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Mass Generation Rates of Ammonia, Moisture, and Heat Production in Mouse Cages with Two Bedding Types, Two Mouse Strains, and Two Room Relative Humidities

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ABSTRACT

Mass generation rates of ammonia, moisture, and heat production were measured for mice that were housed in standard shoebox cages at a density of five mice per cage and maintained in either a 35% or 75% room relative humidity. Two mouse strains kept on two different types of bedding materials were compared when bedding material had been in the cages for two, six, and ten days. Gas exchanges directly measured were ammonia, water vapor, oxygen, and carbon dioxide. Water balance was calculated from gain in bedding weight, drinking water consumption, partial pressure of water vapor, and metabolism (CO_2 produced and O_2 consumed). All measurements were taken on the second, sixth, and tenth day of each test. All weight and volume measurements were obtained in the home environmental chamber in which the mice were housed. Gas exchanges were obtained after four cages of five mice each had been placed into one of three open system calorimeters. Relative humidity was not controlled in the calorimeters because it would have led to erroneous ammonia generation measurements.

Relative humidity had a dramatic influence on ammonia generation rate from the mouse cages. Generation rates at 35% relative humidity were essentially negligible across all treatments. At 75% relative humidity, it took around six days bedding age for ammonia generation to start, but it increased rapidly by ten days bedding age. Ammonia mass generation rate was 12.4 and 0.154 (mg/h)/20 mice (2.7E-5 and 3.4E-7 lb/h/20 mice) in the 75% and 35% humidity treatments, respectively. Mouse strain and bedding type had a significant effect on ammonia generation rates with CD-1 mice producing larger amounts of ammonia than BALB/c mice, and pulp bedding producing larger amounts of ammonia than chip bedding. Relative humidity significantly influenced ammonia emission interactions between strain, bedding material, and bedding age. Carbon dioxide and oxygen exchange were not affected by relative humidity. Heat production on a body mass basis was calculated to be 17.9 and 15.7 W/kg BW (27.7 and 24.3 Btu/h/lb BW) for CD-1 and BALB/c mice, respectively.

INTRODUCTION

Regulation of environmental conditions in laboratory animal facilities is essential to obtain biomedical research information that can be reliably interpreted. In addition to providing research information, the laboratory must control a variety of environmental conditions that have been established for the care and use of laboratory animals (National Research Council 1996). Regardless of the complexity of animal facility requirements, certain baseline information is essential in the design and management of systems that regulate the physical environment of laboratory animals.

There have been a variety of studies on ammonia, relative humidity, and temperature levels in mouse cages (Riskowski et al. 1995), and on heat production of mice (ASHRAE 2001; Gordon 1993). However, none has covered the interactions between mass generation rates of ammonia, moisture, and heat production in mouse cages as affected by bedding type, mouse strain, and room relative humidity. Hardwood chip bedding is very common, but other commercial products are available and may affect ammonia production. The main purpose of this research effort was to evaluate how a small number of environmental variables (biological and physical) can influence mass ammonia generation rates from mice housed in shoebox cages that were not mechanically ventilated. Heat and moisture production and baseline information that influences biological and physical facility management decisions were also studied.

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EXPERIMENTAL PROCEDURES

General Procedures and Experimental Setup

The experiment was designed to measure mass generation rates of ammonia and moisture and heat production rates of groups of mice in a standard shoebox cage (plastic open topped box with wire rack on top but without a filter top). The variables were bedding type (two treatments), mouse strain (two treatments), and room relative humidity (two treatments). The two bedding types were commercially available hardwood chips and commercially available virgin wood pulp of an unknown wood type. The wood pulp had a fine consistency similar to a processed paper product. The two mouse strains were CD-1 (Hsd:ICR) and BALB/c (AnNHsd). CD-1 mice are an outbred stock and BALB/c are an inbred strain. CD-1 mice tend to be larger (average body mass in this study was 27.2 g/mouse (0.060 lb/mouse) for CD-1 and 19.6 g/mouse (0.043 lb/mouse) for BALB/c) and have more genetic variation than BALB/c mice. The two room relative humidities were 35% and 75%. The mice were housed in a home chamber (environmentally controlled chamber) for most of this study. During their respective test days, groups of mouse cages were moved into calorimeters for approximately seven hours. The three indirect-convective calorimeters were housed in a separate environmentally controlled chamber (calorimeter chamber). The calorimeters were smaller chambers with precise control of air exchange rates, which allowed calculation of mass generation rates of ammonia and moisture production and oxygen consumption. Heat production rates were calculated from oxygen consumption.

