Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin,
Akademicka 13 Street, 20-950, Lublin, Poland
e-mail: jacek.sokolowski@up.lublin.pl

KAMILA JANICKA, JACEK SOKOŁOWSKI

Impact of socialization on the possibility to conduct behavioral tests in sheep

Wpływ socjalizacji na możliwość przeprowadzania testów behawioralnych u owiec

Summary. The aim of the study was to assess the impact of socialization with humans on the possibility to conduct tests evaluating problem-solving in sheep. The research included 30 Świniarka sheep aged from 2 to 8 years. The sheep were kept in the barn-pasture system. The observations included three trials carried out on three consecutive days for each sheep. The behavior of the sheep, i.e. exploration and approach to equipment elements of the test arena, was analyzed. The experiment was conducted on groups of sheep with low (G1) and high (G2) levels of socialization. The study showed that the test environment was challenging for the G1 sheep, so they were unable to complete the task successfully.

Key words: socialization, behavioral tests, small ruminants, cognition

INTRODUCTION

Animals can adapt their behavior to changing conditions, which plays a key role in their survival and evolution [Baragli et al. 2011]. This ability is a result of the behavioral flexibility of each individual regulated by both genetic and non-genetic mechanisms [Rowell et al. 2021]. The question of how and why animal cognitive abilities evolve is still an unresolved issue [Johnson-Ulrich et al. 2020, Rowell et al. 2021]. There is a certain variation between populations and individuals within species, probably caused by the different conditions prevailing during their development [Rowell et al. 2021]. In recent

years, the number of studies on animal cognition has increased owing to the development of intelligence assessment methods [Langbein et al. 2004]. The traditional approach based on comparison of the animal abilities with human skills is currently abandoned. Different species have developed certain skills for a specific reason, and comparing it to the abilities of humans, who have different anatomy, may be limiting [Doyle 2017]. Problem solving is one of the most widely studied animal skills. Interestingly, such studies are most often conducted in social species models [Rowell et al. 2021]. It is commonly assumed that the social environment generates crucial cognitive challenges. As proposed by the social intelligence hypothesis, such challenges as creation and maintenance of social bonds as well as anticipation of the actions of other individuals are the main drivers of cognitive evolution [Ashton et al. 2018]. Group living animals (like sheep) have increased opportunities of acquiring or improving new behaviors through observation and interaction with each other [Briefer et al. 2014]. In addition, sheep have large brains with human-like basal ganglia and well developed, convoluted cerebral cortices. Thus, it seems that sheep can be an appropriate animal model in studies of cognitive functions [Morton and Avanzo 2011]. However, as potential prey animals, they exhibit the fear of isolation, new situations, or unfamiliar stimuli, e.g. experimental conditions that they do not experience on a daily basis [Dwyer et al. 2009]. Therefore, it is important to understand the impact of the test environment on cognition and behavior in sheep [Mendl 1999]. The relationship between stress and cognition has long been investigated, but still little is known about this phenomenon. Its variability may depend on the personality and temperament of the animal [Valenchon et al. 2013, Wat et al. 2020, Rowell et al. 2021]. The flock is the basic defense strategy employed by sheep to cope with a stressful or threatening situation by staying in close contact in the group [Doyle 2017]. The specificity of cognition assessment tests in most cases requires separation of an individual from the group, which is extremely stressful for social animals and may thus hinder correct and reliable assessment of their cognitive performance [Rosenberger et al. 2021]. Under high stress, e.g. social isolation, sheep may activate a more automatic survival strategy mode instead of controlling the environment. Consequently, their behavior becomes less flexible and precludes effective problem-solving processes [Doyle 2017].

It may raise the question about the legitimacy of employing testing procedures in assessing cognitive abilities of sheep. To reliably evaluate such skills, certain solutions like appropriate habituation to the test conditions should be incorporated during experiments [Rosenberger et al. 2021]. A solution that seems to have the greatest importance and should be considered is to ensure an adequate level of socialization [Zulkifli 2013, Sokołowski et al. 2022]. The attitude of sheep to humans can potentially be responsible for considerable differences in the results of testing different individuals [Rosenberger et al. 2021]. Positive human-animal relationships can effectively reduce the level of fear and thus facilitate handling of animals [Mastellone et al. 2020]. Contact with humans has an impact not only on animal's behavior towards the handling person but also on animal's reaction to new stimuli [Zulkifli 2013].

