ spreadsheet. For comparison, there were 40 complete pre- and post-talk female survey responses, and 2 males, that could be matched up for analysis using the word supplied by participants.

2.2. Statistical Analysis

Microsoft Excel™ and Minitab 2021™ were used for data analysis. Descriptive analysis was used to summarize the demographic information of female participants' bra bands, cup sizes, and types of bras worn for horse-riding. Cup sizes A to C were classed as smaller-breasted females and cup sizes D and above were classed as larger-breasted **[8]**. Score data for the comfort of talking to others; knowledge of bra fit, breast support, and breast pain, and overall knowledge when the three categories were combined, were analyzed using a generalized linear model as the residuals were normally distributed. Cup size and survey type (pre/post) and experiencing pain when riding were fixed factors and cup size nested in survey type, respondent was included as a random factor. Post hoc Tukey tests were run to compare the results with an alpha set at 0.05.

3. Results

The modal age of participants was 18 years (70%). Female participant bra (UK sizing) cup size ranged from an A cup to a G cup and under band size ranged from 26 to 48 inches **(Table 1)**. The modal bra size was 34B. Of the 63 female participants, 63% were classed as smaller-breasted (n = 40) and 37% were classed as larger-breasted (n = 23). Only 34% of female participants wore a sports bra during equestrian activities and 63% wore an everyday bra of varying styles **(Table 1)**. Of riders (n = 23) who reported a bra size in the larger-breasted group (cup size > D), 30% exclusively wore a sports bra when riding. Experiencing pain when horse-riding was reported by 17% of female participants (n = 11). Of these, 55% were larger-breasted and 45% were smaller-breasted, and only 18% (n = 2) exclusively selected a sports bra when completing horse-riding activities.

All participants were more comfortable talking to others about breast health after the talk ($F_2 = 4.62$, $p = 0.016$) and this was significantly more for respondents reporting larger breast sizes (T = 2.88, p = 0.032) **(Figure 1)**.

Post-talk knowledge of breast health issues; bra fit, breast support, and breast pain all significantly increased compared to pre-talk knowledge levels ($F_2 = 20.04$, $p < 0.01$; $F_2 = 25.94$, $p < 0.01$; $F_2 = 35.22$, $p < 0.01$, respectively) **(Table 2)**.

Table 1: Distribution of female participants' self-reported UK bra size.

	Cup size							
Underband inches	A	B	C	D	DD	E	G	Total
26		1						1
28	2	1	1		2			6
30	1	1		1				3
32	2	8	6	2	1	1		20
34	1	7	6	1	4	2	2	23
36	1	1	2	3	2			9
38						1		1
40				1				1
Total	7	19	15	8	9	4	2	64

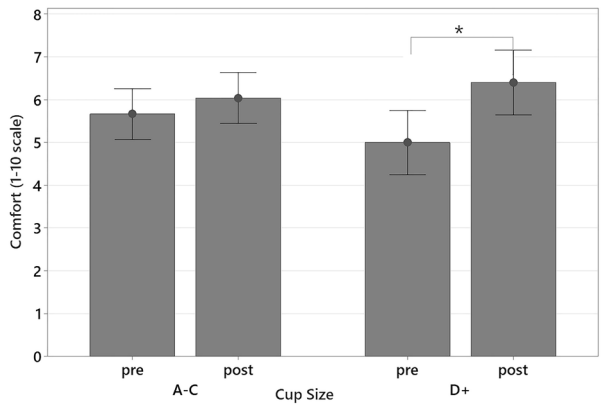

Figure 1: Pre- and post-talk comfort scores when talking to others about breast issues (1- not comfortable, 10 - very comfortable) * indicates a significant difference.

There was no significant difference in knowledge of breast health issues between breast size groups.

When asked whether breast pain was experienced when horse-riding there was no significant difference in pre- and post-talk levels of comfort of talking to others (F_2 = 0.61, p = 0.548), though a significant difference was found for knowledge of breast issues (F_2 = 8.55; 15. 46; 14.04, $p < 0.001$). When further compared by whether pain was experienced when horse-riding, those that said NO had a significant increase in knowledge of breast pain (T = 5.07, $p < 0.001$), breast support (T = 5.41, $p < 0.001$) and bra fit (T = 4.03, p = 0.001) post talk **(Figure 2)**.

Table 2: Mean knowledge of breast issues pre and post talk (1 = very poor, 2 = below average, 3 = average, 4 = above average, 5 = excellent).

Breast Issue	Pre-talk mean (±SD)	Post-talk mean (±SD)
Bra fit	2.7 (0.98)	3.5 (0.79)
Breast support	2.6 (0.97)	3.5 (0.82)
Breast pain	2.3 (1.08)	3.5 (0.79)
Overall knowledge	2.5 (1.01)	3.5 (0.79)

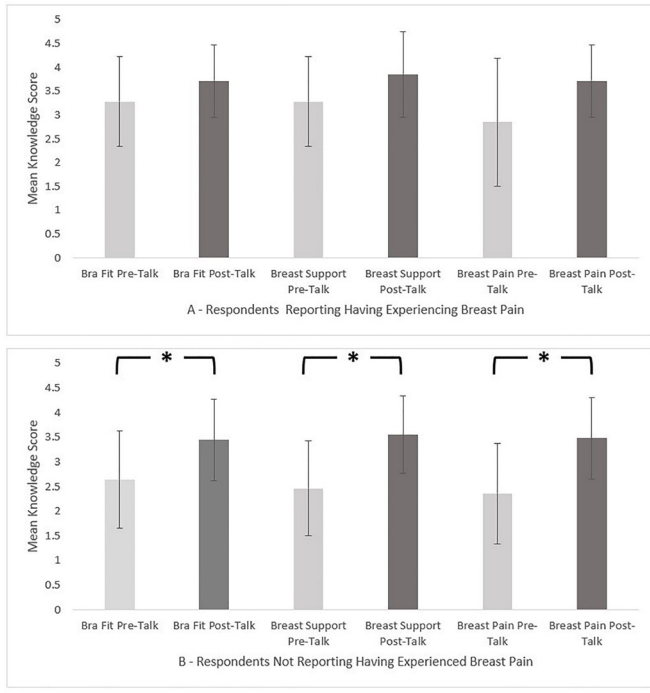

Figure 2: Mean scores of pre- and post-talk knowledge (1 = very poor, 2 = below average, 3 = average, 4 = above average, 5 = excellent) of breast issues for participants that said YES to experiencing breast pain **(a)** and NO to experiencing breast pain **(b)** * indicates $p \leq 0.001$.

The two male participants who completed both the pre- and post-talk surveys felt that access to online resources about breast issues in female horse riders would be useful. Of the 40 females who completed both surveys, 83% (n = 33) felt access to online resources would be useful and 17% (n = 7) felt they would not be useful. The most frequent post-talk response to whether female participants felt they would be able to give advice to others on breast issues was 'yes, but they would like further information' (45%), followed by 'no, but I could advise them where to find information' (35%). Nearly half of female participants (48%) were encouraged to rethink their choice of bra for horse-riding, 23% reported they would change their bra type, while another 23% reported they were happy with their current choice. Of those who would change their bra type choice, all already chose a sports bra for riding, but not exclusively and none had reported experiencing breast pain in the pre-talk survey. The usefulness of the talk was given a score of 6 and above on the Numeric Rating Scale (1 – not useful 10 – very useful) by 78% of all participants.

4. Discussion

The key aim of this study was to assess the impact that giving online educational talks on the topic of breast health issues in female horse riders had on participants' comfort in talking to others about these issues and their knowledge of breast health issues.

Participants felt significantly more comfortable talking to others about breast issues post-talk, particularly those with larger cup sizes. The problems relating to the breast (such as feelings of embarrassment, not being able to find a correctly fitting bra, and breast pain) are very personal subjects to discuss and having an opportunity to discuss these issues

may have helped increase this comfort. There continue to be stigmas surrounding women's health matters [26]. Vrinten *et al.* [27] found that cancer stigma exists, particularly surrounding breast cancer, which can negatively influence behavior toward breast screening. This may mean that it is likely that women are also worried about talking about general breast health issues. Although the current study was specific to breast issues in horse riders, it has highlighted that there is a need for greater education about breast issues to increase confidence and break down social stigmas. If talking about these less serious, but equally important issues of bra fit, breast pain, and breast support, becomes normal, women may then be more willing to raise health concerns or access breast screening services in future.

There seems to be a gap in school curriculums and public health sources for more generalized information about breast health. In this study, the majority of participants were between 18 and 21 years old. This may explain the significant increase in comfort when talking about breast issues post-talk; older females may be less impacted by the educational talk as they are already more comfortable in discussing these issues although further research is warranted. However, these results show that, similarly to Brown *et al.* [25], female adolescents are an ideal target group for promoting breast health awareness.

Participants' knowledge of bra fit, breast support, and breast pain significantly increased after listening to the educational intervention; those classed as larger-breasted increased their knowledge more than those classed as smaller-breasted, but only by 0.1 of a score. Generally, larger-breasted women experience more breast issues such as pain and embarrassment from excessive breast movement [24]. It was therefore assumed that larger-breasted participants would have already had better knowledge of the issues because of personal experience, however, this research highlights that educational interventions are required for female riders of all breast sizes. Those respondents who reported no breast pain when horse-riding increased their knowledge more than those who did report breast pain, suggesting that female riders who had not experienced breast pain may be more in need of educational interventions at a younger age. Of those reporting breast pain when riding, only 18% (n = 2) exclusively wore a sports bra when riding, suggesting that further intervention is warranted in this group as, although they may already have a good knowledge of breast health issues, their bra choices are not necessarily mitigating their breast pain. An enhanced educational intervention would make both groups more aware of the importance of appropriate breast support when horse-riding, and may encourage changes in bra choices.

Of those riders who reported a positive choice to change their bra choice for riding, all reported already choosing a sports bra for horse-riding, but not exclusively, and none had reported experiencing exercise-induced breast pain previously. The educational talk may have made them reconsider their reporting of pain in the pre-talk survey, or it is possible that these participants were influenced by some of the links between breast support and rider position [19] presented within the educational talk, so were prepared to change their bra choice to enhance their horse-riding performance.

There was a strong female bias in this study (95%), which is representative of the sample population of students in equestrian further and higher education programs. Future studies should aim to increase male respondents as, although the vast majority (91%) of British Horse Society Accredited Professional Coaches (BHS APC) are female, at higher levels of equestrianism nearly 40% of coaches are male [28]. Increased awareness of the breast as a potential problem may enable them to direct their clients to online resources for example where they can find the necessary information to be able to help themselves, thus avoiding difficult conversations. The majority of female participants (83%) in this study also thought online resources would be useful. Encouragingly, nearly half the female participants felt that after hearing the educational talk they would be able to advise others on breast issues, but would still like further information and resources. It may be that this educational intervention was too short, the online nature of the talk did not provide the most effective discussion forum. Or it may be that more supporting resources are required for participants to access at a later date, and further research establishing the best method to impart breast health and issues information is warranted for the equestrian community.

The majority of participants wore an everyday bra for horse-riding (63%) with only 34% wearing a sports bra, however, this was an increase on the previously reported figure of 21% of women that exclusively wore a sports bra for horse-riding reported by Burbage and Cameron [8] and maybe reflective of the differences in age within these two studies with younger equestrians being more familiar with sports bras in general. This is despite research showing that a sports bra is the most suitable bra type to wear when horse-riding to reduce relative breast movement and EIBP [18,19]. Nearly three-quarters of the female participants would rethink or definitely change their bra choice following the educational talk, which confirms the talk had an impact, and those who did not want to change their bra were mostly those who already exclusively wear a sports bra when riding. Results suggest that more dissemination of breast health research results within the equestrian community would increase sports bra wear, however, consideration needs to be given to the development of equestrian-specific bras as many feel that the bras currently available on the market do not meet their needs for horse-riding in terms of support, fit and style [12].

A limitation of this study was that the talks were given online, which makes it challenging to interact and connect with the audience fully. It is clear from the optional post-talk surveys that there was a level of disengagement and although online delivery can be utilized to reach a wider audience, in-person presentation might be more effective in increasing listener participation and the efficacy of both methods should be tested in future. Post-talk surveys were also completed immediately after the educational talks. Ongoing recall memory of the information from the educational talks may be negatively impacted by any emotional response to the subjects covered [29], therefore the addition of a follow-up survey after a period of time has elapsed would be beneficial in any future breast issues educational research.

Although on a much smaller scale compared to other similar studies on breast education interventions [22] and tailored specifically to breast health in horse riders, this study has shown that an educational intervention can improve equestrians' knowledge and understanding of breast health issues, however further research is required to then gauge any subsequent changes in bra choices when riding and any resultant reduction in exercise-induced breast pain. This was a short online presentation compared to Omrani *et al.* [22] in-person, longitudinal study, and should also be expanded in future research, with any long-term behavior change monitored. Bra fit and breast support information is rarely included in school health programs [24] and the inclusion of breast health education is recommended by Brown *et al.* [25]. For females engaged in horse-riding, there is further potential to educate young equestrians through Pony Club events, British Horse Society campaigns and their coaching pathways, as well as in college and within University equine courses, once the best method of dissemination is established.

5. Conclusion

There continues to be stigma and feelings of awkwardness surrounding the topic of breast health issues in female horse riders affecting people's willingness to talk to others about the breast-related challenges they may face. This short educational intervention has been shown to be effective in increasing equestrians' knowledge on breast health issues of bra fit, breast support, and breast pain, and increasing their comfort in discussing these issues, although what impact these changes may have on equestrian bra choice is unknown. Further research is needed to assess the best dissemination method for educational interventions to the wider equestrian population to enable equestrians of all ages and disciplines to improve their knowledge of breast issues and health when horse-riding.

Authors' Contributions

L.C. and R.S. planned and designed the study. R.S. collected the data and carried out the study. L.C., R.S., and M.F. carried out the statistical analysis. R.S., L.C., M.F., and V.L. wrote the manuscript. N.S., L.D., and J.B. reviewed it. All authors read and approved the manuscript.

Data Availability

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

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

This study received ethical from the Sparsholt Research and Ethics Group. The study complied with the guidelines of the Declaration of Helsinki.

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What is Welfare? A Qualitative Study into Perceptions of Equine Welfare of the Dutch Equestrian Community

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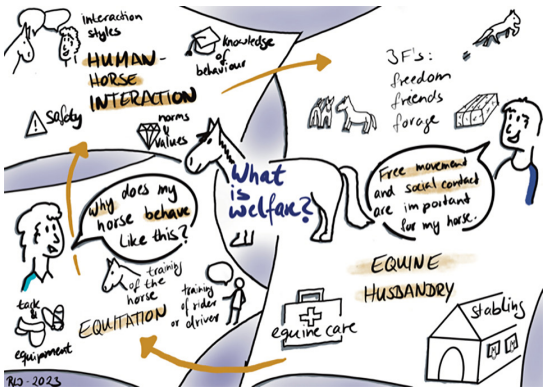
Abstract

Equine welfare is an increasingly important topic in the Netherlands and abroad. While existing literature broadly captures equine welfare concerns, research focused on Dutch horse enthusiasts is sparse. This study aims to identify what aspects horse enthusiasts in the Netherlands consider essential to safeguard equine welfare. An online survey comprising four binary yes-no questions, and 12 open questions was disseminated via social media. Survey data were analyzed qualitatively using inductive thematic analysis. The survey received 875 complete responses. Thematic analysis led to the identification of three higher-order themes: Equine Husbandry, Human-Horse Interaction, and Equitation. Aspects relating to Equine Husbandry were mentioned most, suggesting that Dutch equine enthusiasts consider aspects relating to housing and management more important to overall equine welfare. Within this theme, lower-order themes such as the ability to perform natural behavior and feeding were most prominent. On the theme of Human-Horse Interaction, respondents highlighted the importance of understanding horse behavior and human-horse communication to ensure their welfare, as well as ethical considerations for treating horses as sentient beings deserving respect. On the theme of Equitation, various aspects of training the rider and the horse were highlighted, including a sound knowledge of tack and equipment. Respondents emphasized the importance of qualified instruction on horse care and welfare. Despite evident awareness of a wide variety of welfare aspects among equestrians, considerable discrepancy between knowledge and practice persists, pointing to the need for tailored education and training to facilitate better application of welfare principles.

Keywords

Horses; social license to operate; equine husbandry; human-horse interaction; equitation; Netherlands

Graphical Abstract



1. Introduction

In recent years, against the backdrop of high-profile incidents of equine mismanagement and abuse [1–4], the topic of equine welfare and how to safeguard it has become increasingly important. This growing concern is understandable, given the significant role horses play in society, through a wide range of activities, from recreational pursuits to competition at the highest level, from breeding, training, and trading of horses to coaching and therapeutic practices [5–9]. Unsurprisingly therefore, the issue of how equestrianism is to preserve its "Social License To Operate", i.e. the dynamic, largely intangible, unwritten contract between society and the equestrian sector [8,10–13], occupies much of the political agendas of national and international governing bodies [14–16].

Nevertheless, horse owners themselves play a crucial role in engaging in responsible husbandry and training practices that align with the intrinsic needs of horses and safeguard and promote their physical and mental well-being [17–19]. As such, the knowledge and care provided by humans directly influence the quality of life and welfare experienced by horses. However, intentional malpractice aside, what is thought to be routine management or accepted training practices at home have been shown to – inadvertently – put the welfare of horses at risk [20,21]. Research by Dyson *et al.* [22] into the prevalence of gait abnormalities in leisure horses from the United Kingdom thought to be sound by their owners demonstrated that 28% of 148 horses were lame in hand and 62% were lame when ridden. Similarly, in a study by Rhodin *et al.* [23] more than 70% of "owner-sound" horses presented with above-threshold asymmetries. Common management practices such as stabling horses, feeding substantial amounts of concentrate feed, and allowing no/reduced time at pasture are all known risk factors for colic [24], a condition that is believed to affect up to 10% of horses annually [25], with approximately 20% of cases involving intensive medical treatment, surgery, and/or death of these animals [24].

