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EFFECTS OF HORSE TRAINING LEVEL ON PHYSIOLOGICAL AND -ETHOLOGICAL STRESS INDICATORS DURING SHOEING

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Abstract: Shoeing is an essential procedure for working horses but can provoke significant stress responses, compromising animal welfare and handler safety. Training for regular hoof manipulation may reduce these reactions, yet quantitative data are limited. Forty-two healthy horses of both sexes were assessed for stress responses to shoeing. Horses were classified as trained (n=24) or insufficiently trained (n=18) for routine hoof handling. Physiological indicators (heart rate, defecation frequency) and ethological indicators (leash tightening, object biting, leg pulling) were recorded in the stable and during shoeing. Data were analyzed using Mann-Whitney U and Spearman's correlation tests. Shoeing elicited stress in both groups, evidenced by increased heart rate and stress-related behaviors. Insufficiently trained horses exhibited significantly higher heart rates ($p < 0.001$), more frequent defecation ($p = 0.025$), and markedly elevated behavioral responses, especially leg pulling ($p < 0.001$). Strong correlations were observed between heart rate and leg pulling ($\rho = 0.53$), and between leash tightening and leg pulling ($\rho = 0.81$). The results of this study indicate that training for regular manipulation with extremities and hooves can reduce stress responses during shoeing. These findings highlight the value of early and consistent training to improve welfare and safety during routine farriery procedures. The results of this study also indicate that combining physiological and ethological indicators can provide a more reliable approach for assessing equine stress reaction.

Key words: horses, shoeing, stress, training

INTRODUCTION

Unlike most other species of domestic animals, which have been differentiated into breeds adapted to various production purposes, horses have primarily been used as working animals since their domestication, and this mode of use has remained largely unchanged to this day (Eser and Erat, 2022). Horses generally live much longer than other domestic animals-sometimes spanning several decades-allowing them to learn,

gain experience, and adapt to different situations (Cooper, 2007; McGreevy and McLean, 2007).

In horses, as working animals used for various types of movement-based work (walk, trot, gallop, and other forms of locomotion), the key systems for performing their functions are the musculoskeletal and cardiovascular systems, with a particular emphasis on the health of the hooves and distal limbs (Egenvall et al., 2009; Floyd and Mansmann, 2007). The use of horses for any kind of work (sport, recreation, draft, transport) requires shoeing as a routine procedure, aimed at protecting the hooves and the locomotor system from damage caused by hard terrain and increased loading. Horseshoes also provide firmer footing and support during movement (O'Grady, 2008). Horses that do not work or that move primarily on soft terrain (e.g., broodmares used only for reproduction and kept on pasture) do not require regular shoeing, or the intervals between shoeing can be much longer compared to working horses (Clayton et al., 2011). For working horses in regular use, shoeing is typically performed every four to six weeks to accommodate hoof growth, improve biomechanical performance, and preserve working ability (Leśniak et al., 2017). Proper and regular shoeing can correct limb structure irregularities, stance, and gait, thereby preparing the horse for work while also preserving health and extending working life (O'Grady, 2016; O'Grady and Poupard, 2003; Taylor et al., 2002).

Although shoeing is a routine procedure intended to protect the hooves and facilitate the horse's performance, the initial reaction to presence of horseshoes may be stressful, as they are not naturally part of the horse's body. In addition, handling during shoeing—including restraining, lifting the legs, approaching from blind spots, restricting movement and escape, the presence of unfamiliar people or animals, and exposure to new environments, sounds, and objects—can be significant stressors (Borstel et al., 2017). Shoeing can also involve pain and discomfort, whether due to injury (e.g., hoof damage, laminitis) or the use of restraint tools such as twitches, all of which can contribute to resistance during the procedure (Mansmann et al., 2011).

The use of horses for purposes as sport, draft, recreation or load carrying and the intensity of that use—along with sex and age, during which experience accumulates—can all influence stress responses to handling, including shoeing. These factors should be considered when evaluating the horse's stress in different situations (Jacquay et al., 2023; Baragli et al., 2014). Furthermore, although not objectively measurable, horses—being intelligent and capable of learning—may be affected by prior experiences with shoeing or other handling events (e.g., trailer loading), which can influence the occurrence and intensity of stress responses (Murphy & Arkins, 2007). The ability of horses to learn from experience enables training for specific handling procedures (i.e. learning from often repeated procedures), which can reduce the intensity of stress responses (Dai et al., 2019; Hendriksen et al., 2011; Hanggi and Ingersoll, 2009).

