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Assessment of stress levels in trotting horses in conjunction with age, performance and management

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Abstract

Young Standardbred racehorses start their careers as two-year-old, when their muscular-skeletal system is still growing. Racehorses end their careers earlier than most other performance horses. They have injuries related to the high speeds they travel and the intensity of the training as well as the repercussions from stress, such as a high prevalence of gastric ulcer. It is not completely understood whether this is caused by the early and demanding start into training, but research indicates that there is no positive correlation, and that early training might even be beneficial for a longer and sounder career. However, it remains unclear how this affects stress levels and performance of young racehorses.

We hypothesised that young horses that start training early show more signs of stress compared to older horses and that stress negatively affects the performance of Standardbred racehorses.

Horses (n = 45) of different age groups (two to eleven years) from three successful German Standardbred trainers were assessed by their behavior towards humans in the stall, their acceptance of harnessing and their willingness in training. This assessment was combined with a salivary cortisol concentration measurement and correlated with the horses' performance.

In this study no difference was found between the age groups regarding salivary cortisol concentrations and behavior. There was a significant difference between the three trainers regarding the behavior assessment, but this did not affect the horses' performance.

We conclude that young Standardbred racehorses appear to have similar stress levels as older ones have whereas environmental factor play a more important role than age, since horses in quieter, stables in rural areas with more pasture time showed less signs of stress.

Zusammenfassung

Junge Trabrennpferde starten Ihre Karriere als Zweij hrige, w hrend ihr muskuloskelettales System noch im Wachstum ist. Rennpferde beenden ihre Karriere r als die meisten Sportpferde. Typische Verletzungen stehen in Zusammenhang mit der hohen Geschwindigkeit und der großen Belastung im Training sowie den Auswirkungen von Stress, wie zum Beispiel die Entstehung von Magengeschw n. Ob es einen Zusammenhang zwischen dem n Trainingsbeginn, der Intensit t des Trainings und der Verletzungsinzidenz gibt, ist noch nicht vollst ndig gekl rt. Allerdings haben bisherige Untersuchungen keine positiven Korrelationen ergeben, sondern eher das Gegenteil; ein r Trainingsbeginn scheint eher eine l ngere und ges re Karriere zu fördern. Wie das das Stressniveau und die Leistung junger Rennpferde beeinflusst ist jedoch noch nicht gekl rt.

Unsere Hypothese war, dass junge Pferde, die h mit dem Training starten, mehr Stress zeigen als ltere Pferde und dass Stress die Leistung von Trabrennpferden negativ beeinflusst. Um dies zu untersuchen wurden Pferde (n = 45) verschiedener Altersgruppen (zwei bis elf Jahre alt) von drei erfolgreichen deutschen Trabertrainern hinsichtlich ihres Verhaltens gege r Menschen im Stall, der, Kooperationswilligkeit und der Leistungsbereitschaft beim Training beurteilt. Dies wurde mit Speichelcortisolmessungen kombiniert und im Ve ltnis zur Leistung der Pferde betrachtet.

Es wurde kein Unterschied im Verhalten und den Cortisollevel zwischen den Altersgruppen gefunden. Es gab einen signifikanten Unterschied zwischen den Trainern dieser war aber nicht ausschlaggebe ie Leistung der Pferde.

Wir kommen zu dem Schluss, dass junge Trabrennpferde hnliche Stressanzeichen wie ltere Pferde zeigen, wobei Umweltfaktoren eine größere Rolle spielen als das Alter, da Pferde in ruhigeren St llen in l ndlicher Umgebung, mit mehr Auslauf weniger Stressanzeichen zeigten.

1 Introduction

Before horses became companion animals "only", they were part of humans' daily life, in transport, agriculture and war. In the 16th and 17th century the harness horse was very important and there was a necessity to breed good horses for pulling carriages. These horses had to have good endurance and move at a uniform pace. This is best achieved at a trot, which led to selection of a new horse breed according to its ability to trot (1). To choose the best horses for breeding, races were held which formed the basis for today's harness racing.

Standardbred racing is heavily criticized by the public. The equine industry is being closely watched by animal welfare organizations. Their criticism of the sport varies, many condemn whipping, the use of animals for humans' entertainment and the stress the horses experience. Particularly the high level of performance, frequent and long transports to and from races and the tension during the race itself may contribute to the strain. Supporting this perspective, a notable prevalence of equine gastric ulcer syndrome (EGUS) is observed in high-performance horses, particularly in racehorses (2).

Cortisol serves as a widely recognized indicator of stress in animals, and its dynamics during competitive events, particularly in young horses, have been a subject of investigation. A research group in Spain conducted a study that shed light on cortisol levels in horses at rest and during competitions, comparing them to baseline values in non-competitive settings. The findings revealed that cortisol levels during competitions were notably higher than those observed at rest and exceeded the baseline established for non-competitive horses. Additionally, the study highlighted a trend associated with experience. Specifically, as horses matured and gained experience in competitive environments, a decrease in cortisol values was observed during competitions (3).

Although all equine sports are closely watched, racehorses are additionally singled out because their career starts earlier than in most other sport horses. Both Standardbred and Thoroughbred racehorses are mostly actively racing between two and five years of age. This is heavily criticized by the public, because the skeletal system is not completely developed in the young horses and may be damaged by the strain of intense training (4).

As an answer to the public discontent with the sport, the German Trot Association has prohibited tongue ties, made regulations about whipping over the years and has recently increased the age of horses competing in the German Trotting Derby, one of the most important races, from three to four years. Now horses and trainers have one year more to develop and prepare for the big race.

This might reduce stress for the equine athletes and thus also enhance their performance. In humans it was proven that swimmers were faster without being stressed before the race, compared to if they were exposed to acute psychological stress just before (5). In Standardbreds, a study showed that horses with lower cortisol levels before a race were more likely to win than those with higher values. In this study, blood cortisol values, taken five minutes before the warm-up were lower in older, more experienced horses (6). While stress before and during a race constitutes only a brief episode in a horse's life, its impact can be substantial. This prompts the intriguing question: How does the day-to-day stress experienced by horses influence their racing performance?

Therefore, we aimed to investigate the stress level of Standardbred horses of different ages and training status by assessing their behavior and measuring cortisol levels in the saliva. Our first hypothesis was, that young horses that start training early show more signs of stress compared to older horses, which might be due to their inexperience and the short time they had to get used to the environment, training schedules and sporting events. Secondly, we hypothesized that stress negatively affects the performance of harness racing horses.

2 Literature review

2.1 Training of trotting horses

Over the last decades the horses have become more athletic, intelligent and innately faster, with a better predisposition for gaits. The conformation changed from a heavy boned horse to a more refined one, with higher withers, longer legs and finer bones, creating faster horses (7). The work of the trainer has shifted more towards being a conditioner. Horses tend to be fast already and easier to gait, making fitness and soundness the most important job of the trainer, while maintaining a good attitude and the willingness to work (7).

