Praca oryginalna **Original paper**

Effect of selected factors on features of short-term social isolation of horses: a pilot study*

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Received 03.11.2022 *Accepted 30.12.2022*

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Summary

This study analysed the effect of the sex of the horses as well as the type of their maintenance and use at the centre on the behavioural and physiological expression of short-term social isolation, which was assessed on the basis of heart and respiratory rates. A total of 27 clinically healthy horses were examined. The horses had social contact of various durations, were used with different regularity and were not equally accustomed to isolation. An isolation test was conducted. The heart and respiratory rates were measured at rest and after isolation, and the differences between the values were calculated. Moreover, points were assigned for the effect of isolation. The behaviour in subsequent stages of isolation was also observed. It was found that the sex of the horses, the period spent daily in the paddock in the herd, the regularity of use and habituation to staying in isolation can all be classified as factors that have no effect on the heart rate, respiratory rate or behaviour during short-term isolation. It was suggested that the resting respiratory rate and the effect of isolation could be classified as features that are potentially useful in assessing horses' adaptability to short-term social isolation.

Keywords: horse, isolation, heart rate, breaths, behaviour

The basic needs of horses include free movement, access to bulky feed and, importantly, social contact (8, 23). Not only does satisfying these needs affect the mental and physical comfort of animals, but it also helps humans to train them. It is believed that keeping horses in groups optimally satisfies their physical and behavioural needs, especially the need for social contact with other horses (15). It also has a beneficial effect on interactions between the horse and humans during training. Horses should be released into paddocks every day in order to make their living conditions as similar to natural conditions as possible. These measures are essential to their welfare. Staying on paddocks offers horses a sense of freedom and enables them to relax, reducing the risk of behavioural disorders, such as stereotypies (15, 30, 40).

Unfortunately, social isolation is one of major problems that currently affect horses (4). During the foaling period, the effects of longing for the mother are alleviated by allowing adult, balanced horses near the weaned foals. Positive social behaviours then intensify, while agonistic interactions decrease. A young, socially unstable individual is thus given support in the form of the herd leader. It is commonly known that during the successive stages of the development of horses in the process of domestication, they are often isolated or form small groups of the same sex, breed and age, which may serve to reduce the occurrence of injuries. Keeling et al. (22) only note the advisability of limiting the breed diversity in horse herds. They also demonstrated that the sex and age of horses had no effect on the frequency of injuries among then.

This finding is of particular importance, as according to Stanley et al. (41) the current practice of keeping horses in herds of unrelated individuals may provide sufficient benefits in terms of social support.

* Project financially supported by the Minister of Education and Science (Poland) under the 418 program entitled „Regional Initiative of Excellence" for the years 2019-2023, Project No. 419 010/RID/2018/19, amount of funding 12.000.000 PLN.

This can, therefore, be comparable to relationships in a natural horse herd, especially because the social system of horses is atypical of ungulates, but similar to that of some primate species, e.g. the gorilla (5, as cited in 27). Membership in the herd is so stable that some mares live in the same group for most of their adult lives. Similar to highly social primates, horses also have a good ability to recognise other individuals (5, as cited in: 28). Linklater and Cameron (27) are of a similar opinion and note that horses (*E. przewalskii*, *E. caballus*) and certain zebra populations (*E. zebra*, *E. burchelli*) are structured by long-term social and reproductive relationships in polygamous groups (26). These relationships are long-term in relation to reproductive life, and membership in the group is stable compared with that of other polygamous ungulates. However, with regard to herds of unrelated horses, it is important to add a new horse to a group of individuals already known to each other so as to reduce stress to the minimum (14). It is therefore suggested that a new horse be incorporated into the group of all horses staying together in the pasture because aggression towards an unknown horse is then minimised thanks to the singularity effect.

