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Effect of 2 Bedding Materials on Ammonia Levels in Individually Ventilated Cages

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This study sought to identify an optimal rodent bedding and cage-change interval to establish standard procedures for the IVC in our rodent vivarium. Disposable cages were prefilled with either corncob or α-cellulose bedding and were used to house 2 adult Sprague–Dawley rats (experimental condition) or contained no animals (control). Rats were observed and intracage ammonia levels measured daily for 21 d. Intracage ammonia accumulation became significant by day 8 in experimental cages containing α-cellulose bedding, whereas experimental cages containing corncob bedding did not reach detectable levels of ammonia until day 14. In all 3 experimental cages containing α-cellulose, ammonia exceeded 100 ppm (our maximum acceptable limit) by day 11. Two experimental corncob cages required changing at days 16 and 17, whereas the remaining cage containing corncob bedding lasted the entire 21 d without reaching the 100-ppm ammonia threshold. These data suggests that corncob bedding provides nearly twice the service life of α-cellulose bedding in the IVC system.

For many animal facilities, IVC are an increasingly popular rodent housing option. These cages offer several benefits over traditional cage systems, including better containment, simplified handling, and increased protection from allergens.[9] Disposable IVC systems might also provide labor and cost savings by eliminating the need to clean and sanitize reusable cages. In addition, IVC systems have been shown to reduce cage ammonia levels and extend cage change intervals compared with static cage systems.[7]

As facilities make changes to IVC systems, the type of bedding to use and cage change frequency are important considerations. In accordance with the *Guide for the Care and Use of Laboratory Animals*,[10] bedding must be replaced and the microenvironment cleaned often enough to keep animals clean and dry and to keep pollutants (for example, ammonia) below irritating levels.[10] Due to a lack of directly comparable published data on this topic, conflicting advertising by bedding and IVC manufacturers, and marked differences in design and performance among IVC systems, choosing the right bedding and cage-change interval can be difficult.[3] Although high bedding absorbency is often associated with its ability to better neutralize ammonia, this situation is not always the case, and few published data are available to support these claims.[13] The absorbencies of some bedding types have been measured, but results vary greatly depending on whether absorbency is measured relative to the mass or the volume of bedding.[2]

In the current study, we sought to compare the accumulation of intracage ammonia between IVCs using 1/4-in. of corncob or an α-cellulose paper bedding in a commercially available IVC system for 21 d. Corncob and α-cellulose beddings were selected for this study because they are available in prefilled disposable cages directly from the IVC rack manufacturer. This study sought to identify the optimal bedding choice and cage-change interval for use in the IVC system in our vivarium. Similar studies have been performed by using various types of bedding and cage systems but report inconsistent results. Two studies report significant accumulation of intracage ammonia in IVC after only 1 wk when using recycled paper bedding,[12,15] whereas another reports no measureable intracage ammonia after 2 wk when a similar bedding was used.[7] In addition, many bedding and cage combinations have not been tested in IVC systems. It is important to note that the manufacturer of the α-cellulose paper bedding claims significant performance differences between recycled paper beddings and those of engineered α-cellulose paper.[14]

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Materials and Methods

Rats and husbandry. This study was approved by the IACUC of the US Army Center for Environmental Health Research, an AAALAC-accredited facility. The 2 contact beddings used in this study were corncob and α-cellulose beddings (ALPHA-dri, Shepherd Specialty Papers, Watertown, TN). Innocage Rat Pre-Bedded cages (141-in.^{2} of floor space; Innovive, San Diego, CA) with external water bottles were used, which were filled to a depth of 1/4 in. with the selected bedding. All cages were housed in the Innorack IVC Rat 3.5 system (Innovive) at 60 air changes hourly in negative pressure mode, in accordance with manufacturer's recommendations. Male Sprague–Dawley rats (Hsd:Sprague–Dawley SD; n = 14; weight, 450 g; age, 18 wk; Harlan, Indianapolis, IN) were used for this study, to maximize cage biomass. All rats were screened by using the institution's health monitoring program and were free from the following pathogens: Kilham rat virus, rat parvovirus, Toolan H1 virus, Sendai virus, pneumonia virus of mice, reovirus type II, murine encephalomyelitis virus, sialodacryoadenitis virus, rat minute virus, Hantaan virus, lymphocyctic choriomeningitis virus, cilia-associated respiratory bacillus, mouse adenovirus types 1 and 2, rat rotavirus, rat coronavirus, *Mycoplasma pulmonis*, *Clostridium piliforme*, *Pasteurella* spp., fur mites, and pinworms. Each cage housed 2 randomly distributed rats. Rats of this size were chosen to maximize the amount of animal biomass per cage, following the animal mass and space guidelines described in the *Guide*.[10]