Yearlings start their training when they are about a year and a half old (8). After that they are schooled for about 7 to 9 months before their first race (7). First, they are introduced to the harness and bridle, then line driven at a walk. When they are comfortable with that, they will be hitched and jogged on the track. They will start out with 1.5 - 3 km per day and then incrementally increase the distance (8). They start out going clockwise, which is opposite to the direction they will be racing on most tracks and as they increase in speed and improve their stamina, some speed sessions will be built into training, which then will be counter-clockwise (7). Quite soon, hobbles and shoeing will be fitted on the horse, to encourage them to train in the best gait possible (8).

Many trainers have a similar schedule of training their horses. Twice a week they work at a jog and twice at a faster pace. The other days the horses have off or go in the walker. This keeps similar throughout the years of training, but the relation between the two different excercise types changes: at first the horses do less jogging because they have less stamina, but this starts to shift over the months of training. At the beginning of training they are also trotted slower in the fast training units than the older horses (10). When compared with human athletes that run 1500 m, trotting horses train over less distance in a month but do more fast training units. The hypothesis behind this is that the heart rate frequency in horses in training can be higher than in humans to get a conditioning effect (10).

Some trainers also train in heats, where they do two fast sessions in a day, with a 60 minutes break in between. Well trained horses should be as fast in their second session as they were in the first (8).

2.2 Career of a Standardbred racehorse

Many Standardbred horses start their racing career as two year olds (11) which means that they start harness training as yearlings. The horses used to come straight from the fields. Nowadays young horses are schooled before going to the trainer, preparing them better for the training that awaits them when they start being harnessed (7). This means that they are quicker to go through the breaking process and get into race training earlier.

At the beginning of their careers, all Standardbreds have to complete a qualification before starting in their first race. This also called the standard, which is the minimum requirement for starting as a trotter or pacer. An early standard was 2:30 minutes for a mile, but as horses became faster it was adapted (8). In the United States, pacers have to run the mile in 2:06 and trotters in 2:05 minutes (12). In Germany, two year olds have to achieve a minimal "Kilometertime" of 1:25.0 minutes over 1600 m. As three year olds or older, they have to run the same speed as the two year olds, but over a 1900 m distance (13). To maintain their qualification for races they have to run at least every six months. If they have not raced for over six months, they have to requalify (13).

Most Standardbred racehorses race between two and six years of age. In a Canadian study, only 33% of the horses that started as two year olds were still racing at eight years of age (14). The numbers are even lower for horses that start racing later. Only 23% of horses that started their first race as three year olds and 16% of those that started racing as four year olds were still racing at the age of eight (14). In comparison to riding horses which are mostly broken in as three year olds, Standardbreds are started and raced a lot earlier, putting strain on a still growing body (15). Even though Standardbreds are a fast developing breed, they are not fully developed by the time they start training. Young Standardbred race horses tend to have longer and more successful careers when they are raced as two year olds, even when the results from the first year are not taken into consideration (14). However, it is important to state that horses that race later normally do so because they cannot compete earlier because of injury, late development or other health issues. It is costly to bring up a young horse, so owners want to see results as early as possible, making it a poor economical choice to wait with training and racing if the horse is sound (11).

2.3 Common diseases in Standardbreds

2.3.1 Injuries of the muscoskeletal system

In humans, early high levels of training can lead to skeletal diseases (16). In children and young adults high athletic performances increase the risk of developing osteochondrosis dissecans (OCD) (16) and it is believed to be the same in horses, even though a study has shown that exercise can be beneficial for skeletal health, even in young horses (17).

Horses that start racing younger seem to have less injuries. An Australian study showed that 77% of horses that raced with still open epiphysial plates remained sound, while only 55-56% of horses with closed or intermediate ephyseal plates stayed sound (15). Similar results were shown for the superficial digital flexor tendon in foals, which can still adapt to strain and increase in diameter, but even at the age of 19 months it shows no reaction to training. This would indicate that light forms of training at a young age are rather beneficial for tendon health. The deep digital flexor tendon can still enlarge and strengthen to withstand the new workload, even later in life (18).

According to Wolff´s law bones get stronger under strain and the cross section of long bones increases (18). It has also been observed that the risk of fracture is higher in older horses (19). The most common causes for lameness in young Standardbred racehorses are OCD, splint exostoses and curbs. Splints happen when horses have difficulties learning the racing gait and by making breaks, and particularly pacers hit themselves by breaking because galloping is difficult with hubbles (20). Interferance injuries in trotters ocurr when the toe of the ipsilateral front foot strikes the shin, pastern or coronary band of the hindlimb. Pacers on the other hand, strike the medial aspects of their forlimbs together (20). Curbs often develop in two year olds as they begin speed training (9).

Standardbred racehorses in general have a predisposition for foot pain. They run on hard ground and often present a long-toe and short-heel foot conformation which predisposes them for bruises and corns (9). The hard and uneven ground, sometimes paired with a bad medio-lateral foot balance, makes Standardbreds more susceptible to stress-related fractures of the distal phalanx. This is more common in aged horses (20).

Carpal lameness is one of the most common reasons for one month or more of rest (9) and is the most important career-limiting cause of lamness in trotters (20). In a study with 114 horses entering their first year of racing, 28% developed a carpal lameness and carpal lameness was present in 56% of all forelimb lameness. Poor forelimb conformation and intense speed training was predisposing (21).

The hock undergoes increased stress during intense exercise, especially when driving turns and experiencing rapid accelerations. This heightened strain has the potential to contribute to the development of osteoarthrosis and fractures in the area (20).
Suspensory desmitis is the most common soft tissue injury. Most of the time it is caused by an overload over weeks or months. The hindlimb suspensory desmitis is the second most common career-limiting cause of lameness, it has a better prognosis in pacers than in trotters (20).

2.3.2 Gastrointestinal diseases

In humans and in horses, stress has been found to be a cause for the development of gastric ulcer (22, 2). Environmental stressors, coupled with limited social contact that is common in many competition stables, are also recognized as contributors to the etiology of EGUS. However, it is essential to note that these stress-related factors, though significant, do not offer exclusive explanations for the heightened prevalence of gastric ulcers in racehorses. Additional considerations encompass the impact of high speeds in both training and competition, potentially influencing gastric health (2).

