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Article

Feed preference and feeding behavior of different mouse species in laboratory housing

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ABSTRACT – The feed preference of two species of wild mice, the house mouse (*Mus musculus*), and the mound-building mouse (*Mus spicilegus*) was investigated (kept in our laboratory for 25 generations). Our interest focused on the feed preference (i.e. if the mice choose the type of feed closest to their natural food). The proximate composition of the two granulated feeds offered to mice differed minimally; the crude fat and crude fibre content of the natural feed mixture was higher, and only this feed contained insect protein. Based on the obtained results, both wild mice species approached the natural feed mixture more frequently than the two other granulated feeds. The same tendency was observed for feed consumption where the animals mostly consumed the natural feed mixture. During the 5-day long study, the consumption of the natural feed mixture increased continuously, while the consumption of the granulated laboratory feed decreased significantly. The average feed consumption was also influenced by room temperature. Our studies can help to develop the optimized indoor keeping and breeding of small domesticated mammals.

Keywords: wild mouse species, *Mus musculus*, *Mus spicilegus,* feed preference, natural feed mixture

INTRODUCTION

Mice have been used in biomedical research since the 17th century, and since the 19th century, they have been bred in many places in Europe and the United States based on different fur colors (*Hedrich*, 2004). Although today's classical laboratory mouse strains probably originate from several subspecies of *Mus musculus* (*Bonhomme* et al., 1987; *Wade* et al., 2002), genetic studies have revealed four parental components, *Mus m. domesticus*, *Mus m. musculus*, *Mus m. castaneus* and *Mus musculus molossinus* (*Wade* et al., 2002). Since the beginning of the 20th century, mice have been sold in large numbers to research

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institutes and universities, thus necessarily bringing with them the development of keeping and feeding mice. The feeding and nutrient requirements of laboratory mice from the species *Mus musculus* were determined based on numerous research results by the Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council (*Coates*, 1987). The house mouse (*Mus musculus*) is generally known to eat various plant and animal-origin foods (*Calhoun*, 1941). However, mice are opportunistic omnivores and eat both materials of both plant and animal origin. Wild mice eat a wide variety of seeds, grains, and other plant matter, as well as invertebrates, smaller vertebrates, and carrion. In the course of Whitaker's (1966) studies, we can get accurate information on the food composition of the house mouse, where it was shown that about 42% of the wild house mouse's food is composed of seeds of wild grasses (*Setaria sp*). The seeds of cultivated cereals, such as wheat, corn, and sorghum, make up about 23% of the mice's natural diet, and they consume 15% of animal food, mainly insects and their larvae, and 20% of plant roots, fruits, and fresh green vegetable parts are consumed. In general, it can be stated that the feed composition determined based on Whitaker's (1966) studies and the composition of the feeds intended for laboratory mice do not entirely overlap. The protein content of laboratory feeds mainly comes from plants and contains minimal animal components.

In the present study, we investigated two species of mice native to Hungary and belonging to the *Mus* genus, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*), kept in the laboratory for 25 generations, focusing on the question: which of three offered feeds they prefer in a free-choice test. Among the three feeds offered, one was a laboratory rodent feed in granulated form, the other is a feed containing tall green plant parts also in granulated format, and the third is a seed mix with fruit and dried insect ingredients.

MATERIAL AND METHODS

Animals and experimental design

The study was conducted in the Rodent House of the Kaposvár Campus of the Hungarian University of Agricultural and Life Sciences. The rodent house has its own mouse farm, where individuals of known age, sex, and origin can be found. The current population consists of laboratory-born offspring of wild individuals captured from several parts of the country. For the feed preference study, we used house mice and mound-building mice; a total of 72 individuals were randomly selected, 36 house mice and 36 house mice, within which we selected equal sex ratio. The test took place simultaneously at temperatures of 10 and 21°C in two separate test rooms with similar lighting (Light:Dark=12:12) for five days. During the study, the mice were housed individually in a T4 laboratory mouse box, provided with litter and nest-building material. On the test days, at 8:00 a.m., the three types of feed were placed in the feeding bowls in amounts between 5 and 6 grams, weighed in hundredths of a gram, and after 6 hours, the feeds were re-weighed every day.

Test feeds

Table 1
The name and composition of the feeds used for the test

Feed name	**Components**
Versele-Laga **Nature** Mouse	grain (wheat) derivatives of plant origin vegetables (peas, beets, carrots) seeds fruit vegetable protein extracts insects nuts minerals herbs MOS FOS marigold grape seed
Versele-Laga **Complete** Rat and Mouse	grain derivatives of plant origin vegetables (peas) friuts oils and fats seeds montmorillonite clay yeast eggs and egg derivatives fructo-oligo-saccharides marigold yucca rosemary green tea

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SAFE® 132 **Laboratory** mouse feed	wheat barley corn soy flour extruded soybeans wheat bran calcium-carbonate vitamin premix minerals inactivated brewer's yeast L-lysine DL-methionine

Table 2
Analytical composition of feeds.