According to Fraser et al. (1975), stress is a condition in which an individual must make abnormal or extreme physiological or behavioral adjustments to cope with adverse or unexpected environmental events. In general, stress is defined as a non-specific, active response of the organism to challenges arising from changes in the

environment (Borstel et al., 2017). The most commonly used indicators to assess the intensity of a horse's stress response include changes in heart rate and cortisol concentration in biological samples, both of which are objective measures (Nowak et al., 2025; Dlugosz et al., 2020; Von Borell et al., 2007; Rietmann et al., 2004; Toutain et al., 1995). More recently, changes in ethogram have been used as reliable, non-invasive indicators of stress. Studies linking ethogram changes to potentially stressful events often point to general signs of anxiety and stress, whether defensive (tightening the leash, fleeing, pushing, biting objects) or offensive (lunging, kicking, biting, showing teeth, attacking) (Young et al., 2012; Visser et al., 2010). However, studies specifically associating particular procedures with distinct ethogram changes are limited. To our knowledge, the only study directly linking the shoeing procedure to ethogram changes in horses was conducted by Mansmann et al. (2011). These authors categorized ethogram changes into two groups: evasive reactions (e.g., leg snatching, bucking, pulling back, farrier fear, trembling, restlessness) and offensive reactions (e.g., charging, biting the farrier, kicking, biting the leash, lunging at the handler, tail whipping, front leg stomping).

When evaluating stress responses to potentially stressful situations, it is important to use multiple parameters, both physiological and behavioral, to gain a complete and reliable understanding of the horse's condition (Dawkins, 2003; Mason and Mendl, 1993). Using multiple indicators allows different aspects of the stress response to be identified and linked to specific triggers, facilitating the development of strategies to reduce stress during handling.

Taking the above into consideration, the aim of this study was to examine the differences in stress response on shoeing between groups of horses of different training levels, as expressed by physiological and ethological parameters. This aim of study is based on the hypothesis that training level may influence the intensity of the horse's stress response to shoeing.

MATERIALS AND METHODS

The study was conducted from March 2023 to May 2025 on horses used in equestrian clubs. The criterion for the inclusion of the horse in the study was that at the time of study horse had no psychophysical disturbances and that in the last six months before the day of the examination they had farriery intervention. A total of 42 horses (18 mares, 42.86%, and 24 male horses, 57.14%, 22 geldings and two stallions) were included in the study. The average age of the examined horses was 10.71 ± 6.10 years. The group of horses trained for regular manipulation with extremities and shoeing (57.14%, $n=24$), marked as „trained“, consisted of horses that had regular manipulation with their extremities, i.e. hooves (lifting the leg, cleaning the hooves with a cleaning hook, brush or other suitable tools, hoof trimming, simulation of shoeing, slight blowing with a hammer on the hoof capsule and horseshoe) on daily level and which were regularly shod every four to six weeks, so these procedures can be marked as „often repeated“. The group of insufficiently trained horses (42.86%, $n=18$) consisted

of horses in which regular manipulation of the extremities, i.e. hooves, was not performed on daily level and which had hoof trimming and shoeing done as needed, but at least twice in a period of six months before the day of examination.

Animal procedures complied with Directive 2010/63/EU and IASP guidelines. Ethical Committee of the Faculty of Agriculture, University of Banja Luka, approved the this study (number of Approval 10/1.1883/25), as the procedures used to assess stress response to shoeing—heart rate measurement, visual observation of defecation frequency, and ethogram evaluation—as well as the shoeing itself, were routine, non-invasive, and did not compromise animal welfare or health.