Rats were conscious and freely moving for the duration of the experiment and were given an irradiated, certified chow

designed for toxicological studies (Harlan 2016, Teklad Global, Harlan, Indianapolis, IN) and water (prefilled 500-mL water bottles, Innovive, San Diego, CA) ad libitum. Animal holding rooms were maintained at 69.8 ± 0.1 °F (21.0 ± 0.1 °C), 49.5% ± 4.4% humidity, and a 12:12-h light:dark cycle, as recommended by the *Guide*.[10] Two rolls of certified and irradiated thick rolled tissue paper (Diamond Twists, Harlan, Indianapolis, IN) were provided as enrichment and destructible bedding material. For the duration of the study, enrichment was added only during cage changes, to minimize cage opening. All cages and cage materials were new at the beginning of the study.

Cage setup and ammonia measurement. Six experimental cages, each containing 2 rats, were used: 3 with corncob bedding and 3 with α-cellulose bedding. To establish baseline ammonia levels, 6 control cages, not containing any animals, were used: 3 with corncob bedding and 3 with α-cellulose bedding. We placed 2 control cages, 2 corncob cages, and 2 α-cellulose cages on each level of the rack. The cage types were put into the rack in alternating order so that no 2 adjacent levels were the same, to avoid any bias resulting from position in the rack due to differences in light, air flow, or noise. A small (1/2-in.) hole was drilled into the upper right hand corner of each cage, to use as a sample collection port. The sample-collection ports were sealed with white laboratory tape between samplings. Adding collection ports allowed ammonia measurements to be performed daily without opening the cage or removing it from the rack. Intracage ammonia levels were measured (nos. 6400000 and CH20501 5/a), Accuro pump and ammonia tubes (Item numbers respectively, Draeger Safety Diagnostics, Irving TX) once daily, for each experimental and control cage, between 0800 and 1000. The ammonia tubes have a range of 0 to 70 ppm or 5 to 600 ppm, depending on the scale used. The lower range was used until intracage ammonia exceeded 70 ppm, at which time another measurement was taken by using the 5 to 600 ppm scale. The pump and ammonia tubes were used according to the manufacturer's instructions. The tip of the ammonia tube was inserted approximately 3 inches into the cage, at an upward angle so that the tip of the tube was above the wire bar at the top of the cage, to prevent the rats from chewing on the ammonia tube during sampling (Figure 1).

Once a cage reached an ammonia level of 100 ppm, it was changed immediately. There are no ammonia exposure limits or guidelines for rodents, and we chose 100 ppm because of other studies reporting adverse health effects in rats exposed to higher levels of ammonia.[1,6] In addition, 130 ppm ammonia is highly irritating to humans and can cause adverse respiratory and pulmonary health effects.[4,5]

Water consumption and cage biomass. All rats were weighed on a digital scale (Olympia Plus, Solenhe, Hamburg, Germany) prior to beginning the experiment. Rats were randomly placed into experimental and control cages, and the total cage biomass did not differ between cages. Water consumption was measured over a 1-wk period; fresh water bottles were placed at the beginning of the study on a Monday, and water consumption was measured by carefully weighing the water bottle from each experimental cage on a digital scale (Solenhe) daily, for Tuesday through Friday (4 data points total). Water weights were recorded to the nearest whole gram for all experimental cages, but no water consumption data were taken for control cages. Water bottles were handled carefully to avoid spillage, and cages were observed daily and monitored for leaks. Water consumption was then averaged for each bedding group. Removing the water bottles did not require the cages to be opened and did not interfere with ammonia measurements.

Figure 1. Ammonia measurement through sample port. Pump and ammonia tube are shown, with ammonia tube inserted through the sample port and into the cage, above the wire bar, for ammonia measurement.

Data analysis. Data, ammonia levels, rat weights, and water consumption was averaged for each experimental bedding group. The Student t test was used to determine whether 2 groups of data differed significantly from each other. A P value of 0.05 was chosen as the threshold for significance.