Two separate 15-day experimental periods served to replicate all experimental variables at the two room relative humidities. The same home chamber was used for housing the same experimental mice during each experimental period.

All facilities and procedures for the use with laboratory mice were approved by the campus animal care committee prior to the conduct of this research. In both chambers, the light period was 12 hours of light and 12 hours of darkness. In order to allow data collection during the dark period, the light cycle was shifted from standard times, so lights were on at 3:00 p.m. and off at 3:00 a.m. The mice were allowed to acclimate in the home chamber for at least four days prior to sampling.

Temperature in the home chamber and calorimeters was at 24$\pm$1.5°C (75$\pm$3°F), which is a common operating temperature in laboratory mouse facilities. Relative humidity (RH) was 50%±5% prior to the start of the two static RH treatments. The temperature and relative humidity in each chamber were continuously monitored with hygrothermographs, which were calibrated with a psychrometer.

During each RH treatment period, 48 cages were used to evaluate the experimental factors (two mouse strains × two bedding types × three bedding ages × four cages per experimental factor × three replications each). A total of 240 female mice (120 Hsd:ICR [CD-1] and 120 BALB/cAnNHsd) were randomly placed in 48 shoebox cages at a density of five mice per cage. Five mice per cage is a common density and the maximum allowed by the guide for this size mouse. Four cages were the maximum that would fit into a calorimeter, so that amount was used to give more mouse mass and more accurate results. The mice were initially seven to nine weeks of age and received a commercial rodent diet and water ad libitum. The diets were 22% crude protein, 5% crude fat, and not more than 4.5% crude fiber. Water was provided in standard bottles placed in the cage rack. The same mice were used during both RH periods, and each set of mice had the same bedding type for both periods. Since the 35% RH experimental period followed the 75% RH period, the mice were two weeks older and 2-4 g (0.0044-0.0088 lb) heavier.

Prior to the initial bedding change, all mice were bedded on wood chips. At the beginning of each relative humidity test period, mice were placed into clean cages containing a premeasured volume (720 cm^3 [44 $in.^3$]) of experimentally designated bedding. Cages and accessories were washed with 88°C (160°F) water prior to the start of each experimental period. Neither of the bedding types was autoclaved prior to the study. Bedding was not changed for ten days, and sampling was done two, six, and ten days after bedding change, which were the calorimetry (gas exchange) test days. Consequently, the bedding ages (time since last bedding change) were two, six, and ten days. Mice, in their cages, were randomly assigned to locations on three levels of the same stainless-steel cage rack in the home chamber. On a calorimetry test day, mice were moved to the calorimeter chamber where they remained in their respective cages and were assigned to one of the three calorimeters based on strain and bedding type (i.e., strain and bedding type were not mixed in a calorimeter). In the calorimeter, the cages were located on two rack levels (two cages per level). The mice, feed, water, and bedding were weighed separately before and after being housed in the calorimeter on each calorimetry test day.

Three replications of four cages with five mice each were measured in each of the three identical calorimeters during each test day. During each RH treatment period, each mouse strain and bedding type was replicated six times at each of the three bedding ages. Strain and bedding type variables were assigned to a different calorimeter at each bedding age to prevent a possible calorimeter bias. The mice were placed in the calorimeters at around 8:00 a.m. on each of the test days and removed around 3:00 p.m. Data collection periods were at approximately two, three, four, five, and six hours after the mice were placed in the calorimeters. Ammonia generation reported for each day was calculated as the mean of the five separate collection periods. Since the lights shut off at 3:00 p.m., all of the data were obtained during the daily scotophase (dark cycle) when mice are generally the most active and gas exchange rates the highest (Memarzadeh 1998). Mouse strain/bedding-type groups were started on four consecutive days, which allowed for three replications of each bedding type-bedding age-mouse strain-relative humidity

combination. The schedule for each of the test periods was the same. Experimental treatments were staggered to start each on successive days so that each RH treatment evaluation was on the same day following the initial treatment start day.