2.4 Behavior

Domestication is generally accepted to be a fluid process. The first remains of horses being used for meat date back to 5000 before Christ (BC) in Europe, while horse riding seems to have its origin east of the Ural mountains 3500-3000 BC. By the middle of the second millennium BC, horses were already widely used to pull chariots (23). There are no more wild horses outside of zoos and wildlife conservations, but there are large groups of feral horses. The successful adaptation of domestic horses in the wild suggests that the behavior of horses is relatively unaltered from the ancestral state, which means that they are still adapted to their original natural habitat and social structure. Horses are meant to graze for the largest part of their day and live in herds. This is mostly not possible in modern horse keeping, and some horses that struggle with this environment develop sterotypies (24). Even though they seem well-adapted to wild conditions, the domestic horse fulfils all the critera for domesticated animals (25).
Stereotypies are repetitive non varying behavioral patterns that seem to have no function (26). In a four year retrospective study with 225 Thoroughbred and part-Thoroughbred horses, 34.7% showed abnormal behavior. Horses housed in barns while weaning and after had an increased insidence of sterotypies compared to horses with acces to paddocks or fields (27). It

can be seen as an attempt to adapt to modern husbandry, or the failure of this adaptation (26). For example crib eating and windsucking might be a way to help regulate stress or a way to gain more control over their environment (26). It can also be an expression of natural behavior, for example bed eating, which is mostly seen in horses that are fed small forage quantities (26). If these repetitive behaviors are prevented, they try to do the same movement in a more restricted version or stress increases. This however, does not prove that stereotypies are caused by stress (26). Social interactions have been shown to reduce the incidence of sterotypies (25). As herd animals, horses feel safer in a large group (25). Although horses are social animals, owners are afraid of injury and keeping horses in stalls is often more convinent for humans. The horses that are probably most socially isolated are stallions. Many show behavioral disorders, stereotypies or abnormal behavior during mating or semen collection, which are not seen in feral stallions (25).

Like other flight animals, horses are neophobic, which means that they fear the new (28). This is beneficial as a prey animal, because everything new can potentially be a threat, but it contributes to challenges in horse training. Specially for racing and competition horses, that are expected to achieve high performances in a sometimes loud and distracting environment (24). But luckily they habituate easily to objects they previously avoided (29). This was also shown in a study that analyzed blood cortisol concentrations in competition horses. They found that horses that were less experienced had higher blood cortisol concentrations than their more experienced counterparts (3).

2.5 Stress

2.5.1 Impact of stress on physiology and psychological health

Acute stress in humans improves immunity against pathogens, but chronic stress has the opposite effect; it downregulates the immune response. Chronically stressed people are also more susceptible to inflammatory disorders (30) and stress, both physical and emotional, has been linked to the development of type II diabetes (31). There has also been research supporting the notion that persistent and chronic stress exposure might contribute to tumor initiation in specific cancers (32). In animals, this has not been investigated as intensely, but physiological changes have been reported as well.

Wild animals in captivity, that are under chronic stress, exhibit weight loss, glucocorticoid and adrenomedullary regulation changes, a compromised immune function and altered reproductive behavior (33). Our farm animals are also affected by stress. This altered state is a limiting factor for animal productivity and negatively affects health if they are not able to adapt to the circumstances (34).

In horses, performance is influenced by genetics, adaptation to anaerobic exercise and effective heat dissipation capacity as well as external factors. The most dominant external factors are nutrition, husbandry and the quality of the rider and coach. Besides, there are other factors such as stress that influence performance (35). In a study investigating how horses' and riders' stress influenced performance, it was found that horses with a higher blood cortisol concentration performed better, while riders with high cortisol levels seemed to do worse. It is important to note that riders' cortisol levels rose a lot higher that those of the horses and that for horses the observed 160 % increase might still be under the threshold of what becomes a distress (36).

Stress also influences learning. Horses with higher salivary cortisol concentrations were found to need more repetitions to learn something new. A study with three groups, one that rested, one that exercised and one that was stressed before a learning session, found that horses that had exercised before the learning session were faster to learn, although their cortisol concentration right after excersise was higher than those in the groups that rested or were stressed. But their cortisol was much lower at the end of the learning session. This means that their blood cortisol concentrations were lower for the largest part of the training than in the other two groups (37). This might indicate that even though cortisol concentrations rise while exercising it might not be a psychological stressor for the horse as it does not compromise the ability to learn. More research is necessary in this field.

2.5.2 Quantification of stress

For research in animals, there was a need to quantify stress without being able to talk to the subject and because of the diversity of research projects, that take place in different settings, a variety of methods has been developed. One of the most frequently used methods is the measurement of the cortisol concentration, which can be done in different biomatrices. The sample material can be divided into three types: fluid (blood, saliva, urine, milk), semid-solid (earwax, bone marrow, feces) and solid (hair, wool, feathers, nails, scales). Fluid matrices can be used to obtain information about the stress level during the last minutes. Blood is the most eminent, followed by saliva and finally urine. Bone marrow is only convienent for dead animals, so it is not broadly used. Earwax has been used in recent studies, but it has not been widely used, in contrast to feces which has been used a lot, but has the disadvantage that there are many substances in it that can cause false results (38).

The solid matrices are used to determine chronic stress. Hair accumulates cortisol according to its concentration in the blood during hair growth phases. But it is also exposed to the elements which can influence the results (38).

The variability of heart rate has also been shown to be a reliable method to assess chronic stress (39) as well as eye temperature (40) and muscle tension measurement via surface electromyography (EMG) (41).

3 Materials and Methods

3.1 Animals

In this study, 45 Standardbred racehorses from three German trainers were assessed twice during the racing season 2022 (trainer A: N = 14 horses, trainer B: N = 10 horses, and trainer C: N = 21 horses). The age distribution of the horses is detailed in Table 1.

Horses were randomly selected from those present at the training facilities on the day of the first assessment. Training programs were tailored to age and training status, with horses trained four times a week - twice at a slower pace and twice at a faster pace. Of the 45 horses, 38 participated in the 2022 racing season.

Tab.1 Age distribution of the 45 horses in this study

Trainer	Total	2-year-olds	3-year-olds	4-year-olds	5-year-olds	5+ years old	Median age	Age range
A	14	4	5	2	1	2*[1]	3	2-11
B	10	3	4	2	1	0	3	2-5
C	21	0	8	6	6	1*[2]	4	3-8
Total	45	7	17	10	8	3	3	2-11

*[1] Eight and eleven years-old
*[2] Eight years old

The horses from the three trainers were housed in different environments. Trainer A trains on an active racetrack, the yard is busy all day long and the horses had only occasional paddock time until new paddocks were built a month before the second assessment. Then the horses were outside every night. Trainers B and C are located in more rural areas. Trainer B has fewer staff, and the stables are quieter than the others. The horses go out on big sand pens in groups of three or four. Only a few horses go out alone. They have a racetrack next to the stable and do the slow trainings on a path through the forest which they share with other forest users.

Trainer C trains the horses on a track a few hundred meters away from the stable. On the way to the track horses are also confronted with other users of the path such as pedestrians and riders. They have daily paddock time either in the morning or in the afternoon.

3.2 Behavior assessment

To assess the strain horses are undergoing and the influence of training and age, the behavior of the horses was assessed both from afar as well as in interaction with humans. This assessment was done in spring 2022 and repeated during the racing season (summer 2022).