The level of stress of horses in the stable and during farriery intervention, was assessed by same, trained and experienced person, based on the measurement of the heart rate and the number of defecations, as physiological parameters that indicate the presence of stress, and by recording changes in the horse's behavior, i.e. parameters of the ethogram. The procedure of preparing the horse for shoeing in the stable, bringing it to the shoeing place, processing the hooves and shoeing itself were carried out in the same way for all horses, in a previously prepared area, well-known by horse, protected from sound and visual stressors and other animals, and in the presence of a known person who manages the horse. The first heart rate measurement (heart rate in box, HRB) was performed in the box where the horse lives, during the preparation for shoeing, with the presence of a known person handling the horse, by using a veterinary double-membrane stethoscope, during one minute. At the same time the number of defecations in box (excretion of feces in the box, DB) was recorded. Also, the parameters of the ethogram that indicate the presence of a stress reaction were recorded, namely the number of tightening of the leash with which the horse was tied in the box (TLB), the number of biting of objects (biting of the leash, salt holder, box fence, etc., BOB), and the number of leg pulling during manipulation with the horse in the box, for all four legs together (LPB). After preparing for shoeing in the box, the horses were brought to the shoeing place and tied, and hoof processing and shoeing itself were then performed. All horses included in the study were shod with the cold method, in the same way, by a trained farrier, with the presence of a known person who manages the horse. The second heart rate measurement (heart rate on shoeing, HRS) was performed using a veterinary double-membrane stethoscope, lasting one minute, during the intervention of farrier. During farriery intervention, the number of defecations (excretion of feces on the shoeing, DS) and ethogram parameters that indicate the presence of a stress reaction, namely the number of leash tightening on the shoeing (TLS), the number of biting objects on the shoeing (BOS), and the number of leg pulling on the shoeing, for all four legs together (LPS), were also recorded.

SPSS19 statistical package was used for data processing. The data were processed using descriptive statistics methods, and the normality of data distribution for the total sample and each of the groups was tested with the Kolmogorov-Smirnov test. The statistical significance of the differences in the values of the examined parameters between the groups was evaluated by the Mann-Whitney U test. The mutual correlation of the examined parameters on shoeing was evaluated by using the Spearman

correlation coefficient ρ . Differences at the $p < 0.05$ level were considered as statistically significant.

RESULTS

The values of descriptive statistics for the examined physiological and ethological indicators of stress reaction of horses measured in box and during shoeing are shown in Table 1.

Table 1. Descriptive statistics for the examined parameters in trained and insufficiently trained horses, measured in the box and during shoeing ($\bar{X} \pm SD$)

Parameter	Trained horses (n=24)	Insufficiently trained horses (n=18)
HRB	34.92±4.72	34.11±4.35
DB	0.00±0.00	0.00±0.00
TLB	0.00±0.00	0.00±0.00
BOB	0.00±0.00	0.00±0.00
LPB	0.00±0.00	0.00±0.00
HRS	40.25±6.35	52.72±11.62
DS	0.21±0.42	0.72±0.83
TLS	0.58±0.78	4.39±2.64
BOS	0.04±0.20	1.56±2.06
LPS	1.54±1.50	14.50±9.18

Legend: n - number of samples, \bar{X} - Mean value, SD - Standard deviation, HRB - heart rate in box, DB - number of defecations in box, TLB - number of tightening of leash in box, BOB - number of biting of objects in box, LPB - number of leg pulling in box, HRS - heart rate on shoeing, DS - number of defecations on shoeing, TLS - number of tightening of leash on shoeing, BOS - number of biting of objects on shoeing, LPS - number of leg pulling on shoeing

In both groups of horses the average heart rate values measured in the box (Table 1.) were within the physiological values (34.92±4.72 bpm for trained and 34.11±4.35 bpm for insufficiently trained horses). During recording in box, there was no observed changes of ethogram parameters that can indicate the existence of stress (TLB, BOB and LPB), regardless of the group, which was also the case with the number of defecations (DB), as another physiological indicator of the horse's stress reaction. Average heart rate values measured during shoeing showed an increase compared to the values recorded in box in both groups of horses, which indicates the presence of a stress reaction, regardless of training level. The average value of the heart rate in trained horses remained within the physiological limits (40.25±6.35 bpm), while in insufficiently trained horses, it was above the upper physiological limit (52.72±11.62 bpm). In trained horses lower average number of defecations (0.21±0.42) during shoeing was found, compared to insufficiently trained horses (0.72±0.83). The obvious

presence of a stress reaction to shoeing in both groups of horses is indicated by changes in the values of the examined ethogram parameters during shoeing, compared to the values recorded in the box. The stress reaction to shoeing in trained horses had lower intensity compared to insufficiently trained horses, as indicated by lower average values for tightening the leash (0.58 ± 0.78 vs. 4.39 ± 2.64), biting of objects (0.04 ± 0.20 vs. 1.56 ± 2.06), and pulling the leg during shoeing (1.54 ± 1.50 vs. 14.50 ± 9.18). From all examined ethogram parameters recorded during shoeing, as the one that particularly indicates a higher level of stress in insufficiently trained horses compared to trained ones, leg pulling should be singled out, as it was present in all insufficiently trained horses, while in trained horses it was sporadic.

