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CHANGE OF PULSE FREQUENCY IN HORSES AS AN INDICATOR OF STRESS CAUSED BY LOADING INTO THE TRAILER

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Summary

Transport and loading into a trailer is a potential source of stress for horses, which can consequently undermine the concept of animal welfare. The study aimed to determine the presence of stress reaction in horses during the loading into the trailer, expressed through a change in pulse frequency as an indicator of stress reaction. The study included 25 horses between the ages of 4 and 20. Heart rate verification was performed using a veterinary stethoscope with a double-sided membrane and controlled by a heart rate monitor. When the horses were resting in the stable (first measurement), a pulse frequency of 29 to 40 bpm was found. The second measurement was performed at the moment of loading when the horses stepped onto the loading platform of the trailer, and pulse frequency values from 42 to 72 bpm were found. The results of the t-test indicate that there is a statistically significant difference in the pulse frequency between the first and second measurements. The results of this study indicate that loading horses into the trailer can cause stress, which is manifested by an increase in pulse frequency. The tendency of horses to easily form habits allows their proper training to reduce the level of stress while loading into trailers, which would contribute to improving the well-being of horses, and at the same time contribute to reducing stress and the risk of injury to horses and staff.

Key words: horse, loading, pulse frequency, stress.

INTRODUCTION

The evolution of horse transport ranged from their movement in herds, by carts, through rail and road traffic, all the way to the use of ships and aeroplanes (Cregier, 1982). The diverse use of horses, once in war, today in sports, intensive breeding, recreation, equestrian tourism, riding schools, use in healthcare, participation in fairs and the meat industry increased the frequency of transporting horses, which are transported more often than other types of animals (Fazio et al., 2013). Unlike other types of animals, for example, beef cattle, pigs or other farm animals, which are transported from the place of breeding to the place of use mostly once, horses, as working or sport animals, are transported often and repeatedly during their life, which can be a source of stress, with various consequences. Friend (2001) states that horse transport is "generally associated with lower reproductive rates, increased incidence of disease, temporary reduction in athletic performance and changes in many other physiological traits indicative of stress". The same author states that transportation is associated with an increased frequency of health disorders and that stress can have negative effects on the general health of horses, including digestive disturbances, reduced immune response, and behavioural changes. In general, stress can be defined as a non-specific active response of the organism to various challenges, caused by changed conditions and events in the environment (Borstel et al., 2017). Considering the need to respect the principles of horse welfare, as well as to respect strategies for improving the welfare of horses during their use in work, for sports or for other types of horse use, it is important to understand that horses, as intelligent animals, are capable of connecting transport itself with their experiences during loading, transport and unloading (Leadon et al., 2008).

One of the key stages of transporting horses is their loading into the trailer, with which every transport begins, regardless of the purpose of the transport. The horse's refusal to enter the trailer is a stressful experience accompanied by the combined effects of evasive behaviour and neophobic reactions of the horse. Horse loading into the trailer, from the point of view of horse psychology, combines the effects of claustrophobia with neophobia, including social isolation, effects of unfamiliar surroundings and new individuals, narrowed field of vision, and limited movement, which can cause anxiety and a high level of stress (Hartmann et al., 2011). In addition to possible physical injuries of the horse and the persons participating in the loading into the trailer at a given moment, the negative experiences that a horse acquires on that occasion can be a significant limiting factor in further training and work with a horse, especially bearing in mind the need for repeated transportation of horse during its life (Padalino, 2015). Neophobia, as the fear of

new circumstances, is innate in horses and can be manifested by an increase in fear during each subsequent loading into the trailer, due to inappropriate or rough handling of the horse. The use of physical punishment, inappropriate environment and resistance of the horse during loading, alltogether can increase the already existing stress state of the horse, which already arises as a result of loading into the trailer (Hendriksen et al., 2011). McGreevy and McLean (2007) report that by applying appropriate methods of horse handling, the stress that occurs when loading into the trailer, as well as during transport itself, can be reduced. In their research, Hendriksen et al. (2011) point to the presence of stress in horses during loading, followed by undesirable behaviour, highlighting the unnaturalness of the situation of loading into a trailer, due to neophobia and claustrophobia to which horses are prone. Lee et al. (2001) point to the delicacy of the loading process, finding that 53.4% of transport-related problems are initiated at the loading stage. A common source of stress for the horse's body is precisely the resistance that horses offer when being loaded into a trailer (Shanahan, 2003).

