THESES OF THE DOCTORAL (PhD) DISSERTATION

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES KAPOSVÁR CAMPUS

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DEVELOPMENT OF KEEPING TECHNOLOGY OF SMALL MAMMALS

DOI: 10.54598/004470

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Kaposvár

2024

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1. Background of Research, Objectives

One of the greatest environmental challenges of our time is the preservation of biodiversity. Among the various nature conservation measures, the most effective method for the long-term preservation of biological diversity is the protection of habitats (in situ protection). However, protecting certain species cannot be solved only in their original habitat, so protection outside the habitat, i.e. ex situ, is necessary.

Ex situ protection has improved greatly in recent years; these facilities can perform their tasks much more efficiently, as capture/collection strategies and knowledge of keeping technology have greatly improved (Maunder and Byers, 2005). However, it is still not efficient enough for many less researched species. For ex situ protection, it is essential to know the species, both from an ecological and ethological point of view, so that we can properly keep and reproduce the individuals in captivity. That is why it is important to adapt the husbandry technology to the species' ecological needs. The concept of animal welfare is closely related to husbandry technology. In order to create appropriate housing technology from an animal welfare point of view, we need to understand the natural behaviour and needs of animals (Baumans, 2005); this is just as true for experimental animals as it is for farm animals. The traditional care and maintenance of laboratory animals does not usually include species-specific needs related to their environment. Rodents and rabbits have partially adapted to a captive lifestyle but still show a high degree of similarity to their wild counterparts (Baumans, 2005; Stauffacher, 1995).

For this reason, the environment of laboratory animals should be partially adapted to their innate physiological and behavioural needs, such as social relations, resting, nesting, hiding, exploring, foraging, and chewing. Environmental conditions greatly impact animals throughout their life and thus influence the results of animal experiments (Baumans, 2005). However, systems for housing laboratory and farm animals are often designed based on

economic and ergonomic considerations (e.g. equipment, costs, space, workload, the ability to observe the animals and the ability to maintain a certain degree of hygiene), with little consideration of animal welfare (Baumans, 2005 ; Van de Weerd et al., 1997a). One way to improve the living conditions of animals kept in closed spaces is to provide the animals with opportunities to perform a species-specific behavioural repertoire. Environmental enrichment provides a good opportunity for this, which can be defined as follows: any modification in the environment of captive animals that tries to improve the animals' physical and psychological well-being by providing stimuli that meet the species-specific needs of the animals (Baumans, 2005; Newberry, 1995).
In my doctoral work, I investigated the possibilities of ecological enrichment of rodents kept in the laboratory.

1.1. OBJECTIVES OF THE RESEARCH

During my research, I sought answers to the following questions.

1. Investigation of factors affecting the nesting material preference of wild mouse species kept in laboratory conditions.

2. Investigation of factors influencing the feed preference of wild mouse species kept in laboratory conditions.

3. Examination of the preference of the common ground squirrel nest material in indoor housing.

4. Examination of common ground squirrel feed preference in indoor conditions.

5. Analysis of the composition of the European rabbit's nest.

6. Investigation of the factors influencing the nest building of the domestic rabbit.

2. Material and methods

2.1. Factors influencing nest material preference of wild mouse species kept in laboratory conditions

The tests were carried out in the Rodent House of the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences. In the rodent house, there were individuals of known age, sex and origin. The stock is laboratory-born offspring of wild mice captured in different parts of Hungary. Mound-building mice and house mice were housed in T4 (600 × 200 × 380 mm) laboratory mouse boxes in small groups (1 male, 2-3 females), provided with deep litter and nesting material. Water and feed (SAFE® 132 laboratory mouse feed) were available to the animals ad libitum. The animals were placed in a darkened room with inverted daylight; from 8 am to 8 pm, a 10 lux red light was on, and then from 8 pm to 8 am, a 220 lux yellow light bulb was on. The locality had a temperature of 20-22 degrees Celsius and a humidity of 50-55%.