We conducted a pilot study to check whether sheep exhibiting different levels of socialization with humans could cope in test environment conditions. We hypothesized that a high level of socialization with humans can enable the sheep to cope with the test conditions. We predicted that, in the case of highly socialized sheep, separation from the flock would not pose problems and the animals would actively explore the test arena and approach its elements.

MATERIAL AND METHODS

Ethical approval

All experimental procedures were approved by the Local Ethics Committee for Animal Experimentation of Lublin, Poland (Approvals No. 72/2022 and No. 83/2022).

Animals, housing, and treatments

The research was conducted at the Small Ruminant Research Station, which is part of the University of Life Sciences in Lublin. The experiment was conducted on 3 different dates (July, August, September). In each month, 5 individuals from group 1 (G1; low level of socialization) and 5 individuals from group 2 (G2; high level of socialization) participated in the study. Since the timing of the experiment did not affect the results, as confirmed by the results of the statistical analysis, the study was treated as a whole. Accordingly, the experiments involved 30 Świniarka sheep, ranging in age from 2 to 8 years. Group 1 (G1) consisted of 15 sheep, kept under a conventional farming system. Group 2 (G2) consisted of 15 sheep, kept under conditions of intensive socialization with humans. The animals were cared for by two caretakers, plus the animals had occasional contact with other humans. Interactions between humans and sheep were not limited to feeding and medical care. The animals were treated as companion animals: they were allowed to roam freely around the farm. They were also given names and stroked regularly. Both groups (G1 and G2) were kept in a barn-pasture system, with constant zootechnical and veterinary supervision. The pen area was in accordance with the current requirements for sheep kept in groups (at least 1.5 m^2 per animal) [Regulation of the Minister of Agriculture and Rural Development dated December 14, 2016]. To prevent misidentification, each sheep had a different color collar.

Experimental design

Acclimatization to the experimenter (E) and the test arena (TA) with its elements

Acclimatization to test conditions is usually carried out prior to testing various animal species [Valenchon et al. 2013, Mastellone et al. 2020, Rosenberger et al. 2021]. In stage 1, the sheep underwent 2-week acclimatization to the experimenter (E; unknown person) and the test arena (TA) with its elements (container with lid, movable fence, and two U-shaped transparent barriers). Acclimatization was carried out for each flock, during which there was no social isolation. At this time, the sheep were introduced to the test arena, which they were free to explore and approach the elements inside. The sheep were also allowed to approach, sniffing the experimenter (stationary position), who was located in different areas of the arena. The test arena (TA; 7 m × 5 m; external walls constructed of opaque synthetic shading mesh) used in both groups was located directly at the sheepfold.

Assessment of sheep behavior in test conditions

The study consisted of three experiments, with each successive trial characterized by an increased degree of difficulty. In the first experiment, the difficulty was associated exclusively with social isolation. In the second test, the use of a barrier separating the test

arena was a stressor that increased the level of fear. The third test consisted in introduction of another element, i.e. space limitation (maze). Behavioral responses that demonstrated signs of stress: increased alertness, locomotion, vocalization. Each animal was tested separately but with constant auditory and olfactory contact with the flock. All these pilot tests were performed in the test arena (TA) in the presence of the experimenter (E).

Test 1: Operant problem-solving task (OPST)

The operant problem-solving task is an instrumental manipulation test in which the animal is expected to open a familiar free-standing container with a reward (feed). The container is covered with a lid, which is an element of novelty to the animal. It allows assessment of the animal learning rate.
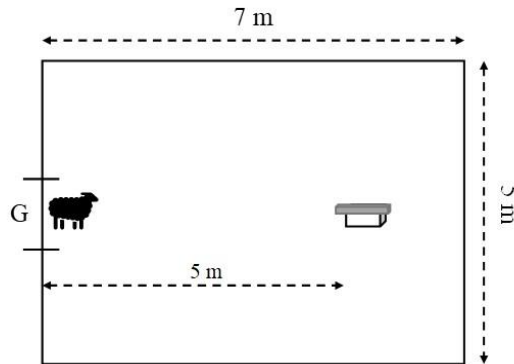

Fig. 1. Test arena adapted for the operant problem-solving task (OPST). G – entrance gate

Before the test, the experimenter placed the container with the lid (40 cm × 25 cm × 15 cm) at the end of the arena (TA) and brought the sheep (it moved freely along the designated "corridor") to the specified place located opposite the container (entrance gate) (Fig. 1). The sheep was allowed to move around the arena (TA) for 5 minutes. Each animal was tested in three trials on three consecutive days. The following parameters were assessed: exploration (yes/no; defined as entering at least 3 of the 4 corners of the arena) and the approach to the container (yes/no; defined as touching the lid of the container).