Nutrient	Versele-Laga **Nature**	Versele-Laga **Complete**	*SAFE*® 132 **Laboratory**
Crude protein	16,5%	14,9%	18,6%
Crude fat	8,2%	4,1%	4,0%
Crude fiber	6%	3,6%	4,2%
Raw ash	5,2%	5,7%	5,5%
Moisture content	8,8%	6,3%	8,7%

Statistical analyses

To determine whether the choice rates of different feeds were similar, a Chi-square test was used, assuming equal contribution. Afterward, the effects of species and room temperature were evaluated with Generalized Linear Mixed Model. In case of the latter analysis, the distribution was multinomial and the generalized logit link function was set. The five-days-test was analyzed in Repeated Measures ANOVA The SAS 9.4 software was used for all types of analysis applying the PROC FREQ, the PROC GLIMMIX, and the PROC CORR procedures, respectively.

RESULTS AND DISCUSSION

Based on the Chi-square test, we showed a significant difference between the con-sumption of the three feed types ($p<0.005$). The mice chose the natural feed type (Nature) in 73% of the three feeds, laboratory (Laboratory feed) in 26% and complete feed (Com-plete) in only 1%.

Based on the Generalized Linear Mixed Model, we found no significant difference between the choice of feed types for the two mouse species ($p=0.57$). Between the two species, we found no significant difference in the choice of natural feed type (p=0.68), nor in the choice of complete feed (p=0.67), nor did we find any difference in the choice of laboratory feed (p=0.52) for the two species.

Based on the estimated probabilities, mound-building mouse and house mouse also chose the natural feed in the highest proportion (Table 3.).

Table 3
Probability of choosing feeds during the choice test for the two species

Feed	Species	Probability	Standard Error
Nature	mound-building mouse	0.7247	0.0340
	house mouse	0.7689	0.0320
Complete	mound-building mouse	0.000024	0.0049
	house mouse	0.000036	0.0075
Laboratory feed	mound-building mouse	0.2753	0.0338
	house mouse	0.2311	0.0320

We found a significant difference in feed choices between the two different room temperatures (p=0.04), within feed type (laboratory feed). At the two different temperatures, we did not find a significant difference in the choice of the natural feed type (p=0.97), nor in the complete feed type (p=0.88), only a significant difference was found in the choice of the laboratory feed (p=0.01). At a temperature of 10 °C, the probability of consuming laboratory feed increased (Table 4).

Table 4
During the choice test, the probability of choosing the feeds at the two different room temperatures

Feed	Temperature	Probability	Standard Error
Nature	10	0.7247	0.0340
	21	0.7689	0.0325
Complete	10	0.0252	0.0235
	21	0.0267	0.0122
Laboratory feed	10	0.3156	0.0347
	21	0.1932	0.0295

Based on the Repeated Measures ANOVA, we found a significant difference in the daily consumption of natural feed during the 5 days (p=0.001), the consumption of natural feed increased on consecutive days (Figure 1, Figure 2). The amount consumed from natural feed showed no significant difference between the species (p=0.791), as well as 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 the 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).

A significant difference was found 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. We found no significant difference between the species (p=0.329) in the consumption of the laboratory feed, but we found a significant difference in the consumption of the laboratory feed between the two different temperatures (p=0.001). The consumption of the laboratory feed increased at colder temperature. No significant difference was found in the interaction between time, species, and temperature (p=0.405).

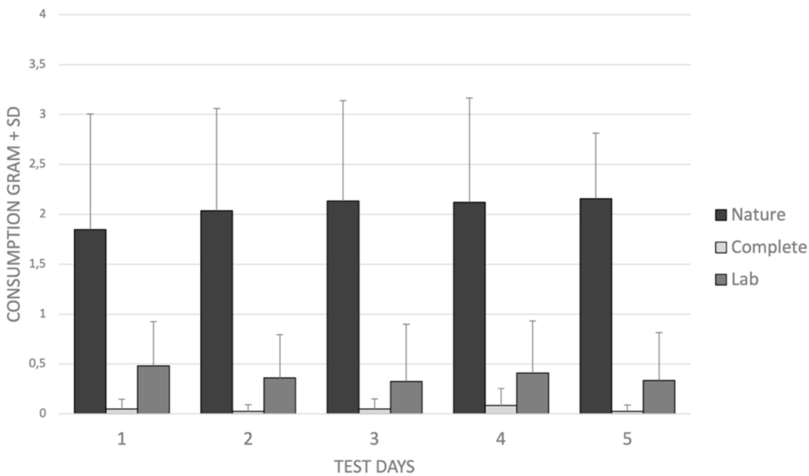

Figure 1. The daily consumption of different types of feed in grams at 21 degrees Celsius during the 5 days.