The assessment of the horses' behavior towards humans in their stall was done at three distances:

1) Standing four meters away from the box (trying not to be seen by the horse and drawing no attention towards the assessor)
2) Standing by the door
3) Approaching the horse in the stall

The horses' behavior was graded from zero to four, according to their behavior towards the examiner and their behavior while being driven (Table 2). The criteria are detailed below.
This test was done by the same person, that all horses did not know at the time of the first assessment. All contact was brief and there was no significant time for them to get to know the assessor.

In stall assessment protocol:
From furthest away (about four meters):
Overall behavior of the horse while being alone in the box (grading is given in brackets)

(4) Relaxed, eating, sleeping or looking out
(3) Slightly nervous, tense posture
(2) Nervous, whinnying, stops eating frequently
(1) Stereotypies (box walking, eating wood, cribbing, weaving, headshaking)

The person stands at the (closed) door of the stall for 30 seconds, after 10 seconds talking to the horse to get attention, after that, wait and observe its behavior.

(4) The horse comes to the door with ears forward.
(3) The horse looks at the person at the door but stays back.
(2) The horse stays back and puts its ears back.
(1) The horse comes at the door with ears back.

The next test is done by going into the stall and approaching the horse, with the arm stretched out at a 90° angle and having the body turned 45° away from the horse, approaching at the shoulder.

(4) The horse comes towards the person, with ears forward, stays while being touched.
(3) The horse has ears forward and lets the person approach and touch it.
(2) The horse has ears back but lets the person touch it.
(1) The horse has ears back and doesn't let the person approach.

Tab. 2 Behavior assessment sheet

	4	3	2	1	0
Behaviour from afar					
Behaviour at door					
Respiration rate					
Behaviour with contact					
Pulse rate					
Condition of hooves/skin					
Body condition					
Behaviour when tacking up					
Behaviour while harnessing/training					

Once in contact with the horses they were stroked, and their pulse rate was measured at the *Arteria facialis*. After that they underwent a swift physical examination to evaluate the body condition, the skin and hooves and to look for skin lesions or alopetic regions.

The condition of the hooves and skin was also rated from zero to four:

(4) Very good condition

(3) Good condition

(2) Poor condition

(1) Extremely poor condition

Lesions and alopetic regions were drawn into horse shapes, marking the exact location (Fig. 1).

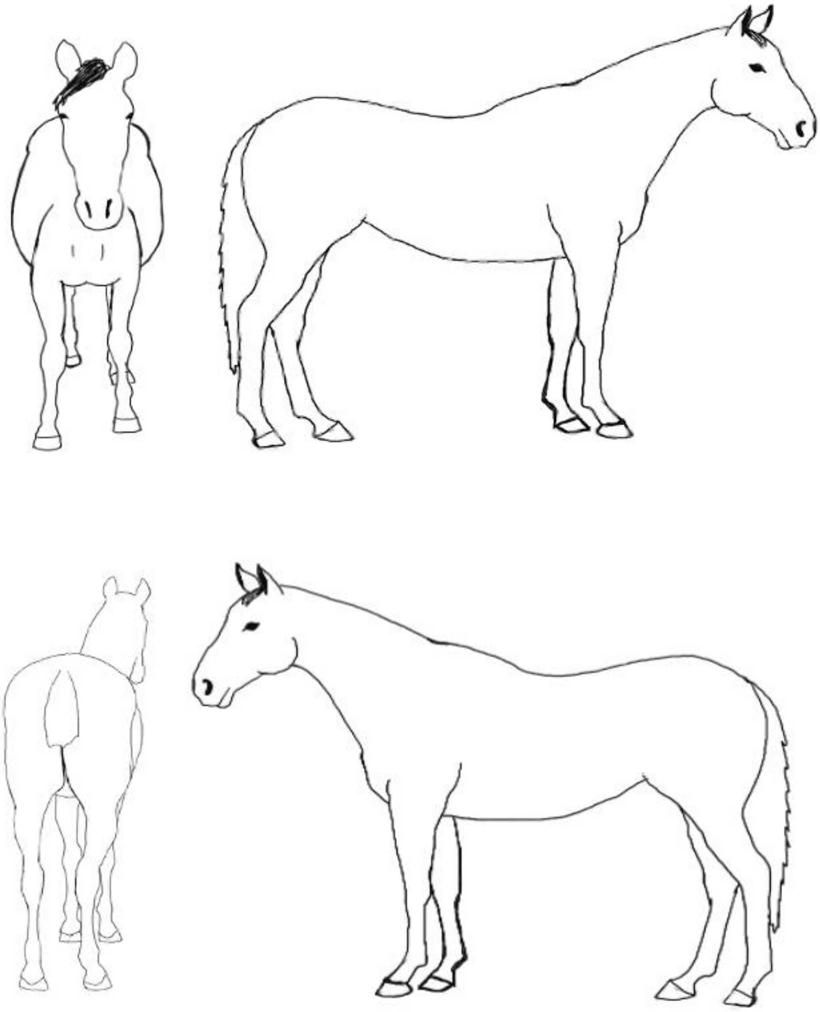

Fig. 1: Lesion diagram

Finally, the horses' behavior at work was assessed.

Assessment while tacking up:

(4) Horse is calm and shows no sign of discomfort while being tacked up.

(3) The horse is mildly nervous but shows no adverse behavior.

(2) The horse is moderately nervous and shows some discomfort.

(1) The horse is nervous and shows adverse behavior when being tacked up.

Adverse behavior was defined as biting, putting ears back or kicking.

Assessment while driving:

(4) Horse is calm and obeys to the aids from the driver.

(3) Horse is nervous or mildly disobedient.

(2) Horse is nervous and mildly disobedient or is moderately disobedient.

(1) Horse is nervous and severely disobedient.

This was assessed by watching the horses in training and asking the drivers, as not everything is visible from afar and the drivers know the horses very well. The scoring system was described to them and then a mean value between their score and the one from the assessor was given to each horse.

In the end, all behavior scores were summed up for each horse and assessment day. A high score indicates a relaxed horse willing to work and a good horse-human interaction.

3.3 Cortisol measurement

Saliva swabs were taken during the second assessment of the horses in summer right after their behavioral examination. Some horses had already trained at that time while others were trained later that day. The collection of saliva swabs was approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna in accordance with the University's guidelines for Good Scientific Practice.

The swabs were taken with Salivette® for cortisol determination from Sarstedt (N brecht, Germany). They were kept cold in a cooling bag with ice for transport. After that they were frozen at -20°C until measurement. Saliva samples underwent analysis utilizing a cortisol enzyme immunoassay (EIA), employing an assay antibody specifically designed against cortisol-3-CMO: BSA (42).