Test 2: A-not-B detour task (ABDT)

The A-not-B detour task test is intended to assess spatial skills with the use of a simple obstacle. In turn, the analysis of the perseverative behavior of animals observed after changing the direction of walking shows the rate of learning.
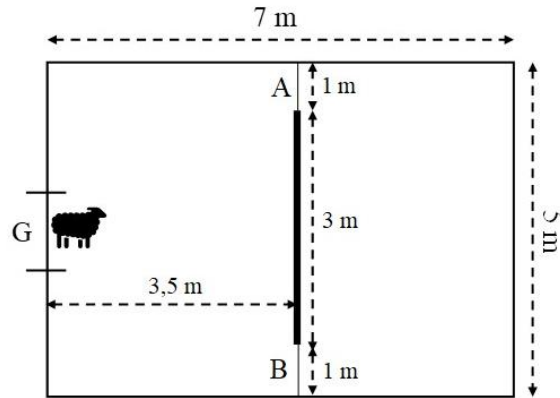
Fig. 2. Test arena adapted for A-not-B detour task (ABDT)
A – gap A; B – gap B ; G – entrance gate

The test arena (TA) was divided using a movable fence into two equal parts with a gap on one (A) or the other (B) side. The experimenter brought the sheep (moving freely along the designated "corridor") to the specified place (entrance gate G). Next, the gate was opened and the sheep was allowed to move around the arena (TA) for 5 minutes (Fig. 2). Each animal was tested in three trials on three consecutive days. The following parameters were assessed: exploration (yes/no; defined as entering at least 3 of the 4 corners of the arena) and the approach to the movable fence (yes/no; defined as approaching the fence for 50 cm).

Test 3: Double detour task (DDT)

The double detour task test is designed to assess the spatial reasoning ability. It consists in temporary moving away from the reward and finding the way to reach it.
The double-detour task test was set up using two nested U-shapes. Plastic poles and transparent stretch film were used to construct the barriers in the maze. The entrance gate was the starting point. The experimenter brought the sheep (moving freely along the designated "corridor") to the designated place (entrance gate). Next, the gate was opened and the sheep was allowed to move around the arena (TA) for 5 minutes (Fig. 3).
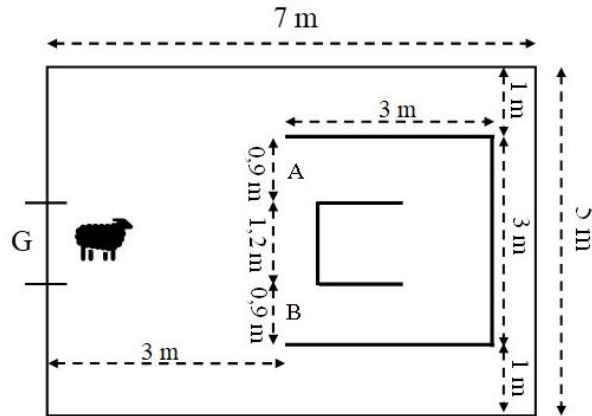
Fig. 3. Test arena adapted for double detour task (DDT)
G – entrance gate; A – entrance A; B – entrance B

Each animal was tested in three trials on three consecutive days. The following parameters were assessed: exploration (yes/no; defined as entering at least 3 of the 4 corners of the arena) and entrance to the U-shaped maze (yes/no; defined as reaching place A or B).

RESULTS

The analysis of the sheep behavior showed differences between the groups. The results indicated that the sheep with a high level of socialization with humans (G2) were more courageous, which was reflected in their behavior. The G2 sheep exhibited an increased level of curiosity, as 100% of the animals explored the test arena and approached the equipment elements in each test (OPST, ABDT, DDT). Their behavior did not change during the consecutive trials (T1–T3). Different results were obtained in the group of sheep with a low level of socialization with humans (G1). All G1 animals (100%) were character-ized by increased alertness, as they did not undertake exploration and did not approach the equipment elements. Such behavioral reactions were observed in the consecutive repetitions (T1-T3) of each test (OPST, ABDT, DDT) (Tab. 1).

