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The impact of bedding volumes on laboratory mice

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ABSTRACT

Environmental refinement is considered to be an improvement in housing conditions for laboratory animals. Previous preference tests showed that female BALB/c and C57BL/6 mice prefer deeper bedding in comparison to shallow bedding (Freymann et al., 2015). In order to give a comprehensive insight into the impact of bedding depths on laboratory mice, we continued to examine the influence of three different bedding volumes (0.5 l, 1.5 l, 6 l) on the preference of male mice (experiment 1), home cage behaviour (experiment 2) as well as body temperature, food intake, food conversion efficiency (gram food intake per gram weight gain), intra-cage ammonia and corticosterone levels (experiment 3) of females and males. Experiment 1 used an automatic system to assess the preferences of male BALB/c and C57BL/6 mice. The bedding volumes were tested in pairs, which resulted in three test conditions (A = 0.5 l vs. 1.5 l; B = 0.5 l vs. 6 l; C = 1.5 l vs. 6 l). The results revealed significant preferences for cages containing large bedding volumes (test conditions A, B: $p < 0.0001$ for both strains; C: $p = 0.0110$ (BALB/c), $p = 0.0511$ (C57BL/6)). The second experiment analysed the home cage behaviour of female and male BALB/c mice between 18:00 – 20:00 (CET) using instantaneous sampling. No significant differences regarding the behavioural patterns locomotion, grooming, agonistic interaction, feeding, drinking, nest-building, resting, digging and burrowing were detected. However, animals housed on shallow bedding (0.5 l) engaged more in nest-building behaviour compared to groups housed larger volumes (1.5 l or 6 l). Experiment 3 demonstrated that bedding volumes (0.5 l, 1.5 l or 6 l) have profound effects on mice's physiology. BALB/c and C57BL/6 mice kept on deep bedding showed higher body temperatures ($p < 0.05$ (0.5 l compared to 1.5 l, or 6 l)), lower food intake $p < 0.01$ (6 l compared to 0.5 l, or 1.5 l) as well as reduced intra-cage ammonia levels compared to groups on shallow bedding. In addition a larger bedding volume increased food conversion efficiency and reduced corticosterone levels in female mice. The trend became particularly obvious in female BALB/c mice ($p < 0.05$ (0.5 l compared to 1.5 l, or 6 l) for both parameters). Our results underline the importance of a sufficient amount of cage bedding in the husbandry of laboratory mice.

1. Introduction

"Environmental refinement implies an improvement in quality of the life of animals" (Baumans et al., 2011). Refinement in the husbandry of laboratory animals can be achieved by allowing them to perform more elements of their natural behaviour. Digging, burrowing and burying belong to mice's natural behavioural repertoire (Adams and Boice, 1981; Berry, 1970). Rodents employ those behaviours for food storage and to create nesting sites protected from ambient temperatures and predators (Harper and Batzli, 1996; Webster et al., 1981). Different consumer demand studies indicated that laboratory mice are highly motivated to gain access to burrowing substrate (King and Weisman, 1964; Sherwin et al., 2004) or nest box (Hackbarth et al., 2009). Comparable to wild mice, laboratory mice perform digging and burrowing behaviour regardless of age and gender (Masuda et al., 2000). In order to satisfy these needs, laboratory mice should be provided with an appropriate bedding depth (Deacon, 2009; Jennings et al., 1998). The use of about 1 cm of bedding is common practice in the husbandry of laboratory mice. However, this is not sufficient to promote distinct digging and burrowing behaviour (Deacon, 2006). Previous preference tests demonstrated that group-housed female BALB/c and C57BL/6 prefer deeper bedding in comparison to shallow bedding (Freymann et al., 2015). This underlines the importance of bedding depth in the husbandry of laboratory mice.

Intra-cage ammonia levels are dependent on different factors, including strain (Smith et al., 2004), caging systems (Ferrecchia et al., 2014; Smith et al., 2004) as well as bedding material

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(Ferrecchia et al., 2014; Potgieter and Wilke, 1996; Smith et al., 2004). Although there is no definite maximum limit of ammonia concentration in rodent cages, 25 ppm has been used as a guideline (Domer et al., 2012; Ferrecchia et al., 2014; Reeb-Whitaker et al., 2001). The bedding material may vary in absorbency (Burn and Mason, 2005), processing and treatment (Domer et al., 2012). For individually ventilated cages (IVCs) it has been demonstrated that a larger amount of bedding reduces intra-cage ammonia levels (Rosenbaum et al., 2009). We want to provide more data regarding the impact of bedding volumes on ammonia levels in open rack caging system, especially regarding the differences within the cage.

Laboratory mice are usually housed between 22 °C–24 °C, but the animals prefer temperatures closer to their thermoneutral zone (approximately 30 °C, (Gordon, 1993)) (Gaskill et al., 2009; Gordon, 1985). A standard ambient temperature of 20 °C leads to profound changes in mice's physiology (Maher et al., 2015; Yamauchi et al., 1983), including a reduced antitumor immune response compared to animals housed at thermoneutral temperatures (Kokolus et al., 2013). It is well documented that lower ambient temperatures increase animals' metabolic demands which results into an increased food consumption in order to counteract a higher heat loss (Cannon and Nedergaard, 2009). Beneficial effects of larger bedding volume concerning heat loss have been reported for female CD-1 mice (Gordon, 2004), however its impact on food consumption and weight gain remains unclear.