Horses need at least auditory and olfactory contact with companions (43). Since it is common practice to keep horses in single stalls, these contacts must be ensured as a substitute for a real social life. However, according to Yarnell et al. (45), stalls result in semi-isolation and limit social interactions to such an extent that they pose a serious hazard to the welfare of horses. Therefore, isolation-induced stress emerges in young horses that are moved from collective rooms to individual stalls (9). Any events that result in separation or confrontation with conspecifics can induce severe psychological stress, which manifests itself in behavioural and physiological changes, including an increased number of vocalisations, accelerated heart and respiratory rates or elevated plasma cortisol levels (23, 24). Opinions on changes in locomotor activity, however, are divided (24, 40). The anxiety associated with social isolation can also disturb the expression and interpretation of behaviours related to suffering (35). Moreover, social isolation is such a significant stressor for a horse that it can actually moderate the behaviour and changes in the heart rate associated with mild somatic pain.

It is also important to note that any movement stereotypies, such as pawing at the litter, walking around the stall or weaving, can be triggered by separation-induced stress (29, 42). When this happens, the animal puts excessive strain on its joints, bones and muscles, which may lead to lameness. In the paddock, on the other hand, the horse starts to run restlessly along the fence while calling nervously to the other individuals (45). Moreover, intensified movement stimulates intestinal peristalsis, which results in defecation, and the elevated body temperature causes sweating, which can even lead to dehydration. However, cribbing may result in problems with colic and bloating (44). In extreme cases, isolation-induced stress can also lead to self-harm and other abnormal behaviours (11).

The modern use of horses makes isolation unavoidable (20). However, the proper management of horses can help them endure this distressing situation in a relatively safe and trouble-free manner. Not only proper management, but also hanging toys, photographs of other horses and mirrors in the stalls are recommended to reduce isolation-induced stress (7, 21, 29).

The study assumed that the features of a short-term social isolation of horses depend on their sex (mares and geldings), the maintenance method at the centre (duration of social contact with the herd, regularity of use, degree of being accustomed to isolation) and the type of use (regular use for sports and regular or non-regular leisure use). It was also assumed that typical features of the isolation-induced behaviour of horses had an effect on their heart rate and respiration rate at that time. Moreover, the heart rate and respiration rate at rest can be determinants of the features of short-term isolation of horses (18-20).

The present study aimed to analyse the effect of the sex, the maintenance method at the centre, and the type of use on the behavioural and physiological expression of stress related to short-term social isolation. We also sought to assess features of this isolation using the heart rate and respiratory rate.

Material and methods

The project was financially supported by the Minister of Education and Science under the program „Regional Initiative of Excellence" for the years 2019-2022 (project no. 010/RID/2018/19, amount of funding 12.000.000 PLN).

The research material consisted of 27 clinically healthy Polish Warmblood sports and leisure horses (16 and 11 animals, respectively). The study group comprised 15 mares and 12 geldings. The horses did not exhibit behavioural abnormalities, which was determined by one of the authors through an interview with the keeper of the horses. Before the study, a behaviourist conducted a passive human test (12). The results did not differentiate the horses: all animals approached a human with confidence immediately after the beginning of the test.

The horses stayed at three different centres under similar atmospheric conditions: average annual air temperature of 8.2 ± 0.29°C (centre 1 – 8.0°C, centre 2 – 8.2°C, centre 3 – 7.8°C), atmospheric pressure of 104.97 ± 2.12 hPa (centre 1 – 103.0 hPa, centre 2 – 107.2 hPa, centre 3 – 104.7 hPa), and precipitation of 609.67 ± 10.34 mm (centre 1 – 612.9 mm, centre 2 – 597.6 mm; centre 3 – 618.3 mm) (https://klimat.imgw.pl/pl/biuletyn-monitoring/#2021/rok, 2022). Horse management was generally similar at all centres. The stalls in the stables had an area of approximately 12 m², with wall dimensions of 3 × 4 m or 3.5 × 3.5 m, and were equipped with a feeding trough, an automatic watering trough, and a salt-lick on a stand. The horses were fed three times a day with meadow hay and nutritive fodder (a mixture of oats and industrial feed granules). Once a day, they were admin-

Tab. 1. Differences in horse maintenance at the centres

Centre	Type of use	The duration (h) of social contact with the herd (the time spent in the paddock by day)	Number of horses	Age	Breed	Regularity of use	Habituation to social isolation
1	leisure	6-7	8	4-12	Polish warmblood, Wielkopolski, Hanoverian	moderately regular (holidays, weekends, occasionally on other days)	occasional (a maximum of 2-3 times a month)
2	leisure	8-12	9	5-15	Polish warmblood, Hanoverian	irregular (only during holidays)	occasional (a maximum of 2-3 times a month)
3	sports	2-3	10	5-14	Polish warmblood, Oldenburger	regular (6 days each week)	frequently (at least 5-6 times a week)

istered a vitamin mixture designed for horses. The quantity of the feed given was determined by individual needs of each animal. The authors described the condition of each horse as very good.