Results

Intracage ammonia levels. Ammonia levels were measured daily for each experimental and control cage in both the corncob and α-cellulose groups. By day 8, all 3 experimental α-cellulose cages had significantly ($P < 0.05$, Figure 2) elevated ammonia levels, relative to the corncob cages, whereas all 3 experimental corncob cages maintained undetectable ammonia levels until day 11. All 3 experimental α-cellulose cages had significantly higher levels of intracage ammonia than did the experimental corncob cages from days 8 through 11. Although all 3 α-cellulose cages exceeded 100 ppm ammonia by day 11, all 3 experimental corncob cages had undetectable ammonia levels until day 14. Please note that no measurements were taken on days 5, 6, 12, 13, 19, and 20, which fell on weekends, because measurements were taken on weekdays only (Monday through Friday). After 14 d, 2 of the corncob cages registered very low levels of ammonia (3 and 17 ppm), which slowly increased until day 17, when they both exceeded 100 ppm. The final corncob cage had its first measurable ammonia level (5 ppm) on day 17; the ammonia level within this cage rose to 90 ppm by day 21. There was no detectable ammonia in any control cage over the course of the 21 d experiment (Figure 2). Although intracage ammonia levels varied significantly between the 2 beddings types, no adverse signs were observed in any of the rats.

Water consumption. Water consumption in each experimental cage was measured over 1 wk (5 business days) by weighing the water bottles daily. Daily water consumption was calculated and averaged for each bedding group. Although the rats in the α-cellulose cages consumed slightly more water daily (64 g) than did the rats in the corncob cages (58 g), the difference was not statistically significant (Table 1). Food consumption was not measured by weighing or counting pellets; due to the brittle

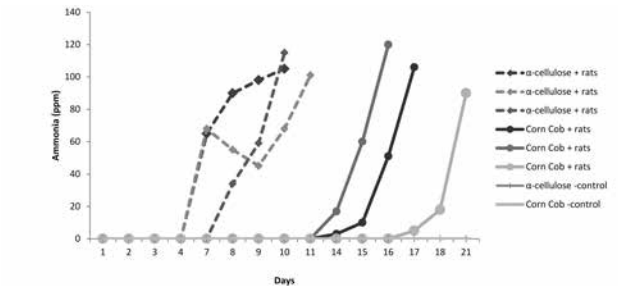

Figure 2. Effect of corncob and α-cellulose bedding on intracage ammonia levels (ppm) in IVC. Intracage ammonia levels were measured once daily for 21 d. Each bedding group contained 3 control cages (without animals) and 3 experimental cages, each of which housed 2 adult rats each. All three experimental cages with α-cellulose bedding had significantly ($P < 0.05$, 2-tailed t test) higher ammonia levels than the experimental corn cob cages from day 8 to the last day that the α-cellulose cages were used. Once the ammonia level reached 100 ppm, the cage was changed immediately, and subsequent ammonia levels are not shown. All 3 experimental α-cellulose cages exceeded 100 ppm ammonia by day 11. One corncob cage lasted the entire 21 d without reaching the 100-ppm ammonia threshold; however the other 2 corncob cages required changing at days 16 and 17. Although each individual experimental cage is plotted separately, the corncob and α-cellulose control groups are represented by one line each, because all control cages remained at 0 ppm ammonia throughout the experiment.

nature of the pellets, it is common for a partially eaten pellet to break or crumble and fall from the wire feeder onto the cage floor. However, food levels were checked daily by an animal technician, and no noticeable differences in food consumption were reported for any of the experimental cages.

Cage biomass. All rats were weighed at the beginning of the study (day 1); all had approximately the same mass. Rats were randomly distributed throughout experimental cages, and total cage biomass was calculated for each group. Total cage biomass at the beginning of the study did not differ between groups. All rats were weighed again at the conclusion of the study (day 21), with no significant difference between groups. Consistent cage biomass between the corncob and α-cellulose groups rules out biomass as a factor contributing to the intergroup differences in intracage ammonia levels.

Discussion

In this study, we compared the ability of corncob and α-cellulose beddings to control ammonia levels in IVC over a 21-d period. Our data suggest that, when biomass is maximized, α-cellulose bedding was effective in the IVC systems for a maximum of 7 d. After 1 wk, the levels of accumulated intracage ammonia will be high (100 ppm or greater). Whether such a level of ammonia causes adverse effects in the rats or confounds experiments has yet to be determined.[1,4,5,6,] In contrast, all 3 experimental corncob cages had relatively low levels of intracage ammonia after 2 wk (14 d), therefore doubling the interval between cage changes compared with that for α-cellulose.