In addition to the gap in equine-related knowledge [26], various international studies have suggested that the way in which horse enthusiasts understand the concept of welfare will likely impact their assessment of equine-related practices [12,27–29]. In an analysis of in-depth interviews with British equine stakeholders, Horseman *et al.* [29] categorized welfare problems according to health-related, management-related, and riding- and training-related welfare problems. Health-related problems most commonly discussed were horses being under- and overweight, foot problems (e.g. abscesses or horses with overgrown hooves), internal parasites, and laminitis. The most prevalent management issues related to the 24-hour stabling of horses are under- or overfeeding, inappropriate rugging, limited access to water, and social isolation. Riding and training-related welfare problems centered on the inappropriate use of training aids and poorly fitting tack [29]. Dubois *et al.* [30] investigated welfare problems at the individual horse level as experienced by Canadian equine professionals. The most critical issues identified were horses being denied access to important psychological or physical resources, together with inappropriate drug use [30]. Other aspects that were ranked relatively high were lack of proper professional care, inappropriate training practices, lack of knowledge or education, overpopulation, and improper dietary practices [30]. Using the Five Domains Framework, McGreevy *et al.* [31] assessed expert perceptions of horse welfare across different categories. Expert panelists from Australia, the United Kingdom, Ireland, Canada, and the United States identified key concerns including abrupt weaning, exclusive concentrate feeding, isolated indoor tie-stalls, forced flexion of the neck (i.e. Rollkur or hyperflexion), constrictive nosebands, ear twitches, and transportation with unfamiliar horses as the most significant risks to equine welfare [31]. A study investigating perceptions of horse welfare at shows in the United States [32] identified training aspects like excessive pulling on the reins, excessive spurring, inducing unnatural movements, excessively repetitious aids or movements, and excessive continued pressure on the bit as the most prevalent welfare issues. A recent study by Williams *et al.* [33] captured the opinions of leading experts actively involved in national and international horse sports with regard to the management of sport horse health and welfare. While participants unanimously considered the key areas of training management, competition management, young horse management, health status and veterinary management, and the horse-human relationship essential, they failed to reach a consensus on the importance of stable and environmental management and welfare assessment. In fact, the majority felt that these areas were already well managed, and thus not considered essential. These findings are evidence of the difficulty in defining equine welfare clearly and unequivocally [33]. Yet as the growing body of literature would suggest, there is an increasing focus on what the concept of equine welfare means to different individuals, and how this might translate into practice at various levels of the industry as well as across various countries.

The Netherlands is considered one of the most prolific equestrian nations worldwide [34,35], with Dutch riders regularly winning medals at the Olympic, World, and European stages, and the Dutch warmblood is considered to be the most successful breed in the world [36]. Approximately 500,000 horse enthusiasts participate regularly in a range of horse-related activities, either during formal competitions or at a recreational level [35]. As it stands, equestrianism continues to be of considerable importance to the Dutch sporting culture. At the same, there has been increasing concern from the Dutch general public regarding equine welfare standards, with several TV reports and main stream newspapers covering aspects ranging from sport horse welfare [37,38] to equine husbandry practices in riding schools [39] and horses kept privately at home [40]. However, little targeted research exists examining the current perspectives of Dutch equine enthusiasts regarding equine welfare. Therefore, the current study aimed to gain an in-depth understanding of which aspects Dutch horse enthusiasts consider important contributors to equine welfare.

2. Materials and Methods

2.1. Survey Recruitment and Distribution

In order to engage as many equine enthusiasts as possible across the Netherlands a cross-sectional study design was used. In line with Dutch regulations for survey-based research, individuals aged 16 years or older, involved at all levels of equestrian activities were targeted. An a priori sample size calculation with a confidence level of 95% and a 5% error margin determined that a minimum sample of 384 was required. Recruitment took place via Facebook, LinkedIn, various online equine news sites, and through email newsletters of partner organizations from the 27th of April to the 21st of August 2023 using SurveyMonkey, an online survey platform (SurveyMonkey Inc., California, USA). Following an introduction of the aim of the study at the start of the survey, respondents were asked to provide active informed consent to participate. All respondents were assigned unique numerical identifiers to ensure anonymity. The survey was conducted according to the Netherlands Code of Conduct for Research Integrity and followed the guidelines of the Declaration of Helsinki.

2.2. Survey Design

The survey was available in Dutch and consisted of two parts with a total of 19 questions; **Supplementary Material 1**

(Dutch) and **Supplementary Material 2** (English translation). The first part of the survey collected demographic information of respondents (gender, age, and type of involvement with horses). In addition to a number of pre-defined options, participants were also given the option to provide an answer in an open-text response. The second part consisted of four binary yes-no questions, determining the relevant routing of the survey, and 12 open questions concerning equine husbandry, riding and driving, and horse handling. In order to elucidate as much detail as possible regarding which aspects participants find important when it comes to equine welfare while staying as close to reality as possible, the questions were phrased as scenarios, e.g. "Imagine a friend of yours has bought a new horse and is now looking for a yard. What would you advise her to look for in a new yard."; "Imagine the daughter of an acquaintance has just started to ride/drive. What should she know or be able to do to become a good rider/driver, who is able to safeguard the welfare of her horse(s)?" and "Thinking back to the start of your life with horses: What would you have liked to have known/been able to do then, that you know/are able to do now?"

2.3. Data Analysis

Data were imported into IBM SPSS for Social Scientists 28.0 to obtain descriptive statistics, such as frequencies and proportions for the available categorical data. Open-text responses to the demographic questions were reviewed and systematically categorized into one of the pre-existing categories. Any information contained in the open-text responses that might result in the personal identification of the participant was removed from the data set prior to analysis.

For the qualitative analysis, a thematic approach was used **[41]**. Responses were imported into NVivo 14 (QSR International, Melbourne, Australia) for thematic coding and subsequent analysis. The first stage of data analysis involved an immersive engagement with the collected data. The second author read through the responses to the open questions multiple times to gain a deep understanding and familiarity with the content. Initial codes were generated by systematically analyzing the data line-by-line and were drawn directly from words or phrases used by participants. Codes were tagged to specific and relevant data extracts that appeared significant to the research question. The initial codes were discussed by the first and second authors and any discrepancies in understanding were resolved through consensus. Following coding, three overarching higher-order themes were identified by clustering together similar or related codes. This process was iterative and involved constant comparison between codes, themes, and the data itself. Each theme was rigorously reviewed to ensure coherence and distinctiveness. This involved re-examining the coded data extracts for each theme and considering their overall narrative. To avoid repetition, similar answers by the same respondents across different questions were coded only once. In these cases, where multiple responses fit a particular category, the response that most comprehensively represented that theme was selected.

After this initial process of selection and higher-order theme development, survey answers were coded manually per higher-order theme into thematic categories. Coding was reviewed at intervals, approximately after every hundred responses, leading to adjustments in themes and the reassignment of previously coded data where necessary. A 'to be determined' category was set up to temporarily contain significant codes that did not clearly fit existing categories. Upon concluding the coding for each question, these provisional codes were integrated into existing categories or served as the foundation for new ones. Throughout the coding process, the first and last authors conferred on the meaning of each thematic category to enhance the reliability and validity of the research findings. Although data saturation is often considered a stopping point in qualitative research **[42]**, analysis was continued even after saturation was achieved. This decision was made to allow for the examination of the frequencies of certain responses, in addition to identifying themes and patterns. Such quantitative insights would not necessarily be captured if data analysis was stopped at the onset of saturation. Thus, by extending the analysis, a more comprehensive view of the participants concerning equine welfare could be gained.

Finally, all categories were examined for similarities in content and clustered into lower-order themes. These lower-order themes were then named to accurately reflect the depth and nuances of the data.

3. Results

The survey yielded 1181 responses. Participants who only completed the demographic multiple-choice questions or who only answered the first of the open-ended questions were excluded from further analysis. A total of 875 responses were included in the final analysis.

Over half of the respondents were in the age cohorts of 35-44 (N = 205; 23.4%) and 45-54 (N = 251; 28.7%). Approximately half of the respondents (N = 465; 50.4%) were recreational riders not engaged in official competitions, while a further 21.6% (N = 199) participated only at a basic amateur level. A total of 21.6% (N = 200) of respondents listed equine industry-related professions and of these, 67.6% (N = 135) work in the primary sector (e.g. riding school, boarding stable, breeding, training stable, or horse trade). A full overview of demographics can be found in **Table 1**.

3.1. Thematic Analysis

In the course of the thematic analysis, 53 unique codes were identified, which were organized into a hierarchical structure consisting of 3 higher-order themes, 11 lower-order themes, and 39 descriptive categories. The primary themes were: Equine Husbandry, Human-Horse Interaction, and Equitation. Detailed definitions of the lower-order themes and descriptive categories can be found in **Tables 2a**, **b**, and **c**.

4. Discussion

The current study aimed to investigate the perspectives of Dutch equine enthusiasts on which aspects contribute toward equine welfare. The following discussion focuses on the most salient aspects and what they might mean to the practical application and interpretation of equine welfare in the Netherlands.

Table 1: Demographic characteristics of respondents.

Demographic Variable	Category	N	Percentage (%)
Gender	Male	106	12.1
	Non-binary	6	0.7
	Female	752	85.9
	Missing	11	1.3
Age	18-24	81	9.3
	25-34	132	15.1
	35-44	205	23.4
	45-54	251	28.7
	55-64	150	17.1
	65-74	51	5.8
	75 and older	4	0.5
	Missing	1	0.1
Type of Involvement with Horses	Owner/employee of horse-related products	4	0.4
	Owner/employee of horse-related services	61	6.6
	Owner/employee of horse-related businesses	135	14.6
	Recreational rider with one or more horses	465	50.4
	Competitive rider; amateur level	199	21.6
	Competitive rider at national or international level	12	1.3
	No own horse, ride at a riding school	46	5.0
	Missing	13	1.5
Primary Activity	Driving	137	15.7
	Riding	725	82.9
	Missing	13	1.5

4.1. Higher-Order Theme Equine Husbandry

Equine Husbandry emerged as one of the three central themes in this survey, incorporating four lower-order themes: Ability to perform natural behavior, Equine care, Feeding, and Stabling. Responses pertaining to Equine Husbandry were more prevalent than those concerning the other two main themes within the survey. This could signify that horse enthusiasts consider husbandry practices as more important for equine welfare than aspects of training or interaction with the horse. Such a perspective is supported by research suggesting that the welfare of recreational and sport horses is primarily influenced by husbandry and management practices [17,43,44]. Research by Furtado *et al.* [45] also showed that horse enthusiasts' perceived improvements in training practices yielded fewer welfare benefits compared to enhancements in overall management. Interestingly, equestrian experts involved at the highest level of the sport were reported as considering stable and environment management as less important compared to aspects relating to training and competition [33], highlighting the potential dichotomy in viewpoints between different types of stakeholders in the equine industry. For a balanced understanding of equine welfare, it is therefore essential to acknowledge that along with husbandry, appropriate human-horse interactions and training practices are equally critical, as corroborated by various studies highlighting their significant impact on the well-being of horses [46–49].

The lower-order theme Ability to perform natural behavior was mentioned by the majority of respondents, mirroring other studies that showed respondents' propensity to emphasize the importance for horses to engage in natural behaviors [50–53]. Bornmann *et al.* [54] were able to demonstrate horse owners' belief that access to turnout and social contact make horses happier. Interestingly, while respondents in the current study considered Free movement to be important, their opinions varied regarding what constitutes sufficient free movement for horses. Many respondents advocated for 24/7 free movement, yet others suggested durations ranging from 2 to 12 hours per day. These differing opinions on the specific duration of free movement indicate that even though horse enthusiasts seem to understand the importance of allowing the horse to perform innate behaviors, their interpretation of the practical implications differs considerably. This could be because horse owners might be motivated by other factors, such as fear of their horses getting injured [55], or spatial restrictions of the accommodation they keep their horses in [56]. These findings highlight the need for communication and educational strategies that focus on helping horse owners make the most appropriate decisions that maximize equine welfare within the context of their particular situation.

Table 2a: Detailed description of higher-order theme Equine Husbandry, descriptive categories, and definitions of participants' perceptions of important welfare components, including the percentage of respondents who mentioned the theme.

Higher-order theme: Equine Husbandry (96%)		
Lower-order theme	Descriptive Category (% of mentions)	Definition
Ability to Perform Natural Behavior (91%)	Free Movement (88%)	Frequency and duration of the horse's ability to move freely within its environment.
	Social Contact (55%)	Opportunities for the horse to interact with conspecifics.
	Protection Against Weather (19%)	Measures to protect horses from climate and weather conditions, such as rain or sun, e.g., presence of a shelter or trees.
	Physical Space (7%)	Aspects of the environment in which a horse is kept, allowing the horse to display natural behaviors, e.g., space to roll or lie down.
	Enrichment (5%)	Activities or objects that provide physical and mental stimulation to the horse, e.g., offering branches or shrubs to browse.
Feeding (72%)	Forage (54%)	Feeding of hay, haylage, or silage.
	Water (11%)	Presence of sufficient and clean drinking water.
	Concentrates and supplements (5%)	Feeding of concentrated feed such as pellets or muesli, as well as additional nutritional supplements.
Stabling (39%)	Stable Size (27%)	The size and space of the stabling available for the horse.
	Stable Climate (22%)	Conditions within the stable, such as ventilation, daylight, cleanliness, and temperature.
	Bedding (3%)	Material used on the floor of the stable, such as straw or sawdust.
Equine Care (39%)	Veterinary Care and Health (25%)	Medical care and treatments given to the horse, and the importance of knowledge of health aspects.
	Grooming (16%)	Basic skills of coat care, getting the horse ready, or not further specified.
	Hoof Care (7%)	Maintenance and care for the horse's hooves.

Many respondents also indicated that horses should have access to free movement year-round, including during winter months. Previous research has shown that horses in the Netherlands have less access to free movement during the winter [57]. Unsurprisingly, therefore, the use of paddocks during winter months, especially when pastures are too wet, was a frequent suggestion by respondents. Respondents also highlighted the importance of dry patches for horses to stand on, especially when conditions are wet. Respondents also emphasized the need for ample Physical space in paddocks or pastures that provide sufficient room for natural behaviors like galloping and rolling. These suggestions demonstrate a solid understanding of participants of horses' intrinsic needs, as research suggests that horses housed in larger paddocks display increased movement and foraging activity, and less time passively standing [58]. Such a behavioral pattern is more congruent with their natural behavior, underscoring the significance of providing adequate space for horses [56]. Protection against the weather was also frequently mentioned, with (artificial) shelters most often suggested as a means of protection.

More than half of respondents highlighted the need for Social contact with other horses. The majority advocated for direct physical contact with conspecifics, but the number of recommended social companions varied, ranging from just one to at least six. This emphasis is supported by research that indicates that social contact is crucial for equine welfare [59,60]. Social isolation is also mentioned as a primary welfare concern to horse enthusiasts in other studies [30,31,51]. If direct contact is not feasible, some respondents indicated that indirect contact—such as through bars or across fences— might also be considered acceptable. Again, these findings seem to indicate that equine enthusiasts tend to consider welfare as part of the environmental context. In the Netherlands, where geographical restrictions can be a challenge, equine enthusiasts will likely look for appropriate alternatives that promote equine welfare as much as possible [13,20].

Enrichment was occasionally mentioned, often in the context of feeding, such as providing horses with slow feeders or branches and shrubs to browse. Some respondents also linked enrichment to periods of stabling and suggested the use of straws to counteract boredom. The provision of enriched feed, incorporating a variety of forages, as well as other types of edible and non-edible enrichments has been observed to engage horses in more foraging behavior and decrease stereotypical behaviors compared to horses fed solely on hay [61–63]. Moreover, such enrichment strategies have been associated with decreased cortisol levels and neophobia, and an increase in the time horses spend lying down [64]. The relative lack of emphasis on enrichment by respondents highlights a potentially overlooked opportunity for advancing equine welfare. These findings seem to mirror some of the issues encountered in the zoo and aquarium sector, where the need for evidence-based environmental enrichment has been widely recognized [65,66], but where practical implementation continuous to be a challenge [67].

Table 2b: Detailed description of Higher-order theme Human-Horse Interaction, descriptive categories, and definitions of participants' perceptions of important welfare components, including the percentage of respondents who mentioned the theme.

Higher-order theme: Human-Horse Interaction (90%)		
Lower-order theme	Descriptive Category	Definition
Knowledge of Behavior (60%)	Human-Horse Communication (43%)	The ability to understand how and why horses respond to different stimuli or situations. Includes the importance of recognition of (body) language and communication between horses and humans.
	Natural Behavior (20%)	Insight and understanding of the natural behavior, the ability to recognize and interpret the instinctive behavior of horses.
	Recognizing Abnormalities (17%)	The ability to identify specific physical or behavioral indicators in a horse that point to well-being or health problems.
Norms and Values (47%)	Respect (36%)	Recognition and treatment of horses as sensitive and intelligent individuals.
	Patience (10%)	The willingness to take time and not to rush, especially when the horse is learning something new.
	Trust (6%)	A mutual relationship of reliability between humans and horses.
Interaction Styles (45%)	Gentleness (24%)	Being soft in both hand and voice, with the overarching sentiment of being clear without being strict. Causing pain or using violence is considered unacceptable.
	Calmness (8%)	Remaining calm, particularly in potentially dangerous situations or whenever the horse does not follow commands. The ability to instill calmness in oneself and the horse.
	Sense of Responsibility (5%)	Awareness of the duty of care, including financial aspects, one has toward the well-being and health of the horse.
Safety (24%)	Safety in Off-site Environments (13%)	Actions and behaviors to ensure safe public interactions, like obeying traffic rules and anticipating other road users' behavior.
	Safety of the Horse (8%)	Measures to ensure the horse's physical well-being, such as proper tack and safe environments.
	Safety of the Human (4%)	Measures and behaviors aimed at protecting people who interact directly with horses, like using safety gear and following safe riding and handling protocols.

