Original scientific paper
Оригинални научни рад
UDC 636.1.082.25:159.9.019.4
DOI 10.7251/AGREN2504257G

University of Banjaluka, Faculty of Agriculture
Agro-knowledge Journal

The analysis of horse interactions depending on group size through the novel object test

Maja Gregić [1], Iva Malenica[2], Vesna Gantner [1], Tina Bobić [1]

[1] *University of Josip Juraj Strossmayer in Osijek, Faculty of Agrobiotechnical Sciences, Osijek, Croatia*
[2] *Abstract factory Ltd., Tordinci, Croatia*

Abstract

The study analyses equine responses to novelty, focusing on how the size of a horse group affects interactions with unfamiliar objects and the degree of curiosity exhibited by the horses. The novel object test (NOT) was used as a method employing three different objects (with / without food). The aim is to demonstrate a link between the object-directed curiosity and group size. Being herd animals by nature, horses derive a sense of security from their social structure, but the development of cognitive abilities in each individual can vary depending on the herd size and environmental factors that foster curiosity. In smaller groups, horses tend to integrate more equally, which allows them to confront new challenges more swiftly and cohesively. Conversely, larger groups may lead to increased individualism. Smaller groups facilitate a more collaborative environment, particularly in scenarios involving intensive human-horse interactions through training, which provides both stimulation and a secure foundation for work. In larger groups, curiosity stimulation is enhanced through positive reinforcement during interactions. The smaller group approached the objects more quickly, the object without a reward (food) was the least interesting, and the approach time varied between the first and second testing. These findings are expected to enhance equine welfare within horse breeding practices. Notably, considerable individual variation has been observed in the horses' responses, indicating a need for further research to deepen our understanding of both equine interactions and their interactions with humans.

Key words: Equine, Curiosity, Novel object test (NOT), Welfare

Introduction

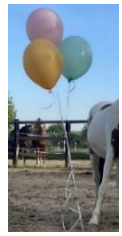
Numerous studies have recently emerged regarding the human-horse relationship, the dynamics of horses with their own species, and the nature of their curiosity. Kelly et al. (2021) underscored significant gaps in our understanding of the emotional states that horses experience during interactions with humans. Freeman (2019) identified factors related to both horses and their owners that can influence this relationship, while Brubaker et al. (2013) and Lloyd et al. (2008) examined the inherited genetic personalities of horses in their interactions. The ability of horses to adapt to novel objects is crucial for ensuring safety in the horse-human relationship. Horses, being herd animals, are naturally curious; however, the variability in their curiosity and interactions with the unknown may be influenced by the size of their herd, especially in today's context of intensive human interaction. To what extent does herd size offer individuals a sense of security in exploring the unknown and enhancing their cognitive abilities? Christensen et al. (2021) suggest that curiosity directed toward novel objects could be central to cognitive performance in horses across various learning tasks, a phenomenon that has previously been demonstrated primarily in humans and primates. According to Liehrmann et al. (2022), the development of the horse-human relationship can take time and is shaped by various factors, including the horse's past and present interactions with humans that impact their daily lives. Klitzing et al. (2025) recommend conducting novel object tests in the same location and under identical conditions, while also emphasizing the importance of considering the individual behaviour of each horse. According to Hartmann et al. (2021), attachment and bonding are essential components of interactions, making horses excellent subjects for studying the dynamics of these processes due to their social nature and contemporary use. However, specific factors that contribute to successful bonding remain largely unclear. Horses have demonstrated the ability to habituate to various colours and shapes simultaneously (Christensen et al., 2011). The novel object recognition test is one of the most commonly used behaviour assessments as it is quick to carry out and can be customized (Drayson et al., 2023). In horses a novel object test assesses their temperament and stress by observing their reaction to an unfamiliar object in a defined environment (stall or pen). It helps to evaluate curiosity, fear, and adaptability, with a horse's level of interaction with the object indicating its state. Common objects used include traffic cones, and balls, and the test can be conducted in the horse's stall or pen to minimize environmental influences (Bulens et al., 2015). The novel object test is a behavioural test designed to investigate exploratory and fear behaviour of unknown objects when animals are confronted with an unfamiliar object (Klitzing et al., 2025). Initially, they tend to avoid unfamiliar objects (Christensen, 2013), a behaviour significantly