The differences in horse maintenance concerned the duration of social contact with the herd, the regularity of use and the degree of habituation to social isolation (Tab. 1).

Isolation test. The horses were released into rectangular-shaped paddocks, which were similar at all centres with the following dimensions: 176 m² (centre 1), 182 m² (centre 2), and 175 m² (centre 3). The horses were then left in the paddocks without human interference for 60 minutes after the release. This was followed by an isolation test, which involved walking each horse separately from the paddock to the stable located 90-120 m away from the paddock exit (depending on the centre). Each horse was walked to its own stall by the keeper. No other horses were present in the stable. The horse stayed in the stable in silence for five minutes. There was no feed in the stall during the test. While the horse was in the stall, only an experimenter was present in the stable corridor, at a distance of 5 m from the stall. After five minutes of isolation, the horse was led out to the stable corridor by the keeper. It was then led out from the stable and let loose on a path leading to the paddock, so that it could return to the herd. Another keeper was waiting for the horse near the paddock and opened the gate at the right moment to allow the horse to rejoin the herd. After 20 minutes, another horse was subjected to the same isolation test. Figure 1 shows the course of the isolation test.

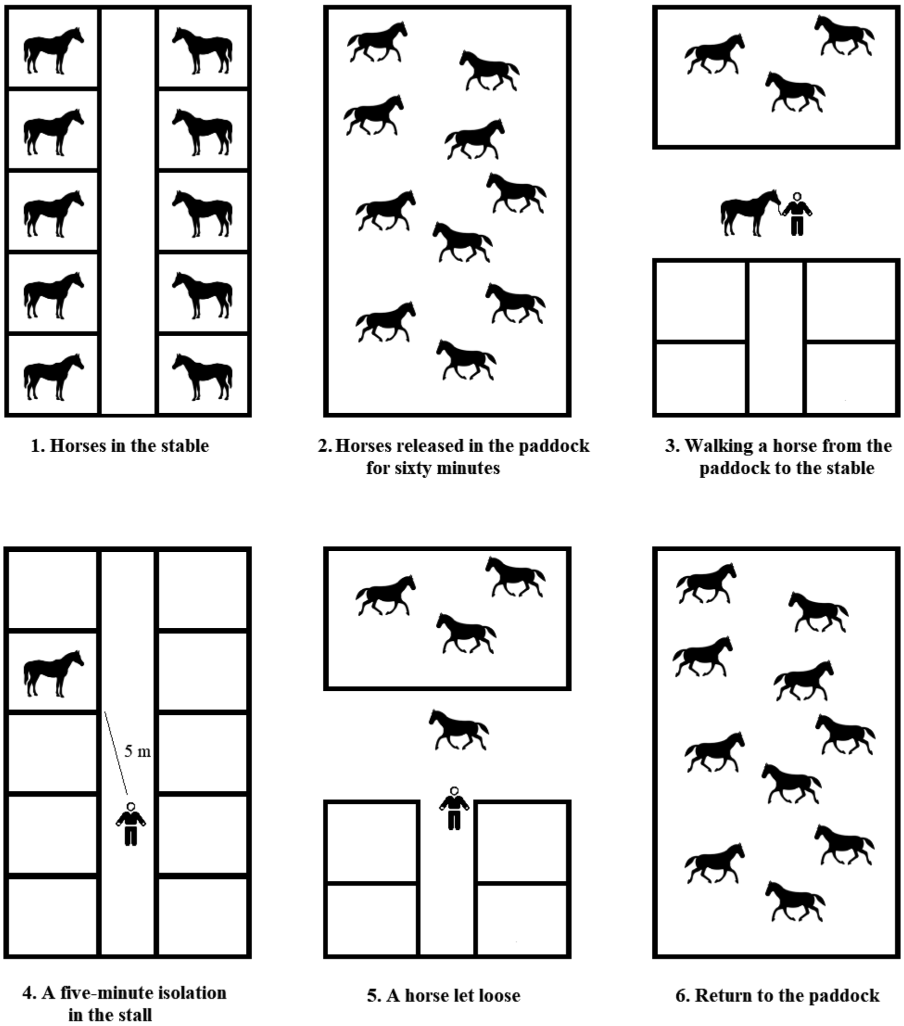

Fig. 1. The course of the isolation test

Measurement of the heart rate and respiratory rate. The heart rate and respiratory rate were measured twice. The first series of measurements (resting heart rate and resting respiratory rate) were carried out on the day of the isolation test, 30 minutes after the first feeding, which took place in the morning, between 06:00 AM and 08:00 AM, depending on the centre. The second series of measurements (heart rate after isolation and respiratory rate after isolation) were carried out in the stable corridor following the five minutes of isolation in a stall. The rising of the sides of the horse's body was observed at a point behind the ribs, and breaths taken during one minute were counted to determine the respiratory rate. The heart rate was measured with a stethoscope at a point behind the horse's elbow on the left side of the body. Based on the values obtained, the following indicators were calculated: the difference in the heart rate, i.e. the difference between the heart rate after isolation and the resting heart rate, and the difference in the respiratory rate, i.e. the difference between the respiratory rate after isolation and the resting respiratory rate. These indicators made it possible to consider individual physiological differences in determining the resting level of the parameters examined (18).

Time measurements and behavioural observations. The time between