The stress reaction to stimuli from the external and internal environment of the organism is manifested by a series of changes in the organism, of which the secretion of catecholamines (adrenaline and noradrenaline) should be singled out as an initial reaction, and in a later phase the secretion of glucocorticosteroids, of which cortisol should be singled out, whose increased concentration in the body is an indicator of the stress reaction. Although changes in hormone concentrations that participate in the body's response to stressful factors are a very reliable indicator of a stress reaction, their determination is related to blood sampling, an invasive diagnostic method, which represents an additional source of stress for the individual, which is why it is necessary to establish an alternative method for assessing the intensity stress reactions, which would be non-invasive and at the same time sufficiently reliable. Physiological changes in the horse's organism related to stress can be manifested through an acceleration of the heart rate, i.e. a change in the pulse frequency (Borstel et al., 2017). An increase in pulse frequency when loading a horse into a trailer, compared to physiological values, may indicate the activation of the sympathetic nervous system, which is responsible for the "fight or fly" response (Yngvesson et al., 2016) and is a simple and reliable indicator of the horse's stress reaction, which can be monitored by non-invasive methods. The horse's fear of being loaded into a trailer and circumstances related to loading, as a psychological stressor, can cause stress, which increases the heart rate (Kay and Hall, 2009). Waran and Cuddeford (1995) report that heart rate during loading into a transport vehicle is higher than during various types of physical exertion, concluding that this is the most stressful phase of transport. Shanahan (2003) indicates that in the phase

of horse loading into the trailer, there was a significant increase in pulse frequency compared to the initial values, measured at rest or in the stable.
Bearing in mind all of the abovementioned, study aimed to show the changes in the horse's pulse frequency, as an indicator of the intensity of the stress reaction caused by loading into a trailer.

MATERIALS AND METHODS

The study included a total of 25 horses aged from 4 to 20 years ($\bar{X}$=9,04±5,623), from the equestrian clubs Čokorska polja, St. Đorđe and Arion, during the period from November 2020 to December 2022. According to the age structure of the examined group of horses, 60% were seven years old and under, 20% were from eight to 15 years old and 20% were 16 years old and older. Of the total number of horses examined, 56% were geldings, 32% mares and 12% stallions. Of the entire population of horses included in study, 12% were purebred Arabian horses, 12% Bosnian-Herzegovinian mountain horses, 8% Holstein horses, 8% Lipizzaner horses, 4% KWPN, 4% Hanoverian horse, 32% half-breed horses, 8% Posavac, 4% Swedish trotter, and 8% of horses are mixed breed, namely Lipizzaner x Arabian horse and Lipizzaner x half-breed horse. According to use, 72% of horses were used for recreation and 28% for sports. The criterion for the selection of horses included in the study was that they were all locomotory sound at the time of examination and that they were able to be led by their handler from the resting position to the loading ramp of the trailer. The horses included in the research were not loaded into the trailers at least three months before the day of the examination.
The horses' pulse frequency in the stable (the first measurement, BSFB) was measured at rest for one minute, after which they were brought from the stable while walking on a halter and on a lead to the line from which they headed towards the loading platform of the trailer. The starting loading line was located 10 meters from the loading platform. At the starting line, the horses were kept at rest for a minimum of three minutes or longer, until the pulse frequency equalled the value at rest in the stable, intending to eliminate the increase in pulse due to the effect of the horse's movement from the stable to the starting line. The second pulse frequency measurement was performed from the moment of stepping on the loading platform of the trailer (BSFU) for one minute. The order of pulse frequency measurement in the stable and on the loading platform within the examined group of horses was in the same order. Heart rate verification was performed using a veterinary stethoscope with a double-sided membrane and controlled by a heart rate monitor "Polar RS800CX N G3 watch, Polar Electro Oy, Finland". For all the horses

included in the study, the same car trailer for two horses, "Böckmann Comfort Vollpoly Anhänger für 2 Pferde mit Sattelkammer" brand was used.