influenced by the stimuli presented in the novel object test (NOT). This testhas been recommended by various institutions. For instance, the Wageningen Welfare Assessment Protocol for horses suggests a novel object test (NOT) (Klitzing et al., 2025). Powell et al. (2023) suggest that a focused evaluation of heart rate cluster phenotypes concerning behavioural reactions could aid in identifying genes that influence startle reflexes to novel stimuli, facilitating the selection of desired genetic variants. Notably, most studies employing the novel object test (NOT) involve testing horses individually in their interaction with humans (Forkman et al., 2007). After undergoing habituation training with an object, horses generally exhibit a diminished reaction (Leiner and Fendt, 2011). The novel object test is one of the three most common fear tests in veterinary science and employed in several different species. Although having been applied in several different studies in horses, it is surprising that there is no standardized test procedure available for these kinds of tests (Klitzing et al., 2025). This raises the question of how herd size impacts interactions with unfamiliar objects during the novel object test (NOT), particularly when considering the perception of the horse in light of breeding changes. The aim of this paper was to determine how herd size influences horses' interactions with unfamiliar objects and the extent to which it stimulates their curiosity. The aim of the study was to demonstrate a link between the object-directed curiosity and group size.

Material and Methods

A total of nine horses participated in the study. All horses were housed and trained under the same conditions, were in good health, and did not receive any treatments. They were kept in individual boxes and fed a uniform diet. Due to an uneven age distribution among the horses, they were categorized into two groups according to age: the first group (1) consisted of six horses, while the second group (2) included three horses. Each group comprised horses of various age (7 to 16 years old) that interacted with one another and the test leader. Horses of different breeds and breeding types were used for riding. The novel object test (NOT) for horses was conducted on familiar terrain. The test was performed on nine horses, all handled by the same individual. The experimental procedures adhered to the guidelines for the ethical treatment of animals in applied animal behaviour research (International Society for Applied Ethology, 2025). The methods used in this study were photography, video, and observation with notes of the time of access to the subjects (P1, P2, or P3). Testing was carried out on several horses at once in groups. Observation was not enough, so each test was recorded to make it easier to notice certain changes in the horses' behaviour and record the exact time. Three static novel object tests (NOT) (P1, P2, and P3) were used in the testing, as presented in Table 1.

Tab. 1 - Static new objects (P1, P2, and P3) in the novel object test (NOT)

OBJECT 1 (P1)		OBJECT 2 (P2)		OBJECT 3 (P3)	
	Black basket: filled with hay, carrots placed in the basket holes , pink Pilates ball placed on top of the basket It contained a prize.		Three balloons: different colours, on the same white string, filled with helium It did not contain a prize.		Merger of objects 1 and 2 Black basket: filled with hay, carrots placed in the basket holes, a pink Pilates ball on top of the basket Three balloons: (different colours, on the same white string, filled with helium; It contained a prize.

The testing was conducted over a period of 10 days in a secured area, free from the distractions of other people or animals. The horses were assessed in a familiar environment and were given 10 minutes before each test to acclimatise to the terrain. For three consecutive days, the horses were introduced to the objects P1, P2, and P3, each presented daily, with a repeat of the tests occurring seven days later in the same sequence. Between testing days, the horses remained in their regular environment and routine. Throughout the assessment, ethological data were collected, focusing on the amount of time spent approaching objects P1, P2, or P3.