Table 1. Behavior of G1 and G2 sheep during three consecutive trials of the pilot tests

Test	Exploration						Approach to the object					
	G1			G2			G1			G2		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
OPST	n	n	n	y	y	y	n	n	n	y	y	y
ABDT	n	n	n	y	y	y	n	n	n	y	y	y
DDT	n	n	n	y	y	y	n	n	n	y	y	y

OPST – operant problem-solving task; ABDT – A-not-B detour task; DDT – double detour task; G1 – sheep with a low level of socialization; G2 – sheep with a high level of socialization; T1 – first trial; T2 – second trial; T3 – third trial; n – no, y – yes

DISCUSSION

Experimental conditions differ from those experienced by animals on farms [Doyle 2017]. They are usually quite specific and strictly controlled [van Horik et al. 2017]. Many experiments comprise an element of novelty, which can be a source of fear experienced by sheep and may influence the outcome of the experiment [Mendl 1999, Dwyer et al. 2009]. The present study was focused on determination of sheep reactions in three test environments with varying degrees of difficulty.

The comparison of the behavior of both groups showed that even the simple version of the test arena was a source of potential stress for sheep with a low degree of socialization, as manifested by increased alertness, locomotion and vocalization. This confirms that fear does not allow sheep to control certain situations encountered by these animals.

The possibility to compare the abilities of individuals with different personalities contributes to better understanding of animals; hence, the issue of problem solving is most often studied in social species. However, real skills can be observed when the animal is separated from the group [Rosenberger et al. 2019]. Social isolation is an extremely stressful challenge for social animals, as it can worsen their welfare and introduce undesirable variables into the experiment [Siebert et al. 2011]. The presence of the experimenter is another factor that may have a negative effect on animal's motivation [Rowell et al. 2021]. A solution that may minimize or eliminate the stress accompanying experimental procedures is to habituate the animals to the research environment, the presence of humans, and the experimental equipment. Additionally, if isolation is necessary, the animal should be allowed to have visual, auditory, or olfactory contact with the herd [Siebert et al. 2011, Rowell et al. 2021]. As demonstrated in the present study, this type of preparation of the animal for tests is not always sufficient, especially when the level of fear is high. The differences in the behavioral response between the two analyzed groups were remarkably pronounced: no activity at all in one group and evident curiosity and exploration in the other. Assessment of such different reactions is extremely difficult, as the sheep that did not explore and approach the equipment elements did not complete the task and thus failed the tests. Cognitive performance can only be assessed in individuals that actively participate in tests. The fact that some individuals participate/do not participate in tests suggests that these animals may have specific characteristics that facilitate or hinder participation.

Undeniably, all behavioral studies require the presence of an experimenter [Coulon et al. 2015, Mastellone et al. 2020, Rosenberger et al. 2021]; therefore, the human seems to be the key factor that should be considered. Many farm animals perceive contact with humans as a disturbing event associated with fear [Zulkifli 2013]. It is worth noting, however, that effective learning and memory formation can be achieved at an optimal level of stress [Meehan and Mench 2007]. The stressor may have a modeling or harmful effect on the individual, depending on the duration of exposure [Schwabe et al. 2010]. Developmental differences between animals may modulate their ability to cope with environmental pressure [Rosenberger et al. 2021] and some sheep cope with stress better, as shown by Sokołowski et al. [2022]. Regular and positive contact with humans is an effective method for suppressing stress and fear reactions [Zulkifli 2013]. There has long been a strong relationship between humans and animals thanks to the process of domesti-

cation [Guesdon et al. 2016]. Gentle handling of animals facilitates establishment of strong interspecific bonds characterized by approaching rather than avoiding [Nowak and Boivin 2015]. Human-animal interactions and the associated stress depend on the level of socialization [Mastellone et al. 2020]. As reported by Mastellone et al. [2020], even short-term socialization of goats with humans was effective in increasing the frequency of interactions. Concurrently, the authors emphasize the importance of the period in which socialization is carried out; as evidenced by the example of the wolf and the wild dog, probably only early socialization allows humans to be accepted as social partners. This was contradicted in the present study analyzing the flock of socialized sheep. In their case, socialization with humans was carried out at different stages of life. Nevertheless, the animals established a bond with the humans and the level of fear decreased, which made it possible to conduct the experiment.