The lower-order theme of Feeding was another prevalent topic, mentioned by 72% of respondents. Quality of feed was a common concern, with a dominant viewpoint advocating for unlimited access to roughage, considered vital for both health and behavioral well-being. If unlimited access to roughage was not feasible, respondents highlighted the importance of providing ample amounts of roughage as an alternative. When unlimited access was not specifically mentioned, respondents generally suggested multiple feeding sessions throughout the day—typically recommending 3-4 times daily or ensuring a maximum interval of 2-6 hours between feeds. Many showed a preference for hay over other types of roughage like silage or haylage. Concentrate feeds were mentioned by a small percentage of respondents, and when it was mentioned, it was often specified that these were not necessary and could even be detrimental to equine health and well-being. Respondents' focus on these aspects not only points toward an awareness of the close link between diet and equine health, and tallies with scientific insights on the importance of roughage for welfare in horses **[68–70]**.

In the lower-order theme of Stabling, stable size was most frequently mentioned by respondents. Some respondents commented that the stable should have specific dimensions such as 3 by 3 meters, but other respondents mentioned that it should be large enough to enable the horse to comfortably turn around and lie down within their stables. The height of the stable was occasionally brought up as a point of concern. An increase in the duration that horses spend lying down has been correlated with larger stable sizes, indicating that more spacious stables may enhance welfare **[56,71]**. Despite this, the literature on optimal stable dimensions is sparse and some researchers suggest that confinement to a stable is invariably detrimental to equine welfare, irrespective of its dimensions **[46,72]**. Given the high percentage of respondents who highlight the importance of stable size, this suggests a significant concern for equine welfare and points toward a need for more comprehensive research to establish welfare-friendly stabling standards.

Veterinary care and health took precedence in the lower-order theme of Equine care, as over half of the responses within this theme centered around this topic. General knowledge relating to equine health and the most common diseases were most frequently mentioned. Respondents emphasized the importance of understanding when to consult a veterinarian and how to manage minor wounds or sore spots themselves. They also expressed a desire to gain more knowledge about first aid and preventative equine health measures for their horses. Grooming a horse also featured prominently in this subtheme. Knowledge of basic grooming techniques was considered essential for daily horse care and well-being. Respondents stressed the importance of being knowledgeable in saddling a horse properly, underlining the connection between proper equipment usage and both rider safety and equine comfort, mirroring findings within the literature **[47,73,74]**.

Table 2c: Detailed description of higher-order theme Equitation, descriptive categories, and definitions of participants' perceptions of important welfare components, including the percentage of respondents who mentioned the theme.

Higher-order theme: Equitation (80%)		
Lower-order theme	Descriptive Category	Definition
Training of Rider or Driver (53%)	Lessons and Formals Instruction (38%)	Formal training sessions led by an instructor.
	Posture, Seat, Balance (14%)	The rider's physical positioning and how it affects the horse.
	Use of Cues (9%)	How a rider uses reins, hands, and legs to communicate with the horse.
	Rider/Driver Fitness or Weight (3%)	The importance of the rider's or driver's physical condition.
Training of the Horse (45%)	Groundwork (16%)	Training techniques executed from the ground to promote mutual respect and trust between horse and human, including (Natural) Horsemanship.
	Anatomy and Biomechanics (16%)	Understanding the horse's movement, informed by anatomy and biomechanics.
	General (11%)	Miscellaneous comments on the training of the horse, e.g. the importance of a proper warm-up.
	Equine Learning Theory (9%)	Understanding the learning processes and mechanisms in horses.
	Equine Fitness (8%)	Knowledge of the physical demands placed on a horse during training and how to prevent overworking.
	Training of Young Horses (4%)	The initial phase where a young horse is taught basic skills and behaviors.
Tack and Equipment (28%)	Knowledge of Tack (20%)	Understanding the different types of equipment and their proper use.
	Use of Training Aids (11%)	Using equipment like saddles, auxiliary reins, or bits during training.

In summary, the current study's findings on themes relating to Equine Husbandry underline how much horse enthusiasts value good management practices. However, these findings warrant a nuanced interpretation. For example, Luke *et al.* [75] highlight that concentrating solely on certain aspects can lead to a 'welfare blind spot,' overlooking other important aspects such as the horse's mental and emotional states. In the context of this study, it may be valuable to investigate further whether the high emphasis on the ability to perform natural behavior and feeding is symptomatic of a similar welfare blind spot among respondents, which might cause participants to ignore, or pay less attention to, other aspects that could influence equine welfare. These might include aspects relating to riding, training, or general interactions.

4.2. Higher-Order Theme Human-Horse Interaction

Human-horse interaction emerged as the second higher-order theme, with Knowledge of behavior most frequently mentioned by the respondents. This focus on horse behavior aligns with the literature that stresses the importance of understanding horse behavior to enhance their welfare, as well as human safety around equids [46,47,76]. Respondents emphasized the importance of 'speaking the horse's language' (Human-horse communication) which entailed accurately reading their mood and responding appropriately. The significance of being able to interpret the horse's signals correctly was often cited, emphasizing that effective communication between horse and human is essential to respondents.

Respondents also highlighted the critical role that horse owners play in correctly interpreting signs of discomfort, illness, stress, or pain in their horses. The ability to Recognize abnormal behaviors and correctly interpret them was stressed as crucial for timely intervention and the maintenance of equine well-being. This emphasis echoes current research on this topic, which identifies misinterpretation of horse behavior as a significant welfare issue [47,49,77]. Behaviors that are considered problematic by owners, such as aggression, are often dismissed or misattributed to the horse's character [75,78,79]. The frequent mentions in the survey that a horse's reactions are not designed to frustrate humans, or that a horse who misbehaves is not 'naughty,' shows that at least some horse enthusiasts are aware of these misconceptions.

However, the high incidence of hyperreactive behaviors observed in ridden horses, as reported by Luke *et al.* [47], suggests that awareness alone might not be sufficient. A problem with recognizing abnormal behavior is that overexposure could desensitize horse owners, making it more difficult to identify, and act upon, signs of poor well-being [80–83]. Many horse owners reportedly struggle to detect signs of stress [43,84] and pain, such as back soreness or facial expressions [73,85,86]. The emphasis by respondents on the importance of recognizing pain and stress signals in horses underlines their awareness of the crucial role that horse owners play in these aspects of equine welfare.

Abstract values such as respect, patience, and trust were also mentioned relatively frequently and formed the lower-order theme of Norms and values. The fact that a horse is a sentient being with feelings and cognition was mentioned often, as well as that each horse is a unique individual with a distinct character, deserving of understanding and respect. Another key point was the necessity to know and respect a horse's boundaries. These comments may indicate that instead of a utilitarian ethical framework, horse enthusiasts think of the

horse-human relationship as a symbiotic one, facilitating mutual friendship and emotional bonds [28]. Additionally, respondents urged against anthropomorphizing horses, stressing that horses have different needs and perceptions compared to humans. McGreevy *et al.* [31] elaborate on the dangers of anthropomorphism, describing it as "unhelpful at best and may promote poor welfare at worst," especially when it inaccurately ascribes human-like motives to horse behavior. However, anthropomorphism can also help humans to develop empathy and insight into their horse's experiences [7]. Given the evident significance of this issue among Dutch horse enthusiasts, further research on this topic could provide valuable insights into whether such anthropomorphic perspectives ultimately contribute to or detract from horse welfare.

Patience was also considered an important aspect of human-horse interactions. Those discussing this topic emphasized not only the unfairness of rushing a horse through the learning process but also the need for humans to avoid feelings of frustration or anger during interactions. They highlighted that horses need time to acquire new skills or behaviors and that a patient approach is essential for a successful relationship. These considerations are in line with the theoretical underpinnings of the revised 5 domains model by Mellor *et al.* [49]. Domain 4, "Behavioral Interactions", in particular, stresses the impact of human behavior on an animal's affective experiences.

Related to such concepts was the lower-order theme of Interaction styles, including the categories of Cooperation, Gentleness, and Calmness. Respondents focused on the notion of teamwork and the importance of training the horse in a harmonious manner. This included aspects like being a leader and being able to assist the horse in stressful or difficult situations. In addition, respondents emphasized that cooperation with the horse should not equate to domination or forced submission. Rather, respondents advocated for asking or motivating the horse to cooperate rather than to force them into compliance. Participants also stressed the importance of being soft in both hand and voice. The overarching sentiment was that one can be clear without being strict and that causing pain or using violence is unacceptable. The fact that respondents valued calmness and gentleness when interacting with horses is encouraging given that they form the basis of an equitable human-horse partnership [27,49,87] and the lack of these qualities can have a detrimental impact on equine welfare [46,48,49].

The lower-order theme of Safety emerged as a diverse but often mentioned subtheme, encapsulating a range of concerns affecting the safety of both horses and humans. With regards to the Safety of the horse, respondents stressed the importance of a secure environment—free from sharp edges in the stable, a safe terrain, and (daily) supervision to promptly identify injuries or illness. Safety of the human centered around the use of secure tack and proper equipment, such as appropriate footwear and headgear during riding or training sessions. Knowledge of safe handling practices, such as not walking behind a horse unexpectedly, was underscored.

Where participants mentioned aspects relating to Safety in an Off-site environment, the emphasis was on knowledge of traffic rules and best practices for handling horses in stressful and potentially dangerous situations. Respondents also noted that riders or drivers on public roads should be aware of the general lack of equine awareness among other road users. While road safety is undeniably critical (see e.g. [88]), who states that over 60% of UK horse riders report having experienced a road-related near-miss or accident), its relative impact on equine welfare due to physical injury or psychological effects remains to be fully explored. However, current results show that safety for horses and humans is important to almost a quarter of respondents for safeguarding equine welfare.

In essence, according to survey respondents, the theme of Human-Horse Interaction is essential in understanding equine welfare. More than half of respondents mentioned the importance of understanding horse behavior, which seems to indicate that horse enthusiasts are motivated to understand how their horses think and feel, arguably to enhance the well-being of the horse as well as to promote human safety [27,89,90]. Additionally, ethical considerations such as respect, patience, and trust underscore the evolving understanding of horses as sentient beings deserving of ethical treatment, an aspect that [28].

4.3. Higher-Order Theme Equitation

The third higher-order theme Equitation encompasses the practice of riding or driving horses. In the lower-order theme of Training of Rider or Driver, lessons and formal instruction were frequently mentioned, with many respondents suggesting that the role of an instructor is to not only focus on the technicalities of riding or driving but also on establishing harmony and teamwork between horse and rider, a sentiment that has been widely acknowledged within the field of equitation science [80,91,92]. According to participants, the type of topics to be covered by educational activities include equine care, management, husbandry, and welfare. This suggests that instructors play a pivotal role in enhancing animal welfare, as it shows respondents are open to receiving instruction on a host of subjects, encompassing a holistic approach to the care of horses.

The Use of cues emerged as another important topic in this theme, including the use of hand and leg aids, with the emphasis on maintaining a quiet hand and leg. Participants often mentioned that the goal is to establish gentle contact with the horse's mouth. A clear consensus emerged that being too forceful could lead to discomfort or pain for the horse. Respondents also highlighted the necessity of Correct posture, and a quiet and balanced seat to facilitate better communication and avoid hindering the horse. It is encouraging that horse enthusiasts seem to appreciate the importance of using correct and unequivocable signaling. However, studies indicate a high incidence of oral injuries in horses due to the harsh use of bits and hand cues (e.g. [93]) as well as increased levels of resistance to acceleration and deceleration cues in horses with multiple riders [94] suggesting a gap between ideal practice and actual execution. Interestingly, this implicit need for better education is mirrored in participants' responses on the

importance of formalized instruction, suggesting that Dutch equine enthusiasts are highly motivated to learn.

Only a small percentage of respondents discussed Rider's or driver's fitness and weight, which contrasts with a study by Challinor *et al.* [95] in the UK, where a majority of participants recognized the potential adverse effects of increased rider weight. Research suggests that rider weight is a significant welfare issue, as it can induce temporary lameness and behaviors consistent with musculoskeletal pain in horses [96]. This discrepancy suggests a need for increased awareness of the potential impact of rider weight on equine health and welfare, which should also be incorporated into educational activities.

On the lower-order theme of training the horse, respondents commented on various aspects of training, such as the importance of a structured regimen that includes proper warming up and cooling down, the value of introducing variation in exercises to keep the horse engaged, and methods to encourage the horse to use its body effectively. Good training practices were acknowledged as crucial not just for performance but also for the horse's overall well-being, which implies that respondents are aware that poor training techniques can lead to stress responses in horses [48]. Knowledge of Anatomy and biomechanics was considered vital for good training practices according to some respondents, as well as for identifying health-related issues. Other important aspects were knowledge and proper application of Groundwork techniques and knowledge of Equine learning theory. McLean and Christensen [48] suggest that "the correct use of learning theory should be established as a 'first principle' in equestrian coaching," but other researchers have established that knowledge of learning theory does not necessarily equate to better welfare for the horse [75]. While it is positive that respondents appreciate the role of learning theories in horse training, the practical application thereof needs to be integrated into more formalized teaching and education for equestrians [27,90,91,97,98].

Lastly, respondents touched upon the intensity of training and how to gauge and build up Equine fitness. This was particularly highlighted in the context of driving, as several respondents raised the issue of the weight of the carriage potentially being too much for some horses. Respondents also noted that the age of the horse should be considered when determining the appropriate level of physical strain. The attention given to a horse's fitness and age indicates that respondents are mindful of the physiological limitations of horses, and aligns with what researchers found in other studies [52,99].

The responses on the aspect of Tack and equipment were rich in content and diversity. The importance of having a comprehensive understanding and Knowledge of tack, the different types, and how to use them safely to avoid causing pain or injury to the horse was highlighted. Respondents emphasized the importance of properly fitting tack, aligning with other studies identifying this as a critical welfare issue [31,51,79]. The use of training aids, such as whips, spurs, and auxiliary reins, was mentioned relatively often, but elicited differing opinions. While some emphasized the importance of employing them correctly, a significant portion of respondents argued against their use altogether. Interestingly, there was notable sentiment against the use of bits, possibly suggesting an emerging trend or preference among horse enthusiasts [75,100]. The diverging opinions on the use of training aids point toward a growing ethical debate within the equine community, mirror findings by DuBois *et al.* [30], who found no clear opinion on whether artificial aid use is welfare-compromising or not. However, the outspoken critical stance concerning the use of auxiliary reins by some respondents resonates with increasing concerns about their use in sports and leisure activities [29,45,46,74,101].

To summarize, the focus of the higher-order theme Equitation highlighted three lower-order themes—Training of the Rider or Driver, Training of the Horse, and Tack and Equipment. Respondents emphasized the importance of qualified instructors who are able to convey not only the technical aspects of riding but are able to incorporate other aspects of equine welfare into their teachings. Other aspects raised revolved around the proper use of cues, and diverging opinions on training aids, which underlined the obvious gap between theoretical knowledge and practical application. These findings underscore the need for ongoing education on these subjects.

4.4. Limitations

The sampling methods used in this study could have introduced several biases that should be considered when interpreting the results. The online format of the survey may inadvertently have excluded individuals with limited literacy skills or those who lack access to digital resources [102]. Reliance on a web-based sampling method could also lead to an over-representation of more privileged social groups [103]. The majority of respondents were female. While this is consistent with previous surveys on attitudes toward equine welfare [32,52,53,74], it may nevertheless skew the results of the survey. Females may have a heightened interest in animal welfare, making them more likely to participate in such surveys [104]. Further research could help to determine the specific reasons for the female predominance in these surveys, as understanding the underlying factors could enhance the design and interpretation of future studies on equine welfare.

Additionally, as the survey was voluntary, it likely attracted participants who already have an interest in equine welfare, potentially over- or underestimating certain opinions, similar to the study by Visser and Van Wijk-Jansen [53] on equine welfare in the Netherlands. However, while the sample may be skewed toward certain demographics or opinions, the data still provides valuable information for understanding key aspects of the subject.

Interpretation bias is an inherent risk in all qualitative research due to the significant role played by the researcher in data analysis [105]. We have sought to minimize this effect by adhering to recommended guidelines for qualitative reviews [106], which included coding the data as one cohesive dataset (instead of summarizing responses to each question separately) and employing peer-reviewed coding.

Open-ended questions pose the issue of potentially giving more prominence to respondents who provide more extensive answers. Andrews [107] found that male respondents and respondents over the age of 50 provided shorter answers in open question-surveys than female and young participants. In the qualitative part of the study, this

was not a significant concern as the analysis was theme-based rather than volume-based.

The current study used common scenarios to elucidate participants' thoughts and ideas. While every effort was made to devise scenarios that reflected common equestrian practices, it is possible that the way scenarios were phrased biased respondents. What is more, the order in which the scenarios were presented may have caused participants to pay comparatively more attention to the first question, compared to subsequent questions. This would be in line with the primacy effect, which shows that proportionally more attention is paid to information presented first [108]. However, seeing that the survey relied on routing to encourage ease of completion, no alternative way of presenting the scenarios could have been devised. Lastly, participants are likely to have varying levels of expertise and experience when it comes to dealing with horses, leading to potential variations in data quality and consistency of the statements. However, seeing that the study was aimed at gaining a comprehensive insight into which aspects a representative sample of Dutch equine enthusiasts considers important to ensuring equine welfare, such divergence in expertise may also be considered an essential aspect of the study.