Tab. 2 - Testing schedule by day, during the novel object test (OBJECT P 1, 2, or 3, testing (1 or 2), and group of horses (1 or 2))

Day	Test OBJECT 1 (P1)	Test OBJECT 2 (P2)	Test OBJECT 1 (P3)
1.	Presented 1 time (P1_1_1, P1_1_2)		
2.		Presented 1 time (P2_1_1, P2_1_2)	
3.			Presented 1 time (P3_1_1, P3_1_2)
8.	Presented 2 time (P1_2_1, P1_2_2)		
9.		Presented 2 time (P2_2_1, P2_2_2)	
10.			Presented 2 time (P3_2_1, P3_2_2)

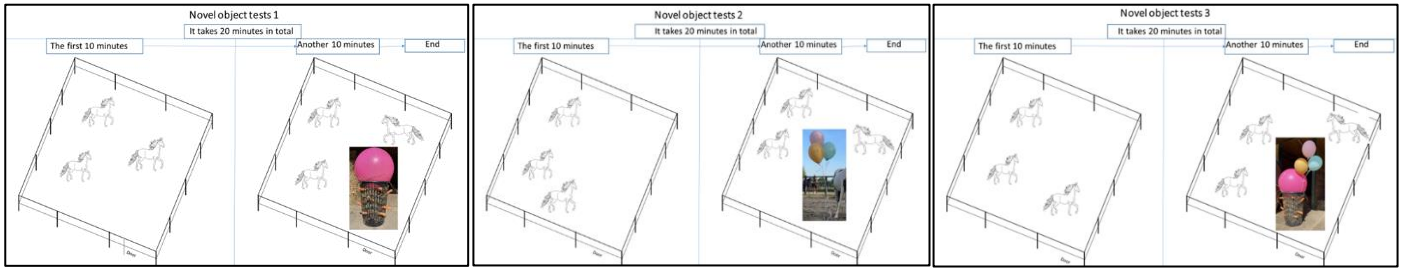


Fig. 1 - The novel object test (OBJECTS 1, 2, or 3)

Tab. 3 - The novel object test of tests and results in measured time by object (P 1, 2, or 3), testing (1 or 2), horse (1 – 9), group (1, 2)

Test / Horse	**P1_1**	**Group**	**P1_2**	**Group**	**P2_1**	**Group**	**P2_2**	**Group**	**P3_1**	**Group**	**P3_2**	**Group**
1	P1_1_1		P1_2_1		P2_1_1		P2_2_1		P3_1_1		P3_2_1	
2	P1_1_2		P1_2_2		P2_1_2		P2_2_2		P3_1_2		P3_2_2	
3	P1_1_3	**P1_1_1**	P1_2_3	**P1_2_1**	P2_1_3	**P2_1_1**	P2_2_3	**P2_2_1**	P3_1_3	**P3_1_1**	P3_2_3	**P3_2_1**
4	P1_1_4		P1_2_4		P2_1_4		P2_2_4		P3_1_4		P3_2_4	
5	P1_1_5		P1_2_5		P2_1_5		P2_2_5		P3_1_5		P3_2_5	
6	P1_1_6		P1_2_6		P2_1_6		P2_2_6		P3_1_6		P3_2_6	
7	P1_1_7		P1_2_7		P2_1_7		P2_2_7		P3_1_7		P3_2_7	
8	P1_1_8	**P1_1_2**	P1_2_8	**P1_2_2**	P2_1_8	**P2_1_2**	P2_2_8	**P2_2_2**	P3_1_8	**P3_1_2**	P3_2_8	**P3_2_2**
9	P1_1_9		P1_2_9		P2_1_9		P2_2_9		P3_1_9		P3_2_9	

On the first testing day, the horses encountered a novel object containing food (OBJECT 1 (P1)). The second day featured a new object without any feed (OBJECT 2 (P2)), and on the third day, they were presented with an object that included both stimuli (OBJECT 3 (P3)). The same testing procedures were replicated seven days later. In total, three distinct tests involving three different objects (P1, P2, and P3) were conducted (Table 1), with the horses being organized into two groups and each test repeated twice (Tables 2 and 3). The tests were performed by a single individual familiar to the horses, which aided in the interactions. The methodologies used included photography, video recording, and observational notes on the horses' access time to objects (P1, P2, or P3). The testing involved several horses simultaneously in groups 1 and 2. Due to the limitations of observation alone, each session was recorded to better capture and analyse any changes in the horses' behaviour. Figure 1 shows the flow of testing the novel object test of each group through OBJECTS 1, 2, and 3 over a period of time.