The difference in the values of the examined parameters between trained and insufficiently trained horses was analyzed using the Mann-Whitney U test.

Table 2. Difference in the values of the examined parameters between trained and insufficiently trained horses (Mann-Whitney U test)

Parameter	n=42	Mean Rank	p value
HRB	Trained horses	21.65	0.928
	Insufficiently trained horses	21.31	
DB	Trained horses	21.50	1.000
	Insufficiently trained horses	21.50	
TLB	Trained horses	21.50	1.000
	Insufficiently trained horses	21.50	
BOB	Trained horses	21.50	1.000
	Insufficiently trained horses	21.50	
LPB	Trained horses	21.50	1.000
	Insufficiently trained horses	21.50	
HRS	Trained horses	15.38	0.000
	Insufficiently trained horses	29.67	
DS	Trained horses	18.46	0.025
	Insufficiently trained horses	25.56	
TLS	Trained horses	13.75	0.000
	Insufficiently trained horses	31.83	
BOS	Trained horses	17.23	0.000
	Insufficiently trained horses	27.19	
LPS	Trained horses	12.50	0.000
	Insufficiently trained horses	33.50	

Legend: n - number of samples, Mean Rank - mean rank, p - level of significance of differences, HRB - heart rate in box, DB - number of defecations in box, TLB - number of tightening of leash in box, BOB - number of biting of objects in box, LPB - number of leg pulling in box, HRS - heart rate on shoeing, DS - number of defecations on shoeing, TLS - number of tightening of leash on shoeing, BOS - number of biting of objects on shoeing, LPS - number of leg pulling on shoeing

Using the Mann-Whitney U test (Table 2.), it was found that there was no statistically significant difference between trained and insufficiently trained horses in the values of physiological parameters and ethogram parameters during measuring in the box ($p=0.928$, $p=1.000$, $p=1.000$, $p=1.000$ and $p=1.000$ for HRB, DB, TLB, BOB and LPB, respectively). During shoeing, a statistically significant difference ($p<0,05$) was found between trained and insufficiently trained horses in all examined parameters, indicated by the lower heart rate ($p=0.000$), lower number of defecations ($p=0.025$), less tightening of the leash ($p=0.000$), less biting of objects ($p=0.000$) and less leg pulling ($p=0.000$) in trained compared to insufficiently trained horses.

The mutual correlation of the examined parameters that indicate the stress reaction of horses to shoeing (regardless of group) were analyzed using Spearman’s correlation coefficient, which shows the strength and direction of the correlation between individual parameters that represent indicators of the horses’ stress reaction to shoeing and its intensity (Table 3).

Table 3. Mutual correlation between the examined physiological and ethogram parameters during shoeing for all horses (n=42) (Spearman rank correlation coefficient ρ)

Parameter	HRS	DS	TLS	BOS	LPS
HRS	1,000	0,477	0,341	0,369	0,534
DS	0,477	1,000	0,248	0,198	0,387
TLS	0,341	0,248	1,000	0,539	0,807
BOS	0,369	0,198	0,539	1,000	0,520
LPS	0,534	0,387	0,807	0,520	1,000

Legend: HRS - heart rate on shoeing, DS - number of defecations on shoeing, TLS - number of tightening of leash on shoeing, BOS - number of biting of objects on shoeing, LPS - number of leg pulling on shoeing

Using Spearman’s correlation coefficient ρ (Table 3), it was found that heart rate during shoeing (HRS) had a strong positive correlation with the leg pulling on shoeing (LPS) ($\rho = 0.534$, $p = 0.000$), and a moderately strong positive correlation with the number of defecations on shoeing (DS) ($\rho = 0.477$, $p = 0.001$), the number of biting of objects on shoeing (BOS) ($\rho = 0.369$, $p = 0.016$), and leash tightening on shoeing (TLS) ($\rho = 0.341$, $p = 0.027$). Leash tightening on shoeing (TLS) was in a very strong positive correlation with leg pulling on shoeing (LPS) ($\rho = 0.807$, $p = 0.000$) and in a strong positive correlation with biting of objects on shoeing (BOS) ($\rho = 0.539$, $p = 0.000$), while its correlation with the number of defecations on shoeing (DS) was weakly positive and not statistically significant ($\rho = 0.248$, $p = 0.113$). The leg pulling on

shoeing (LPS) was in a strong positive correlation with biting of objects on shoeing (BOS) ($\rho = 0.520$, $p = 0.000$), and in a moderately strong positive correlation with the number of defecations on shoeing (DS) ($\rho = 0.387$, $p = 0.011$). Between the number of defecations on shoeing (DS) and the number of biting of objects on shoeing (BOS) a weak positive correlation was found, but without statistical significance ($\rho = 0.198$, $p = 0.209$).