Tab. 2. Points assigned to the horses for behaviour noted during isolating a horse from the herd and the time needed for this isolation (the effect of isolation)

Score	Behaviour
0	the horse escapes at a trot or a canter, does not allow the human to approach; catching and leading the horse away from the herd is impossible at six attempts
1	upon the approach of the human, the horse runs a few steps away at a trot or a canter, allows itself to be caught at the fifth attempt, walks or trots towards the exit with resistance, tries to stop or break free, looks back at the herd and vocalises
2	upon the approach of the human, the horse runs a few steps away at a trot, allows itself to be caught at the fourth attempt, walks or trots towards the exit with resistance, tries to stop or break free gently, looks back at the herd, and vocalises
3	upon the approach of the human, the horse walks or trots a few steps away, allows itself to be caught at the third attempt, walks towards the exit with no resistance, looks back at the herd, and vocalises
4	upon the approach of the human, the horse walks a few steps away, allows itself to be caught at the second attempt, walks towards the exit with no resistance, looks back at the herd, and does not vocalise
5	upon the approach of the human, the horse stands still, allows itself to be caught at the first attempt, walks towards the exit with no resistance, does not look back at the herd, and does not vocalise
Score	**Time (s) needed for isolating a horse from the herd (value interval)**
0	≥ 900
1	600-899
2	300-599
3	120-299
4	60-119
5	0-59

the moment the keeper entered the paddock through the gate to catch the horse and walk it to the gate and the moment of exiting the paddock was measured. The behaviourist also assessed the horses' behaviour during that time. The results of time measurements and behavioural observations were grouped into value intervals, and points ranging from 0 to 5 were then assigned (Tab. 2). The final score was the sum of the points assigned, so the horse could obtain from 0 to 10 points. This feature was described as the effect of isolation.

From the moment the horse was led out of the paddock to the moment of return into the paddock, its behaviour was observed, including the number of defecations and vocalisations. During the horse's stay in the stall, the number of acts of pawing at the ground with the front leg and walking around the stall was determined, noting the short-term occurrence of this feature, i.e. not exceeding 150 s (less than half of the time spent in isolation – short-term activity) and the continuous occurrence of the feature, i.e. exceeding 150 s (more than half of the time spent in isolation – continuous activity). The prevailing type of gait during the return of the loose horse from the stable to the paddock gate (the manner of returning to the herd) was also noted: walk, trot, canter (over 50% of the distance) or failure to join the herd by the horse's choice.