Not every change in cage environment has a positive effect on animal welfare. While nesting material reduced stress-related parameters (Van Loo et al., 2002, 2004), rigid forms of cage enrichment increased aggressive interaction and corticosterone levels in male mice (Haemisch et al., 1994; Marashi et al., 2003). However, apart from the enrichment itself, the impact of different housing conditions is strain (Van Loo et al., 2004) and sex (Tanaka et al., 2014) dependent. It has been demonstrated that type of bedding material can affect mice's behaviour (Tanaka et al., 2014), but the impact of bedding volume on behaviour including stress levels has not yet been evaluated.

These studies clearly demonstrated that, although bedding is invariably used the husbandry of laboratory mice, there is still a lack of knowledge regarding its impact on animal welfare and experimental results. The overall aim of this study was to evaluate the role of bedding volumes in the husbandry of laboratory mice more precisely. In particular we want to analyse the preference of group-housed male BALB/c and C57BL/6 mice for different bedding volumes (Experiment 1). Furthermore, our study examines the effect of bedding volumes on behavioural patterns of group-housed female and male BALB/c mice (Experiment 2) as well as the influence of bedding depths on intra-cage ammonia levels, body temperature, food intake, food consumption in relation to weight gain and corticosterone levels of female and male BALB/c and C57BL/6 mice (Experiment 3).

2. Experiment 1

Housing and experimental procedures of all experiments (1–3) conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe, ETS No. 123, Strasbourg 2006) and approved by German authorities.

2.1. Material and methods

2.1.1. Animals

All animals (Experiment 1–3) were obtained from Janvier Labs, Le Genest Saint Isle, France. The specific-pathogen-free (SPF) status of the animals was documented by the breederís health certificate. At the end of the experiment the animals were health monitored according to the Federation of European Laboratory Animal Associations' (FELASA) recommendations (Nicklas et al., 2002).

A total of 144 inbred male mice (72 BALB/cByJRj and 72 C57BL/6NRj mice) were used in the study. The animals arrived at 6 weeks of age: they were weighed and divided into groups of four (average weight: BALB/cByJRj: 22.5 ± 1.4 g; C57BL/6NRj: 20.6 ± 1 g). After a two-week adaptation period, the experiments started when the mice were 8 weeks of age. Due to aggression interaction during adaptation phase and technical problems four C57BL/6 mice (two animals each for condition B and C) needed to be excluded from preference testing.

2.1.2. Housing conditions

During the entire experiment the animals were housed in Type III (37.5 cm × 21.5 cm × 5.0 cm, Bioscape, Castrop-Rauxel, Germany, with standard wire tops) Makrolon double cages (two Type III connected by a Perspex tube) in an open rack. The same amount of food (Ø10 mm pelleted diet, Altromin No. 1324, Altromin, Lage, Germany) and tap water were available *ad libitum*, in every cage during adaptation as well as test period. Coarse-gained aspen chips were used as bedding (ABEDD; LAB & VET Service GmbH, Vienna, Austria). None of the components was autoclaved prior to the use. During the adaptation period l l of aspen chips was provided for all cages, within the experiment 0.5 l (≈0.5 cm bedding depths), 1.5 l (≈1.5 cm bedding depths) or 6 l (≈6 cm bedding depths) of bedding were used, depending on the test condition. Solely bedding, cages and water were changed weekly during acclimatization. To avoid that a familiar smell influenced the animals' preferences, all housing components, including food, water, water bottles, cage tops, cages and bedding were changed prior to the testing. Cages, cages top and water bottles were washed with a cage washer (Meiko 250 FV 250B, Iserhagen, Germany).

2.1.3. Environmental conditions

The animals were housed in conventional holding rooms at a temperature of 22 ± 2 °C with 55 ± 10% relative humidity and 10–16 changes of air per hour. The room was maintained at a 12/12 h light/dark cycle (lights on 06:00 CET) with artificial light (140 ± 10 lx in the rack, 1 m above the floor).

2.1.4. Experimental procedure and design

Three bedding volumes (0.5 l, 1.5 l, 6 l) were tested in pairs using three test conditions (A = 0.5 l vs. 1.5 l; B = 0.5 l vs. 6 l; C = 1.5 l vs. 6 l). The experiments were performed in twelve replicates, respectively. In every replicate the three test conditions were tested for one week (in total 24 mice per test condition per strain). Each group of mice was only used once. In order to allow a comparison to the data of female mice, experimental procedure and design were chosen according to previously published methods (Freymann et al., 2015). Prior analysis revealed that, despite the fact that the mice had the same preferences, they moved independently between the two cages (Freymann et al., 2015). Therefore, the data of the individual animals were used for evaluation (n = number of mice). Additionally, the results of the datasets (n = number of cages) are also provided.

2.1.5. Statistical analysis

The data of all the experiments (1–3) were tested for normal distribution using the Shairop Wilk test and further analysed with the StatView computer program (Version 5.0; SAS Institute Inc., Cary, NC).

Mean differences between dwelling time as well as food and water intake were evaluated for each cage using the paired *t*-test. The analysis of variance (ANOVA) was used to assess strain dif-