3.4 Racing data

Information on all horses was retrieved from the German Trotting Association online portal (43). This data encompassed annual and total earnings, the fastest run, first qualification, race placements, and the current racing status of activity. The study considered all results up until September 30th, 2023. From the detailed list of races each horse ran, earnings were assigned to each age group. For example, a horse that is five years old will be in the list for the first to fourth year of racing each time with the earnings they achieved in that year and their total earnings until the end of the observation period, or their retirement from racing.

3.5 Statistical Analysis

The statistical analysis was done with SigmaPlot 14.5 (Systat Software, Inc., Germany). A one-way Analysis of Variance (ANOVA) was applied to test for potential differences among the three trainers or age groups. First the Shapiro-Wilk test was employed to assess the normality of the data. The results determined whether the data met the assumption of normal distribution. If this was true, a Brown-Forsythe test was conducted to assess the equality of variances among the groups. This test examined whether the assumption of equal variances was met. Post-hoc analyses, using the Holm-Sidak and Dunn's method to control for Type I error, were performed to identify specific group differences. Pairwise comparisons were conducted between the groups to explore potential variations. Last, a power analysis was carried out to assess the sensitivity of the statistical test. The calculated power of the test, with an alpha level of 0.050, provided insight into the likelihood of detecting true effects. Correlation analysis was performed using a Pearson Product Moment Correlation.

4 Results

4.1 Behavior

There was a significant difference in the horses' behavioral assessment scores between trainer A (median $(\tilde{x})_{spring}$ = 14.00; $(\tilde{x})_{summer}$ = 15.50) and trainer B ($(\tilde{x})_{spring}$ = 20.00; $(\tilde{x})_{summer}$ = 18.00) in summer and spring, while there was only a statistically significant difference between trainer A and C ($(\tilde{x})_{spring}$ = 18.00; $(\tilde{x})_{summer}$ = 18.00) in spring. There was no difference between trainers B and C at any time point (Fig. 2).

A negative correlation between the behavior in summer and total earnings was found (correlation coefficient (r) = -0.376; p = 0.0484), and the behavior in spring (r = -0.400; p = 0.0191) and summer (r = -0.0466; p = 0.0218) negatively correlated with the earnings in the second year as racehorses.

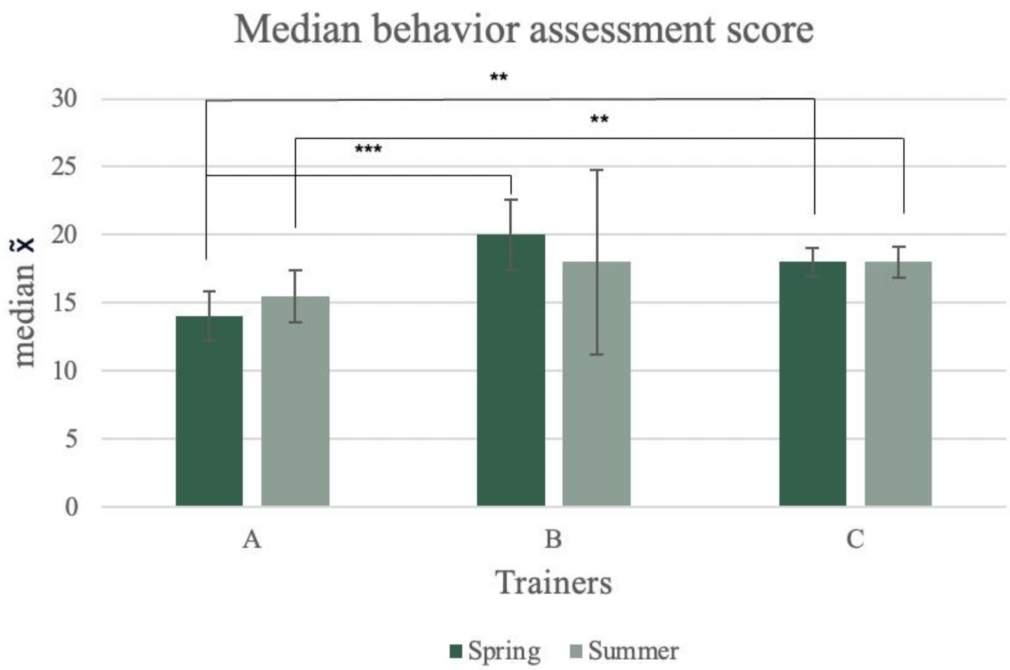

Fig. 2: Behavior assessment scores in horses located at three different training facilities at two different points, spring (dark green) and summer (light green). The bars show medians and standard deviation. **p <0.01, ***p < 0.001

There was no statistically significant difference between total behavior (the sum of the scores in spring and summer) among the different age groups (p = 0.587, Fig. 3).

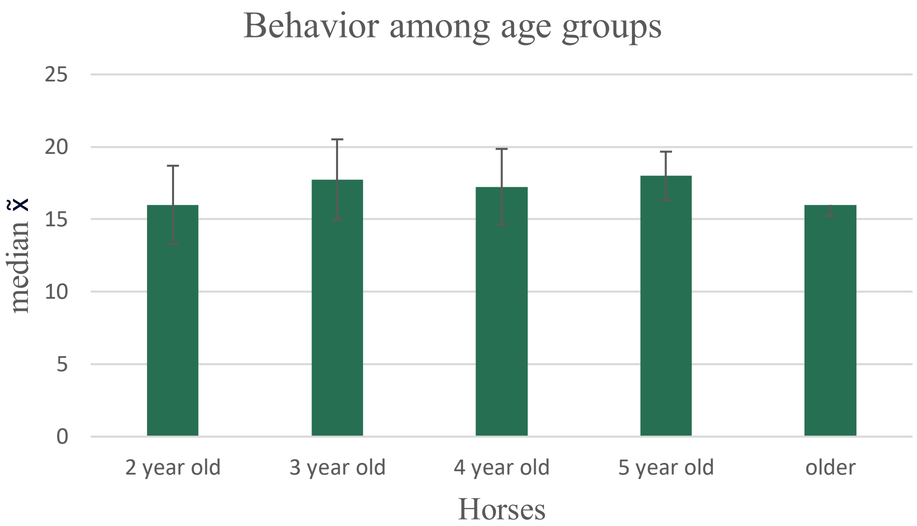

Fig. 3: Comparison of behavioral scores between age groups. The bars show medians and standard deviation. There was no statistically significant difference between the groups ($p = 0.587$).

4.2 Cortisol measurement

There was no statistically significant difference regarding the cortisol levels in the horses' saliva between the three trainers ($p = 0.132$, Fig. 4).