DISCUSSION

Various events in a horse's life, including both routine procedures and emergency situations, can be sources of stress and may impair its well-being in both the short and long term (Borstel et al., 2017; Budzyńska, 2014). Shoeing, even though it is a routine procedure intended to preserve hoof health and improve the biomechanical performance of the hooves (Leśniak et al., 2017), can also be a significant source of stress. Although many studies have investigated the occurrence and intensity of a horse's stress response to various events, only a limited number have examined the relationship between shoeing and the stress response, the factors that influence its intensity, and the development of strategies to mitigate it. Additional factors that may influence the results of this type of research include the number and characteristics of the animals included in the study, as well as the homogeneity of the sample itself, which can be a limiting factor regarding the validity of the conclusions drawn. It is important to note that the sample size in this study was limited by the specific characteristics of the available horse population in equestrian clubs. Unlike other domestic animal species, horses are usually kept in small numbers and mainly for non-commercial purposes. Therefore, the number of horses that can be included in studies of this type within one or more equestrian clubs is regularly small and heterogeneous in terms of sex, age, purpose, breed, training level, and other grouping criteria. Even at stud farms or equestrian clubs with larger numbers of horses of the same breed, forming homogeneous research groups of 30 or more horses—taking into account their sex, age, purpose, previous training, and other criteria—represents a serious challenge. An additional limitation is the fact that horse owners often show little willingness to allow their horses to participate in research. Furthermore, some horses were excluded from the study because accurate measurements could not be taken due to excessive resistance or restless behavior, which further reduced the total number of horses included. Considering all these factors, the sample used in this study represents the available horse population in equestrian clubs and should provide insight into the influence of training on the stress response during shoeing, although the conclusions drawn should be interpreted with caution, bearing in mind the limitations imposed by the sample size.

Most sources point to heart rate increase as the primary indicator of stress, followed by other physiological responses (tremors, sweating, defecation), changes in other physiological parameters such as cortisol concentration in blood and other body fluids (Ilić et al., 2023; Von Borell et al., 2007; Rietmann et al., 2004; Toutain et al., 1995), and behavioral changes (Borstel et al., 2017; Young et al., 2012). The visible influence

of shoeing as a stressor on heart rate, found across all horses in the study, regardless of training level, can be explained by the rapid activation of the autonomic nervous system in response to acute stress, triggering adrenaline release to initiate a "fight or flight" response (Yngvesson et al., 2016; Von Borell et al., 2007). This adrenaline effect is quick but short-lived, often followed by the slower but longer-lasting effects of glucocorticoids like cortisol, which are more readily measurable (Dlugosz et al., 2020). Other stress-related physiological signs during shoeing, such as defecation, sweating, and trembling, are also linked to sympathetic nervous system activation (Yngvesson et al., 2016).

In this study, horses trained for regular manipulation with extremities and shoeing maintained physiological heart rates during shoeing, unlike insufficiently trained horses, suggesting that training for regular manipulation with extremities and shoeing can reduce stress responses, though it does not eliminate them entirely. Also, horses trained for regular manipulation with extremities and shoeing defecated significantly less than untrained ones, which can indicate their reduced stress response and higher level of tolerance on manipulation and shoeing due to training. Statistically significant differences were also found in all examined ethogram parameters, which also can indicate the positive influence of training for regular manipulation with extremities and shoeing to reducing stress response, in order to make horses safer to handling and manipulation, and also to preserve their welfare.

Despite domestication, horses predominantly respond to stress by fleeing rather than fighting—a pattern rooted in their evolution (Yngvesson et al., 2016; Cooper and Albertosa, 2005). Mansmann et al. (2011) categorized stress-related ethogram changes during shoeing as either evasive (e.g., rearing, pulling back, tightening the leash, leg pulling) or offensive reactions (e.g., lunging, biting, kicking). These behaviors are influenced not only by the immediate experience but also by prior painful or stressful encounters, which may not be easily measurable.