5. Conclusion

The current study provides a detailed exploration into the perspectives of the Dutch equestrian community on what is important to safeguard equine welfare. Thematic analysis indicates a strong awareness among Dutch horse enthusiasts of the multifaceted nature of equine welfare, which encompasses a broad spectrum from husbandry practices to the nuances of human-horse interactions and equitation. Although this survey was not meant to test participants' knowledge of equine welfare, the frequent mention of key aspects suggests a significant level of awareness on this subject among Dutch equestrians. On the other hand, the persistence of welfare issues implies a discrepancy between the theoretical importance of these themes and their practical application, presenting an opportunity for future research to bridge this divide.

The insights gathered in this study can be used to inform the educational practices that are culturally relevant to the Dutch equestrian community. Such initiatives should strive to transform knowledge into action, ensuring that the principles of equine welfare are integrated into the daily lives of horses and horse owners. The study confirms a genuine concern for equine welfare in the Dutch horse-owning community, and the next step should be to bring this concern and knowledge into practice to safeguard equine welfare in the Netherlands.

Supplementary Materials

Supplementary Material 1: Original Survey Questions in Dutch; **Supplementary Material 2:** Survey Questions Translated into English.

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Ethical Approval

The survey was conducted according to the Netherlands Code of Conduct for Research Integrity and followed the guidelines of the Declaration of Helsinki.

Authors' Contributions

Conceptualization, I.W.; Methodology, I.W.; Formal analysis, F.L.B. and I.W; Writing—original draft preparation, F.L.B.; Writing—review and editing, I.W. and Y.E.; Supervision, I.W. and Y.E.; All authors have read and agreed to the published version of the manuscript.

Data Availability

Data storage was conducted according to the Research Data Management policy framework of the University of Applied Sciences Van Hall Larenstein. Data management will adhere to the principles of Open Science and data is accessible on request.

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Conflicts of Interest

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Original Article Open Access

Postbiotic Activity of *Enterococcus asini* EAs 1/11D27 Strain Originating from the Norik of Muráň Breed

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Abstract

The Norik of Muráň breed is a Slovak horse breed mainly intended for forestry and agriculture-related work. It is also a unique type among the cold-blooded horse breeds. In general, the microbiota influences animal health status. However, limited information regarding the microbiota of this horse species is available. Similarly, few aspects are known about the species *Enterococcus asini* bacteriocin (postbiotic) potential. Therefore, this study investigated the *E. asini* strain EAs 1/11D27, isolated from mucosal samples obtained from horses, along with the evaluation of its molecular characteristics and bacteriocin (postbiotic) activity. Taxonomic allocation of the strain EAs 1/11/D27 was confirmed using the sequencing method, reaching 99.86% similarity (match) with the nucleotide sequence of the strain *E. asini* NR113929.1. *E. asini* EAs 1/11D27 has been assigned the GenBank accession number (AN) MN822908. This strain is hemolysis-negative, deoxyribonuclease-negative, and gelatinase-negative. EAs 1/11D27 lacks genes encoding for virulence factors such as gelatinase, enterococcal surface protein, adhesins, hyaluronidase, and aggregation substance. It does not produce damaging enzymes and is susceptible to antibiotics. Additionally, it produces bacteriocin-like substances with inhibitory activity against 165 out of 170 indicator bacteria tested (97%). The highest inhibitory potential was recorded against staphylococci (88), enterococci (57), lactococci (7), and streptococci (4/8). The growth of 9 out of 10 Gram-negative strains was also inhibited. These results suggest a valuable postbiotic potential of the studied bacteriocin substance, and further studies are needed to establish its applications in horses.

Keywords

Equine; Norik of Muráň breed; postbiotic; antimicrobial effect; *Enterococcus asini*

1. Introduction

The Norik of Muráň breed is a unique cold-blood horse that resulted from original cold-blooded mares of different genetic backgrounds that were bred by Norik stud horses in Slovakia [1]. This breed is primarily used for work in forestry and agriculture due to its good temperament and excellent constitution. Additionally, it is frequently utilized for hippotherapy and recreational riding [2]. The breeding locations for this breed are Veľká Lúka and Dobšiná in the National Park Muráň Plain, located in the central Slovakia region encompassing the districts of Brezno, Revúca, and Rimavská Sobota. The National Park Muráň Plain is a part of the Spiš-Gemer Karst within the Slovenské rudohorie (Slovak Ore Mountains).

Previous studies have indicated the influence of microbiota on the overall health status, particularly in horses, where the

intestinal microbiota significantly impacts equine health and performance [3]. Along with traditionally confirmed common bacteria, gut microbiota dysbiosis is increasingly associated with diseases [4]. Horses are susceptible to various disorders, many of which have a bacterial etiology and manifest as gastrointestinal clinical signs [5]. To optimize the microbial status and preserve normal microbiota in horses, the use of beneficial bacteria-probiotics and their antimicrobial substances-bacteriocins-postbiotics could be an important approach [6,7]. Lactic acid bacteria (LAB) are among the frequently used beneficial bacteria [8]. Enterococci have been allotted as a part of LAB. Nowadays, the genus *Enterococcus* comprises approximately 61 validated species. Individual strain species as representatives of the genus *Enterococcus* have been found to have beneficial/probiotic properties [9] and the ability to produce bacteriocins-enterocins [10–12]. Bacteriocins-enterocins are substances of proteinaceous character produced by mostly enterococcal species with antimicrobial effects against more or less related bacterial species [9]. Production of enterocins by horses strains has been already reported in our previous studies [13,14]. These strains produce enterocins which have demonstrated beneficial effects under *in vivo* conditions in horses [13,14]. They have shown a reduction in Gram-negative bacteria and an increase in non-specific immunity parameters, specifically phagocytic activity [13,14]. These bacteriocin substances align with the concept of postbiotics [7], which refers to non-viable bacterial products produced by individual bacterial strain species that have biological activity in the host [8].

Among enterococci, *E. faecium* strains have been identified as the most frequently enterocin producers [9–11]. However, strains of *E. mundtii* isolated from Norik of Muráň horses [12], as well as strains from the skin mucosa, such as *E. moraviensis* [15], have also been found to produce enterocins. The species strain *E. asini* was first isolated and described from caecal samples obtained from donkeys (*Equus asinus*) [16]. However, the bacteriocin potential of this species has not been previously reported. To the best of our knowledge, this is the first report describing the postbiotic potential based on the inhibitory activity of the species strain *E. asini* EAs 1/11D27, which was isolated from the inner mucosa of the auricle in the Slovak horse breed Norik of Muráň.

2. Materials and Methods

2.1. Strain Isolation

A mucosal swab was taken from the inner part of the auricle of a Norik mare of the Muráň breed and placed in Amies agar gel without charcoal (Copan, Italy) [15]. The swab was then placed in one ml of isotonic saline solution with a pH of 7.0 and processed using the standard microbiological method (ISO, 1:9). An aliquot of 100 µl from the appropriate dilutions was spread onto De Man-Rogosa-Sharpe agar (MRS, pH 6.4; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and cultivated under partially anaerobic conditions (Gas Pak Plus, BBL, Microbiology Systems, Cockeysville, USA) at 37 °C for 48 hours, as previously described by Lauková *et al.* [15]. Morphologically distinct colonies were selected from the individual dilutions under the same growth conditions to obtain a pure culture. Each picked colony was checked using Gram-positive staining. For further testing, the isolate was stored at -70 °C using the Microbank™ system (Pro-Lab Diagnostics, Ontario, Canada) [15].

2.2. Strain Identification Process: DNA Extraction, PCR Amplification, and Sequence Analysis

The genomic DNA was extracted from a pure colony using DNAzol direct (Molecular Research Center Inc., Cincinnati, USA) according to the manufacturer's instruction, as previously reported by Focková *et al.* [12]. The 16S ribosomal RNA (rRNA) gene from the isolate was amplified by PCR using the universal primers: Bac27F(5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGYTACCTTGTTACGACTT-3 (Merck-Sigma Aldrich, Darmstadt, Germany). The PCR reaction was performed in a 50 µl PCR mixture containing 2 µl of DNA shield, 46 µl of a reaction mixture comprising One Taq 2x Master Mix with Standard Buffer (New England Biolabs, United Kingdom) diluted with water for molecular biology (PanReac AppliChem, Darmstadt, Germany) to 1x concentration, and 1 µl of each primer (concentration 33 µm). The following PCR protocol conditions (thermocycler- TProfesional Basic, Biometra GmbH, Göttingen, Germany) were as follows: 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and primer extension at 72 °C for 3 min, and finally at 72 °C for 10 min as previously reported by Focková *et al.* [12]. The aliquot PCR product was separated by horizontal 3% (w/v) agarose gel electrophoresis in Tris-acetate-EDTA buffer (pH 7.8) and visualized with GelRed (Biotium, Inc., Hayward, CA, USA). The amplified product was sent (in a low bind tube at a minimal volume of 15 µl) for purification and sequencing in both directions using 1492R and Bac27F primer (Microsynth, Wien, Austria). The obtained 16S rRNA sequence was validated and assembled using Geneious 8.0.5 (Biomatters, Auckland, New Zealand) and subjected to BLASTn analysis (https://BLAST.ncbi.nlm.nih.gov/BLAST.cgi).

2.3. Additional Phenotypic Test (Gram-Positive BBL Crystal)

Additional tests were performed using the Gram-positive ID system (BBL Crystal GP, Becton and Dickinson, Sparks, Maryland, USA). This panel includes 29 enzymatic and biochemical parameters, following the reference strain *E. asini* DSM 11492^T [16]. Among these tests, the preferred ones were carbohydrates fermentation tests, such as arabinose, xylose, lactose, sucrose, mannitol, ribose, as well as the enzymes β-glucuronidase and alkaline phosphatase. The strain was cultivated on M-Enterococcus agar at 37 °C for 48 hours. The picked-up colony was suspended in a labeled tube of inoculum fluid to a turbidity equivalent to a 0.5 McFarland standard. The tube was vortexed for 15 seconds, and the entire content was poured into an appropriately labeled panel base. The inoculum was gently rolled along the tracks of the base to fill the wells. A lid was aligned over the base and snapped into place. The panel was placed in an incubation tray at 37 °C for 24 hours. The results were visually read using the BBL Crystal Panel Viewer. A 10-digit profile number was generated and recorded on a pad listing the results. The profile number and Gram stain reaction were entered into a computer with the BBL Crystal ID System Electronic Codebook installed. The computer program generates a single genus and species identification or several differentiated identifications. The identification of the tested strain was derived from a comparative analysis of the reaction patterns of the tested strain with the reference strain in the database.

2.4. Bacteriocin Activity Testing (Postbiotic Potential)

The inhibitory activity of antimicrobial substance produced by the strain *E. asini* EAs 1/11D27 was checked using the quantitative agar spot method [17]. The 18-hour culture (60 ml, A_{600}=0.797) of EAs 1/11D27 strain in Brain Heart Infusion (BHI broth, pH 7.0, Difco, USA) was centrifuged at 10.000 × g for 30 minutes at 4˚C. The pH was checked and adjusted to 4.5. Then, the cell-free supernatant was treated with EDTA/Chelaton III (Sigma, Germany) and heated at 80˚C for 10 minutes to eliminate the effects of other organic substances. The supernatant was concentrated using Concentrator Plus (Eppendorf, Hamburg, Germany) to obtain a concentrated substance in a final volume of 6 ml. The inhibitory activity (IA) was tested against indicator strains *Enterococcus avium* EA5 (from our laboratory, the principal and most sensitive indicator) and *Streptococcus equi* subsp. *zooepidemicus* CCM 7316 (kindly provided by Dr. Styková, UVMP in Košice) as well. The IA was expressed in arbitrary units per ml (AU/ml). It is defined as the highest dilution of the substance which inhibited the growth of the indicator strain. In addition to those two indicator bacteria, other indicator bacteria were used, including 13 faecal strains of *E. mundtii* (isolated from Norik of Muráň breed, [12]), 7 human strains of *Str. pneumoniae* (Spn58, Spn51, Spn49, Spn57, Spn922), and *Streptococcus pyogenes* (Sp117, Sp113) from *otitis media* [18], 21 human strains of *E. faecium* with high resistance to aminoglycoside antibiotics (kindly provided by Dr. Aleksandra Troscianczyk from the University in Lublin, Poland), 9 faecal poultry-originated strains of *E. faecium*, and 13 faecal *E. faecium* strains from wild-living animals (Dr. Troscianczyk). Lactococci (7 strains) isolated from raw goat milk were also included in the testing. Moreover, staphylococci were used as indicators, including 32 strains of *Staphylococcus pseudintermedius* from canine faeces, 13 *S. chromogenes* from cows, 15 strains of *S. felis* from cats, and *S. aureus* from pigs (28) (kindly provided by Dr. Troscianczyk). Furthermore, 10 faecal Gram-negative species strains isolated from horses and roe deer were used (isolated in our laboratory), such as *Acinetobacter johnsonii* K17/PL2, *Ac. lwofii* ACl K8/3, *Serratia liquefaciens* K2PL/1, *Citrobacter freundii* K10PL/2, *Pantoea agglomerans* PATK4/2, *Yersinia enterocolytica* 12/111/2, *Serratia fonticola* 11/91/1, *Escherichia coli* 12/111/1, *E. coli* 10/116/2, and *E. coli* 10/139/2. In total, 170 indicators were used, including 160 Gram-positive strains (57 enterococci, 7 lactococci, 8 streptococci, and 88 staphylococci), and 10 strains of various Gram-negative species.

2.5. Virulence Factor Checking: Hemolysis, Nuclease, Gelatinase Activity, Genes for Gelatinase, Enterococcal Surface Protein, Adhesins, Hyaluronidase, and Aggregation Substance

Hemolysis activity was analyzed on BH agar (Difco, USA) supplemented with 5% defibrinated sheep blood according to Semedo-Lemsaddek *et al.* [19]. The agar plate was incubated at 37 ºC for 48 h in an incubator. The presence/absence of a cleared zone around the colonies was interpreted as α/β-hemolysis; negative hemolysis is indicated as γ-hemolysis.

Deoxyribonuclease activity was evaluated as previously described by Lauková *et al.* [20]. The strain was inoculated onto the surface of DNase agar (Oxoid, USA) and incubated at 37 ºC for 24 hours. Colonies producing DNase hydrolyze the deoxyribonucleic acid (DNA) within the medium. After flooding and acidifying the medium with 1 N HCl (hydrochloric acid), the DNA precipitated, and the medium became turbid with cleared zones around DNase-positive colonies. *Staphylococcus pseudintermedius* SPs 948 served as a positive control (our strain from ruminant).

The gelatinase phenotype test was analyzed by streaking single colonies onto Todd-Hewitt agar (Difco, USA) supplemented with gelatin (Biomark, 30g/l) and incubating at 37 °C for 48 hours. After flooding the medium with 1.5% $HgCl_2$ in 2.0% HCl, the medium became turbid with cleared zones around gelatinase-positive colonies. *Staphylococcus aureus* ATCC 25923 served as the positive control.

Virulence factor genes tested were *gelE* (gelatinase), *agg* (aggregation substance), *EfaAfm* (adhesin *E. faecium), EfaAfs* (adhesin *E. faecalis)*, *esp* (enterococcal surface protein), and *hylEfm* (hyaluronidase). The PCR product was separated using agarose gel electrophoresis (1.2% w/v, Sigma-Aldrich, Saint Louis, USA) with 1 µl/ml content of ethidium bromide (Sigma-Aldrich) using 0.5 × TAE buffer (Merck, Darmstadt, Germany). The PCR fragment was visualized with UV light. The strain *E. faecalis* 9Tr1 (our strain from beaver) *E. faecium* P36 (Dr. Semedo-Lemsaddek, University of Lisbon, Portugal) were used as positive controls. The PCR was carried out in a 25 µl volume, with a mixture consisting of 1x reaction buffer, 0.2 mmol/L of deoxynucleoside triphosphate, 3 mmol $MgCl_2$, 1 µmol/l of each primer, 1 U of Taq DNA polymerase, and 1.5 µl of DNA template with the cycling conditions as previously reported by Kubašová *et al.* [21]. The PCR conditions for *gelE*, *agg*, *esp*, $EfaA_{fm,}$ and *EfaAfs* were as follows: denaturation at 95 °C for 3 minutes followed by 35 cycles for 30 seconds at 95 °C, 30 seconds at 55 °C, 30 seconds at 72 °C, and 5 minutes at 72 °C. The PCR condition for *hyl* gene was as follows: denaturation at 94 °C for 4 minutes, followed by 30 cycles for 30 seconds at 94 °C, 30 seconds at 50 °C, 30 seconds at 72 °C, and finally for 4 minutes at 72 °C.

2.6. Metabolic Enzyme Activity Testing and Antibiotic E-Test

For this test, the API-ZYM panel system (BioMerieux, Marcy l'Etoile, France) was applied according to the manufacturer's recommendations, as previously reported by Lauková *et al.* [22]. The following enzymes were tested using this panel: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. A volume of 65 µl of McFarland standard 1 inoculum was transferred into each well of the test panel plate. Incubation was performed at 37 °C for 4 hours. Reagents Zym A and Zym B were added to each well. Enzyme activity was evaluated based on color intensity values ranging from 0 to 5. Then their relevant values in nanomoles (nmol) were assigned for each reaction according to the color chart supplied with the kit.

Antibiotic susceptibility was evaluated using the EUCAST (European Committee on Antimicrobial Susceptibility Testing) [23] E-test strip diffusion process. The minimum inhibitory concentration (MIC) was also established. The antibiotic strips used were as follows: penicillin (0.016-256 µg/

ml), chloramphenicol (0.016-256 µg/ml), gentamicin (0.064-1024 µg/ml), rifampicin (0.032-32 µg/ml), streptomycin (0.064-1024 µg/ml), and erythromycin (0.015-256 µg/ml). Agar plates (Mueller-Hinton agar, BioRad, United Kingdom) were seeded with an overnight broth culture (BHI, Difco) of tested strain (100 µl), and the appropriate antibiotic strips were placed there. *E. faecalis* ATCC29212 was included as the positive control strain.