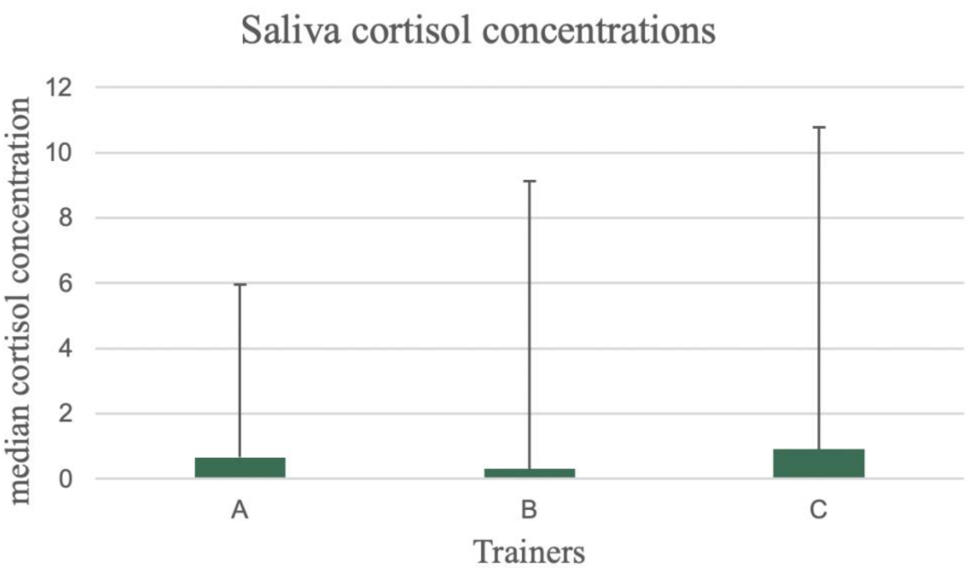

Fig. 4: Median saliva cortisol concentrations in horses at three different training facilities (trainer A, B and C). The bars show medians and standard deviation.

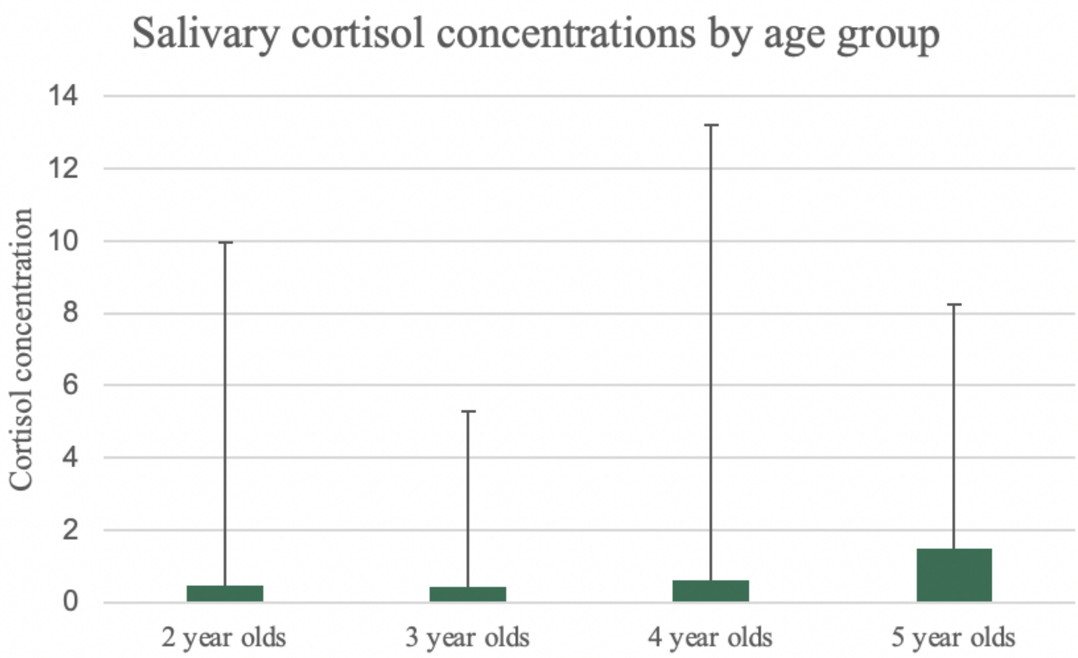

Fig. 5: Median saliva cortisol concentrations by age groups. There was no statistically significant difference between the groups (p = 0,795).

No correlation between behavior and cortisol concentration was found (r_{spring}= 0.151; p_{spring} = 0.410; r_{summer} = 0.0634; p_{summer} = 0.730).
There was also no correlation between cortisol concentrations and total earnings (r = 0.186; p=0.344), speed (r = 0.0136; p = 0.945) nor age (r = 0.0958; p = 0.596).

4.3 Racing data

In the first year of racing (when the horses are two years old), trainer B earned significantly less than the two other trainers (p = 0.003, Table 3, Figure 6). There was no statistically significant difference between trainers A and C.
In the second and third year, as well as in the total earnings, no statistically significant differences were found among the three trainers.

Tab. 3: Median earnings for trainers A, B and C in Euros for each age cohort.

	1st year x̃	2nd year x̃	3rd year x̃	Total earnings
A	1460.00 €	5800.00 €	12792.00 €	14700.00 €
B	0.00 €	3010.00 €	11165.00 €	12740.00 €
C	1660.00 €	4100.00 €	10350.00 €	14030.00 €

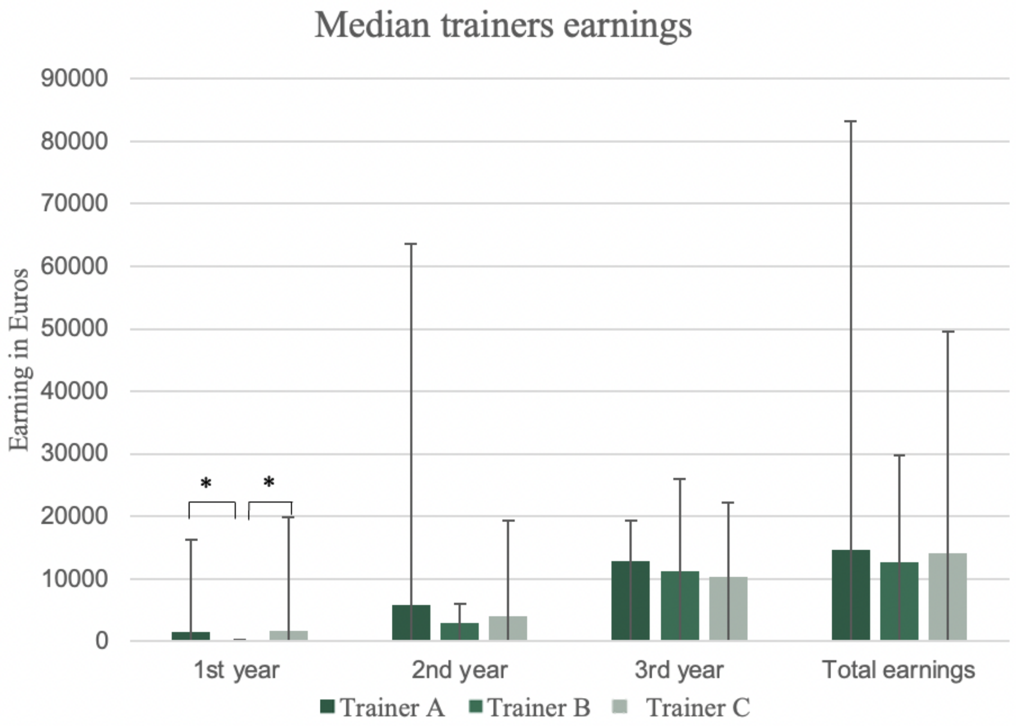

Fig. 6: Median trainer earnings classified by age.