A total mice were selected 104, 52 mound-building mice and 52 house mice, were selected for the nesting material preference study, with an equal proportion of male and female individuals. During the study, the mice were housed individually in a T4 laboratory mouse box and provided with food and water ad libitum. The nest material preference test lasted 7 days; the mice could choose from 3 nest materials: paper (Enviro-Dri rodent bedding 20 kg), cotton (MultiFit small rodent cotton bedding 30 l) and hay (Versele-laga mountain hay 50 l), which they were placed in hay pockets attached to a mouse box. At the beginning of the test, 100-150 grams were measured per nest material. On the last day of the study, the finished nests were removed, and the mice were returned to their original place in small group housing. Szenczi et al. (2011) studies described the composition of the completed nests. We homogenized the nest and then randomly pulled out 20 fibres to estimate the composition of the entire nest based on the proportion of each nest-building material in the nest. Before the nest analysis, we determined the quality of the nests according to Gaskill et al. (2013), where the completed nests were given a score of 2 to 5. The completed nest received a score of 2 if only a small amount of nest-building material was brought together, but no nest form was formed from it. The cup shape, where the nest already has a small rim, received a score of 3; where this rim is already higher but does not yet close at the top, received a score of 4; and which forms a completely closed sphere, the nest received a score of 5. For the statistical evaluation of the study, SAS 9.4. we used version number statistical software. A chi-square test was used to determine whether the choice rate of different types of nest material differed. After that, we analyzed the effect of species and sex on the composition of the nest with a generalized linear mixed model, the distribution was multinomial and a generalized logit link function was set. The relationship between the composition of the nest (percentage of paper, cotton, hay) and the quality of the nest was investigated using polyserial correlation.

The effect of temperature on the mice's choice of nest material and nest quality was investigated in two test rooms with similar lighting but different temperatures. One room had a temperature of 10 degrees Celsius, while the other had a temperature of 21 degrees Celsius. 52 mice were selected as their temperature, 26 mound-building mice and 26 house mice. During the study, the mice were housed individually in a T4 laboratory mouse box and provided with food and water ad libitum. The nest material preference test lasted 7 days, and the mice could choose from 3 nest materials: paper (Enviro-Dri rodent bedding 20 kg), cotton (MultiFit small rodent cotton bedding 30 l) and hay (Versele-laga mountain hay 50 l), which was placed in hay pockets attached to a mouse box. The composition of the completed nests was determined, as mentioned above, by the study of Szenczi et al. (2011). The quality of the nest was determined by Gaskill et al. (2013) based on the investigations. For the statistical evaluation SAS 9.4. version number statistical software was used, a generalized linear mixed model was set for the effect of temperature on nest composition, the distribution was multinomial, and a generalized logit link function was used.

2.2. Factors influencing the feed preference of wild mouse species kept in laboratory conditions

The placement of the animals was the same as described in chapter 2.1. For the feed preference test, we used mound-building mice and house mice, a total of 72 individuals were randomly selected, 36 mound-building mice and 36 house mice, male and female mice were selected in equal proportion. During the study, the mice were housed individually in a T4 laboratory mouse box and provided litter and nest-building material at 21 degrees Celsius in reverse daylight. On the test days, at 8:00 a.m., all three feed types were placed in the feeding bowls in amounts between 5 and 6 grams, measured to the nearest hundredth of a gram, and after 6 hours, the feeds were remeasured on each test day. The following feeds were used for the test: Versele-Laga Nature Mouse feed mix, Versele-Laga Complete Rat and Mouse granulated feed and SAFE® 132 Laboratory mouse feed, which is also a granulated feed. For the statistical evaluation of the study, SAS 9.4. we used version number statistical software. To determine whether the choice ratio of the three types of feed was different, we used a Chi-square test. After that, the effect of the two species on the choice of feeds was analyzed with a generalized linear mixed model; the distribution was multinomial, and a generalized logit link function was set. The data of the five-day test were analyzed with Repeated Measures ANOVA.

The effect of temperature on the feed choice of mice was investigated in two test rooms with similar lighting but different temperatures. One room had a temperature of 10 degrees Celsius; the other had a temperature of 21 degrees

Celsius. For the study, we used mound-building mice and house mice, 36 individuals were randomly selected for each temperature, 18 house mice and 18 house mice, male and female mice were selected in equal proportion. During the study, the mice were housed individually in a T4 laboratory mouse box, provided litter and nest-building material, under inverted daylight. The test was conducted based on the above; on the test days at 8:00 a.m. all three feed types (we used a feed mixture: Versele-Laga Nature Mouse and two granulated feeds: Versele-Laga Complete Rat and Mouse, SAFE 132 Laboratory mouse feed) were placed in the feeder into bowls in an amount between 5 and 6 grams, measured to the nearest hundredth of a gram, and after 6 hours, the feeds were remeasured on each test day. To evaluate the results SAS 9.4. we used version number statistical software. The effect of temperature on feed selection was analyzed using a generalized linear mixed model; the distribution was multinomial, and a generalized logit link function was set. The data of the five-day test were analyzed with Repeated Measures ANOVA.