3. Results

3.1. Identification of EAs 1/11D27 Strain and Assessment of its Characteristics

The pure bacterial isolate was analyzed using sequencing (BLASTn analysis). BLASTn analysis assigned this strain to the species *Enterococcus asini*. The percentage identity (match) of BLASTn 16S rRNA sequence for the strain reached up to 100% (99.86% similarity was detected with the nucleotide sequence of the strain *E. asini* NR113929.1). The strain EAs 1/11D27 has been assigned the GenBank accession number (AN) MN822908.

Phenotypization using the BBL Crystal GP system confirmed individual characteristics typical for this species, such as acid production from lactose and xylose. However, no acid production from arabinose was observed by the EAs 1/11D27 strain. The esculin reaction was positive.

E. asini EAs 1/11D27 is hemolysis-negative (ɣ-hemolysis), deoxyribonuclease-negative, and gelatinase-negative. Moreover, this strain was also absent of genes for virulence factors such as gelatinase, enterococcal surface protein, adhesins, hyaluronidase, and aggregation substance. Evaluation of the API ZYM kit results confirmed that *E. asini* EAs 1/11D27 did not produce damaging enzymes (e.g., β-glucuronidase), but also no production of other enzymes was found. This was a reason to test beneficial property-bacteriocin activity. Following the E-test evaluation, *E. asini* EAs 1/11D27 was found to be susceptible to antibiotics with MIC = 2 µg for chloramphenicol, MIC = 16 µg for gentamicin, MIC = 0.012 µg for rifampicin, MIC = 0.12 for erythromycin, and MIC = 1 µg for penicillin. Resistance to streptomycin was observed (MIC = 192 µg).

3.2. Postbiotic Potential of Concentrated Substance EAs1/11D24

The concentrated substance (CS) of EAs1/11D27 strain was active against the principal indicator strain *E. avium* EA5 (1600 AU/ml). Moreover, the growth of 13 *E. mundtii* strains from the Norik of Muráň horse breed was inhibited **(Table 1)** with inhibitory activity ranging from 200 to 400 AU/ml. *E. faecium* strains from various sources, including 21 human strains with high resistance to glycosamide antibiotics, were inhibited with inhibitory activity of 200-400 AU/ml **(Table 2)**. Similarly, *E. faecium* strains (9) derived from poultry with similar resistance were inhibited with activity ranging from 100 to 400 AU/ml, as well as the growth of *E. faecium* (13) from wild-living animals, which was inhibited with activity 100 AU/ml. Seven strains of lactococci were inhibited with activity of 100 AU/ml **(Table 2)**.

Table 1: Inhibitory activity of concentrated substance EAs 1/11/27D against *Enterococcus avium* EA5 and *E. mundtii* in arbitrary units per ml (AU/ml).

Indicator	Inhibitory activity
EA5	1 600
EM12/1	200
EM13/3	200
EM22/1	200
EM23	200
EM24/1	200
EM29/1	200
EM31/2	200
EM32/3	400
EM34/2	400
EM40/2	200
EM37/1	200
EM38/1	200
EM41/3	400

EM - *Enterococcus mundtii* - faecal strains from the Norik of Muráň breed

Table 2: Inhibitory activity of concentrated substance EAs 1/11/27D against *E. faecium* strains and lactococci in AU/ml.

Indicator	Tested/Inhibited strains	Inhibitory activity
EF human HLAR	21/21	200-400
EF poultry HLAR	9/9	100-400
EF wildlife animals	13/13	100
Lactococci	7/7	100-400

EF human HLAR - high resistance to aminoglycosides, *Enterococcus faecium strains*, human-derived; EF poultry HLAR-high resistance to aminoglycosides, *Enterococcus faecium strains* from poultry; EF wildlife animals, *E. faecium* from wild-living animals; lactococci from raw goat milk (MK2/1-MK2/8)

In terms of species strains, one strain of *Str. pyogenes* (Sp117) was inhibited with inhibitory activity of 100 AU/ml, and two strains of *Str. pneumoniae* (clinical human isolates) were inhibited with activity of 100 AU/ml **(Table 3)**. *Str. equi subsp. zooepidemicus* (from horses) was inhibited at 200 AU/ml. Surprisingly, all tested staphylococci (88) were inhibited regardless of the species, with inhibitory activity of up to 200 AU/ml **(Table 4)**. When Gram-negative indicators of different species (10 strains) were used, the growth of 9 strains was inhibited **(Table 5)**. In total, 170 indicator bacterial strains were involved in testing, and the growth of 165 strains (97%) was inhibited. The principal indicator strain EA5 showed the highest susceptibility among enterococci. Enterococci, in general, were inhibited with almost the same inhibitory activity, as were lactococci (**Table 1** and **Table 2**). All tested enterococci (57) and lactococci (7) were inhibited. Among streptococci, 4 strains were resistant to CS EAs1/11D27, while 4 strains were susceptible, mostly with an activity of 100 AU/ml, and one strain showed an activity of 200 AU/ml **(Table 3)**. Most staphylococci (88) **(Table 4)**, were inhibited at 100 AU/ml. Five strains of *E. coli* were inhibited

at 100 AU/ml, as were *Acinetobacter lwofii*, *Ac. johnsonii*, and *Serratia liquefaciens* **(Table 5)**. However, *Pantoea agglomerans* PATK4/2 exhibited the highest susceptibility among Gram-negative bacteria, with inhibitory activity of 400 AU/ml.

Table 3: Inhibitory activity of concentrated substance EAs 1/11/27D against streptococci in AU/ml.

Indicator	Inhibitory activity
CCM	200
Sp117	100
Sp113	ng
Spn49	100
Spn51	ng
Spn57	100
Spn58	ng
Spn922	ng

CCM 7316 - *Streptococcus equi* subsp. *zooepidemicus*, Sp. - *Streptococcus pyogenes*, Spn - *Str. pneumoniae*, ng-negative - non inhibited

Table 4: Inhibitory activity of concentrated substance EAs 1/11/27D against staphylococci in AU/ml.

Indicator	Tested/Inhibited strains	Inhibitory activity
S. pseudintermedius	32/32	100
S. aureus	28/28	100-200
S. felis	15/15	100-200
S. chromogenes	13/13	100

Staphylococcus pseudintermedius - canine faeces; *S. aureus* - faecal strains of pigs; *S. felis* - faecal strains of cats; *S. chromogenes* - faecal samples of cows

Table 5: Inhibitory activity of concentrated EAs 1/11D27 against Gram-negative bacterial strains from roe dear and horses in AU/ml.

Indicator	Inhibitory activity
Ac.j. K17/PL2	ng
Ac. lwofii K8/3	100
S. lq.K2PL/1	200
Ac. sp.K10PL/2	100
P. aggl.PATK4/2	400
Ec 12/111/2	100
Ec 11/91/1	100
Ec 12/111/1	100
Ec 10/116/2	100
Ec 10/139/2	100

Acinetobacter johnsonii K17/PL2, *Ac. lwofii* ACl K8/3, *Serratia liquefaciens* K2PL/1, *Citrobacter freundii* K10PL/2, *Pantoea agglomerans* PATK4/2, *Yersinia enterocolytica* 12/111/2, *Serratia fonticola* 11/91/1, *Escherichia coli* 12/111/1, *E. coli* 10/116,/2, *E. coli* 10/139/2

4. Discussion

Only very limited information exists regarding the occurrence of the species *E. asini* **[16]**. This species belongs to the phylum Firmicutes, Family Enterococcaceae, and genus *Enterococcus*. Based on 16S rRNA gene similarity analysis, enterococci are divided into several principal groups. The species *E. asini* was allotted in *E. dispar* group together with the species *E. dispar*, *E. caninintestini*, *E. hermaniensis*, and *E. pallens* **[9]**. Regarding the basic taxonomic allocation of EAs 1/11D27, de Vaux *et al.* **[16]** described similar properties for *E. asini* strain from caecum of donkeys (*Equus asinus*) as formerly mentioned.

Furthermore, knowledge about the use of beneficial bacteria in horses is limited **[6,13,14]**. Our previous studies in horses have documented the use of bacteriocins produced by faecal enterococci **[14,24]**. Enterocin (Ent) M was applied in horses which led to a statistically significant reduction of coliforms and campylobacters ($p < 0.05$), and clostridiae ($p < 0.001$) in faeces. A beneficial effect on non-specific immunity (phagocytic activity values, PA) was also noted ($p < 0.0001$). When Mundticin-like substance EM 41/3 was applied to horses of the Norik of Muráň breed, a decrease in staphylococci was noted in individual horses, along with a statistically significant difference in the decrease of coliforms and pseudomonads. Tendency to increase PA was also noted **[24]**. However, to date, bioactive substances from the species *E. asini* and/or its postbiotic potential have not been mentioned. Although it is necessary to study other properties of a newly discovered active substance, the substance produced by *E. asini* EAs1/11D27 appears to have a broad antimicrobial spectrum.

Lauková *et al.* **[15]** reported the production of a bacteriocin-active substance by the species strain *E. moraviensis* EMo 1-1Nik from the buccal mucosa of Slovak warm-blood horses. The Norik of Muráň breed was found to be a source of bacteriocin-active strains of *E. mundtii* **[12]**. However, that substance showed a bacterial-related inhibitory spectrum. Bacteriocin substances produced by enterococcal species strains mostly belong to thermo-stable bacteriocins-enterocins **[10]**. Some of them exhibited a broad inhibitory activity spectrum, while others demonstrated a limited inhibitory activity spectrum, as also presented by Kubašová *et al.* **[25]** in the case of Ent B from canine *E. faecium* strain. Additional *in vitro* and *in vivo* studies are required to better understand the mechanisms involved.

Currently, the advantages of postbiotic use are frequently discussed. From the general perspective, postbiotics play an important role in the host's health status and their unique advantages include targeted regulatory effects that are not limited to the intestinal tract but also extend to the oral cavity, skin, and other parts **[26]**. Based on this information, it can be supposed that postbiotics also benefit the health status of horses health. Postbiotics are currently recommended as preventive and curative tools **[26]**. Mosca *et al.* **[27]** reviewed the use of postbiotics in clinical treatment. They showed evidence that postbiotics have a more pronounced effect in improving acute/chronic diarrhea, immune function, allergic reactions, and neurodegenerative diseases with good stability and safety. Considering this evidence and our previous conclusions based on postbiotic application (from the species *E. faecium*) in horses, these are the benefits of postbiotics that can be expected regarding the horses' health status.

Recently, for example, a bacteriocin-active substance from the strain species *Lactobacillus plantarum* was reported to be used as a complementary and adjuvant therapy for human colorectal cancer [28].

5. Conclusion

Enterococcus asini EAs 1/11D27, isolated from a mucosal swab of the Slovak breed Norik of Muráň, was taxonomically allocated based on a sequence match of 99.86% with the nucleotide sequence of the strain *Enterococcus asini* NR113929.1 in GenBank. The strain EAs 1/11D27 has been assigned the GenBank accession number MN822908. This strain is hemolysis-negative, deoxyribonuclease-negative, and gelatinase-negative, and it lacks genes for virulence factors such as gelatinase, enterococcal surface protein, adhesins, hyaluronidase, and aggregation substance. It does not produce damaging enzymes and is susceptible to antibiotics. The most important is its bacteriocin-like substance production with a broad antimicrobial spectrum, mostly against staphylococci (88). In total, the growth of 165 out of 170 indicator bacteria was inhibited (97%). This strain exhibits a broad postbiotic potential, and further studies are intended to explore its use in horses. To the best of our knowledge, this is the first report describing the postbiotic potential of species strain *E. asini* isolated from horses.

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Ethical Approval

Ethical approval was not required to conduct this study. However, we obtained kind agreement for sampling from Štátne Lesy a s. Dobšiná (Norik of Muráň breed) - director. Samples from live animals were taken by veterinarians and/or responsible persons involved in the project. They were analyzed at both institutions for diagnostic purposes and approved by the relevant Ethics Committee (permission code: SK U 0716).

Authors' Contributions

A.L. Conceptualization, Investigation, Data Curation, Writing, Project Administration; E.S. Resources, Methodology; V.F. Methodology; M.M. Methodology; A.T. Indicator resources, Methodology.

Data Availability

All data presented are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Comparative Evaluation of 2-Port Laparoscopic Ovariectomy Using LigaSure versus Standard 3-Port Laparoscopic Ovariectomy with a Bipolar Electrode in Mares

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Abstract

Ensuring fast and efficient hemostasis is crucial for achieving optimal outcomes in laparoscopic ovariectomy surgery. This study compared the clinical outcomes of standing laparoscopic ovariectomy for medium-sized granulosa cell tumors (≤15 cm in size) using a 2-port LigaSure versus a 3-port bipolar electrode, focusing on operating time, mean blood loss, intraoperative and postoperative complications, and the duration of the prospective hospital stay. Twelve mares were divided into two groups: six underwent standing laparoscopic ovariectomy with LigaSure through a 2-port approach, while the remaining six underwent the standard 3-port procedure with the bipolar electrode. Our findings demonstrated that 2-port laparoscopic ovariectomy using LigaSure was not only technically feasible and safe but also offered several advantages, including shorter operating times, simplified procedures, decreased postoperative analgesic requirements, and improved cosmetic appearance of surgical wounds. Moreover, this technique proved to be a reliable method for achieving hemostasis of the mesovarium while also being technically straightforward, time-saving, and cost-effective. Overall, our study suggests that 2-port laparoscopic ovariectomy with LigaSure is a promising alternative to the standard 3-port approach. This approach not only benefits patients by potentially reducing postoperative discomfort and enhancing recovery but also provides advantages for surgeons in terms of efficiency and resource utilization.

Keywords

Laparoscopic; Ligasure; minimally invasive; mare; ovariectomy; granulosa cell tumor

1. Introduction

Granulosa cell tumors (GCTs) are the most common ovarian tumors found in mares, accounting for more than 85% of reproductive neoplasms and approximately 2.5% of all neoplasms in horses [1]. These tumors originate from the follicular granulosa, with a subset known as granulosa-theca cell tumors (GTTs) also containing a distinct theca-derived component [2]. This distinction can be identified through histopathological examination and is functionally characterized by the capacity for androgen secretion [3].

The majority of cases of GCT are unilateral, and concurrent atrophy of the contralateral ovary provides support for the presumptive diagnosis of GCT [4]. Various surgical techniques have been utilized for the removal of ovaries, particularly those affected by granulosa thecal cell tumors, in mares [5]. These methods include the ventral midline approach [5], the flank approach (potentially involving laparoscopy) [6,7], and colpotomy via the vaginal wall [8]. The choice of approach is influenced by factors such as ovarian size, surgeon preference, and the overall health condition of the animal [9,10], while smaller and medium ovaries are extracted through the flank

using either an open, laparoscopic, or laparoscopic-assisted approach [2,9].

Standing laparoscopic ovariectomy has emerged as a more frequently employed technique for ovarian removal in mares [11–13]. Compared to traditional laparotomy-based procedures, laparoscopic methods offer several advantages, such as reduced complications due to better visualization, less invasiveness, efficient hemostasis, shorter recovery times, fewer postoperative complications, and secure vessel ligation within the mesovarium [14–21]. However, a significant challenge with laparoscopic ovariectomy is determining the best approach for ligating the ovarian pedicle and achieving hemostasis [22,23]. Consequently, various techniques have been utilized, including stapling instruments [23,24], laser methods [25], ligature application [23], vascular clips [26], and electrocoagulation [27]. However, these hemostatic techniques often prove to be time-consuming and challenging to apply and may lead to intraoperative hemorrhage [28]. The use of precise techniques and instruments is crucial for achieving optimal outcomes in laparoscopic surgery [29].

Advances in energy-based vessel sealing technologies, including bipolar sealing devices, ultrasonic devices, and nanotechnology-based devices, have expanded options for achieving hemostasis [30,31]. Among these, LigaSure stands out for its superior burst pressure and fast sealing time, effectively sealing vessels up to 7 mm in diameter while minimizing thermal spread [31–33].

LigaSure has been used with a high success rate in equine surgery [34,35]. Furthermore, employing reduced-port laparoscopic surgery optimizes the surgical procedure, minimizes instrument interference, reduces surgical incisions, and promotes faster postoperative recovery [20,36,37].

This study aimed to (1) evaluate the clinical application and outcomes of a 2-port laparoscopic ovariectomy in mares using LigaSure compared to a standard 3-port laparoscopic ovariectomy and (2) conduct a comprehensive histological evaluation of these soft masses to enhance the understanding of their nature and characteristics.

2. Materials and Methods

The present study adhered to ethical protocols and received approval from the Faculty of Veterinary Medicine at Damanhour University (Ref. No. A02/2023). Surgical procedures were performed in the Animal Panorama Center of Excellence, Faculty of Veterinary Medicine, Damanhour University. Between October 2018 and April 2022, a total of twelve mares of the local breed were included in the study and underwent elective standing unilateral laparoscopic ovariectomy. These mares exhibited unilateral ovarian pathologies characterized by soft tissue masses, as diagnosed by ultrasound examination **(Figure 1)**.