Descriptive characteristics of the examined group of horses were obtained using the methods of descriptive statistics, while the normality of the distribution of the results was tested using the Kolmogorov-Smirnov test. The t-test of paired samples was used to examine whether there was a statistically significant difference in pulse frequency in the same group of horses at rest in the stable and in the phase of loading into the trailer. The relationship between pulse frequency as an indicator of stress reaction and loading into a trailer as a potential source of stress was investigated by eta square. A value of $p<0.05$ was taken as the limit of statistical significance of differences. The statistical program "SPSS 19" was used for data processing.

RESULTS

The results of the pulse frequency before and during loading into the trailer (Table 1) indicate a higher pulse frequency when loading to the trailer, compared to the pulse frequency measured in the stable. The pulse frequency measured in the stable (BSFB) was in the range from 29 bpm to 40 bpm, and the one measured during loading into the trailer (BSFU) from 42 bpm to 72 bpm. The normality of the distribution of pulse frequency values was tested by the Kolmogorov-Smirnov test, where the results of the distribution of pulse frequency values in stable ($p=0.147$) confirm the normal distribution, while the results of the distribution of pulse frequency values during loading ($p=0.004$) showed that the assumption of normality of the distribution of results is not confirmed and should be rejected.

The negative value of the asymmetry of the pulse frequency measured in stable (Skew=-0.04) showed that it was among the higher values, while the flattening values (Kurt=0.337) showed that it was sharper than normal, i.e. that there were more results clustered around the centre of the distribution. The positive value of pulse asymmetry during horse loading into the trailer (Skew=1.798) reveals that the values of the pulse frequency were among the lower values, while the flattening value (Kurt=6.322) indicates that it was more pointed than normal with more results grouped around the distribution centre.

Table 1 Descriptive statistic for obtained pulse frequency values

	N	X̄	Min	Max	K - S	Sig.	Skew.	Kurt.
BSFB	25	35,12	29	40	0,153	0,147	-0,044	0,337
BSFU	25	52,28	42	72	0,217	0,004	1,798	6,322

Legend: BSFB – pulse frequency measured in the stable, BSFU –pulse frequency measured during loading, N – number of samples, X̄ - mean value, Min – minimum, Max – maximum, K – S – Kolmogorov – Smirnov test, Sig. – significance level ($p < 0.05$), Skew. – the value of asymmetry, Kurt. - flattening value.

The normality of the distribution of pulse frequency values measured in stable (Figure 1) is also indicated by the appearance of the Normal Q - Q Plot curve, i.e. the normal probability of the data, which are closely distributed along a straight line. The Detrended Normal Q – Q Plot curve (Figure 2) with symmetrically distributed deviations of data from the horizontal line and the absence of data grouping indicates the normality of the distribution of results.

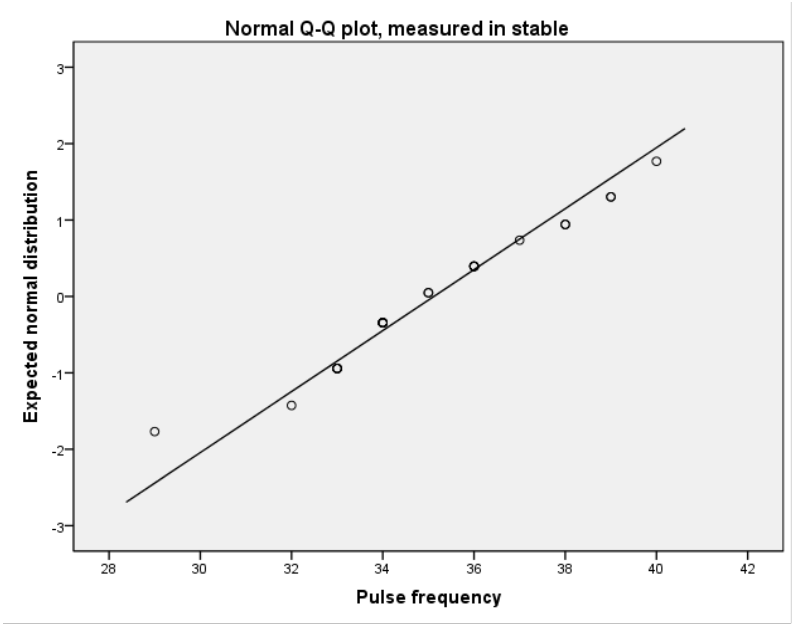

Figure 1 Curve of the normal probability distribution of the pulse frequency values measured in the stable