The test results were divided into groups according to the size of the group of subjects in testing P1, P2, and P3 through data analysis. Ethological indicators are presented textually. Time periods of access to the subject are presented graphically. Statistical processing was performed and graphical displays were made. The results are presented plots and estimated marginal means.

Results and Discussion

Based on the size of the group and the novel object test, the horses were divided into two groups. Figure 2 represents the approach times for different object, P1, P2, or P3, categorized by groups 1 (a) and 2 (b), along with the mean values for each group. From Figure 2, it is evident that the larger group took the longest to approach the objects, averaging 295 seconds, while the smaller group averaged 218 seconds. The first group exhibited a decrease in approach time during repeated testing. Notably, they were least inclined to approach object P2, which also took them the longest time to reach. In contrast, they approached object P3 (268 seconds) faster than object P1 (370 seconds). The second group showed a quicker approach to the objects during repeated trials, except for object P2, which saw an increase in approach time from 15 seconds in the first test to 63.3 seconds in the second. Object P2 consistently took the longest for the horses to approach, with some even refraining from approaching it altogether. This disinterest in interacting with object P2 appears to stem from a lack of motivation, as it does not present a food incentive. The results of this study have shown considerable differences in the behaviour of horses during the novel object test. Moreover, the behaviour of horses differs in particular whether it contains food or not.

To promote accurate results, Klitzing et al. (2025) suggested choosing a time frame with expected minimal interference with other horses, personnel, or agricultural machinery and a duration extending 5 min, which was done. The novel object test was conducted at the same location (Klitzing et al., 2025) comparing several tests (P1, P2, and P3). According to Bulens et al. (2015), the presence of roughage influences a horse's reactions and should be considered, a finding that is also evident in this research. The mean values indicate that a smaller group of horses approached the unfamiliar object more quickly on average, which, as noted by Christensen et al. (2021), suggests they exhibit greater cognitive success. Weak or rapid reactions, or even the lack of response to P2 during repetitions, indicate the horse's habituation, a phenomenon corroborated by Leiner and Fendt (2011). The low incentive for both groups in the P2 test stems from the item itself, which does not provide a reward to the food bank, a conclusion that aligns with other research on negative reinforcement (Christensen, 2013). In contrast, the objects in P1 and P2 experienced positive reinforcement through food and approached the object more swiftly in the second repetition, signalling the development of cognitive abilities, according to Christensen et al. (2021).

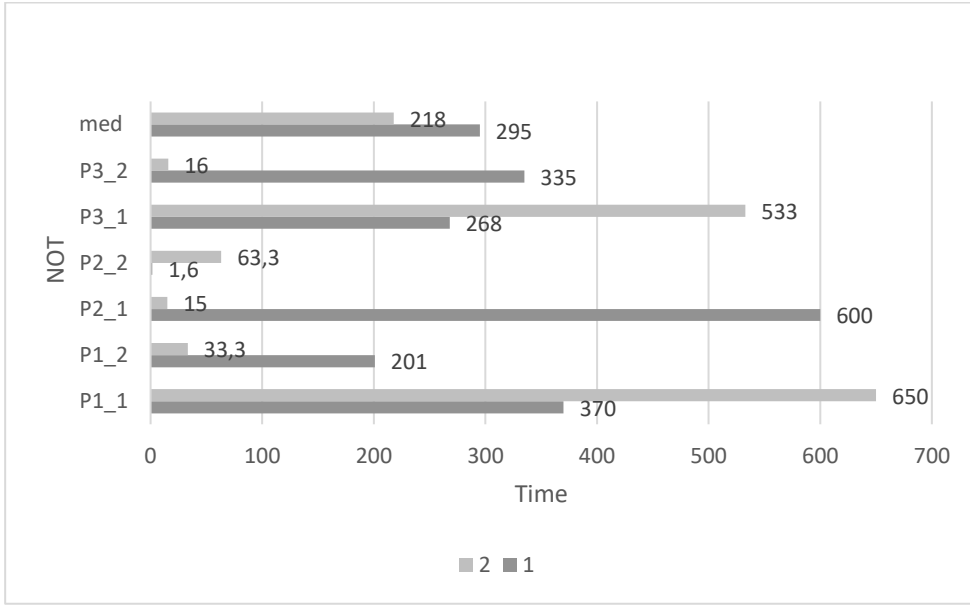


Fig. 2 - Approaching time period of approach in the novel object test (NOT) of horses to the object (P1, P2, or P3) according to the first (1) or repeated (2) tests in the first