This study confirms a clear link between shoeing and ethogram changes. Training for regular manipulation with extremities and shoeing showed a statistically significant effect on all ethological indicators. A particularly strong correlation was found between physiological stress indicators and leg pulling—a specific, shoeing-related behavior—highlighting its relevance for future research. Unlike leash tightening and object biting, which are signs of general discomfort and resistance in horse, and may be influenced by various environmental factors, leg pulling appears to be directly related to shoeing. It reflects discomfort from an unnatural posture, immobilization, and potential pain, exacerbated by prior negative experiences (Mansmann et al., 2011). Although stress reactions were observed in both groups of horses, their intensity was greater in untrained horses. The highly reduced incidence of leg pulling in horses trained for regular manipulation with extremities and shoeing (more than nine times, 1.54 ± 1.50 vs. 14.50 ± 9.18) underscores training's effectiveness in lowering stress.

The results of this study indicate mutual relationships and a clear correlation between the examined physiological and ethogram parameters that reflect the stress reaction of horses during shoeing, that is, their frequent joint occurrence in stressful situations. An

increase in heart rate during shoeing, a commonly used physiological indicator of a horse's stress response, was accompanied by a higher frequency of leg pulling, defecations, biting of objects, and leash tightening. This shows that this physiological stress indicator is regularly accompanied by changes in the ethogram—i.e., in the horse's behavior—especially leash tightening as a general sign of discomfort and resistance, as well as leg pulling, an ethogram parameter directly associated with shoeing as a potentially stressful event for the horse. An elevated stress level during shoeing (or another stressful event), expressed through increased heart rate, is also indicated by more frequent defecations, as well as more frequent object biting and leash tightening, which are general ethogram indicators of frustration and discomfort. However, the weaker correlation found between the number of defecations during shoeing and both object biting and leash tightening suggests that the number of defecations is a less specific indicator of the stress reaction compared with heart rate. The results of this study point to a high degree of correlation among different ethogram parameters during shoeing, confirming that certain stress-related behaviors in horses often appear together and simultaneously. Thus, the occurrence of one stress-related behavior (e.g., TLS—leash tightening on shoeing) is highly likely to be accompanied by other stress-related behaviors (e.g., LPS—leg pulling on shoeing). This finding may have considerable practical importance for predicting a horse's reaction to potentially stressful situations and for assessing risk during work with horses, thereby enhancing both the safety of personnel handling the horses and the welfare of the animals themselves.

Interpreting these results requires considering not only the stressor itself but also factors like upbringing, previous experiences, breed, and individual temperament of each horse. Horses' longevity and learning ability make them suitable for training to various forms of handling and manipulation, including shoeing (Ilić et al., 2023; Dai et al., 2019; Hendriksen et al., 2011; Hanggi and Ingersoll, 2009; Murphy & Arkins, 2007). Early and consistent training for regular manipulation with extremities and shoeing can reduce stress during shoeing, as supported by results of this study. This can support the development of various training models (e.g., positive reinforcement, reward-based training) aimed at reducing stress during different types of handling and manipulation. Proper handling and shoeing procedures not only benefit equine welfare and longevity but also reduce the risk of injury to handlers and other staff (Ilić et al., 2023).

The results of this study also point to the need for a comprehensive approach—monitoring both physiological and ethogram parameters—when assessing the presence and intensity of a horse's stress reaction in potentially stressful situations, such as shoeing and other forms of handling.

CONCLUSION

This study showed that training can have significant effect on reducing stress during shoeing. Horses trained for regular manipulation with extremities and shoeing displayed lower physiological indicators and fewer ethological signs of stress

compared to insufficiently trained horses. The statistical analysis confirmed mutual correlations between physiological stress indicators and ethogram parameters, with some of them more pronounced (e.g., between elevated heart rate and leg pulling), while others were weaker (e.g., between defecation and other ethological signs of stress response). These findings emphasize the importance of simultaneously assessing both physiological and behavioral parameters to achieve a more comprehensive understanding of the stress response in horses during shoeing.