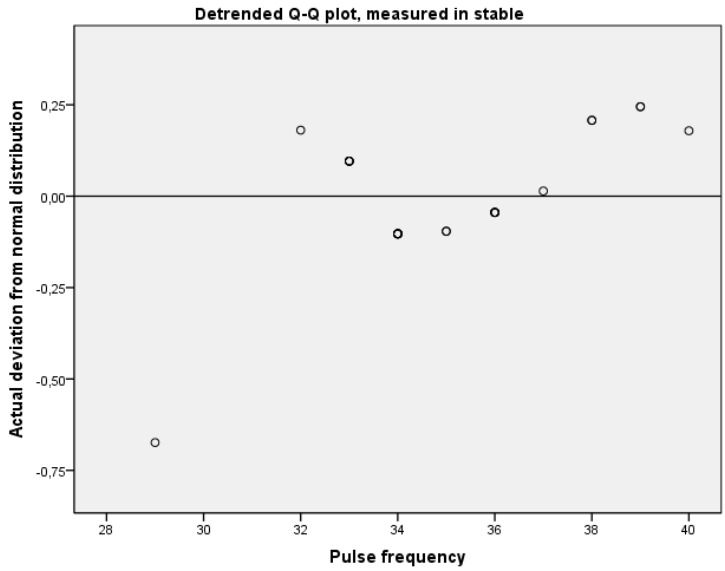

Figure 2 Actual deviation of the observed pulse frequency values measured in the stable

The appearance of the Normal Q – Q Plot curve (Figure 3) shows the deviation of data from the straight line for the established pulse frequency values obtained by measurement during horse loading into the trailer. Also, the asymmetric distribution and grouping of the data in relation to the horizontal line shown in the Detrended Normal Q – Q Plot (Figure 4) show a deviation from the normal distribution.

Figure 3 Curve of the normal probability distribution of pulse frequency values measured during loading into the trailer

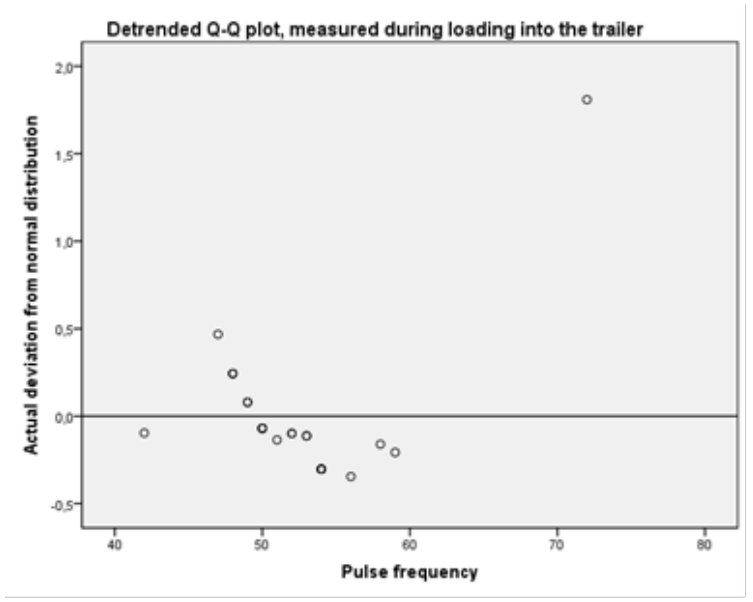

Figure 4 Actual deviation of observed pulse frequency results measured during loading into the trailer

The results of the statistics for the paired samples (Table 2) indicate an increase in the pulse frequency measured during loading into the trailer ($\bar{X}$=52.28±5.512) in relation to the values of the pulse frequency measured in the stable ($\bar{X}$=35.12±2.505).

Table 2 Paired sample statistics for pulse frequency

	N	($\bar{X}$)	SD	SE
BSFB	25	35,12	2,505	0,501
BSFU	25	52,28	5,512	1,102

Legend: BSFB – pulse frequency measured in the stable, BSFU –pulse frequency measured during loading, N – number of samples, $\bar{X}$ – mean value, SD – standard deviation, SE – standard error.