1 (n=6) and second 2 (n=3) groups of horses, and presentation of the mean value according to the group size (med)

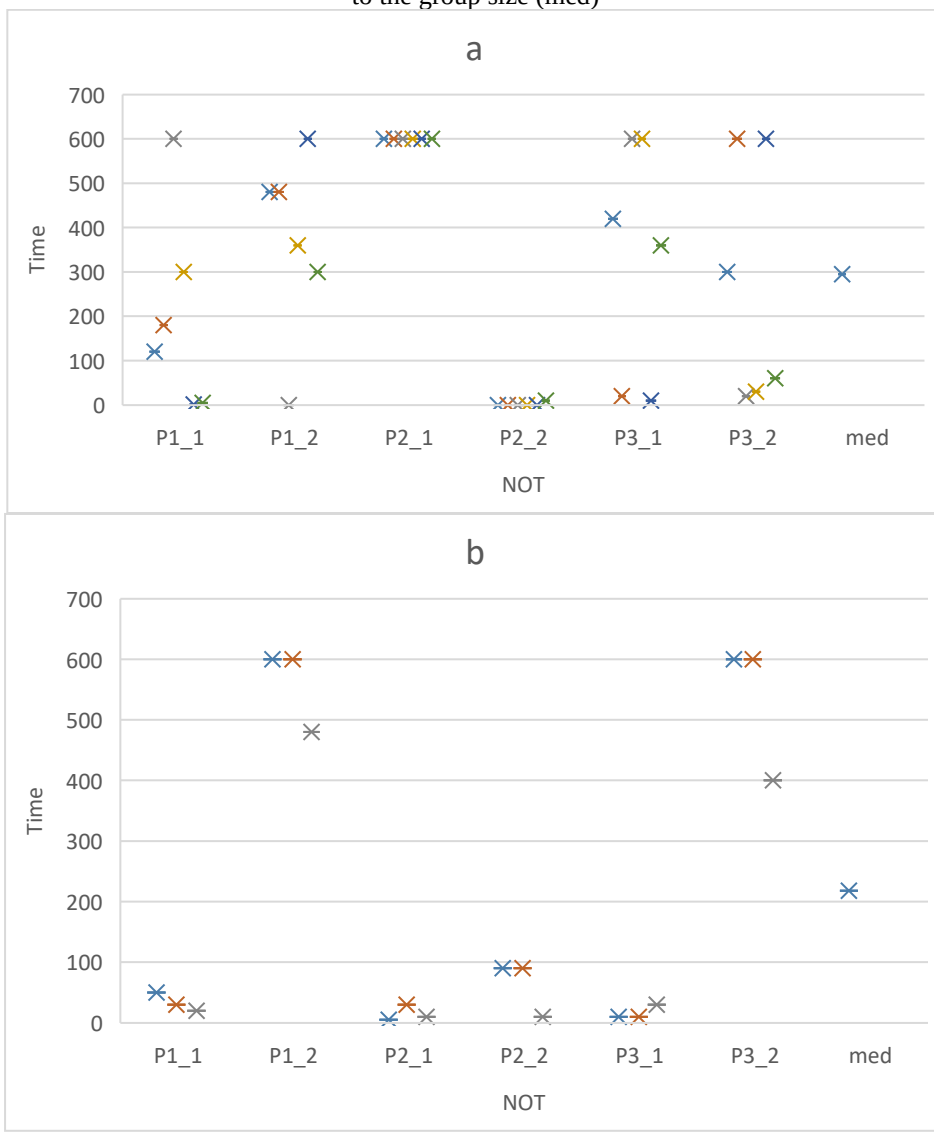


Fig. 3 - Approaching time period of approach in the novel object test (NOT) of each individual horse to the object (P1, P2, or P3) according to the first (1) or repeated (2) tests in the first (a- 1 horses) (n=6) and second (b- 2 horses) (n=3) groups of horses, and presentation of the mean value according to the group size (med)

Figure 3 represents an individual horse within a larger group as it approaches an object, demonstrating how behaviour may vary in different group

sizes. In the first group (a), the horses approached the P1 and P3 objects with a more dispersed formation, where the stimulus was food. In contrast, there was a notable reduction in interest and rapid habituation observed with the P2 object.