2.3. Investigating the nest material preference of the European ground squirrel

The study was carried out in the rodent house of the MATE campus in Kaposvár, on the offspring of common squirrels caught freely from different parts of Hungary and kept in the laboratory. The capture and keeping of the animals was authorized by the Department of Environmental Protection and Nature Protection of the Pest County Government Office, based on registration number PE-KTF/7728-7/2017. The ground squirrels were housed individually in 2-level Ferplast rodent cages with a floor area of 0.55 x 0.39 m and a height of 0.28 m, at a temperature of 21 degrees Celsius, with 12 hours of daylight. The animals were provided with wood chip litter and nesting material, feed (Agroszász Kft. rabbit feed) and water ad libitum.

20 adult animals participated in the study, 10 males and 10 females were randomly selected. During the test, commercially available meadow hay (Bunnynature Freshgrass hay), Lignocel (J. Rettenmaier & Söhne GmbH + Co KG) and paper nest material (SAFE Crinklets Natural) were placed in random order in the hay pockets of the animals' cages, 200 grams of material. The duration of the study was 7 days. We planned the preference test for the summer period to not affect the breeding season and the care of the offspring. Studies by Szenczi et al. (2011) described the composition of the nests. The nest was homogenized, and then 20 strands were randomly drawn to estimate the composition of the entire nest based on the proportion of each nest building material in the nests. Before determining the nest material, we also scored the quality of the completed nests according to Gaskill et al. (2013), where they were given scores from 2 to 5. The finished nest received a score of 2 if only a small amount of nest-building material was collected but no nest shape was formed. The cup shape, where the rim of the nest is already rimmed, was given a score of 3; where this rim was already higher but not yet closed at the top, was given a score of 4, and which was a completely closed sphere form, it received 5 points. IBM SPSS Statistics 27.0 statistical software was used for the statistical evaluation of the study. We used the Chi-square test for the ratio of nest material selection and Pearson's correlation for nest quality.

2.4. Investigation of feed preference of European ground squirrels

The placement of the animals was the same as described in chapter 2.3. 24 adult animals participated in the feed preference study. The test lasted 5 days. The animals' access to drinking water was not restricted. We used 3 different feeds to determine feed preference. The first feed was a commercially available rabbit feed (Agroszász Kft.), the second was Versele-laga Cuni Adult Complete complete rabbit feed, and the third was Versele-laga Nature Cuni rabbit feed. The data were analyzed with the IBM SPSS Statistics 27.0 program package. A chi-square test was used to examine the first choice of feeds. In the multi-day feed preference test, we used Repeated Measure ANOVA to reveal possible differences between the choice of different types of feed.

2.5. Determining the composition of European rabbit nest material

To describe the composition of the original rabbit nests, we searched for rabbit burrows after the calving period in the Kiskunság National Park. The 21 found rabbit nests were collected in sealable sterile bags and stored at 5 degrees Celsius until analysis. The plant and hair components were separated, and further analysis was limited to the plant components only. The plant parts were homogenized, and then 10 sub-samples were randomly taken from each nest with tweezers and identified under a NIKON SMZ1270 microscope with 40x magnification. For each piece, we determined the species identity and colour under a microscope, whether it was green (fresh) or yellow (dry). The length of the blades of grass was measured to the nearest centimetre using a digital calliper.

2.6. Investigating the factors influencing the nest building of the domesticated rabbit

We used randomly selected female chinchilla rabbits from Germany (Standard Chinchilla by Thomae, Biberach) to select the nest material. The test took place in the experimental facility of ELTE in Göd.

Pregnant females weighing 2.7 ± 0.2 kg were placed individually in standard rabbit cages (100 length × 50 width × 45 height cm), where they were supplied with ad libitum laboratory granulated rabbit food (Agroszász Kft.) and water. The cages were equipped with external wooden litter boxes (45 length × 40 width × 35 height cm). During the examination, the breeding box door was open; the mother rabbit was free to visit the chicks. The test room temperature was between 18 and 22 degrees Celsius. The lighting consisted of 14 hours of light and 10 hours of darkness.