Statistical methods. The following fixed factors were defined: sex (two levels), centre (three levels), type of use (two levels) and, additionally (for selected models): the manner of returning to the herd (three levels), the occurrence of defecation (two levels), short-term activity (two levels), continuous activity (two levels), pawing at the ground and walking around the stall (two levels). The variability of the following characteristics was analysed: resting heart rate, heart rate after isolation, difference in the heart rate, resting respiratory rate, respiratory rate after isolation, difference in the respiratory rate, the score for the so-called effect of isolation, and the number of vocalisations. The last two features were not used in all calculations, because of the data structure.

Statistical calculations were performed using the SAS 9.4 package (38). In order to select the optimum data analysis method, the distribution of features was analysed on the basis of the Kolmogorov-Smirnov test.

The effect of isolation on the change in the heart rate and respiratory rate was analysed on the basis of Student's *t*-test for the dependent variables. Coefficients of Spearman rank correlation between the following features were then calculated along with their significance ($\alpha = 0.05$): resting heart rate, heart rate after isolation, difference in the heart rate, resting respiratory rate, respiratory rate after isolation, difference in the respiratory rate, the score for the so-called effect of isolation, and the number of vocalisations. We then examined the impact of the sex, centre, type of use, manner of returning to the herd, occurrence of defecation, short-term activity, continuous activity and pawing at the ground on the values of the resting heart rate, heart rate after isolation, difference in the heart rate, resting respiratory rate, respiratory rate after isolation and difference in the respiratory rate. The analysis included the Wilcoxon (rank-sum) test and the Chi^2 Kruskal-Wallis test. For fixed effects with a significant impact on the level of the features analysed (the score for the so-called effect of isolation, defecation, short-term activity, continuous activity and pawing at the ground), an even analysis of multiple two-sided comparisons was conducted by the Dwass, Steel and Critchlow-Fligner method.

Results and discussion

Significant differences were found between the heart rate and respiratory rate measured after isolation and at rest (Tab. 3).

Tab. 3. The effect of isolation on the heart rate and respiratory rate

Difference in the parameter value after isolation and at rest	μ	SE	t value	Pr. > |t|
Heart rate	–4.38	0.79	–5.57	< 0.01
Respiratory rate	–1.42	0.23	–6.19	< 0.01

The coefficients of correlation between the features analysed proved to be statistically significant in almost 68% (Tab. 4). In the group of significant correlations, 26% were negative. The resting respiratory rate and resting heart rate were correlated with the other features in the smallest number of cases, while the respiratory rate after isolation was significantly correlated with all of the features. The score for the so-called effect of isolation was the only feature to correlate negatively with the other features.

Tab. 4. Coefficients of Spearman rank correlation between the features

Feature	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Resting heart rate	0.72*	0.29	0.49*	0.48*	0.29	–0.13	0.27
(2) Heart rate after isolation		0.83*	0.25	0.68*	0.79*	–0.58*	0.68*
(3) Difference in the heart rate			0.01	0.56*	0.89*	–0.63*	0.80*
(4) Resting respiratory rate				0.73*	0.06	–0.18	0.08
(5) Respiratory rate after isolation					0.69*	–0.59*	0.59*
(6) Difference in the respiratory rate						–0.67*	0.79*
(7) Score for the effect of isolation							–0.53*
(8) Number of vocalisations							

Explanations: * a significant correlation at $\alpha = 0.05$

Tab. 5. Analysis of the impact of fixed effects on the value of the features analysed

Factor / Feature	Sex		Centre		Type of use	
	Chi^2	$Pr > Chi^2$	Chi^2	$Pr > Chi^2$	Chi^2	$Pr > Chi^2$
Resting heart rate	0.51	0.47	4.11	0.25	0.96	0.32
Heart rate after isolation	0.02	0.96	1.50	0.66	0.61	0.43
Difference in the heart rate	0.77	0.38	0.62	0.89	0.04	0.85
Resting respiratory rate	0.01	0.92	1.68	0.64	3.58	0.06
Respiratory rate after isolation	0.04	0.83	1.50	0.68	3.04	0.08
Difference in the respiratory rate	0.07	0.87	0.90	0.82	0.04	0.85

Tab. 6. Analysis of the dependence of the factor "the manner of returning to the herd" on the value of the features analysed