Trainer B earned significantly less than trainers A and C in the first year of racing (*p = 0.008). The bars show medians and standard deviation.

Horses that won more in their first year of racing also did so in their second year (r = 0.717; p < 0.001), but good performance in the first two years was not correlated with high earnings in the third year (r = 0.0560; p = 0.820).

Age (r = 0.355; p = 0.0289) and horses racing for four or more years (r = 0.760; p = 0.0107) was positively correlated to higher total earnings.

No correlation between speed and total earnings was found (r = -0.233; p = 0.166).

There was also no correlation between total earnings (r = 0.153; p = 0.345), cortisol (r = 0.0201; p = 0.918), behavior (r = 0.0249; p = 0.896) and the age the horses first started racing.

4.4 Lesions, skin and hoof condition

All horses had good skin and hoof conditions. There was no statistical difference between the age groups regarding skin and hoof condition (Figure 7). The median values for each trainer were A = 3.75, B = 3.75 and C = 4 and there was also no statistically significant difference between the trainers (Figure 8).

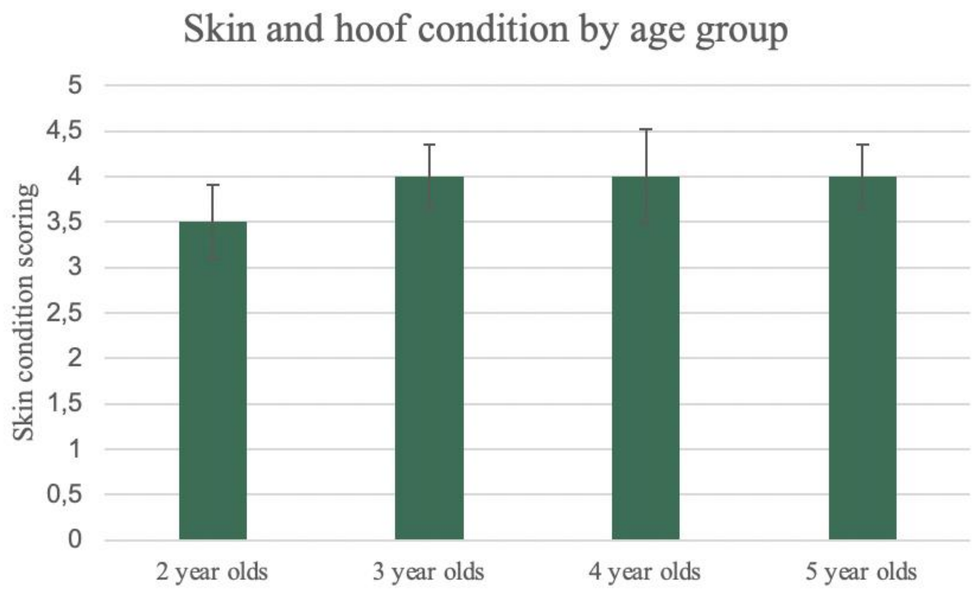

Fig. 7: Skin and hoof condition per age group. There was no statistically significant difference between the age groups.

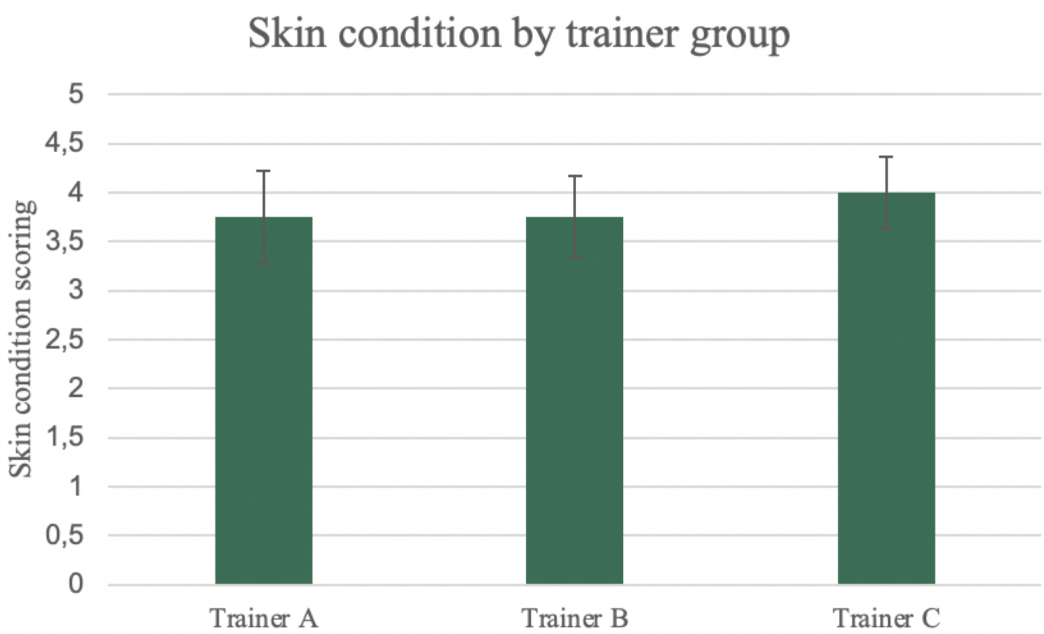

Fig. 8: Skin and hoof condition by trainer group. There was no statistically significant difference between the trainers.

The lesions found were old or only superficial (Table 4). Most injuries occurred to the hind fetlocks (n = 11), then to the metacarpal/metatarsal region (n = 6). There were also some cuts and alopecic regions on the shoulder (n = 1), flank (n = 1), chest (n = 2), head (n = 3), carpus (n = 3) and girth region (n = 2).

Tab. 4: Number of lesions found on the horses of each trainer (if the horse had two lesions, it was counted as two).

Trainer	Number of lesions	Horses assessed	Percentage of lesions
A	13	14	93%
B	2	10	20%
C	15	21	71%

5 Discussion

This study aimed to examine signs of stress in harness racing horses, particularly concerning the timing of training initiation, behavioral signs and its impact on salivary cortisol concentrations. The initial hypothesis that early training in young horses might correlate with heightened stress levels, was contradicted by the observed data. Notably, Trainer B started racing his horses mostly as two-year-olds, while trainers A and C waited until they turned three. Although the difference was not statistically significant, the horses of trainer B had rather lower salivary cortisol concentrations than those from trainers A and C. In contrast, the behavioral assessment resulted in significantly lower scores in the horses from trainer A compared to trainers B and C.