We used seven 30-week-old female chinchilla rabbits to examine fresh and dry plant materials and tested them on the 28th day of pregnancy in a two-way choice test. 200 grams of green (fresh) and 100 grams of dry grass were placed in the cages at 8 a.m. The rabbits' choice of nesting material was recorded with a video camera. The nests made by the rabbits were removed and replaced with the same amount of hay, and the finished nests were stored at 5 degrees Celsius until analysis. The test was repeated 4 times in a row. The plant parts of the completed nests were homogenized, and then the nest composition was deduced by randomly pulling out 20 threads. IBM SPSS Statistics version 27.0 was used for statistical analyses. For statistical analysis, paired Wilcoxon tests were used to compare two-way choice tests.

To examine the short and long nest materials, we tested seven 30-week-old chinchilla dams in a two-way choice test on the 28th day of pregnancy. At 8 a.m., two pieces of dry grass of the same volume but different in length were placed in the mothers' cage. The choice of nest material was recorded with a video camera. The long blades of grass were cut into 30 cm lengths, and the short ones into 10 cm pieces. The nests made by the rabbits were removed, replaced with hay, and then stored at 5 degrees Celsius until analysis. We homogenized the plant parts of the nests, then randomly pulled out 20 threads and weighed the threads to determine the chosen nest material. We used the IBM SPSS Statistics version 27.0 program package for the statistical analysis. The data were analyzed using the paired Wilcoxon test.

3. RESULTS

3.1. Factors affecting the nesting material preference of wild mouse species

Based on the Chi-square test, we showed a significant difference between the three types of nest material ($p<0.005$). Mice chose hay in 76% of the three nest material types, paper in 21%, and cotton in only 3% for nest construction. Based on the generalized mixed linear model, no significant differences were found between the types of nest material between the two mouse species ($p=0.272$). For the two species, no significant difference was found in the choice of cotton nest material ($p=0.54$), nor the choice of paper ($p=0.54$), nor was there a difference in the choice of hay ($p=0.94$). Based on the estimated probabilities, mound-building mice and house mice also chose hay in the highest proportion. Overall, we found no significant difference ($p=0.051$) in the choice of nest material between the two sexes. There was a significant difference between the two genders in the choice of cotton ($p=0.01$), but no difference was found in the choice of paper ($p=0.54$) and hay ($p=0.38$). The male and female similarly preferred the hay type of nesting material.

We found a significant difference between the temperatures and the nest material choices ($p=0.001$). At the two different temperatures, we found a significant difference in the choice of cotton nest material ($p<0.001$), as well as paper ($p<0.001$) and hay ($p<0.001$). At a temperature of 10 degrees Celsius, the use of hay for nests increased, that of paper decreased, and the use of cotton increased.

Regarding the relationship between the nest material composition of the completed nests and the quality of the nest, we found that the amount of hay ($p<0.001$) and paper ($p<0.001$) affects the quality of the nest, but cotton does not ($p=0.161$). A larger amount of hay improves the quality of the nest ($r=0.537$), while the presence of paper worsens it ($r=-0.482$).

We found a significant difference in the quality of the nests at the two different temperatures (p=0.008); the quality of the nests increases minimally (r=-0.258) as the temperature decreases. At warmer temperatures (21 degrees Celsius), the quality of the nests averaged 4 points, while at colder temperatures (10 degrees Celsius), the completed nests received an average of 4.5 points.

3.2. Factors influencing the feed preference of wild mouse species

Based on the Chi-square test, we showed a significant difference between the three feed types (p<0.005). The mice chose the Nature feed type in 73% of the three feeds, Laboratory in 26% and Complete feed in only 1%. Based on the generalized mixed linear model, we found no significant difference between the choice of feed types in the two mouse species (p=0.57). For the two species, no significant difference was found in the choice of the Nature feed type (p=0.68), nor the choice of the Complete feed (p=0.67), nor was there a difference in the choice of the Laboratory feed (p=0.52) in two species. Based on the estimated probabilities, mound-building mice and house mice chose the Nature feed in the highest proportion.