We cannot definitively account for the drop in intracage ammonia seen in an experimental α-cellulose cage on days 8 and 9, but this cage still exhibited higher ammonia levels than any of the corncob cages during this time window. The bedding type, amount, and cage airflow were the same as those for all other cages in the α-cellulose group. This cage was housed in the same rack in the same room as the other cages, so an external factor such as temperature, humidity, or disturbances can be eliminated. The cage was not removed from the rack or opened during this time. We hypothesize that the drop in intracage ammonia can be attributed in some way to animal behavior; the rats' activity level or waste production likely affected the ammonia levels.

The 2-wk cage-change interval will not only markedly reduce labor time and material costs, especially when using disposable cages in an IVC system, but it will also allow studies that require prolonged exposures, treatments, or observations without disturbing the animals. In our study, we maximized the biomass in each cage to create a 'worst-case scenario' for ammonia accumulation and soiled bedding. We will base our bedding choice and cage change interval for our entire rodent vivarium on these data, rather than having different change intervals for every different situation, to simplify and streamline planning and ordering. In light of these data, we will be using corncob bedding and a cage change interval of 2 wk for our entire rodent vivarium.

If fewer or smaller rats were used, we would expect to see an increase in service life for each bedding type. Increased cage biomass (that is, more or larger rats) results in increased ammonia levels,[16] therefore we hypothesize that using fewer or smaller animals might potentially extend the service life of corncob bedding to 3 wk (21 d). In our study, 1 of the 3 experimental corncob cages lasted 21 d without reaching 100 ppm ammonia; however the other 2 cages in this group needed to be changed on days 16 and 17, due to high ammonia levels.

Water consumption was measured in each experimental cage as an indirect way to assess amounts of urination in each group, to determine whether differences in urine production affected the ammonia levels reported. Intracage ammonia results primarily from urease-positive bacteria, which metabolize urea from the urine and feces of the animals.[8] Therefore, ammonia levels are proportional to the amounts of wet urine and urease-positive bacteria present in the cage. IVC systems help to reduce the levels of both urine and urease-positive bacteria by providing sufficiently frequent air changes to dry the cage bedding.[11,7] We found that there was no statistically significant difference in water consumption, and presumably urine production, between the α-cellulose and corncob groups in our study. Therefore, we conclude that the significant difference in intracage ammonia levels between the 2 groups was not due to differences in urination.

Because increased cage biomass results in increased intracage ammonia levels,[16] we ensured that each cage had the same total biomass before the experiment began. We weighed all of the rats at the conclusion of the experiment to see whether the animals in each group had similar growth rates over the course of the 3-wk experiment. A difference in growth rates between the groups might account for some of the difference observed in intracage ammonia levels. We found that total cage biomass did not differ between the 2 groups. Again, this finding suggests that the differences in ammonia levels between the α-cellulose and corncob groups were due to the bedding material and not another external factor.

Choosing the right bedding and cage change interval are important for the wellbeing of the animals and for minimizing the time and expense spent on unnecessary cage changes. Determining the optimal bedding and cage-change interval for a particular study, animal species, and cage setup can be challenging, given the lack of published information, conflicting reports from bedding manufacturers, and differences in the performance of various IVC systems. Although other published studies have compared different bedding materials in both static and IVC cages, we are unaware of any study that has compared the effect of α-cellulose and corncob beddings

Table 1. Water consumption.

		Water consumption (g)				
Bedding	Cage no.	Day 1	Day 2	Day 3	Day 4	Average
α-Cellulose	1	64	64	78	37	61
α-Cellulose	4	59	72	85	65	70
α-Cellulose	6	61	56	63	63	61
Corncob	2	59	61	67	66	63
Corncob	3	52	48	63	65	57
Corncob	5	54	50	58	53	54

Water consumption was an indirect way to assess amounts of urination in each experimental group, to determine whether differences in production between groups affected the ammonia levels reported. Water consumption did not differ between the bedding groups (α-cellulose, 64 g; corncob, 58 g).

on intracage ammonia levels in an IVC system for an extended time period. Other factors to consider when choosing bedding type and change interval for a particular facility or study might include intracage carbon dioxide levels and fecal cortisol but were not included in this study.

The cages used in this study were purchased prefilled with bedding and are designed for a specific commercial rack system. The design of the rack system we used is fairly new, and its popularity is growing quickly, but only a few relevant data are available in the literature. One study compared IVC cages with static cages over 9 d[15] and described various advantages of the IVC system. However, to our knowledge, long-term studies that compare the 2 bedding options (corncob and α-cellulose) available directly from the rack manufacturer are unavailable. Our current study fills this gap and will help other animal facilities to make educated decisions that are based on empirical data rather than common practice, when they need to choose a type of bedding and a cage-change interval.

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