The primary objective of this investigation was to evaluate the technical feasibility, safety, and benefits of performing a 2-port laparoscopic ovariectomy utilizing LigaSure in comparison to the conventional 3-port procedure. A histological analysis of the ovarian masses was conducted. Technical feasibility was defined as the ability to easily conduct laparoscopic ovariectomy using a single visual port for the laparoscope and another instrumental port for the LigaSure device or two ports for the bipolar handle and grasping forceps. Safety was evaluated based on the absence of major complications, such as bleeding and damage to visceral organs. The outcomes of the procedure were evaluated by considering various factors, including operative time, ease of the procedure, postoperative analgesia according to the visual analog scale, duration of hospital stay, and cosmetic appearance of surgical wounds. The operative time was measured from the initial incision to the completion of wound closure. The ease of the procedure was scored by the operating surgeon on a scale of 0-3, where 0 indicated no exertion by the surgeon, 1 indicated an easy procedure, 2 referred to difficulty, and 3 suggested extreme difficulty requiring an additional port for completion. The scoring parameters for ease of assessment were based on observations of the laparoscopic field, accessibility of organs, tissue handling, and the number of operative failures. Postoperative pain was assessed using the visual analog scale (VAS), as per standard practice [38], where 0 on the visual analog scale (VAS) indicated the absence of pain, and 10 indicated severe pain. Postoperative cosmetic outcomes were evaluated by another independent surgeon 30 days after the procedure using a scale ranging from 1 (worst: the wound is infected and inflamed with exudation) to 2 (average: the wound is inflamed or swollen without infection or exudation) to 3 (best: the wound is clean, has completely healed, and has no inflammation or infection). A follow-up assessment was conducted by the operating surgeon 30 days postoperatively through a telephone questionnaire to gather information on any complications that arose after discharge, pain scores, and the cosmetic scale.

2.1. Preoperative Measures

Prior to surgery, routine preoperative measurements were taken, which included a comprehensive physical examination, gynecological examination per rectum, transrectal ultrasound (Sonoscape E2 vet Expert, China), intravenous catheter placement into the jugular, and complete bloodwork. Food was withheld for 24 hours before the surgery, while water intake was not restricted. Two hours before the procedure, the mares were administered Procaine penicillin (22000 U/kg) im and flunixin meglumine (1.1 mg/kg) iv. Immediately before the surgery, the mares were positioned in stock and given detomidine hydrochloride (0.002-0.004 mg/kg IV) as a bolus, with repeat doses as necessary, to provide sedation and analgesia throughout the surgical procedure. The relevant paralumbar fossae were then prepared and draped in an aseptic manner. An inverted-L block was performed using lidocaine, with 50 mL of a 10 mg/mL solution (Lidocaine, Hospira Inc., USA) injected into each fossa.

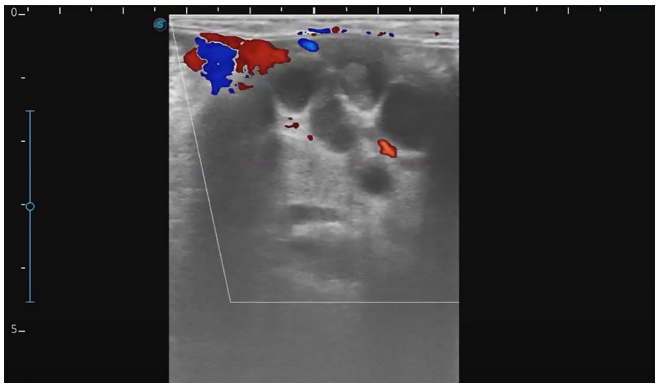

Figure 1: Transrectal color Doppler ultrasound of the ovary revealed a multicystic appearance and active vascularization.

2.2. Two-Port Laparoscopic Ovariectomy (Figure 2)

The 2-port technique was employed on a total of six mares (n = 6). To initiate the technique, one port was inserted into the laparoscope, while another instrument port was placed in the flank. The laparoscope portal, measuring 10 mm, was positioned 3 cm caudally to the last rib and ventrally to the ventral border of the coaxial tuberosity. An approximately 1 cm incision was made in the skin and through the abdominal wall, and a trocar-cannula set of 10 mm (AED, USA) was advanced through the abdominal wall into the peritoneal cavity. A 10 mm 0-degree laparoscope was then introduced (Karl Storz, Germany).

The second operative port, also measuring 10 mm, was placed caudal and ventrally approximately 6-8 cm from the first port. A skin-stab incision of approximately 1 cm was made, and a trocar-cannula unit of 10 mm (AED, USA) was pushed through into the peritoneal cavity after dissection of the abdominal muscles. CO_2 was used to insufflate the abdomen at a rate of 6 L/min, achieving a pressure of 12 mm/Hg, which exposed the ovary and created the operative field. Subsequently, an 18-gauge 30 cm histological needle (HS Medical, China) was inserted through the secondary instrumental port to induce local anesthesia of the ovary and mesovarium.

Infiltration anesthesia of the ovarian pedicle was performed using a 20 mL solution of 1% lidocaine (Lidocaine, Hospira Inc., USA), after which the needle was removed. Two minutes later, a 10 mm-37 cm LigaSure (Covidien, USA) was applied through the secondary port across the mesovarium. The instrument was closed, triggering the cautery to seal the tissue. The second trigger was used to cut the sealed tissue. The LigaSure jaw was positioned approximately 1 cm above the ovary to ensure complete occlusion of the jaws and achieve optimal results. Frequent cycles of full cautery dissection were performed on the mesovarium, mesosalpinx, and appropriate ligaments. Each point of tissue was sealed twice at a distance equal to the jaw width of the device before being cut midway.

Following the complete dissection of the ovary, the LigaSure instrument was removed, and the tissue was observed for any signs of hemorrhage. Prior to releasing the ovary, the LigaSure instrument was used to seal the tissue but not to cut it. This allowed the ovary to remain suspended until it was accessed with a grasping instrument for removal from the abdomen. Once the ovariectomy procedure was completed, the ovary was extracted through the second port's skin incision by grasping it with Babcock grasping forceps. In cases where the ovaries were large, an additional extension of the incision was made to facilitate their removal. The right secondary port was always used to remove the ovaries.

Post ovariectomy, the abdominal incisions were routinely sutured in layers. The abdominal muscles were sutured as a single layer using number 0 Vicryl suture in a simple continuous pattern. The subcutaneous tissue was closed with Vicryl number 0 using a simple continuous pattern, and the skin was closed using number 1 polypropylene in simple interrupted stitches.

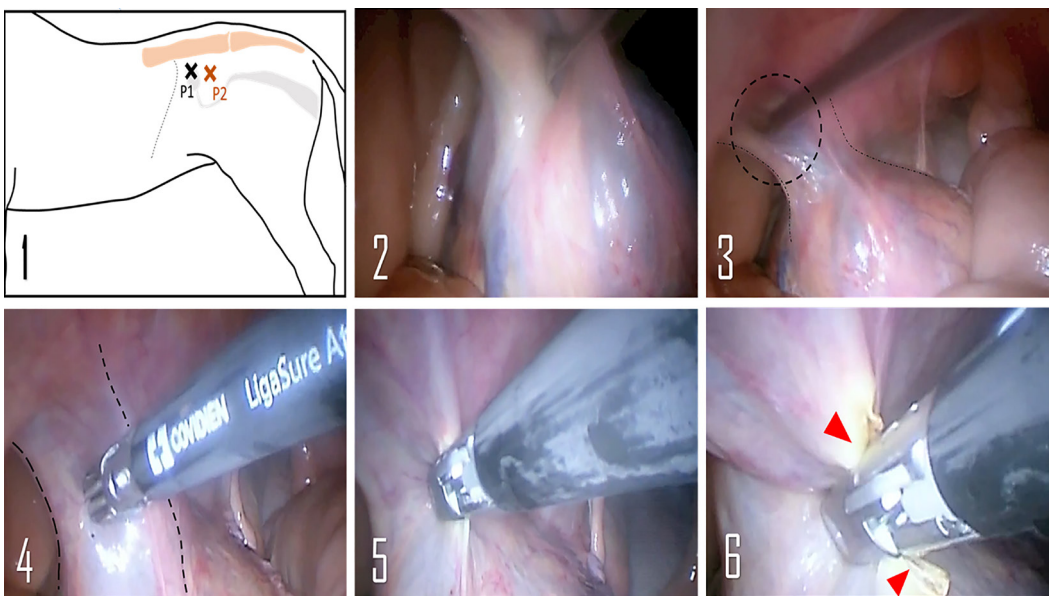

Figure 2: Two-port laparoscopic ovariectomy in a standing mare using LigaSure via a single surgical port. (1) The site for the laparoscope (P1) and LigaSure port (P2), (2) identification of the left ovary, (3) infiltration of the local anesthetic, (4) insertion of the LigaSure into the mesovarium, (5) initiation of the desiccation process, and (6) the cutting process.

2.3. Three-Port Laparoscopic Ovariectomy (Figure 3)

The technique was applied to six mares. A 10 mm main port for the laparoscope was positioned 3 cm caudal to the 18th rib at the level of the tuber coxae. Two access points for instruments were created in the paralumbar fossa. The first portal was situated at the upper border of the internal abdominal oblique muscle, halfway between the last rib and the tuber coxae. The second portal was located 8 cm below the first portal. Three 10 mm trocar-cannula units (AED, Germany) were inserted into the incisions of the main and secondary portals. Carbon dioxide (CO_2) was used to inflate the abdomen at a rate of 6 L/min until a pressure of 12 mm/Hg was achieved.

A 0-degree laparoscope was used to explore the abdomen. An anesthesia needle was inserted through the secondary cannula, and the ovarian pedicle was infiltrated with a 1% lidocaine solution (20 ml). Two 10 mm instruments were utilized: a Maryland grasping forceps (EndoMed Systems GmbH, Germany) held by the surgeon's left hand through the ventral portal and a bipolar cautery device (5×330 mm bipolar flat, U handle, BSC, India) operated by the surgeon's right hand through the dorsal port (the surgeon being right-handed). Additionally, 10 mm curved scissors (EndoMed Systems GmbH, Germany) were alternated with the bipolar electrocautery device. Following anesthesia, the ovarian pedicle was grasped for stabilization, and a bipolar device was used to cauterize the tissue cranially to caudally. The power of the cautery device was adjusted based on the color of the dissected tissue. A white to yellow color indicated a good seal, while a brown to blackish color indicated tissue burning. After each cycle of tissue cauterization, the bipolar device was replaced with scissors to perform dissection. Two to three cycles of cautery and dissection were performed until the ovary was completely freed. Each point where tissue dissection was required was cauterized twice at a distance equal to the jaw of the device, and scissors were used to cut at the midpoint. The ovaries were then removed through the incisions of the secondary ports. The incisions through which the ovaries were removed were enlarged to the needed length to remove the ovaries from the abdomen.

The abdominal wall was sutured in a single layer, encompassing all three muscle layers, using a Vicryl number of 0 in a simple continuous technique. The subcutaneous tissue was closed in a similar manner. The skin was closed with polypropylene number 1 in a simple interrupted pattern. Adhesive dressings were applied over the surgical sites and changed daily.

2.4. Postoperative Measures

The mares were provided with soft food two hours after the surgery, and they were discharged from the hospital 24 hours after the procedure. Postoperatively, systemic antibiotics (penicillin/streptomycin) (8 and 10 mg/kg) were administered intramuscularly, and phenylbutazone (4.4 mg/kg) was administered intravenously once daily for three consecutive days, except for animals that showed general illness or local surgical site infection. Veterinary care and stall rest were advised.

2.5. Evaluation Process

Local veterinarians were asked to monitor the animals during the complete evaluation process. Their precise observations were transferred to the operating surgeon via telephone calls. All the evaluation parameters, including the technical feasibility, operative time, degree of ease, postoperative pain, and cosmetic appearance of surgical wounds, were recorded.

2.6. Histopathological Examination

The excised ovaries were collected, grossly examined, and photographed to record the ovarian structures and/or any alterations. The ovaries were then cut longitudinally into two equal halves and fixed in 10% formalin solution immediately after collection for 48 hrs. The specimens were then processed by the paraffin embedding method, sectioned at 5-7 µm, and stained with hematoxylin and eosin according to Bancroft and Gamble [39].

2.7. Statistical Analysis

Statistical analysis was performed to determine the standard error of the means by using SPSS software (SPSS, Version 28.00, IBM, USA).

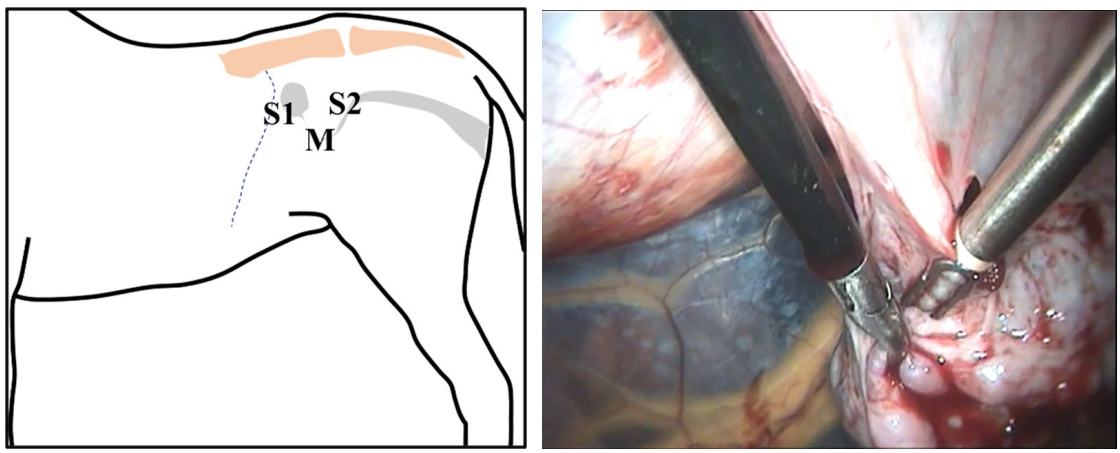

Figure 3: Three-port standing laparoscopic ovariectomy using bipolar electrodes and Maryland grasping forceps. (S1) is the grasping forceps port, (M) is the laparoscope port, and (S2) is the bipolar port.

3. Results

Unilateral laparoscopic ovariectomy was successfully performed on twelve mares using two different techniques: the two-port LigaSure technique (n = 6) and the three-port bipolar technique (n = 6). In terms of technical feasibility, both techniques were feasible, but the two-port technique was more feasible. In terms of safety, the use of LigaSure was safer than the use of bipolar lenses, with no intraoperative complications recorded. However, tissue coagulation until blenching, shrinkage, and burning were observed in all animals treated with bipolar materials, and tissue blood oozing occurred in 3 patients, which was managed by further cauterization. Bleeding was observed in 3 patients, but it was controlled by additional cauterization attempts. The use of bipolar cauterization resulted in smoking and temporary unclear visualization of the target tissue. No injuries to internal organs were recorded with either technique.

The mean operative time for the LigaSure group was 30.65 ± 2.85 minutes, while the bipolar cauterization technique took longer, with a mean surgical time of 43.1 ± 4.25 minutes. Both techniques were considered easy, with the operating surgeon rating all LigaSure procedures and 5 out of 6 bipolar procedures (90%) as easy (score = 1). One of the bipolar surgeries (16.6%) was rated as difficult (score = 2) due to poor tissue manipulation and required the longest surgical time (51.34 minutes).

Regarding postoperative pain, 2 out of 6 animals (33.3%) in the LigaSure group experienced moderate postoperative pain (score = 6) that required intravenous administration of flunixin meglumine for an additional 2 days. One mare (16.6%) experienced severe postoperative pain (score = 9) accompanied by fever and anorexia, which resolved by day 7 after surgery. In the bipolar group, 5 out of 6 animals (83.3%) experienced moderate postoperative pain (score = 6) 5 days after surgery. Two of the 6 animals (33.3%) experienced general illness, fever, or severe postoperative pain (score = 8), which resolved with systemic treatments by day 9 after surgery. An evident increase in pain score was observed with the three-port approach.

In terms of surgical wounds and cosmetic appearance, 2 out of 6 animals (33.3%) in the LigaSure group exhibited wound swelling and seroma, as well as local inflammation without infection. One animal out of 6 (16.6%) showed mild swelling and localized subcutaneous emphysema. Local abnormalities at the surgical site disappeared in all animals by day 14, and the sutures were removed. In the electrocautery group, wound infection was reported in two animals (33.3%) on day 3; this infection was treated topically and fully resolved by day 9 postoperatively. All animals exhibited a good cosmetic appearance three weeks after surgery. Additionally, all the mares were able to resume normal activity within 15-21 days after surgery, and the owners were satisfied with the cosmetic outcomes.

Overall, two-port laparoscopic ovariectomy using LigaSure proved to be a feasible, safe, and beneficial alternative to the three-port electrocautery technique. Grossly, the ovaries were 10-15 cm in diameter. On the cut surface, GCTs were polycystic, solid, or a combination of both. The cyst fluid was sanguinous or serous. The solid areas were white and grayish to yellow and orange, depending on the degree of hemorrhage that occurred within the tumor. According to a microscopic examination of the ovaries, 11 of the examined samples exhibited granulosa cell tumors. They were mostly polycystic and histologically consisted of cysts that resembled disorganized attempts at follicle formation, accompanied by a prominent supporting stroma of spindle cells interpreted as theca cells. Within the follicular structures, multiple layers of cells that resemble granulosa cells, often palisading at the periphery, were observed. The last ovary displayed a multifocal pattern with rosettes, where groups of eosinophilic materials formed Call-Exner bodies with radiating granulosa cells. These cells were arranged in rosettes that clustered together and were bound by stromal cells **(Figure 4)**.