Animal-Environment Gas Exchange and Equilibrium Measurements

Calorimeter Design. Three indirect, convective calorimeters were used for this project. Air temperature and air velocity were controlled in each calorimeter. The calorimeter boxes were constructed from 6.4-mm-thick (0.25-in.-thick) clear plastic and were 0.356 m (14 in.) high × 1.07 m (42 in.) long × 0.585 m (23 in.) deep. Clear plastic was used to allow observation of animals and to allow light into the calorimeter from the environmental chamber. The entire front panel was removable to allow access of workers and to move mice in and out. The inside edges of the front panel were sealed with weather stripping and secured to the calorimeter with ten clamps attached to the perimeter.

Air Temperature Control. The calorimeter box and air recirculation system were completely sealed; therefore, heat generated within the calorimeter had to transfer through the box or recirculation tube surface. To enhance this heat transfer process, all three calorimeters were placed within an environmental chamber that was operated at a lower temperature than the desired calorimeter air temperature. Also, a plastic duct was placed around the outside of the air recirculation tube and thermally conditioned air was forced between that duct and the air recirculation tube to create a heat exchange system. Air from the tube heat exchange surface was recirculated through air-conditioning/heating units to control the temperature of the air passing through the heat exchanger and, thus, the amount of heat leaving or entering the heat exchanger. This heat exchange system allowed for precise control of air temperature in the calorimeter boxes (24°C±1.5°C [75±3°F]). Temperatures within the calorimeters were sensed with one type-T thermocouple placed in the center of the calorimeter box in front of the cages to sense air approaching the cages.

Air Velocity Control. Air moved horizontally through each calorimeter and across the long axis of each mouse cage. Cages were placed two each, side by side, on two cage rack levels (four cages of five mice each per calorimeter). Air movement was created by recirculating air through an air recirculation tube (20 cm [8 in.] diameter clear plastic tube), which exited the top of an end of the long axis of the calorimeter box, went over the calorimeter, and attached to an in-line fan that blew return air into the opposite end of the calorimeter box. This air recirculation system allowed for the control of air velocity past the cages without affecting the fresh airflow exchange rate. The airflow rate through the recirculation fan was controlled by adjusting the fan speed. There was a square air diffuser at the air entry that distributed the air around the calorimeter cross section. To further improve the uniformity of airflow across the calorimeter cross section, an air settling system was placed after the diffuser and before the animal cages. The air settling system consisted of three perforated stainless-steel sheets with 60%, 40%, and 30% open areas. An air velocity meter was used to sample velocity between the air settling system and the mouse cages. The average air velocity approaching the mouse cages was set at 0.25±0.05 m/s (50±10 ft/min) prior to each test.

Relative Humidity Control. Ammonia has a high water solubility coefficient, and ammonia generation rate was a major objective of this research, so we did not attempt to control relative humidity during the calorimetry periods because removing water from the air would also remove ammonia. Separate electronic sensors were used for recording relative humidity in each calorimeter and the environmental chamber in which the calorimeters were housed. Prior to the study, the electronic sensors were calibrated against a psychrometer.

Relative humidity was controlled in the home chamber in which the mice and cages were housed for 95% of the experiment. In the 35% relative humidity treatment period, water vapor was removed from the home chambers with dehumidifiers to control relative humidity to 35%±5%; during the 75% relative humidity treatment period, water vapor was added with humidifiers to control relative humidity at 75%±5%.

