BEDDING SELECTION BASED ON AMMONIA LEVEL MONITORING IN VENTILATED RAT CAGES

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ABSTRACT

In order to determine the most effective bedding selection that would result in decreased ammonia concentrations and thus extended cage change periods in an Individually Ventilated Cage system (IVC), ammonia levels were measured with rats housed on a variety of bedding substrates. Ammonia levels were measured on day 7 and day 14, post-cage change, with animals housed in IVC and static isolator cages. The beddings considered and their approximate volumes were as follows:

(A) Andersons® ¼" corncob 1,200 cm³, **(B)** Andersons® ¼" corncob 2,400 cm³, **(C)** Omega Dri - 2,000 cm³, **(D)** Alpha Dri®/ ¼" corncob mixture (50:50) 2,000 cm³, **(E)** Alpha Dri®/ ¼" corn cob/Omega Dri® (25:25:50) 2,000 cm³, and **(F)** Aspen Shavings 1,800 cm³.

Different volumes of bedding were used so that each cage had approximately a 1" layer of bedding on the bottom of it. We also added cages with a ½" layer of bedding (corn cob 1,200 cm³) as a comparison, as many facilities use this protocol. Static cages with 2,400 cm³ of Andersons® ¼" corncob bedding were also used to compare them to the ventilated system. Four cages were used for each type of bedding. Each cage, measuring 143 in², housed one 500-600 gram male Sprague Dawley® rat. With the exception of the four static cages, all cages were placed on a ventilated cage rack (Allentown Caging Equipment, Allentown, NJ), which supplies air so that the cages are maintained at positive pressure. Upon initiation and completion of the study an anemometer (Edstrom Certiflow®, Waterford, WI) was used to measure the air changes in each cage. The air change rate measured at each interval was approximately 50- 57 air changes per hour. Ammonia levels were measured via a 1" grommet located in the lid of the micro-isolator cage. All readings were taken 5 ½" off the bottom and 3½" from the front wall of the cage. A smoke test confirmed that opening the grommet did not alter the air pressure or the air flow within the cage. Results show that using 1,200 cm³ of corncob bedding gave the lowest ammonia levels at 7 and 14-day intervals. The second best option was 2,400 cm³ of corncob bedding. Although the 1,200 cm³ of corncob bedding in an IVC system is associated with lower ammonia concentrations than the 2,400 cm³ of corncob, it becomes more soiled and prevents the animals from staying clean and dry. Aspen shavings used in an IVC system are associated with the highest ammonia levels. We concluded that ammonia levels could be kept at an acceptable level using a ventilated cage rack with a once weekly cage changes. Individually housed rats could be safely placed on 2,400 cm³ of corncob bedding substrate for 14 days prior to cage change. It was also determined that weekly cage change and every other week cage changing of individually housed animals resulted in a significant cost savings compared to traditional static cage set-ups, which require changes 2-3 times per week.

INTRODUCTION

There is little published information about optimal environmental conditions for housing rats in individually ventilated caging (IVC) systems. IVC systems have been shown to improve intracage microenvironment by controlling the amount of air supplied through the top or wall of a filter top cage. IVC systems are capable of suppressing the increase of ammonia concentration within cages, thereby reducing the frequency of cage changes and subsequent stress to animals. Elevated ammonia levels have been shown to have a role in respiratory diseases in rodents, such as *Mycoplasma pulmonis*[1].

We conducted a study to determine:

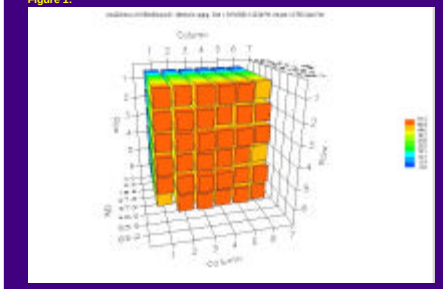
1. The bedding substrate that produces the lowest level of ammonia concentration in an IVC rat cage over a two-week period.
2. The optimal volume of bedding required to produce the least exposure to ammonia concentrations.
3. The frequency of bedding changes required for rats housed in an IVC system.
4. The cost of different types of beddings and how it related to the frequency of cage changing.

By reducing the number of cage changes per week, we could not only reduce the stress to the animals but also reduce costs related to technician time as well as indirect costs.