4. Discussion

The present study aimed to evaluate the feasibility, safety, and benefits of a two-port laparoscopic ovariectomy using LigaSure compared to the three-port electrocautery technique in standing mares. The results of the study demonstrated that LigaSure was a feasible option for performing standing laparoscopic ovariectomy in mares, which is in line with previous studies **[11,40]**. LigaSure provided simultaneous coagulation and cutting of tissue, and its ease of use, along with the microprocessor that adjusts optimal heat and sealing time, made it more feasible than bipolar electrocautery, which requires manual adjustments for optimum tissue sealing **[41,42]**. The single-device approach with LigaSure simplified the procedure and reduced the operative time compared to the use of multiple tools, such as Babcock grasping forceps, bipolar electrodes, and scissors. Moreover, the use of a single operating port for LigaSure was sufficient to seal and cut the ovarian pedicle and broad ligament, eliminating the need for additional grasping tools, reducing invasiveness, and improving cosmetic outcomes. These findings support the convenience and benefits of the two-port approach in terms of feasibility, ease of use, shorter surgical time, minimal postoperative pain, and improved cosmetic appearance, as reported in previous studies **[43–47]**.

The LigaSure vessel-sealing device allowed for simultaneous grasping, coagulation, and cutting of tissue, enabling the surgeon to operate with a single port without the need for instrument exchange. LigaSure utilizes a combination of pressure and energy to effectively seal larger vessels up to 7 mm in diameter with minimal thermal spread, potentially reducing collateral tissue damage and offering faster dissection times **[41]**. In the current study, where the granulosa cell tumors were midsize, ≤15 cm in diameter, and highly vascularized, LigaSure was optimal for removing tumors with minimal intra- and postoperative complications. In contrast, while bipolar electrosurgery is versatile and capable of a wide range of surgical functions, such as cutting, coagulating, and dissecting tissues, it poses a slightly elevated risk of causing tissue charring and inadequate hemostasis. This heightened risk has contributed to intraoperative complications, emphasizing the limitations of bipolar electrocautery for mid-size ovariectomy. The results of the present study aligned with previous reports of using bipolar electrocautery to control bleeding limited to small blood vessels up to 3 mm **[48]**. The anatomical position of the equine ovaries facilitated accessibility without requiring extra grasping instruments, and the use of LigaSure reduced the technical demands and the need for mesovarium dissection, leading to a shorter learning curve and reduced operative time **[21,49,50]**.

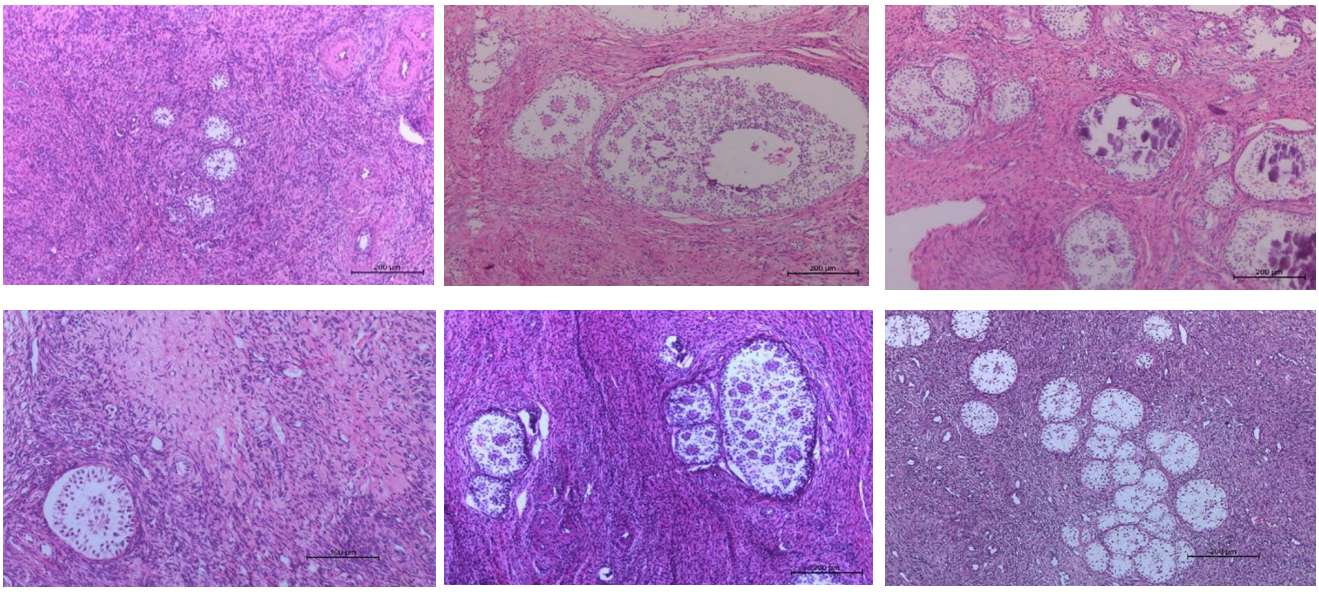

Figure 4: Photomicrographs of ovarian sections from studied mares (different ages) showing multifocal coalesced granulosa cell tumor bounded by fusiform stromal cells in a whorl-like arrangement (H&E; bar = 200 µm).

The study also revealed that the two-port laparoscopic ovariectomy technique using LigaSure resulted in a shorter mean operative time and decreased postoperative pain in the first 12 hours. The reduced number and sizes of the ports contributed to minimal scarring and improved cosmetic outcomes, as observed in other studies on reduced-port surgeries in animals and humans [51–54]. No major intraoperative or postoperative complications were encountered during the study, indicating that LigaSure provided a quick and secure method of vessel hemostasis, leading to successful standing laparoscopic removal of normal ovaries. The device demonstrated superior performance in terms of burst pressure, sealing time, thermal spread, and smoke production compared to bipolar cautery or ultrasonic devices [55–57]. Furthermore, the two-port laparoscopic ovariectomy technique was associated with reduced analgesia requirements and faster recovery time, leading to lower overall costs. The less invasive nature of the technique, with smaller incisions and improved cosmetics, was also advantageous [58–60].

5. Conclusions

In summary, the two-port laparoscopic ovariectomy technique using LigaSure has been proven to be a safe and efficient method for achieving hemostasis of the mesovarium. It has also been shown to be technically straightforward, leading to shorter operative times than the traditional three-port electrocoagulation technique, especially in cases involving medium-sized granulosa cell tumor ovaries.

Authors' Contributions

M.W.E. advised the project, and A.F. performed and interpreted the results of the histopathological examination. M.W.E. and A.N.E. performed the experiments, collected the data, and prepared the figures. M.W.E. and A.N.E. discussed the results and reviewed and edited the manuscript. M.W.E. and A.N.E. contributed equally to this work and shared the first authorship.

Data Availability

All the data collected or analyzed during this study are included in this published paper.

Funding

No funding was received to support this study.

Conflicts of Interest

The authors declare no competing interests.

Ethical Approval

The present study adhered to ethical protocols and received approval from the Faculty of Veterinary Medicine, Damanhour University (Ref. No. A02/2023). Surgical procedures were performed at the Animal Panorama Center of Excellence, Faculty of Veterinary Medicine, Damanhour University.

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Can Repeated Exposure to Music Mitigate Horses' Reactions to Sudden and Unexpected Stimuli?

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Abstract

The living conditions imposed on horses mean that they are inevitably confronted with situations that can induce stress. Music is a promising tool for managing such situations, but its benefits could be attenuated by repeated exposure. In this study, we aim to determine whether music can mitigate horses' reactions to unexpected stimuli and if playing the same music daily leads to a loss of its efficiency. We compared three groups of 12 horses that were led on a route punctuated by potentially stressful stimuli for 10 consecutive days. Each group of individuals wore headphones and was consistently subjected to one of the three experimental conditions: a "music" test condition during which the same music track was played, a "noise" condition during which pink noise was played, and a "no-music" control condition. We found that music has a relaxing effect on horse behavior and heart rate. Interestingly, parameters with the pink noise were intermediate between the music and no-music conditions. Regarding the music's loss of efficiency through repeated playing, our results show that this tool continues to effectively mitigate the behavioral expression of stress after seven consecutive exposures, but this effect was not found every day. Music can therefore help make human-horse interactions safer by limiting the stress of horses faced with unexpected events, but further investigation is needed to understand the underlying mechanisms and ensure a safe and consistently efficient use in the field.

Keywords

Stress; behavior; heart rate; habituation; equitation science; welfare

1. Introduction

It has been shown that music can be beneficial to animal welfare [1]. Music could specifically alleviate stress and fear responses, as shown for chickens [2], rats [3], and cows during milking [4]. Additionally, studies have shown that music has a calming effect on dogs and chimpanzees [5] and promotes play behavior in piglets [6]. Similarly, in rodents, it can also improve physiological parameters such as weight, heart rate, and sympathetic and parasympathetic system activity [7]. Beneficial effects of music on the emotional state have been observed in horses after exposure in the stables without any particular stressful situation [8–11]. In their study, Carter and Greening [8] explored the impact of four different styles of music that they played in the stables: country music, classical music, jazz music, and rock music. They found different stress and relaxation behaviors according to the style of music. Comparing the impact of the different tracks with each other, they observed more resting behaviors and less alertness and

vigilance behaviors when country and classical music were played than when jazz and rock music were played.

The living conditions imposed on horses mean that on a regular basis, they are inevitably confronted with multiple situations that can generate a stressed state. Transport, for example, is clearly recognized as stressful, and several studies have shown it to be a significant stressor, even in experienced horses [12–14]. In addition to these situations, we must consider the occasions where horses are led by humans through environments with unpredictable and random stimuli. Finally, veterinary care is one of the events that generate stress, particularly when carried out in the stall on isolated individuals. All these events, taken separately or cumulatively, can affect the emotional state of the animals. In humans, several teams have demonstrated that listening to music can induce greater relaxation during exposure to stressful events or stop the stress response immediately after exposure [15]. Similarly, in horses, the stress generated by potentially stressful situations such as social isolation, transport, or farriery can be limited by playing Western music [10,16–18], which appears to be an effective solution for improving individual well-being. Regarding cardiac physiology, horses exposed to the playing of the main theme of Forrest Gump displayed a better recovery with a faster return to a basal heart rate after stress compared to horses that were not exposed to music [16,18].

Prolonged and/or repeated states of stress can have harmful consequences in the medium and long term and will jeopardize the well-being of individuals [19]. Improving the management of these situations is a major scientific challenge to improve animal welfare and, in the long term, the safety of human-horse interactions. A more relaxed horse will be less likely to express dangerous behaviors (e.g., defense, escape), thus reducing the risk of accidents. Furthermore, the relationship between a rider and horse is established over time, through repeated interactions and routines. The quality of this relationship is a factor involved in the evaluation of the stressful nature of a situation such as handling or horse van loading [12], and when this relationship is positive, it can be a source of intrinsic rewards for the animals [20]. Thus, limiting negative interactions and reducing the animal's state of stress during routine, recurrent, and necessary interventions (such as transport, veterinary care, etc.) can only be of long-term benefit to the human-horse relationship.

The regular use of music to manage the daily stressful situations encountered by horses inevitably involves the repeated or prolonged exposure of individuals to music. It is therefore essential to address the question of the evolution through time of the calming effect of music on horses to determine if a form of habituation occurs (i.e., a decrease in the efficiency of a stimulus – here the music – induced by multiple exposures to this stimulus).

In the current literature, no study to date has specifically tested habituation to music playing in horses. However, some authors have found that the beneficial effects of music change with prolonged exposure. This is the case, for example, of Stachurska *et al.* [9] in their study on the impact of music when it is played for 5 hours daily in stables. Beyond the calming effect of music through the decrease and stabilization of heart rate, the authors observed a return to the initial cardiac physiology parameters during the fifth month of exposure. Similarly, Wiśniewska *et al.* [11] played music to older horses (over 20 years old) for 3 hours per day in the stable and observed a disappearance of the beneficial effects on cardiac physiology after 3 weeks of treatment. These observations suggest a decrease in the beneficial effects of music that may reflect a habituation phenomenon with extended exposure.

1.1. Study Objectives

With the aim of optimizing the use of music as a stress management tool, this study aims to determine whether music can alleviate horses' reactions to acute stressors and if the daily playing of the same music leads to a decrease in its calming effect. To this end, we compared three groups of 12 horses that were led along a route and were subjected to potentially stressful stimuli (auditory, visual, sudden) for 10 consecutive days. Each group of individuals was subjected to a different experimental condition using headphones: a "music" test condition during which a music track was played continuously through the headphones, a "noise" condition during which pink noise was played continuously through the headphones, and a "no-music" control condition during which the headphones were inactive. The group led in the "music" condition will allow 1) to evaluate the impact of music on the horses' reaction to stressors and 2) to test whether a phenomenon of habituation to music - inducing, therefore, a progressive loss of its calming effect - appears. The group led in the "noise" condition will allow us to test the impact of an auditory track that is not musical. Finally, the group of horses tested in the "no-music" condition will allow us to verify the stressful nature of the route *per se* while being led by a human and to observe whether a decrease in their reactions to the stressors occurs over time.

2. Materials and Methods

2.1. Subjects and Individual Measures and Distribution of Individuals

The 36 horses that participated in the present study were housed in 10m^2 individual stables. They were ridden or taken out to the paddock every day. They were fed daily in the morning, at noon, and at the end of the afternoon with pellets, and some were also provided hay. Water was available *ad libitum* through an automatic waterer located in the stall. It should be noted that the equestrian center did not play any music via the radio.

In order to obtain three comparable experimental groups, their composition was balanced according to the temperament, sex, and age of the individuals. For this purpose, the experimental individuals were subjected to simplified temperament tests [21]: reactivity to an unknown surface, reactivity to an unknown object, and reactivity to suddenness.

We used a semi-random allocation procedure. Random draws were conducted to assign each individual to one of the three experimental groups. Once this was done, we checked the balance of the groups by comparing the mean values of the ages and coordinates of the individual reactions to the unknown surface, unknown object, and suddenness tests.

2.2. Experimental Conditions

The music was played directly into the horse's outer ear using an "audio cap" (see **Figure 1**). This is a traditional earcap

equipped with a pair of earphones in front of the pinnae and a pocket to hold a small MP3 player between the two ears. The use of earmuffs is a common practice in horse riding, so the subjects were already familiar with this type of equipment before the study began. However, we familiarized them with the audio cap by fitting it on the horse and playing music for 15 minutes on four occasions prior to the study and checked for the absence of negative or stressful reactions. Additionally, to prevent any potential association between the cap and stress episodes, we also exposed the horses to the cap and music outside the context of stress. To do this, the horses were fitted with the audio cap between two successive trials when they were alone in their home stable.

Finally, as suggested by Wilson *et al.* **[10]**, simple auditory stimulation implies some attenuation of the horse's sound environment, and this noise reduction could partly explain the relaxing effects of music. We therefore included the "noise" condition to ensure that the differences that might be observed in the "music" condition are indeed due to the acoustic and musical parameters of the audio tracks. In this condition, individuals were exposed to pink noise. Pink noise is a random signal with constant energy in the octave bands and is therefore weighted according to the properties of the human audiogram (and, by extension, also those of horses). Comparisons between the music and pink noise conditions would thus allow us to test the contribution of musical characteristics to the effect of music, with potentially a lower stress response in the music condition compared to the noise condition.

2.3. Experimental Situations

The stressful situation we chose was that of leading the horses along a track and subjecting them to unexpected and potentially stressful stimuli. The routes had a mean duration of 6 min 38 s (± 2 min 10 s) and were each composed of five visual and/or sudden stimuli and five auditory stimuli (10 in total). The details of each visual and sudden stimulus are provided in **Table 1**. For the auditory stimuli, we selected animal sounds (a dog or wolf barking, a rooster crowing, a cow mooing or a donkey braying) and human environmental sounds (a whistle, an ambulance siren, a car or truck horn, a motorbike engine starting or a truck engine running). These are short sound recordings (maximum five seconds) that were recorded and kindly made available by Joseph Sardin **[22]**.

It is possible that repeated exposures to the same stressful situation may induce habituation to this type of stress *per se*, thus gradually diminishing its stressful nature. To limit this risk, the stimuli's position and order of appearance were modified from one exposure to another, and different audio stimuli were selected each time. The aim was to prevent the horses from becoming accustomed to the stressful situation, which would prevent the detection of a potential habituation to the music. In addition, for a given stressor type (e.g., throwing a ball), the intensity was gradually increased after each exposure (first exposure: ball thrown 5m in front of the horse, last exposure: ball thrown 3m behind the horse, **Table 1**. For an example, see **Supplementary Material 1**.

Figure 1: Photograph of the audio cap placed on a halter.

2.4. Data Collection and Analysis

All trials were recorded with cameras (GoPro, JVC, or Canon camcorders). Several cameras were used to increase the number of viewing angles. Thus, behavioral observations were conducted retrospectively from the recorded videos using the focal sampling method **[23]**. All observations were carried out blind; i.e., only the experimenter in charge of handling the horses was aware of the assigned conditions for each horse during the experiment.

For our analyses, using the focal sampling method, we recorded the occurrence of the following behaviors during the presentations of the different stimulus categories (visual, sudden, auditory) as well as the duration of the trial (see **Supplementary Material 2** for the description of the different behaviors):

For visual stimuli: alertness; backing away, blowing sound; defecation; gaze towards door; glances; immobility; outward alertness; running away; scratching; sniffing the stimulus objects; sniffing other objects; startled; stopping; trotting.

For sudden and auditory stimuli (ordered by reaction's intensity, see below): orientation of the ears towards the stimulus (1); raising head (2); glances (3); twitches (4); stopping (5); blowing sounds (6); startled (7); backing away (8); turning around (9); running away (10).

To analyze the stress responses to visual stimuli, as the exposure duration depended on the time spent by the individual horse on the route, we calculated the sum of the occurrences of behaviors weighted by the duration of each individual's route. For this purpose, we calculated a coefficient equal to the duration of each individual's trial, divided by the maximum duration achieved by an individual for the same route. We then calculated the mean occurrence of the behaviors displayed during the different routes, weighted by this coefficient.