These observations align with the findings of Leiner and Fendt (2011), who noted rapid habituation when an object did not serve as a stimulus. The P2 object was approached by the group in both the first and second repetitions, indicating it is negatively reinforced in stressful situations. The group provides a sense of security, and their reactions are synchronized, a pattern that is particularly evident in the smaller group (b).

Conclusion

A herd of individuals offers greater security in smaller groups, allowing for quicker adaptation to new challenges, while larger groups tend to promote individuality. In smaller groups, integration is more consistent, facilitating effective collaboration where intense interactions occur between humans and horses during training. This dynamic adds depth and fosters a safe environment for work. The stimulation of curiosity and the use of positive reinforcement in these interactions enhance outcomes. It is crucial to perform the novel object test at the same location especially if several tests are compared to each other. These findings are expected to improve equine welfare in horse breeding. Notably, there has been considerable individual variation in the responses of horses within the larger group, indicating that further research is essential to deepen our understanding of both horse interactions and human-horse relationships.

Acknowledgement: The paper is the result of collaboration with a student Iva Malenica in preparing her graduation thesis "The role and changes of curiosity through the horse age" at the Faculty of Agrobiotechnical Sciences Osijek.

References

Bulens, A., Sterken, H., Van Beirendonck, S., Van Thielen, J., & Driessen, B. (2015). The use of different objects during a novel object test in stabled horses. *Journal of Veterinary Behavior*, 10(1), 54-58. https://doi.org/10.1016/j.jveb.2014.09.002

Brubaker, A., Berger, J., Willits, N., & Coss, R. (2013). Novelty-seeking and object play behavior in adult domestic horses. Embracing Science to Enhance Equine Welfare and Horse-Human Interactions. In *Proceedings of 9th International Society for Equitation Science Conference* (p. 48). New Bolton Center, PA, USA.

Christensen, J. W. (2013). Object habituation in horses: The effect of voluntary versus negatively reinforced approach to frightening stimuli. *Equine Veterinary Journal*, 45(3), 298-301. https://doi.org/10.1111/j.2042-3306.2012.00629.x

Christensen, J. W., Zharkikh, T., & Chovaux, E. (2011). Object recognition and generalisation during habituation in horses. *Applied Animal Behaviour Science,* 129(2-4), 83-91. https://doi.org/10.1016/j.applanim.2010.11.016

Christensen, J. W., Ahrendt, L. P., Malmkvist, J., & Nicol, C. (2021). Exploratory behaviour towards novel objects is associated with enhanced learning in young horses. *Scientific reports*, 11(1), 1428. https://doi.org/10.1038/s41598-020-80833-w

Drayson, O. G., Vozenin, M. C., & Limoli, C. L. (2023). A rigorous behavioral testing platform for the assessment of radiation-induced neurological outcomes. In A. Sato, J. Kraynak, A. E. Marciscano, & Galluzzi, L. (Eds.), *Methods in cell biology 180: Radiation Oncology and Radiotherapy Part C* (pp. 177-197). Academic Press. https://doi.org/10.1016/bs.mcb.2023.02.015

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. A. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, 92(3), 340–374.. https://doi.org/10.1016/j.physbeh.2007.03.016

Freeman, S. L. (2019). A scoping review of the current literature exploring the nature of the horse-human relationship. *Veterinary Evidence*, 4(4). https://doi.org/10.18849/ve.v4i4.240

Hartmann, E., Rehn, T., Christensen, J. W., Nielsen, P. P., & McGreevy, P. (2021). From the horse's perspective: investigating attachment behaviour and the effect of training method on fear reactions and ease of handling—a pilot study. *Animals*, 11(2), 457. https://doi.org/10.3390/ani11020457