METHODS

Each cage, (floor area 143 in²) contained one 500-600 g male Sprague Dawley® rat (Charles River, Wilmington, MA). Rats were housed in an AAALAC accredited facility and were negative for all rodent pathogens. They were given food (Purina Lab Diet 5002, irradiated, certified) and water ad libitum. Rats housed on the ventilated rack received water via the Edstrom® automatic watering system whereas rats housed in static cages were given water bottles. Animals were on a 12:12 light-dark cycle. Animal rooms in this facility had HEPA filtered air, air changes ranged from 150-180 per hour. Ninety percent of the air was re-circulated through charcoal filters and then HEPA filtered and reused. Animal room conditions were within the recommendations of the *Guide for the Care and Use of Laboratory Animals* (temperature 64ºF-79ºF and humidity 30-70%)[2]. Animal rooms were under positive pressure in relation to the hallways. Four cages were used for each bedding type, a total of 28 cages. All but the 4 static cages were placed on a ventilated cage rack (Allentown Caging Equipment, Allentown, NJ). Any vacant spaces were filled with empty cages to maintain the proper air change rate. The air change rate was measured for each cage location at initiation and completion of the study by an anemometer (Edstrom Certiflow®, Waterford, WI). Ammonia levels were measured by a Toxic Gas Detector (Matheson®, Model 8014-400A) via a 1" grommet located in the micro isolator lid at a level of 5 ½" from the bottom of the cage and 3 ½" from the front wall of the cage (Figure 5). After 14 days the cages were changed and the ammonia testing was repeated.

Figure 1:

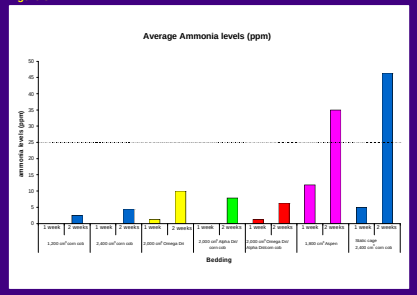


Certiflow® Test Results

Figure 2:

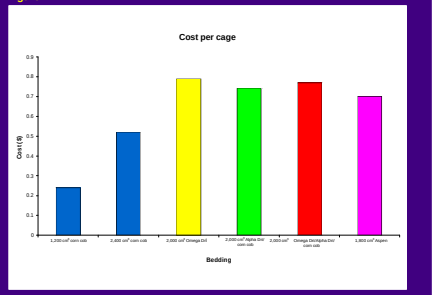
Ventilated Cage Rack

Figure 3:



Average Ammonia Levels

Figure 4:



Cost Comparisions

Figure 5:

Ammonia Level Testing

Figure 6:

Bedding Types

RESULTS

Results showed that using corncob bedding produced the lowest ammonia levels (1,200 cm³; 7 day = 0 ± 0 ppm, 14 day = 2.50 ± 1.33 ppm, and 2,400 cm³; 7 day = 0 ± 0 ppm, 14 day = 4.38 ± 1.75 ppm). We preferred using 2,400 cm³ of corncob because increasing bedding depth allowed the rat more clean bedding to lie in with no significant increases in ammonia levels. The least effective bedding was the Aspen Shavings (7 day = 11.88 ± 2.98 ppm, 14 day = 35.00 ± 7.56 ppm). We concluded that ammonia levels could be kept within an acceptable range using a ventilated cage rack with once weekly cage changes. We also found that rats housed on the ventilated cage rack, one per cage with corncob bedding, could be changed once every 14 days, if necessary. Data showed that housing on the ventilated rack decreases the ammonia level within cages, as the static cages had mean ammonia levels of 5.00 ± 5.00 ppm after 7 days and 46.25 ± 11.17 ppm after 14 days.

CONCLUSION

Ammonia levels could be kept within an acceptable limit of 25 ppm using a ventilated cage rack with a once weekly cage-changing schedule.[3] Individually housed rats could be safely placed on 2,400 cm³ of corncob bedding substrate for 14 days prior to cage change. Although ammonia levels were within acceptable range with 1,200 cm³, the feces build up and it is not aesthetically pleasing to the observer. The bedding that gave the lowest ammonia level readings (Andersons® ¼" corncob) was also the most cost effective bedding. Weekly cage change and every other week cage change resulted in a significant cost savings compared to traditional static cage set-ups. For example, in a facility with 30 IVCs (36 cages per rack), if cages were changed twice per week as opposed to once per week it would cost $29,203.20 more per year in bedding costs. If changed three times per week, the cost would increase to $58,406.40 more per year. These cost increases do not include technician time and indirect costs, which can also be expected to decrease with a decrease in the frequency of cage changes. By reducing the number of cage changes per week, we could not only reduce the stress to the animals but also reduce costs related to bedding, technician time and indirect costs.

REFERENCES

1. **Broderson, J.R., Lindsey, J.R. & Crawford, J.E. (1976). The role of environmental ammonia in respiratory mycoplasmosis of rats. American Journal of Pathology 85, 15-126.**

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3. **American Conference of Governmental Industrial Hygienists (1991) Documentation of the Threshold Limit Values and Biomedical Exposure Indicies. Cincinnati, OH, USA: American Conference of Governmental Industrial Hygienists, pp 58-59.**

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