There are many factors that have to be taken into consideration to explain these results, because stress can be the result of many environmental, physical or physiological challenges (35). One factor that comes straight into mind is that pasture or paddock time was different between the groups. Horses from trainer C had daily paddock time, they were turned out alone, but with horses in sight, some in the morning and others in the afternoon. Trainer B turned out the horses every day on big sand paddocks in groups. All two- and three-year-olds were turned out in groups of three or four, only some horses were turned out alone, because they didn't get along with others. Trainer A had very few paddocks at the beginning of the year and then build new ones for the summer. When the second assessment was done and the cortisol samples were taken, horses had been turned out every night for one month already. This might explain the slight increase in the behavioral assessment score for these horses in summer when compared with the results from spring. Even though this change was not statistically significant, it showed a tendency towards improvement. The horses from trainer B had the most contact with others and the most time outside, and they also proved to be the ones with the highest mean behavioral assessment score and lowest salivary cortisol concentrations, followed by the groups from trainer C and trainer A.

Something that was not considered in this study was how busy and noisy the stables were. At the days of assessment, the stable from trainer A was perceived to be very busy, with shouting and lots of people walking around. The other two stables appeared to be quieter, and although the stable from trainer C was busy, there was a lot less noise. The stable from trainer B was very quiet, with less staff. The consensus statement for EGUS from the European College of Internal Medicine states that even radio music can influence the occurrence of gastric ulcers positively, while people talking has a negative impact on the disease development (2).

The training environments were also very different between the groups. In contrast to the other two trainers, trainer A kept and trained the horses on an active race track in an urban setting, which is also listed as an contributing factor for EGUS (2). A likely interpretation is that horses find these kinds of environments unsettling which increases stress. These horses also only go to the track to train with very limited exposure to other stimuli. Although they will get to know that track well, not being exposed to other scenarios can make them more anxious about new environments. Horses from trainer B go through the woods for slow trainings, where they encounter riders, people, dogs, and wildlife. It is generally accepted in the equestrian community that horses benefit greatly from being exposed to those kinds of things, because it creates a more confident and calmer partner, both in sport and leisure. But trotting horses are not racing in this kind of environment. They have to achieve peak performance on a racetrack, to which the horses from trainer A are more used to. To determine which environment is better for racehorses, more research is necessary. Although in this study horses from trainer A had higher median earnings than trainer B's horses, the difference was not statistically significant. But horses from trainer B had higher behavioral scores, i.e., they had a friendlier attitude towards humans and were more cooperative in training. Together with the lower cortisol levels this indicates that horses from trainer B are less stressed. However, their performance is mildly lower than in the other two groups. Horses might perform better with higher cortisol concentrations as suggested by a study on equestrian athletes and their riders (36), although the opposite was shown in humans (36) and in learning equines (37).

It is also important to note that cortisol concentrations change with exercise. In humans it has been demonstrated that the blood cortisol concentration increases with high-intensity interval exercise (44). In horses exercise had a similar effect on blood cortisol concentrations, with an increase of about 6% (35). In this study, salivary cortisol was analyzed, which is mostly used to measure acute cortisol levels, so many factors in that specific day may have influenced the results. Some horses had already been trained, while others were not or were on a pasture. It is not possible to rule out that a horse might have gotten spooked before taking the sample. Hair might have been a better matrix for this study, although it also has limitations. It can be easily influenced by the elements. Most horses in this study regularly went into the sun and the samples were taken in summer. It was demonstrated that sunlight decreases the glucocorticoid concentration in hair (45). Even though all samples were taken in the morning, the samples were taken within a four-hour span, because the single assessor was not able to probe all horses in less time. This means that the circadian rhythm might have had influence on the results. Salivary cortisol only represents the free cortisol concentration in the blood, i.e., the values are lower than the ones expected by blood samples. In the literature different results on the correlation between serum and salivary cortisol concentration are reported. Some studies showed high correlations while others didn't find one (46). Peeters et al. (47) found a positive and significant saliva to cortisol correlation, but Pell and McGreevy (48) could not find a significant correlation between the two concentrations.

The discrepancy between cortisol concentrations and behavior assessed in this study raises the question if stress is inherently harmful or if there is a certain level of stress that is tolerated well. Some researchers hypothesize a potential distinction between stress and distress, proposing that horses may manage well even with mildly elevated cortisol concentrations, remaining within a stress threshold before transitioning into distress (3). Some horses had elevated cortisol concentrations, but their behavior was not changed, while others had lower cortisol concentrations but lower behavioral scores. This could mean that the threshold to distress might be different between individuals. It could also be an indication that behavior might not be the best parameter to assess stress in horses. As flight animals, horses try to hide much of their fear and pain as a means of survival. They have also undergone training, in which they were taught not to show adverse behavior, making it more unlikely for them to show it (49). Still, some horses showed adverse behavior. A possible explanation could be that these horses are less trained, trainable or so stressed that they cannot hide it any more or they are more daring individuals. These questions would have to be answered before behavior can be used as a sole indicator of stress.

Although behavior has its limitations when it comes to defining stress, it is safe to say that horses that show behavioral signs of stress are stressed at that point and that horses that developed stereotypies have experienced stress at some point (26).

Our second hypothesis that stress negatively affects the performance of harness racing horses could not be confirmed in this study as well, but because of the small sample size and all the factors discussed above that might have influenced the results it is not possible to discard this hypothesis yet. It may be noted that horses from trainer A had the most lesions, followed by trainer C and then B. This raises the question if stress increases the prevalence of injury and therefore has a negative impact on Standardbred racehorses. Research has shown a strong correlation between stress and higher injury prevalence in humans (50). This topic has not been paid attention in horses so far and certainly warrants further investigation, since it could contribute to a great improvement in equine athletes' health and longevity in sport.

This study investigated the impact of training and racing horses at a younger age on behavior, cortisol levels, and racing performance in harness racing horses. Contrary to the initial hypothesis that an early start of the racing career might increase signs of stress in horses, neither salivary cortisol levels nor behavioral scores supported this notion. Factors such as paddock time and training conditions affected the assessed parameters more than age and an early racing start. There was no clear correlation found between salivary cortisol concentrations, behavior, and racing performance. While we cannot dismiss the hypothesis that stress negatively impacts racing performance completely due to the limitations of this study, such as a small sample size, our findings underline the difficulties of equine stress assessment and its effects on performance. Our study highlights the need for more research to achieve a better understanding of the complex relation between stress, behavior, and racing outcomes in harness racing horses.

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