Feature	Chi^2	$Pr > Chi^2$	Multiple comparisons		
			Walk (n = 2)	Trot (n = 14)	Canter (n = 11)
Resting heart rate	5.69	0.13	26.00	27.15	28.40
Heart rate after isolation	7.23	0.04	29.00^{a}	30.31^{ab}	35.00^{b}
Difference in the heart rate	4.79	0.19	3.00	3.15	6.60
Resting respiratory rate	9.88	0.02	7.50^{a}	8.62^{a}	9.70^{b}
Respiratory rate after isolation	14.17	< 0.01	8.50^{a}	9.62^{a}	11.90^{b}
Difference in the respiratory rate	7.58	0.04	1.00^{a}	1.00^{a}	2.20^{b}

Explanations: a, b – the values marked with different letters differ significantly at $\alpha = 0.05$

No significant impact of fixed effects, such as the sex, centre or type of use, on the value of the features analysed was observed (Tab. 5).

The values of the resting respiratory rate, respiratory rate after isolation and the difference in the respiratory rate were significantly higher for horses which cantered to the herd than they were for horses which walked or trotted to the herd. The heart rate after isolation only varied between horses returning to the herd at a walk compared with those returning at a canter (Tab. 6).

Multiple comparisons for the fixed effects are presented in Tables 7-10, including the arithmetic means of the features analysed, where 1 denotes the occurrence of the factor, and 0 denotes its non-occurrence.

The heart rate after isolation, difference in the heart rate and difference in the respiratory rate were significantly higher for horses which defecated during isolation than they were for horses which did not defecate (Tab. 7). In the other cases, this factor did not significantly differentiate the values of the features analysed.

The resting heart rate and the heart rate after isolation were significantly lower in horses with "short-term activity" noted, as compared to the horses in which no effect of this factor occurred (Tab. 8).

For horses with the "continuous activity" noted, the values of the heart rate after isolation, the difference in the heart rate, the resting respiratory rate, the respiratory rate after isolation and the difference in the respiratory rate were significantly higher than the values noted when no "continuous activity" occurred (Tab. 9).

The horses showing the feature of "pawing at the ground" were characterised by higher values of the heart rate after isolation, the difference in the heart rate, the respiratory rate after isolation and the difference in the respiratory rate compared to the horses that did not paw at the ground (Tab. 10). The modern use of horses requires that these animals be isolated for shorter or longer terms (16). Social isolation undermines the principle of the five freedoms, which are the foundation of welfare (33). However, for working animals, such as horses, isolation appears to be unavoidable. Moreover, it appears that at least some horses (e.g. sports horses) may become accustomed to even prolonged social isolation (16).

Nevertheless, this is not always the case, as evidenced by various scientifically described effects of separation, for example, stress expressed in physiological and behavioural changes (32). This bipolarity in horses' response to isolation indicates the need to answer the question as to what determines tolerance or intolerance of this condition. Is it merely the proper horse management when preparing an animal for isolation, or is it individual factors that determine the final effect? Is it possible to predict which horses will cope with isolation better and which worse?

Some of the parameters tested in this study (resting heart rate and respiratory rate, heart rate and respiratory rate after isolation, difference in the heart rate and respiratory rate, points assigned for the so-called effect of isolation, and the number of vocalisations) were most frequently correlated with each other. Actually, in most cases, these correlations were positive. This result is not surprising, as according to Hothersall and Casey (17), changes in the level of each of the features analysed here can be regarded as a typical picture of isolation-induced stress. However, the small number of significant correlations (compared to the others) between the resting heart and respiratory rates and the other features appears to be explainable. Not only are these parameters an expression of emotional excitability, but they also indicate individual physiological properties of the body or simply the physical fitness level (1). It can therefore be suggested that an early assessment of the adaptability of specific horses to isolation should be based on other parameters. This study looks at the difference between the resting and post-isolation values of the parameters. It appears that these values can most effectively assess response to isolation, as they will show the actual condition of the animal after isolation while considering the resting parameter values. The resting parameter values (although they are characterised by low variability, which makes it difficult to assess a specific response of the body to a particular phenomenon) generally determine basic emotional excitability (19). Therefore, they should not be completely disregarded.