We found a significant difference in feed choices between the two temperatures (p=0.04). At the two different temperatures, we did not find a significant difference in the choice of the Nature feed (p=0.97), nor did we find a significant difference in the Complete feed type (p=0.88); we only find a significant difference in the choice of the Laboratory feed (p=0.01). The probability of consuming Laboratory feed increased at a temperature of 10 degrees Celsius.

Based on the Repeated Measures ANOVA, we found a significant difference in the consumption of the Nature feed during the 5 days (p=0.001); the consumption of the feed increased on the consecutive days. The amount consumed from the Nature feed showed no significant difference between the species (p=0.791) and between the temperatures (p=0.128). No significant difference was found in the interaction between time, species and temperature ($p=0.480$).

No significant difference was found in the consumption of Complete feed during the 5 days ($p=0.755$). The amount of Complete feed consumed did not show a significant difference between species ($p=0.921$), just as we did not find a significant difference in consumption between the two different temperatures ($p=0.724$). No significant difference was found in the interaction between time, species and temperature ($p=0.745$).

We found a significant difference in the consumption of the Laboratory mouse feed during the 5 days ($p=0.001$); the consumption of the Laboratory feed decreased during the 5 days. No significant difference was found between the species ($p=0.329$) in feed consumption, but a significant difference in consumption was found between the two different temperatures ($p=0.001$). Laboratory feed consumption increased at colder temperatures. No significant difference was found in the interaction between time, species and temperature ($p=0.405$).

3.3. Investigating the nest material preference of the ground squirrel

Based on the Chi-square test, we found a significant difference between the three nest materials ($p<0.005$). Of the three nesting materials, ground squirrels chose paper nest building material in 28.4%, Lignocel in 1.3% and hay in 70.3%.

Looking at the correlation between the nest material composition of the completed nests and the quality of the nest, we found that the amount of paper ($p<0.05$) and hay ($p<0.05$) affects the quality of the nest, while Lignocel does not ($p=0.26$). A larger amount of paper minimally worsens nest quality ($r=-0.25$), while the presence of hay improves it ($r=0.42$).

3.4. Investigation of feed preference of ground squirrels

Based on the video camera recordings, we found a significant difference ($p<0.005$) between the choices of the three feeds, which feed the animals went to first. Based on the first choice test, the animals chose Nature feed in 74%, Complete in 15% and the rabbit feed they already knew in only 11%.

Based on the 5-day feed preference test, we found a significant difference between the consumption of feed types ($F(1,18)=2.21$ $p=0.032$). Animals consumed the largest percentage of Nature feed.

During the 5-day feed selection, we found a significant difference ($F(1,19)=4.66$ $p=0.044$) in the consumption of Nature feed between the test days; a slight increase was observed. The consumption of Complete feed did not change significantly during the days ($F(1,19)=0.27$ $p=0.608$); the daily consumption of Complete feed stagnated during the 5 days. We found a significant difference in the consumption of rabbit feed between the test days ($F(1,19)=12.21$ $p=0.002$); the consumption of rabbit feed showed a decreasing trend during the test. No significant difference was found between the genders in the choice of feed ($F(1,18)=0.17$ $p=0.952$).

3.5. Determining the composition of European rabbit nest material

European rabbit nests contained an outer layer of vegetation and an inner layer of rabbit fur. The nest comprised 43% hair, 55% plant material and 2% sand and earth. The plant part could be divided into two parts: a dry plant part, which accounted for 84.4% of the total plant part, and a green plant part, which accounted for 15.6% of the plant part. *Agropyron sp.*, *Calamagrostis sp.* and *Carex sp.* species were found among the dry plant materials. *Colchicum sp.*, *Hypnum cupressiforme* and *Polygonatum sp.* species were found in the green plant part.

3.6. Investigating the factors influencing the nest building of the domestic rabbit

The mother rabbits immediately started exploring, collecting the nesting material placed in the cage and carrying it into the nesting box. Females spent more time collecting dry grass than green, fresh grass, which showed a significant difference based on the paired Wilcoxon test ($p<0.05$). However, the animals spent much more time eating green grass ($p<0.05$). During the analysis of the completed nests, it was revealed that they contain significantly ($p<0.05$) more dry plant material than green, fresh grass.

Mothers significantly preferred long grass when collecting nest material ($p<0.05$). The length of the grass did not affect consumption ($p=0.06$). The consumption of grass in this study was low (3.5%), similar to the consumption of dry grass in the previous experiment. This was probably due to a lack of green plant material for consumption.