Table 1: Details of the visual and sudden stimuli along the different routes.

Stimulus	Details
	Visual Category
Unknown surface 1 (SI 1)	Ground tarpaulin (3.5 x 2m) framed by 2 bars
Unknown surface 2 (SI 2)	Ground tarpaulin (3.5 x 2m) framed by 2 bars, with one bar raised by a cube
Framed area 1 (UCPA 1)	Round green carpet (2m diameter) laid on the ground and framed by a 2m high flag (right) and a garden hose (left)
Framed area 2 (UCPA 2)	Round green carpet (2m diameter) laid on the ground and framed by a 2m high flag (right) and a tarpaulin with aluminum foil taped to it, then attached to the wall aluminum attached to the wall
Framed area 3 (UCPA 3)	Round green carpet (2m diameter) placed on the ground and framed by a 2m high flag (right) and a chandelier covered by a tarpaulin with aluminum foil taped to it (left)
Incongruous object 1 (IO 1)	Chandelier covered with a tarpaulin with aluminum foil taped to it
Incongruous object 2 (IO 2)	Passage between IO 1 and two construction site signs
Incongruous object 3 (IO 3)	Passage between two chandeliers surrounded by aluminum
Incongruous object 4 (IO 4)	Two-person tent on the ground
Incongruous object 5 (IO 5)	Two-person tent placed upside down with aluminum foil taped to it
Banner 1 (GP 1)	Orange banner, 7m long creating a 3m wide corridor with the wall
Banner 2 (GP 2)	Orange banner, 8m long in the center of the arena, with two zones marked with studs. Zone 1 = 0 to 2m; zone 2 = 2 to 4m.
Banner 3 (GP 3)	Orange banner, 7m long creating a 2m wide corridor with the wall
Low branch 1 (BB 1)	A 2m bamboo branch suspended 2.5m from the ground by means of two stanchions. Ten 50 cm-long ribbons are attached to each side of the branch
Low branch 2 (BB 2)	A 2m bamboo branch suspended 2.5m from the ground by means of two stanchions. Ten 1m-long ribbons are attached to each side of the branch
	Sudden Category
Flags 1	3 flags of 1m in the center of a construction cone. An experimenter pushes the cone with her foot so that the 3 flags fall in front of the horse, which has been stopped beforehand
Flags 2	Same conditions as "Flags 1," but six ribbons have been attached to the 3 flags
Umbrella 1	Quick opening of an umbrella 3m in front of the horse
Umbrella 2	Quick opening of an umbrella with 20cm-long ribbons at the ends, 3m in front of the horse
Ball 1	Throwing a ball against the arena's boot barrier, 5m in front of the horse
Ball 2	Throwing a ball against the arena's boot barrier, 3m behind the horse

As auditory and sudden stimuli were applied punctually, a simple intensity score was calculated for the analysis of stress responses. To this end, a value was assigned to each behavior in the sudden and auditory categories. Ranging from 1 for ear orientation to 10 for running away, these values increased in incremental steps of 1 according to the order of the behaviors presented above. For example, raising head scores to 2 and turning away scores to 9. In **Supplementary Material 2**, the definitions of the behaviors recorded during the reaction to auditory and sudden stimuli reflected the intensity of the horse response from a simple focus towards the stimulus, to a higher elevation of the head, to the occurrence of body jerks, to a more tense or backward attitude, and ultimately to the flight of the subject.

We also recorded heart rate (HR) which allows the investigation of the activity of the autonomic nervous system (ANS). To achieve this, we used a Polar EQUINE RS800CX heart rate monitor. This tool allows to measure the R-R interval, also referred to as the Interbeat Interval (IBI), which is defined as the time in milliseconds (ms) between two peaks of the R wave of the QRS complex on the trace of an electrocardiogram (ECG). The heart rate measurements started in the stall four minutes before the subject left for the trial and ended in the stall four minutes after it had returned. We considered separately the test phase (i.e., when the horse was being led on the stressful route), the pre-test phase (4 min before leaving the stable), and the post-test phase (4 min after returning to the stable).

2.5. Statistical Analysis

The statistical analyses were conducted using R software version 4.2.1 **[24]**. None of the variables follow a normal distribution, so they were all statistically analyzed using permutation tests for non-parametric data.

For the analysis of the behavioral variables, the data from 18 of the 36 horses initially subjected to the experiment were analyzed (the videos of the 18 remaining individuals were not exploitable), and the physiological variables (pre-test, test, and post-test heart rate) were analyzed for all individuals (N = 36).

To address the questions concerning the impact of music on the stress state of the horses, we first studied the impact of music on all the data collected during the 10-day experiment. We then

extended our analysis within each session to study the impact of music throughout the daily exposure period and explore the potential implementation of a habituation phenomenon.

3. Results

3.1. The Overall Impact of Music

3.1.1. Expression of Stress Generated by the Different Categories of Stimuli

The stress behaviors displayed by individuals during the trials were statistically different between conditions for the visual and sudden stimuli (p = 0.006 and 0.007 respectively) but not for the auditory stimuli (p = 0.4) (see **Table 2**). When comparing conditions for the visual stimuli, the intensity of the reaction was lower for the music condition than for the no-music condition (permutation test, $p < 0.001$), the other comparisons being non-significant (music vs. noise and no-music vs. noise conditions, p = 0.1, post-hoc permutation test). For the sudden stimuli, differences were found between the noise condition and the two other conditions (post-hoc permutation test, music vs. noise, p = 0.004; no-music vs. noise, p = 0.04) while music and no-music conditions did not differ (permutation test, p = 0.2). In the noise condition, horses were more reactive to stimuli that appeared suddenly.

3.1.2. Cardiac Physiology

There was a significant difference between the mean heart rates of individuals during the test and post-test phases (p = 0.005 and 0.001 respectively) depending on the condition, but not for the pre-test phase (p = 0.9) (see **Table 3**). Only the music condition differed from the two other conditions during the test and post-test phases (permutation test, music vs. no-music p_{test} = 0.02 and $p_{post\text{-}test}$ = 0.04; music vs. noise $p_{test} < 0.001$ and $p_{post\text{-}test}$ = 0.005). The mean heart rate was significantly lower in the music condition compared to the non-music condition or the pink noise condition.

3.2. Evolution of Music Impact on Stress Behavior Across Days

To study the potential habituation to music, the behavioral variables (occurrence of stress behaviors and response intensity score) were analyzed day after day.

3.2.1. Visual Stimuli

The evolution of the mean occurrence of stress behaviors generated by the visual stimuli across days is presented in **Figure 2**. The expression of behavioral stress responses only differed between experimental conditions for days 2, 4, and 7 (see **Table 4**). Post-hoc comparisons showed that on days 4 and 7, horses in the music condition expressed significantly fewer stress behaviors than those in the no-music condition (permutation test, day 4: p = 0.01; day 7: p = 0.01). For day 2, stressful behaviors tended to decrease in the music condition compared to the no-music condition (permutation test, p = 0.06). Moreover, there was no difference between the music and noise conditions (permutation test, day 2: p = 0.9, day 4: p = 0.8, and day 7: p = 0.5). This result could reflect an attenuation of the individual's sound environment by the pink noise. Indeed, fewer stress behaviors were observed in the noise condition than in the no-music condition on days 2 and 4 (permutation test, day 2: p = 0.03 and day 4: p = 0.01) and a trend was observed on day 7 (permutation test, day 2: p = 0.06).

3.2.2. Sudden Stimuli

The stress response to sudden stimuli (umbrella, balloon, and flags) across days is presented in **Figure 3**. The only difference in the expression of stress occurred on day 4 (permutation test, p = 0.04, see details in **Table 4**). Post-hoc comparisons showed that the response intensity scores tended to decrease in the music condition vs. the no-music condition (permutation test, p = 0.06, see **Table 4**). Moreover, there was no statistical difference in the expression of stress behaviors between music and noise conditions (permutation test, p = 0.7) or between noise and no-music conditions (permutation test, p = 0.08).

Table 2: Means and standard deviation (±) of stress responses according to the conditions corresponding to the occurrence of stress behaviors for visual stimuli and the response intensity score measured for auditory and sudden stimuli. The significance level was set at 0.05.

	Nb of horses	Visual	Auditive	Sudden
Music	6	6.5 ± 12.5	17.0 ± 20.0	14.2 ± 12.6
No music	6	13.3 ± 24.3	13.2 ± 13.7	16.6 ± 11.8
Pink noise	6	9.6 ± 28.9	15.3 ± 12.5	21.8 ± 15.6
p value		0.006	0.4	0.007

Table 3: Means and standard deviation (±) of the heart rates (in beats per minute) of the individuals during the different test phases and according to the conditions. The significance level was set at 0.05.

	Nb of horses	Pre-test	Test	Post-test
Music	12	35.4 ± 4.2	47.8 ± 8.8	35.8 ± 4.0
No music	12	35.5 ± 3.4	50.8 ± 12.1	37.5 ± 3.5
Pink noise	12	35.6 ± 4.4	50.2 ± 10.0	37.2 ± 3.5
p value		0.9	0.05	0.001

Table 4: Means and standard deviation (±) of stress responses from day 1 to day 10 according to the conditions corresponding to the occurrence of stress behaviors for visual stimuli and the response intensity score measured for auditory and sudden stimuli. The significance level was set at 0.05. Only significant differences are reported.

		Visual			Sudden
	Nb of horses	Route 2	Route 4	Route 7	Route 4
Music	6	3.0 ± 4.6	3.3 ± 3.0	5.3 ± 4.7	10.1 ± 6.7
No music	6	6.1 ± 6.6	13.2 ± 20.6	16.4 ± 25.5	27.0 ± 19.5
Pink noise	6	3.0 ± 2.2	3.0 ± 5.8	6.7 ± 9.7	11.5 ± 7.2
p value		0.03	0.002	0.01	0.04

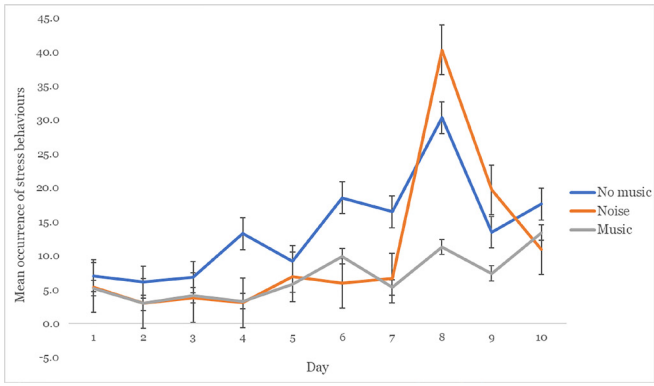

Figure 2: Evolution of the expression of stress behaviors (mean occurrence weighted by the duration of the trial) in response to visual stimuli in the different conditions day after day. Permutation tests, * $p < 0.05$, ** $p < 0.01$.

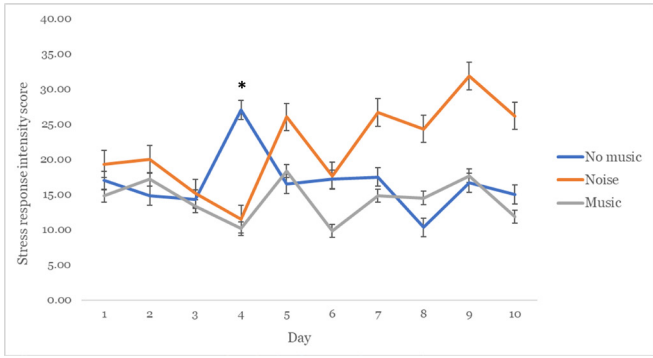

Figure 3: Evolution of the expression of stress behaviors (mean occurrence weighted by the duration of the trial) in response to sudden stimuli in the different conditions day after day. Permutation test, * $p < 0.05$.

4. Discussion

4.1. Benefits of Music Diffusion

In the overall analysis, our results reveal a calming impact of music on the behavioral and physiological components (i.e., heart rate) of horses. This effect is reflected by a decrease in the expression of stress responses of horses to visual stimuli when music was played compared to the absence of music. Interestingly, intermediate levels of stress were observed in case of pink noise, as the behavioral expression in the pink noise condition did not differ from the music or the no-music conditions. Such a gradient of efficiency between the conditions, going from the no-music, the pink noise to the music condition, suggests that the calming effect of music could be explained both by its auditory (such as the pink noise) and musical nature (unlike the pink noise). The auditory stimulation provided by music or pink noise could have distracted the horses from the environmental stimuli (here the visual ones). However, the fact that the behavioral expression differed from the no-music condition only in case of music indeed suggests that the musical features of the music could have an additional calming impact on the horses. The fact that lower heart rates during and after the tests were only observed in the music condition reinforces this hypothesis.

These results confirm previous observations concerning the ability of music to alleviate horses' reactions in a stressful context **[16–18]** and other studies in non-stressful conditions **[8–11]**. Moreover, horses faced with a stressful situation had a lower heart rate in the music condition compared to those in the non-music or noise conditions, supporting the hypothesis of a calming effect of music as already found in rodents **[7]**. Furthermore, we can hypothesize that the beneficial effects of music on a stress state have a long-lasting impact since the effect continued after exposure to the stressful situation.

As regards the difference in responses to sudden stimuli between individuals in the music and no-music condition, the results indicate that music had no effect on the response of individuals to unexpectedness. However, we observed a higher expression of stress for individuals within the noise condition. An explanation could be that pink noise at constant intensity is a monotonous sound that could reduce the alertness of the horse. If this is indeed the case, the horse's reaction when confronted with a sudden stimulus would be stronger than reactions in the no-music condition.

4.2. Evolution of the Effects of Music over Days

The three conditions were tested on routes that were different across days. The calming effect of music was not observed every day, but significant differences were observed between music and no-music conditions on day 4 and day 7 specifically. This result suggests that no habituation occurred until after at least seven exposures. Furthermore, the stress response curves for the visual stimuli had a sawtooth progression that was present for all conditions **(Figure 2)**, showing that we succeeded in limiting habituation to the stressful situation. It is however important to note that music and noise conditions led to similar responses. Conversely, the stress response to sudden stimuli seems to decrease with each exposure, suggesting habituation to this category of stimuli. While some individuals faced the stimuli despite their apprehension, others adopted a strategy of sensory subtraction, i.e., they stood still during the walks and turned away (in terms of their gaze, their attention, and sometimes even their entire body) from the stimulus that the experimenter was inviting them to encounter. As the variations in heart rate due to the presentation of the different stimuli were punctual and the strategies adopted by the horses in response to stress were multiple, a more targeted analysis of the heart rates at the precise moments of the presentation of each stimulus should therefore help us to clarify our question of the impact of music on the individual's state of stress and identify learning phenomena such as habituation that could be involved as a consequence of its use.

5. Conclusion and Perspectives

This study showed that the use of music did not interfere with awareness of and response to environmental noise, which is crucial in case of real danger. The lack of a calming effect in the specific case of auditory stressors could be explained by the fact that auditory stressors might have directly disrupted the perception of the auditory features of the music or pink noise because they are all perceived via the same sense (i.e., hearing). Indeed, as auditory stressors were sudden and intense sounds – such as the sound of a sports motorbike engine or a car horn – they were probably perceived and processed as a priority by the horse in order to face the potential threat they represent. In other words, the attentional

focus might have switched from the music or pink noise to the auditory stressors.

The stressful situation we chose was leading the horses along a track on which they were subjected to unexpected and potentially stressful stimuli designed to simulate real situations encountered by domestic horses. Indeed, in their daily life, domestic horses are confronted with the sudden appearance of objects of all shapes and colors; they may come across cars that have varying levels of noise and speed with drivers who may honk their horns or dogs that may show aggression and bark, etc. [12–14]. All of these situations may lead to the expression of fear, and to flight responses that cause many accidents involving both the horse and humans in the vicinity. Thus, our results can contribute to making human-horse interactions safer by limiting the stress of horses within their environment.

Regarding habituation to music for managing daily stress, we have shown that this tool continues to reduce the behavioral expression of stress even after seven consecutive exposures. However, a clear significant effect was not found every day. It could be explained by slight variations in the intensity of the stimuli perceived by the horse, especially as our experimental situations were not designed to be highly intense but mild, making day-to-day comparisons less powerful than the global analyses discussed earlier. It is also possible that the use of various music tracks could have had a higher and more consistent effect. Moreover, continuous playing of music in the living environment has been shown to lose its calming effect over time [11]. It suggests that playing music continuously throughout the day, as often seen in stables, for instance, might be counterproductive. Altogether, those elements that playing music could indeed be used to mitigate the negative impact of acutely stressful situations, especially when they possess a visual component. However, to keep the music's efficiency, it should be used parsimoniously to target time-limited events. Further investigation is needed to understand the underlying perceptual and emotional mechanisms of the impact of music on horses and to ensure safe and consistently efficient use on the field.

Supplementary Materials

Supplementary Material 1 includes pictures depicting the evolution of the "framed surface" stimulus in the different routes, ranging from the 1st version (on the left) to the 3rd version (on the right). **Supplementary Material 2** provides definitions of the various behaviors.

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Authors' Contributions

M.V., O.P., and C.E. designed the experiments; C.E. and M.C. conducted the experiments; C.E., M.V., O.P., and O.A. discussed the results and interpretations; O.P., C.E., and M.V. wrote the manuscript.

Data Availability

Data are available upon request from the authors.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

All the experiments undertaken in this study complied with the ethical standards of French research practices. The authorization number is AL/25/09/18/02 for the Regional Ethical Comity for Animal Experimentation (CREMEAS). Neither procedure adversely affected the horses in the short term or for the overall period of the study. Additionally, the authors confirm that the study has followed the guidelines of the Declaration of Helsinki.

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