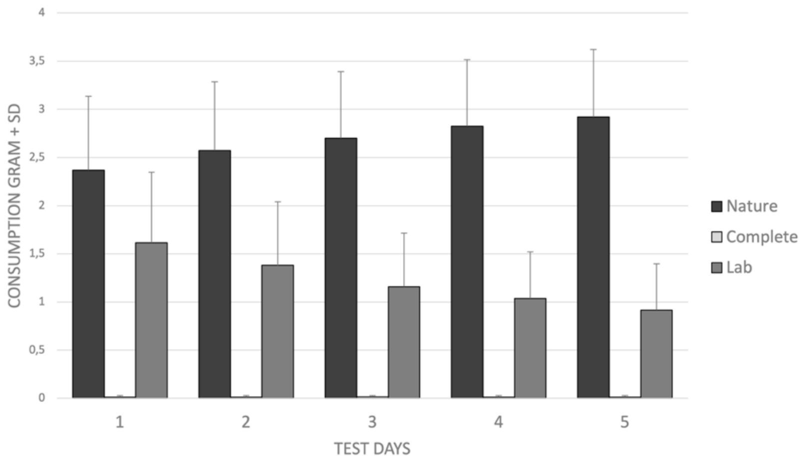

Figure 2. The daily consumption of different types of feed in grams at 10 degrees Celsius during the 5 days.

Our tests revealed that the mice chose the food (Nature) closest to their natural food in the three-way choice test. The chosen feed is closest in both proximate composition and physical structure to the food that mice eat in nature. Both the mound-building mouse and the house mouse chose the natural feed (Nature) mixture under laboratory conditions. Examining the analytical composition of the feeds, we can see that the natural feed mixture is not the highest in protein content, but this feed contains only insect protein, which is an important element of the rodents' natural diet (*Whitaker*, 1966). The natural feed mixture (Nature) has the highest percentage of crude fiber and crude fat. The result of choosing a diet with a higher crude fat composition is consistent with another study in mice, where mice were shown to prefer diets and liquids with higher oil and fat content in a two-way choice test, over fat-free diets (*Rowe* et al., 1974; *Takeda* et al., 2000). According to some studies, fatty appearance contributes to the preference for certain feeds in rodents (*Ramirez*, 1994). Another study reported possible orosensory recognition of fatty acids in rodents (*Gilbertson* et al., 1997; *Gilbertson*, 1998; *Tsuruta* et al., 1999), so the presence of esterified or free fatty acids increases the preference for the given feed, also termed as oleogustus.

In rodents, the percentage of crude fat in the feed is also an essential factor from the point of view of reproductive biology and offspring sex ratio. According to some studies, female house mice fed a low-fat diet gave birth to fewer male offspring (*Rivers & Crawford*, 1974; *Labov* et al., 1986). In addition, studies conducted with golden hamsters (*Mesocricetus auratus*) revealed that females fed a diet of a lower fat content gave birth to smaller litters and that the male offspring from such litters developed more slowly than the male offspring of females fed a diet of higher fat content (*McClure*, 1981).

The Nature feed mix used for our study is a feed composed of seed mixtures; its preference was the same as in the study with laboratory rats, where it was found that the rats prefer grain feed more than granulated feed (*Abdel-Kader* et al., 2014). Based on another study on rats, those preferred mixed feed in choice tests over homogeneous feed containing only one type of ingredient (*Ito*, 2001; *Schein and Orgain*, 1953).

In rodent food preference tests, it is common for the animals to be warier of the unknown food at first, and then the consumption of the unknown food increases as the test days pass (*Pennycuik & Cowan*, 1990). This phenomenon was also observed in our tests; the consumption of the feed mixture most preferred by the animals (Nature) increased with the test days, while the consumption of the laboratory feed known to the animals gradually decreased over the days. During the tests carried out at two different temperatures, it was revealed that in the colder environment, feed intake increased minimally, but overall, which was significantly detectable in the case of laboratory feeds. Due to their small body size and very high metabolic activity, mice are relatively sensitive to heat loss (*Lisk*, 1969); therefore, in colder environments, animals must take in more feed and probably more lipids in t to meet their energy needs due to heat retention (*Lenzhofer* et al., 2020).

CONCLUSIONS

We can say that even the wild mouse species that have been bred and kept in the laboratory for 25 generations have chosen the feed closest to their natural diet, which is identical in composition and structure to the food of their wild counterparts. For small mammals kept in the laboratory, it would be worthwhile to carry out further tests involving insect protein since currently available small mammal feeds do not contain insect protein, even though numerous field studies have shown that insects play an important role in the diet of small mammals.

Institutional Review Board statement: This research was approved by the Committee on the Ethics of Animal Experiments of Kaposvár Campus (permit number: MATE KC MÁB/10-5/2021). The authors declare that all experiments were performed in accordance with approved guidelines and regulations.

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