International Society for Applied Ethology. (2025). *Ethical treatment of animals in applied animal behaviour research*. http://www.applied-ethology.org/ethical_guidelines.html

Kelly, K. J., McDuffee, L. A., & Mears, K. (2021). The effect of human–horse interactions on equine behaviour, physiology, and welfare: A scoping review. *Animals, 11(10),* 2782. https://doi.org/10.3390/ani11102782

Klitzing, L., Kirsch, K., Schindler, M., Merle, R., Hoffmann, G., Thöne-Reineke, C., & Wiegard, M. (2025). Exploring factors that influence the behavior response to novel object tests in young thoroughbred horses: investigating sex, test site and auction history. *Frontiers in Veterinary Science,* 11, 1478350. https://doi.org/10.3389/fvets.2024.1478350

Leiner, L., & Fendt, M. (2011). Behavioural fear and heart rate responses of horses after exposure to novel objects: Effects of habituation. *Applied Animal Behaviour Science*, 131(3-4), 104-109. https://doi.org/10.1016/j.applanim.2011.02.004

Liehrmann, O., Viitanen, A., Riihonen, V., Alander, E., Koski, S. E., Lummaa, V., & Lansade, L. (2022). Multiple handlers, several owner changes and short relationship lengths affect horses' responses to novel object tests. *Applied Animal Behaviour Science*, 254, 105709. https://doi.org/10.1016/j.applanim.2022.105709

Lloyd, A. S., Martin, J. E., Bornett-Gauci, H. L. I., & Wilkinson, R. G. (2008). Horse personality: Variation between breeds. *Applied Animal Behaviour Science*, 112(3-4), 369-383. https://doi.org/10.1016/j.applanim.2007.08.010

Powell, B. B., Horvath, K. C., Gilliam, T. L., Sibille, K.T., Keil, A., Miller-Cushon, E. K., & Brooks, S. A. (2023). Behavioral and physiological reactions to a sudden novel object in the weanling horse: quantitative phenotypes for future GWAS. *Genes*, 14(3), 593. https://doi.org/10.3390/genes14030593

Анализа интеракција коња у зависности од величине групе путем теста нових предмета

Маја Грегић[1], Ива Маленица[2], Весна Гантнер[1], Тина Бобић[1]

[1]*Свеучилиште Јосипа Јурја Штросмајера у Осијеку, Факултет агробиотехничких знаности, Осијек, Хрватска*
[2] *Abstract factory Ltd., Тординци, Хрватска*

Сажетак

Студија је анализирала реакције коња на новине, фокусирајући се на то како величина групе коња утиче на интеракције са непознатим предметима и степен радозналости који коњи показују. Тест нових предмета је коришћен као метода и примијењена су три различита предмета (са/без хране). Циљ је да се покаже веза између радозналости усмјерене на предмете и величине групе. Будући да су по природи животиње крда, коњи црпе осјећај сигурности из своје друштвене структуре, али развој когнитивних способности код сваке јединке може варирати у зависности од величине крда и фактора околине који подстичу радозналост. У мањим групама, коњи имају тенденцију да се равномјерније интегришу, што им омогућава да се брже и кохезивније суоче са новим изазовима. Насупрот томе, веће групе могу довести до повећаног индивидуализма. Мање групе олакшавају окружење за сарадњу, посебно у сценаријима који укључују интензивне интеракције између људи и коња кроз обуку, што пружа и стимулацију и сигурну основу за рад. У већим групама, стимулација радозналости се појачава кроз позитивно појачање током интеракција. Мања група је брже прилазила објектима, објекат без награде (хране) је био најмање занимљив, а време приближавања је варирало између првог и другог тестирања. Очекује се да ће ови налази побољшати добробит коња у пракси узгоја коња. Примјетно је да су постојале значајне индивидуалне варијације у одговорима коња, што указује на потребу за даљим истраживањима како би се продубило разумијевање интеракција коња и њихових интеракција са људима.

***Кључне ријечи**:* коњ, радозналост, Тест нових предмета, добробит.

Corresponding author: Maja Gregić
E–mail: mgregic@fazos.hr

Received: March, 18, 2025
Accepted: September 18, 2025