While these results provide valuable insights in stress response in horses during shoeing, they also underline certain limitations of the study. Although heart rate and ethogram parameters proved useful, future research would benefit from the inclusion of additional biomarkers, such as salivary cortisol or long-term behavioral monitoring, as well as from a larger study population. Consideration of further factors, including breed, temperament, age and previous experiences, would also contribute to the robustness of the findings. Such an approach would enable more reliable conclusions regarding the impact of training on reducing physiological reaction and occurrence of stress-related behaviors during shoeing and other forms of horse handling.

Conflict of interest statement: The authors declare that there is no conflict of interest.

REFERENCES

- Baragli P., Vitale V., Banti L., Sighieri C. (2014): Effect of aging on behavioural and physiological responses to a stressful stimulus in horses. (*Equus caballus*). *Behaviour*, 151(11):1513-1533. DOI: 10.1163/1568539X-00003197
- Borstel U.K.V., Visser E.K., Hall C. (2017): Indicators of stress in equitation. *Applied Animal Behaviour Science*, 190:43-56. DOI: 10.1016/j.applanim.2017.02.018
- Budzyńska M. (2014): Stress Reactivity and Coping in Horse Adaptation to Environment. *Journal of Equine Veterinary Science*, 34(8):935-941. DOI: 10.1016/j.jevs.2014.05.010
- Clayton H.M., Gray S., Kaiser L.J., Bowker R.M. (2011): Effects of barefoot trimming on hoof morphology. *Australian Veterinary Journal*, 89:305-311. DOI: 10.1111/j.1751-0813.2011.00806.x
- Cooper J.J. (2007): Equine learning behaviour: Common knowledge and systematic research. *Behavioural Processes*, 76:24-26. DOI: 10.1016/j.beproc.2006.12.009
- Cooper J.J., Albentosa M.J. (2005): Behaviour adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behaviour. *Livestock Production Science*, 92:177-182. DOI: 10.1016/j.livprodsci.2004.11.017
- Dai F., Dall Costa A., Bonfanti L., Caucci C., Di Martino G., Lucarelli R., Padalino B., Minero M. (2019): Positive reinforcement based training for self loading of meat horses reduces loading time and stress related behavior. *Frontiers in Veterinary Science*, 6:350. DOI: 10.3389/fvets.2019.00350
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- Dawkins M.S. (2003): Behaviour as a tool in an assessment of animal welfare. *Zoology*, 106:383-387. DOI: 10.1078/0944-2006-00122
- Dlugosz B., Prochniak T., Stefaniuk-Szmukier M., Basiaga M., Luszczynski J., Pieszka M. (2020): Assessment of changes in the saliva cortisol level of horses during different ways in recreational exploitation. *Turkish Journal of Veterinary & Animal Sciences*, 44(4):754-762. DOI: 10.3906/vet-2003-57
- Egenvall A., Lönnell C., Roepstorff L. (2009): Analysis of morbidity and mortality data in riding school horses, with special regard to locomotor problems. *Preventive Veterinary Medicine*, 88:193-204. DOI: 10.1016/j.prevetmed.2008.10.004
- Eser E., Erat S. (2022): Domestication and Origins of the Modern Horse Breeds. *Journal of Bahri Dagdas Animal Research*, 11(1):51-61.
- Floyd A., Mansmann R. (2007): Equine Podiatry. Philadelphia: Elsevier Health Sciences. DOI: 10.1016/B978-0-7216-0383-4.X5001-4
- Fraser D., Fraser A.F., Ritchie J.S.D. (1975): The term "stress" in a veterinary context. *British Veterinary Journal* 131:653-662. DOI: 10.1016/S0007-1935(17)35136-9
- Hanggi E.B., Ingersoll J.F. (2009): Long-term memory for categories and concepts in horses [*Equus caballus*]. *Animal Cognition*, 12:451-462. DOI: 10.1007/s10071-008-0205-9
- Hendriksen P., Elmgreen K., Ladewig J. (2011): Trailer-loading of horses: Is there a difference between positive and negative reinforcement concerning effectiveness and stress-related signs? *Journal of Veterinary Behavior*, 6(5):261-266. DOI: 10.1016/j.jveb.2011.02.007
- Ilić P., Jotanović S., Grbić S., Kazazović J. (2023): Change of pulse frequency in horses as an indicator of stress caused by loading into the trailer. *Veterinary Journal of Republic of Srpska*, 23(1-2):333-347. DOI: 10.7251/VETJEN2301333I
- Jacquay E.T., Harris P.A., Adams A.A. (2023): Age-Related Differences in Short-Term Transportation Stress Responses of Horses. *Journal of Equine Veterinary Science*, 128:104879. DOI: 10.1016/j.jevs.2023.104879
- Leśniak K., Williams J., Kuznik K., Douglas P. (2017): Does a 4-6 Week Shoeing Interval Promote Optimal Foot Balance in the Working Equine? *Animals*, 7:29. DOI: 10.3390/ani7040029
- Mansmann R.A., Currie M.C., Correa M., Sherman B., Vom Orde K. (2011): Equine Behavior Problems Around Farriery: Foot Pain in 11 Horses. *Journal of Equine Veterinary Science*, 31:44-48. DOI: 10.1016/j.jevs.2010.11.018
- Mason G., Mendl M. (1993): Why is there no simple way of measuring animal welfare? *Animal Welfare*, 2(4):301-319. DOI: 10.1017/S0962728600016092
- McGreevy P.D., McLean A.N. (2007): Roles of learning theory and ethology in equitation. *Journal of Veterinary Behavior*, 2(4):108-118. DOI: 10.1016/j.jveb.2007.05.003
- Murphy J., Arkins S. (2007): Equine learning behaviour. *Behavioural Processes*, 76(1):1-13. DOI: 10.1016/j.beproc.2006.06.009
-