Interestingly, negative correlations were noted when comparing the score for the so-called effect of isolation with the heart rate and respiratory rate after isolation, the difference in the heart rate and respiratory rate and the number of vocalisations. It means that individuals that tried to avoid isolation had an elevated heart rate, respiratory rate and number of vocalisations after isolation. The horses which better accepted being led away from the herd also better accepted spending time in isolation. Thus, the course of isolating the horse from the herd (effect of isolation) indicates the level of isolation-induced stress.

It also appears that neither the sex of the horses nor the type of use had any effect on the values of the physiological features under study. However, this should come as a surprise. As far as the sex is concerned, mares are expected to respond to isolation more negatively than geldings. Natural horse herds show that it is mares that establish close social relationships (37). Males, at most, live in more or less permanent bachelor groups or serve reproductive and protective functions in a herd of mares and their offspring (13). Therefore, the different functions that horses play in the herd suggested a hypothesis about the effect of the sex on horses' physiological response to isolation, which was rejected in the current study.

Tab. 7. Analysis of the effect of the factor "defecation" on the value of the features analysed

Feature	Chi^2	$Pr > Chi^2$	Multiple comparisons	
			0 (n = 18)	1 (n = 9)
Resting heart rate	1.18	0.27	27.06	28.00
Heart rate after isolation	4.70	0.03	30.35^b	34.44^a
Difference in the heart rate	4.35	0.04	3.29^b	6.44^a
Resting respiratory rate	0.04	0.84	8.82	9.00
Respiratory rate after isolation	1.96	0.16	9.88	11.11
Difference in the respiratory rate	5.00	0.03	1.06^b	2.11^a

Explanations: 0/1 – no occurrence of the effect/occurrence of the effect; a, b – the values marked with different letters differ significantly at $\alpha = 0.05$

Tab. 8. Analysis of the effect of the factor "short-term activity" on the value of the features analysed

Feature	Chi^2	$Pr > Chi^2$	Multiple comparisons	
			0 (n = 16)	1 (n = 11)
Resting heart rate	0.61	0.04	27.93^a	26.73^b
Heart rate after isolation	4.57	0.04	31.87^a	30.24^b
Difference in the heart rate	0.07	0.79	4.73	3.91
Resting respiratory rate	0.20	0.65	9.00	8.73
Respiratory rate after isolation	0.02	0.89	10.40	10.18
Difference in the respiratory rate	0.04	0.85	1.40	1.45

Explanations: as in Tab. 7

Tab. 9. Analysis of the effect of the factor "continuous activity" on the value of the features analysed

Feature	Chi^2	$Pr > Chi^2$	Multiple comparisons	
			0 (n = 18)	1 (n = 9)
Resting heart rate	1.13	0.29	27.00	28.11
Heart rate after isolation	4.02	0.04	29.94^b	35.22^a
Difference in the heart rate	6.55	0.01	2.94^b	7.11^a
Resting respiratory rate	3.37	0.04	8.53^b	9.56^a
Respiratory rate after isolation	6.10	0.01	9.59^b	11.67^a
Difference in the respiratory rate	4.63	0.03	1.06^b	2.11^a

Explanations: as in Tab. 7

Tab. 10. Analysis of the effect of the factor "pawing at the ground" on the value of the features analysed

Feature	Chi^2	$Pr > Chi^2$	Multiple comparisons	
			0 (n = 17)	1 (n = 10)
Resting heart rate	3.47	0.06	26.69	28.50
Heart rate after isolation	8.35	< 0.01	29.50^b	35.40^a
Difference in the heart rate	5.22	0.02	2.81^b	6.90^a
Resting respiratory rate	3.60	0.06	8.50	9.50
Respiratory rate after isolation	11.62	< 0.01	9.31^b	11.90^a
Difference in the respiratory rate	11.12	< 0.01	0.81^b	2.40^a