4. Conclusions and Suggestions

4.1. Factors affecting the nesting material preference of wild mouse species

The choice of nesting material of the wild mouse species for both mouse species (mound-building mouse, house mouse) was the same as the nest-building material used in nature by their wild congeners, i.e. dry grasses. For wild mouse species kept in the laboratory or rodents participating in species conservation programs, it is worth providing the nesting material used by the species in nature or an alternative closest to it, such as commercially available hay. In laboratories where hygiene protocols are in place, it is worth choosing paper nesting material for the animals, as our tests revealed that paper was the second most preferred nesting material after hay. The common feature of hay and paper nesting material is its long, fibrous structure from which mice can easily build their nests. For mice kept in the laboratory, nest building is extremely important; with the expression of natural forms of behaviour, the stress level of the animals decreases; on the other hand, the finished nest also provides shelter for the animal. Furthermore, a nest built from suitable nest-building materials provides good thermal insulation for the animal and the offspring in case of reproduction.

4.2. Factors influencing the feed preference of wild mouse species

The results of the feed choice for wild mouse species kept in the laboratory are the same as the food consumed by their wild counterparts. Wild mice can be said to be omnivores, as they eat everything from green plant parts to seeds to insects and worms. In our study, the feed preferred by mice in the first place is closer to the food of wild mice in its physical characteristics, grain feed, and composition. The most preferred feed contained insect protein as the ingredient. Adequate protein intake is extremely important for mice, as it is essential for pregnancy and proper offspring care. Based on the preference test results, it would be worthwhile to investigate feeds containing insect protein further, thereby revealing possible beneficial physiological effects.

4.3. Investigating the nest material preference of the ground squirrel

The laboratory housing environment for ground squirrels participating in ex situ species conservation should be as natural as possible in terms of the needs of the species. Like their wild counterparts, the animals participating in the study chose dry grasses as nest-building material. One of the basic conditions of a successful species conservation program is that the housing technology of animals kept and bred in closed spaces is adapted to the natural needs of the species. In the case of ground squirrels kept in cages, the nesting material is extremely important, as they cannot dig holes in the cage, protecting them from external environmental factors. Hay, as a nest-building material, has a long, fibrous structure from which ground squirrels can build a nest of adequate quality, which insulates it during hibernation, thus protecting the animal from unnecessary heat loss and thus from losing body weight. Ground squirrels need to wake up from hibernation with the right body weight, as mating takes place immediately after waking up; if the female groundhog's body weight is not adequate, she cannot be fertilized. A nest of the right quality is also extremely important from the point of view of offspring care since starlings are born naked, blind and without independent thermoregulation, so the protection and thermal insulation provided by the nest is also important for the survival of the offspring. Based on our results, we can state that the ground squirrels were able to build nests of adequate quality from commercially available hay, structurally similar to their wild congeners.

4.4. Investigation of feed preference of ground squirrels

The European ground squirrel species protection program was implemented within the framework of the EU LIFE+ project RAPTORSPREY (2014-2018), where rabbit feed was defined as feed in closed enclosures. The feed preference test revealed that the ground squirrels preferred the seed mixture containing fruits, vegetables and animal protein, which are closest to their natural diet, over the granulated rabbit feed. According to literature data, groundhogs in nature feed on green parts of plants, fruits, insects, and seeds. In the case of hibernating small mammals, proper feeding is extremely important; the active period of ground squirrels is very short, and only a few months are available to create adequate fat reserves. An adequate amount of fat reserves is necessary for hibernation and for successful reproduction after awakening. Males who wake up with a higher body weight have better chances to occupy territory in nature, and females with a higher body weight become pregnant and raise their offspring. Our research results can thus contribute to developing a more successful species protection program, where death during hibernation can be minimized with the right feeding, the pregnancy rate can be higher, and the death before selection can be lower, which is particularly important for a highly protected species.

4.5. Examination of European and domesticated rabbit nesting material

The analysis of European rabbit nests revealed that the largest proportion of rabbits living in Hungary use bushgrass (Calamagrostis epigeios) for nest construction, which is a widespread plant species in the wastelands and meadows where European rabbits occur. Based on observations, burrowing rabbits prefer the grasses found in their habitat as food and as nest-building material. The examined wild rabbit nests consisted of long, dry grasses. Like the European rabbit, the domestic rabbits in the laboratory preferred dry and long grass. Based on our results, it can be said that domestication has no significant effect on the choice of nesting material; the rabbits kept in the laboratory preferred the same nesting materials as their wild ancestors. Our results can contribute to providing suitable nesting material for rabbits in the laboratory or for commercial purposes, which can have many positive advantages, such as reducing pre-choice mortality.