The knowledge of the mechanisms of the formation and maintenance of interspecific relationships is still quite limited. It is certain that the relationship between humans and farm animals has a strong impact on animal welfare and ease of handling [Nowak and Boivin 2015]. Given its highly social nature, the sheep is an interesting model for studying interspecific bonds [Guesdon et al. 2016]. The comparison of the behavior of sheep with different levels of socialization in the present study demonstrated their different attitudes towards humans. Sheep whose contact with humans was limited to daily handling avoided interactions with the experimenter and stayed close to the arena exit. In contrast, individuals with a high level of socialization interacted readily and moved freely around the test arena. The results reported by Mastellone et al. [2020] indicated a similar relationship in goats. In a situation where the task became impossible to solve, highly socialized goats interacted more often with the human. As shown by Guesdon et al. [2016], a familiar person, e.g. the handler, can provide social support in potentially aversive situations. It is worth emphasizing that animals tend to associate their positive or negative experience with the handler with their contact with other humans [Destrez et al. 2013]. It is therefore possible that the presence of the experimenter in this study was a positive event for the socialized Świniarka sheep, and the test environment was not a challenge in this group.

CONCLUSIONS

It is essential to elucidate the impact of stressors on sheep's cognitive abilities and behavior. Stress can impair cognitive processes in animals, limiting their capabilities through incorrect processing of information from the environment. It seems that an appropriate level of socialization with humans is a key factor to be taken into account while designing studies to assess cognitive abilities. The level of socialization has an impact on behavioral reactions not only to humans but also to a number of new stimuli and can help animals to cope with emerging stressors. Since investigations of cognitive abilities require controlled conditions, it is worth investing time in habituation of animals, as it can enhance their motivation to solve problems.

REFERENCES

Ashton B.J., Ridley A.R., Edwards E.K., Thornton A., 2018. Cognititve performance is linked to group size and affects fitness in Australian magpies. Nature 554(7692), 364–367. https://doi.org.10.1038/nature25503

Baragli P., Vitale V., Paoletti E., Sighieri C., Reddon A.R., 2011. Detour behaviour in horses (*Equus caballus*). J Ethol. 29, 227–234. https://doi.org.10.1007/s10164-010-0246-9

Briefer E.F., Haque S., Baciadonna L., McElligott A.G., 2014. Goats excel at learning and re-membering a highly novel cognitive task. Front Zool. 11(20). https://doi.org/10.1186/1742-9994-11-20

Coulon M., Nowak R., Peyrat J., Chandèze H., Boissy A., Boivin X., 2015. Do lambs perceive regular human stroking as pleasant? Behavior and heart rate variability analyses. PLoS ONE. 10(2), e0118617. https://doi.org/10.1371/journal.pone.0118617

Destrez A., Coulon M., Deiss V., Delval E., Boissy A., Boivin X., 2013. The valence of the long-lasting emotional experiences with various handlers modulates discrimination and generalization of individual humans in sheep. J. Anim. Sci. 91, 5418–5426. https://doi.org/10.2527/jas.2012-5654

Doyle R.E., 2017. 4 – Sheep cognition and its implications for welfare. Advances in Sheep Welfare. Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, 55–71. https://doi.org/10.1016/B978-0-08-100718-1.00004-2

Dwyer C.M., 2009. Welfare of sheep: providing for welfare in an extensive environment. Small Rumin. Res. 86, 14–21. https://doi.org/10.1016/j.smallrumres.2009.09.010

Guesdon V., Nowak R., Meurisse M., Boivin X., Cornilleau F., Chaillou E., Lévy F., 2016. Behavioral evidence of heterospecific bonding between the lamb and the human caregiver and mapping of associated brain network. Psychoneuroendocrinology 71, 159–169. https://doi.org/10.1016/j.psyneuen.2016.05.020

van Horik J.O., Gangley E.J.G., Whiteside M.A., Madden J.R., 2017. Differential participation in cognitive tests is driven by personality, sex, body condition and experience. Behav. Processes. 134, 22–30. https://doi.org/10.1016/j.beproc.2016.07.001

Johnson-Ulrich L., Holekamp K.E., Hambrick D.Z., 2020. Innovative problem-solving in wild hyenas is reliable across time and context. Sci. Rep. 10, 13000. https://doi.org/10.1038/s41598-020-69953-5

Langbein J., Nürnberg G., Manteuffel G. 2004. Visual discrimination in learning in dwarf goats and associated changes in heart rate and heart rate variability. Physiol. Behav. 82, 601–609. https://doi.org/10.1016/j.physbeh.2004.05.007

Mastellone V., Scandurra A., D'Aniello B., Nawroth C., Saggese F., Silvestre P., Lombardi P., 2020. Long-term socialization with humans affects human-directed behavior in goats. Animals 10, 578. https://doi.org/10.3390/ani10040578