By using the T-test (Table 3), a statistically significant increase in the value of the pulse frequency was found when loading the horse into the trailer to the value of the pulse frequency measured in the stable [t=15.174; p<0.00]. The average increase in pulse frequency was 17,160, while the 95-percent confidence interval extends from the lower 14,826 to the upper 19,494. The result of the Eta square was 0.9, and following Cohen's guidelines, this value indicates that loading a horse into a trailer has a statistically very significant effect on increasing the pulse frequency.

Table 3 Results of T-test for paired samples

	Paired Differences					t	df	Sig (2-tailed)	Eta square
	$\bar{X}$	SD	SE	95 % Confidence Interval of the Differences					
				Lower	Upper				
BSFU - BSFB	17,160	5,654	1,131	14,826	19,494	15,174	24	,000	0,9

Legend: BSFB – number of pulse frequency measured in the stable, BSFU – number of pulse frequency measured during loading, Mean ($\bar{X}$) – mean increase in pulse, SD – standard deviation, t – indicator value, df – degree of freedom, (2-tailed) - level of significance ($p < 0.05$), Eta square - Eta square effect size.

DISCUSSION

Loading a horse into a trailer can significantly affect pulse frequency as an indicator of the horse's organism's response to loading as a stressful event. During the preparation for loading into the trailer, horses may experience stress due to changes in the environment, the presence of unknown objects such as trailers, or the presence of unknown persons and other horses that can be perceived as a potential threat and stressogenic factor. The stage of entering into the trailer can often be the most challenging moment within the process of horse transport, and thus a key stressogenic factor (Yngvesson et al., 2016, Hartmann et al., 2011). Horses, which are naturally timid beings, can perceive entering a trailer as a potential danger. During the entry of the horse into the trailer, an acceleration of the pulse frequency can be observed, as an indicator of stress reaction to the changed circumstances (Kay and Hall, 2009). Driving a horse in a trailer can also have a stressful effect on the horse because the oscillations and vibrations that occur during the ride can cause additional stress in the horse. During transport, especially if it is carried out in an inappropriate manner (inappropriate speed, sudden turns, acceleration and deceleration), horses can have difficulties with maintaining balance inside the trailer, which can be an additional source of stress, and manifest itself in an increase in pulse frequency (Tateo et al., 2012).

The process of unloading the horse from the trailer, after arriving at the destination, can represent another stressful moment for the horse's organism, and a new source of stress. The horse needs to adapt its behaviour to the new environment, and the very act of unloading from the trailer can cause a certain amount of discomfort and, consequently, stress. After unloading the horse from the trailer, the pulse frequency may remain increased for some time, because the horse needs some time to adapt to the new conditions. The stress reaction, expressed through an increase in pulse frequency compared to physiological values, can be maintained even after the horse has left the trailer, especially if the previous loading process, as well as the transport itself, had a stressful effect on the horse's body (Padalino, 2015).

When using horses in work, sports and other activities, it is of great importance to recognize the possible presence of a stress reaction as an indicator of the state of the organism (Mott et al., 2020). In addition to the previously mentioned indicators of the presence and intensity of a stress reaction, such as the level of adrenaline and cortisol in the blood, the presence of a stress reaction and its intensity can be identified through the variability of the heart rate, i.e. the pulse rate (Bukhari and Parkes, 2023; Ohmura and Jones, 2017, Munsters et al., 2013). It should be borne in mind that the key stressogenic factors that affect the horse's organism during its

use in work, competitions and other types of use are those that originate from the external environment, and are in contrast to the natural and usual forms of horse behaviour. Some of these factors are the restriction of the horse's movement during loading and transport, new sounds, contact with unknown people, unknown horses and similar factors and events that can cause stress (Dlugosz et al., 2020).

The results of this study showed that there was a statistically significant difference in the pulse frequency between the measurement in the stable during rest and the measurement when loading the horse into the trailer. The mean value of the pulse frequency was significantly higher during loading compared to the pulse frequency in the stable, and the value of eta square indicates that loading into the trailer has a statistically very significant effect on the increase in pulse frequency.