Explanations: as in Tab. 7

Another factor that had been expected to differentiate the horses was the centre where they were kept, since different durations of social contact in the paddock, degrees of habituation to isolation and regularity of use were regarded as highly relevant to the problem analysed in this study. According to Cooper et al. (6), a long period of staying in the herd provides an opportunity to establish actual social bonds. It was therefore reasonable to assume that horses that stayed in the paddock for a limited period of time would tolerate isolation more readily. Moreover, just as in the current study, those horses were additionally accustomed to isolation due to their being used for sports purposes (the type-of-use factor). It turned out, however, that this aspect did not differentiate the results either. This is rather surprising, but may be variously explained. For example, social relationships are often discussed on the basis of natural horse herds (31). However, good horse management reduces the effects of isolation (25). Moreover, psychological traits of horses correlate with isolation-induced stress level (2). It is therefore difficult to assess what in the present study contributed to such findings. Perhaps it was the psychological traits of the horses that played a decisive role.

Important results were also obtained from the analysis of features which, due to their very low variability, could only be regarded as factors. It should be emphasised that the authors are aware of the limitation of the study, namely, the small number of horses examined, especially considering the number of factors analysed. It appears, however, that the statistical analysis rejected two previously analysed features, namely, the points assigned for the so-called effect of isolation and the number of vocalisations. The number of vocalizations was positively correlated with the heart rate after isolation, difference in the heart rate, respiratory rate after isolation and difference in the respiratory rate, which may indicate greater emotional reaction in horses during isolation. For this reason, they were regarded as factors for the analysis of the other features. However, there is one interesting example in the results regarding the previously mentioned resting values of these parameters. The respiration rate at rest was actually significantly higher in horses cantering to the herd than it was in those walking or trotting. The results were confirmed for the analysis of the "continuous activity," which was also noted in horses with a significantly higher resting respiratory rate. However, since it is only a single parameter in this paper, and the result did not concern all of the behavioural features analysed, it can only be hypothesised that horses with an increased resting respiratory rate can tolerate social isolation less readily. Freeman (10) took a similar view when he concluded that this could have been related to a greater demand for oxygen and increased gas exchange.

Another noteworthy fact is that the occurrence of the behavioural features was often associated with significantly higher values of heart and respiratory rates after isolation and of the difference in the heart and respiratory rates. For example, horses which defecated had a higher heart rate after isolation and higher differences in the heart rate and the respiration rate. Moreover, horses which pawed at the ground during their stay in the stall also had a higher respiratory rate after isolation than did the other horses. Continuous activity in the stall resulted in a significant increase in almost all parameters. Short-term activity, on the other hand, did not result in such an increase, as, surprisingly, a lower value of the heart rate after isolation was noted in horses in which this feature had occurred. Horses studied by Baragli et al. (3) showed low HRs in response to stressful situations despite looking slightly agitated when their behaviour was being analysed. Moreover, Minero et al. (34) found that the HR response to restraint by covering a horse's head with a solid hood was lower in sport horses than in therapeutic riding horses. These observations indicate that some horses can react to stressful stimuli by changing their behaviour or they may mask their reactions, but the heart rate increases. Thus, the low level of excitability observed in some horses is not a genetic or general feature, but rather a result of previous experience and training. However, the other parameters did not differ significantly. Most probably, the horses discharged their emotions in this way. A similar claim was made by Ricci-Bonot et al. (36), who called it behavioural buffering.

Therefore, it appears that the willingness of breeding horses to socialise can be significantly limited and that it cannot be compared to social relationships in a herd of free-living horses. Unnatural maintenance conditions and the nature of use change horses' behaviour irreversibly. Silk (39) takes a similar view, claiming that sociality evolves when the direct benefits of a close relationship with other individuals exceed the costs borne by the body. Humans increasingly do not favour the horses' sociality, which may result in these animals having to learn how to function "normally" without a herd.

The sex of the horses, procedures followed at the horse maintenance centre associated with different durations of daily social contact (horses' habituation to solitude) and the regularity and type of use can be classified as factors that have no effect on the heart rate, the number of breaths or the behaviour during short-term isolation. It can also be suggested that the resting respiratory rate and the effect of isolation (i.e. the time needed for isolating a horse from the herd and the horse's behaviour at that time) can most probably be classified as potentially useful in the assessment of horses' adaptability to social isolation. However, due to the limitations of the present study, this claim is only a hypothesis.

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