- Nowak A.C., Macho-Maschler S., Biermann N.M., Palme R., Dengler F.D. (2025): Investigating the interplay of stressors and health in horses through fecal cortisol metabolite analysis. *Frontiers in Veterinary Science: Sec. Animal Behavior and Welfare*, 12:1545577. DOI: 10.3389/fvets.2025.1545577
- O'Grady S.E. (2008): Basic Farriery for the Performance Horse. *Veterinary Clinics of North America: Equine Practice*, 24(1):203-218. DOI: 10.1016/j.cveq.2007.12.002
- O'Grady S.E., Poupard D.A. (2003): Proper physiologic horseshoeing. *Veterinary Clinics of North America: Equine Practice*, 19:333-351. DOI: 10.1016/s0749-0739(03)00020-8
- O'Grady S.E. (2016): Therapeutic Shoes: Application of Principles. In: *Equine Laminitis*. Eds. J. K. Geor, R. J. Belknap. Hoboken: John Wiley & Sons, Inc., 343. DOI: 10.1002/9781119169239.ch38
- Rietmann T.R., Stuart A.E.A., Bernasconi P., Stauffacher M., Auer J.A., Weishaupt M.A. (2004): Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters. *Applied Animal Behaviour Science*, 88:121-136. DOI: 10.1016/j.applanim.2004.02.016
- Taylor D., Hood D.M., Wagner I.P. (2002): Short-term effect of therapeutic shoeing on severity of lameness in horses with chronic laminitis. *American Journal of Veterinary Research*, 63:1629-1633. DOI: 10.2460/ajvr.2002.63.1629
- Toutain P.L., Lassourd V., Popot M.A., Laroute V., Alunerie M., Bonnaire Y. (1995): Urinary cortisol excretion in the resting and exercising horse. *Equine Veterinary Journal Suppl.*, 18:457-462. DOI: 10.1111/j.2042-3306.1995.tb04973.x
- Visser K., Karlas K., Van Deurzen I., Workel I., Van Reenen K. (2010): Experts' assessment of temperament in sport horse. *Journal of Veterinary Behaviour*, 5(4):214-215. DOI: 10.1016/j.jveb.2009.11.003
- Von Borell E., Langbein J., Despres G., Hansen S., Leterrier C., Marchant J., Marchant-Forde R., Minero M., Mohr E., Prunier A., Valance D., Veissier I. (2007): Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals - a review. *Physiology & Behaviour*, 92:293-316. DOI: 10.1016/j.physbeh.2007.01.007
- Yngvesson J., De Boussard E., Larsson M., Lundberg A. (2016): Loading horses (*Equus caballus*) onto trailers-Behaviour of horses and horse owners during loading and habituating. *Applied Animal Behaviour Science*, 184:59-65. DOI: 10.1016/j.applanim.2016.08.008
- Young T., Creighton E., Smith T., Hosie C. (2012): A novel scale of behavioural indicators of stress for use with domestic horses, *Applied Animal Behaviour Science*, 140:33-43. DOI: 10.1016/j.applanim.2012.05.008
-