Meehan C.L., Mench J.A., 2007. The challenge of challenge: can problem solving opportunities enhance animal welfare? App. Anim. Behav. Sci. 102, 246–261. https://doi.org/10.1016/j.applanim.2006.05.031

Mendl M.,1999. Performing under pressure: stress and cognitive function. Appl. Anim. Behav. Sci. 65, 221–244. https://doi.org/10.1016/S0168-1591(99)00088-X

Morton A.J., Avanzo L., 2011. Executive decision-making in the domestic sheep. PLoS ONE, 6(1), e15752. https://doi.org/10.1371/journal.pone.0015752

Nowak R., Boivin X., 2015. Filial attachement in sheep: similarities and differences between ewe-lamb and human-lamb relationships. App. Anim. Behav. Sci. 164, 12–28. https://doi.org/10.1016/j.applanim.2014.09.013

Rosenberger K., Simmler M., Langbein J., Keil N., Nawroth C., 2021. Performance of goats in
    a detour and a problem-solving test following long-term cognitive test exposure. R. Soc.
    Open Sci. 8, 210656.8. https://doi.org/10.1098/rsos.210656
Rowell M.K., Pillay N., Rymer T.L. 2021. Problem solving in animals: Proposal for an
    ontogenetic perspective. Animals 11, 866. https://doi.org/10.3390/ani11030866
Regulation of the Minister of Agriculture and Rural Development dated December 14, 2016 on the
    minimum requirements to apply to the institution and the minimum requirements for the care
    of animals kept at the institution (Dz.U. 2016 poz. 2139) [Rozporządzenie Ministra
    Rolnictwa i Rozwoju Wsi z dnia 14 grudnia 2016 r. w sprawie minimalnych wymagań, jakie
    powinien spełniać ośrodek, oraz minimalnych wymagań w zakresie opieki nad zwierzętami
    utrzymywanymi w ośrodku (Dz.U. 2016 poz. 2139)].
Schwabe L., Wolf O.T., Oitzl M.S., 2010. Memory formation under stress: quantity and quality.
    Neurosci. Biobehav. Rev. 34, 584–591. https://doi.org/10.1016/j.neubiorev.2009.11.015
Siebert K., Langebein J., Schön P.-C., Tuchscherer A., Puppe B., 2011. Degree of social isolation
    affects behavioural and vocal response patterns in dwarf goats (*Capra hircus*). Appl. Anim.
    Behav. Sci. 131, 53–62. https://doi.org/10.1016/j.applanim.2011.01.003
Sokołowski J., Janicka K., Rozempolska-Rucińska I., 2022. Wpływ poziomu strachu na behawior
    pokarmowy owiec. Med. Weter. 78(11), 577–580. http://dx.doi.org/10.21521/mw.6693
Valenchon M., Lévy F., Prunier A., Moussu C., Calandreau L., Lansade L., 2013. Stress
    modulates instrumental learning performances in horses (*Equus caballus*) in interaction with
    temperament. PLos ONE 8(4), e62324. https://doi.org/10.1371/journal.pone.0062324
Wat K.K.Y., Banks P.B., McArthur C., 2020. Linking animal personality to problem-solving
    performance in urban common brushtail possums. Anim. Behav. 162, 35–45. https://doi.org/
    10.1016/j.anbehav.2020.01.013
Zulkifli I., 2013. Review of human-animal interactions and their impact on animal productivity
    and welfare. J. Anim. Sci. Biotechnol. 4, 25. https://doi.org/10.1186/2049-1891-4-25.

Streszczenie. Celem badań była ocena wpływu socjalizacji z człowiekiem na możliwość przeprowadzenia testów oceniających rozwiązywanie problemów u owiec. Badania przeprowadzono na 30 owcach rasy świniarka, w wieku od 2 do 8 lat. Owce utrzymywano w systemie alkierzowo-pastwiskowym. Obserwacje obejmowały 3 próby, w trzech kolejnych dniach dla każdej owcy. Analizowano zachowania owiec podczas przebywania w arenie testowej: eksploracja i podejście do znajdujących się elementów wyposażenia. Eksperyment przeprowadzono na grupach owiec o niskim (G1) i wysokim (G2) stopniu socjalizacji. Badanie wykazało, że środowisko testowe stanowiło duże wyzwanie dla owiec z grupy G1, dlatego nie były w stanie ukończyć zadania z sukcesem.

Słowa kluczowe: socjalizacja, testy behawioralne, małe przeżuwacze, zdolności kognitywne