5. NEW SCIENTIFIC RESULTS

1. During the tests I carried out, I proved that the wild mouse species kept in the laboratory for generations chose from the offered nesting materials the one that most closely resembled the nesting materials used in nature.
2. I found that the wild mouse species kept in the laboratory chose from among the offered feeds, which, in terms of composition and physical characteristics, were most similar to the natural food of their wild congeners.
3. During my tests, I proved that European ground squirrels bred indoors chose the most natural nesting material.
4. During my tests, I proved the European ground squirrels bred indoors chose the closest to their natural food from the offered feeds.
5. I found that domestication does not significantly affect the choice of nest material for house rabbits; they chose materials with the same characteristics for their nests as those found in wild European rabbit nests.

6. REFERENCES

BAUMANS, V. (2005): Science-based assessment of animal welfare: laboratory animals. Revue scientifique et technique-office international des epizooties, 24(2) 503.

GASKILL, B. N.; GORDON, C. J.; PAJOR, E. A.; LUCAS, J. R.; DAVIS, J. K.; GARNER, J. P. (2013): Impact of nesting material on mouse body temperature and physiology. Physiology & Behavior, 110 87-95.

MAUNDER, M., BYERS, O. (2005): The IUCN technical guidelines on the management of ex situ populations for conservation: reflecting major changes in the application of ex situ conservation. Oryx, 39(1) 95-98.

NEWBERRY, R. C. (1995): Environmental enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science, 44(2-4) 229-243.

STAUFFACHER, M. (1995): Environmental enrichment, fact and fiction. Scandinavian Journal of Laboratory Animal Science, 22 39-42.

SZENCZI, P., BÁNSZEGI, O., DÚCS, A., GEDEON, C. I., MARKÓ, G., NÉMETH, I., ALTBÄCKER, V. (2011): Morphology and function of communal mounds of overwintering mound-building mice (Mus spicilegus). Journal of Mammalogy, 92 852- 860.

VAN DE WEERD, H. A., VAN LOO, P. L. P., VAN ZUTPHEN, L. F. M., KOOLHAAS, J. M., BAUMANS, V. (1997): Preferences for nesting material as environmental enrichment for laboratory mice. Laboratory Animals, 31, 133-143.

7. PUBLICATIONS ON THE SUBJECT OF THE DISSERTATION

Peer-reviewed papers published in foreign scientific journals

Bárdos, Boróka; Kövér, György; Szabó, András; Gerencsér, Zsolt; István, Nagy Feed preference and feeding behavior of different mouse species in laboratory housing ACTA AGRARIA KAPOSVÁRIENSIS 26: 2 PP. 17-26., 10 P. (2022)

Bárdos, Boróka; Nagy, István; Gerencsér, Zsolt; Altbacker, Vilmos Nest Material Preference of Wild Mouse Species in Laboratory Housing APPLIED SCIENCES-BASEL 12: 11 Paper: 5750, 8 p. (2022)

Bilkó, Ágnes; Petróczi, Imre; Bárdos, Boróka; Nagy, István; Altbacker, Vilmos Composition of the Wild Rabbit Nest and Its Implication for Domestic Rabbit Breeding APPLIED SCIENCES- BASEL 12: 4 Paper: 1915, 10 p. (2022)

Bárdos, Boróka; Altbacker, Vilmos; Török, Henrietta Kinga; Nagy, István: Housing European Ground Squirrels (Spermophilus citellus) for an Ex Situ Conservation Program METHODS AND PROTOCOLS 7: 2 Paper: 18, 11 p. (2024)

Peer-reviewed full conference papers, proceedings in foreign language

Bárdos, Boróka; Nagy, István; Altbäcker, Vilmos A fényintenzitás hatása az egerek aktivitására In: Bene, Szabolcs XXVIII. Ifjúsági Tudományos Fórum Keszthely, Magyarország: Magyar Agrár- és Élettudományi Egyetem Georgikon Campus (2022) pp. 173-177., 5 p.