The mean value of the pulse frequency measured in the stable was in the range of expected physiological values for horses without stress and at rest. The value of the standard deviation indicates that all horses were not under stress during the measurement of the pulse frequency in the stable. An increase in the mean value of the pulse frequency during loading into the trailer indicates the presence of stress in horses due to fear during loading, while the value of the standard deviation indicates individual differences in the level of stress reaction during loading. An increase in the value of the pulse frequency can be an indicator of a change in the horse's behavioural state, i.e. a reaction to non-motor changes (Padalino, 2015). Pulse frequency as an indicator of the state of the autonomic nervous system, i.e. the net interaction between parasympathetic and sympathetic activity, can be used as an indicator of stress reaction, ie. the organism's response to a given situation. An increase in heart rate and pulse frequency can be considered a reliable indicator of stress reaction, as well as its intensity (Yngvesson et al., 2016, von Lewinski et al., 2013) and was previously used in studies conducted on horses (Borstel et al., 2017). Schmidt et al. (2010) also claim that acute and chronic stress can be identified through an increase in pulse frequency and a change in heart rhythm.

A statistically significant increase in the pulse frequency during loading compared to the measurement at rest, found in our study, is following the results of Shanahan (2003), who points out that there was a significant increase in the pulse frequency in the phase of loading the horse into the trailer compared to the starting position. Similar results were obtained in the study of Kay and Hall (2009), who state that the fear of loading, as a psychological stressor, can cause stress, which is indicated by an increase in pulse frequency. Loading into a trailer is ethologically unnatural for a horse and as such can be a cause of fear and therefore stress in the horse. A significant increase in heart rate during loading may indicate that loading into a trailer is a stressful activity, which is supported by the study of Waran and

Cuddeford (1995), who found that the heart rate during loading was higher than during transport itself, and concluded that loading is the most stressful phase of transport. The results indicating that loading a horse into a trailer initiates a stressful state are also supported by Tateo et al., (2012), who claim that stress is always identified in horses during loading. The detected stress in the horses included in our study expressed through a significant increase in pulse frequency during loading, can also be explained by the fact that loading has a very large effect (indicated by eta square value) on the increase in heart rate. Padalino (2015) in his study indicates that loading is a "frightening experience" for the horse, and a source of stress, while Dai et al. (2019) indicate that previous negative loading experiences can lead to permanent changes in the horse's behaviour and increase stress level during loading into trailer.

All horses included in the study had previous experience of loading and transport. Considering that the horse can learn and form conceptual behavior following its evolutionary course (Murphy and Arkins, 2007), the increase in heart rate may be the result of previous experiences related to loading, fear of confinement in a confined space or anticipation of transport. Conflicting reactions of the horse during loading into the trailer can lead to injuries to the horse and man. Condensed training period, inappropriate use of aids and inadequate approach to the individuality of each horse can produce unwanted responses in the horse (Hendriksen et al., 2011), and thus injury to the horse and the people who work with them. The correct training method can produce long-term desirable behavioural changes in horses to reduce reactive behaviour (Dai et al., 2019). Non-aversive methods of habituation and horse training to be loaded can reduce the level of stress and risks associated with loading a horse into a trailer (Padalino and Riley, 2020; Yngvesson et al., 2016; Shanahan, 2003). Positive induction through rewards and other types of positive experiences as a method of horse training can give encouraging results in training horses to be loaded in trailers and reducing stress levels (Dai et al.; 2019, McGreevy and McLean, 2007). Bukhari et al. (2021) also point to the importance of positive horse induction for reducing stress levels, and that it is important to establish proper horse management methods to protect their well-being.

CONCLUSION

Changes in the pulse frequency in horses can be used as an indicator of the stress reaction caused by loading into a trailer. The results of this study indicate that the horses had a stressful reaction when being loaded into the trailer, indicated by a statistically significant increase in pulse frequency during loading, compared to the

values found when resting in the stable. When interpreting the results of this and similar studies, in addition to the effect of the horse loading procedure, it is necessary to take into account other factors that can affect the intensity of the horse's stress reaction to manipulation in general, such as the psychosocial aspects of their growing up, the methods of the previous of working with them, their previous experiences in manipulation during previous loading, breed and individual characteristics of each horse. Understanding the causes of a horse's stress reaction to being loaded into a trailer is an important element in the creation of measures that will adapt the loading process to the needs of the horse's well-being, and to reduce the intensity of their stress reaction. Literature data indicate the beneficial effect of positive induction of horses with the help of rewards or other types of positive experiences on reducing the intensity of the stress reaction during various types of manipulation with horses, including when loading them into a trailer, which is why it is necessary to conduct wider research in future, to establish an optimal model of dealing with horses when loading them into the trailer.

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

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