Fresh Air Exchange. Fresh air exchange (ventilation) was provided to each calorimeter for several reasons: (a) to maintain appropriate levels of respiratory gases (O_2 and CO_2), (b) to remove moisture and help maintain appropriate relative humidity, and (c) to provide sample air for gas analysis. Air was removed from the exit end of the 20 cm (8 in.) diameter air recirculation tube and passed through a precision airflow meter (accuracy = 2% of reading). Prior to each test the fresh air exchange flow meters were calibrated against a 1 L (60 $in.^3$) bubble airflow meter. The air exchange volumes were corrected to standard temperature (0°C [32°F]) and pressure (760 mm Hg [29.92 in.], sea level). Pressure was also corrected for the slight negative pressure and water vapor in the calorimeters. It was assumed that any change in air density due to changes in partial pressure of oxygen and carbon dioxide were negligible. Air flowed to the gas analysis instruments, which were located in an adjacent control-recording environmental chamber.

Air drawn out of the calorimeters was used as exchange volume to calculate the O_2 consumption and CO_2, NH_3, and H_2O vapor production. The slight negative pressure that was maintained within the calorimeters would draw in the same amount of fresh air from the surrounding environmental chamber as was removed by the pump. A planned air inlet (8 mm [0.3 in.] diameter hole) was placed in the inlet part of the air recirculation tube, but some fresh air would have entered through leaks. Since the entire calorimeter was at a negative static pressure and a certain amount of air had to enter the calorimeter anyway, the leaks did not create a problem, especially in these calorimeters, where the air was well mixed by the air recirculation system. The level of negative pressure was measured in each calorimeter during each test day with

manometers. The fresh air exchange rates for the calorimeters ranged from 260 to 360 L/h (9.2-12.7 ft^3/h). The higher air exchange was used to help reduce relative humidity during the 35% RH study.

Oxygen, Carbon Dioxide, and Ammonia Analysis. Air flowed through a solenoid valve switching system that directed airflow to where the O_2, CO_2, and NH_3 concentrations were analyzed. O_2 and CO_2 levels were analyzed with infrared analyzers. NH_3 levels were determined with precision colorimetric tubes. The sample volume was 100 mL (6.1 in^3), and sampling time was approximately one minute. Air was analyzed from six sources hourly—the three calorimeters (air Out), the environmental chamber that housed the calorimeters (air In), and two standard gases. Certified standard gases were selected to bracket the ranges of O_2 and CO_2 that were to be analyzed. Standard gas #1 was certified to have 17.3% O_2 concentration and 0.49% CO_2 concentration. Standard gas #2 was certified to have 19.5% O_2 concentration and 1.48% CO_2 concentration. Output from the gas analyzers was continuously monitored on a strip chart recorder. Ammonia concentration (ppm) was read directly from the calorimetric tubes and recorded along with oxygen and carbon dioxide at each sampling interval. The ammonia analysis procedure was calibrated against two separate standard gases (25.6 and 52.5 ppm) that were attached through the same solenoid and sampling system used for the sampling intervals.

Calibration of Calorimeters. Immediately prior to the test period, the calorimeters were calibrated by burning an ethanol lamp in each calorimeter to determine weight/volume recovery ratios of both CO_2 and O_2. This procedure also served as an integrated check on all components of the calorimeter and determined the overall accuracy of the calorimeter. An ethanol lamp was filled with absolute ethanol (EtOH) and placed on an analytical balance that had been leveled on a platform inside a calorimeter. The lamp was ignited and the calorimeter door was sealed shut. After a stable ethanol burn rate (weight change per minute) was established, and a stable volumetric oxygen use and carbon dioxide production (L/min [$in.^3$/min]) exchange was approached (approximately 90 min), recovery ratio data were collected. Recovery ratio data were recorded over several closely timed intervals. Differences in percent O_2 content of air leaving the calorimeter (O_2 Out) was subtracted from O_2 content of air entering the calorimeter (O_2 In) over the time intervals (O_2 In – O_2 Out). The same procedure for CO_2 analysis was simultaneously recorded (CO_2 Out – CO_2 In). Accuracy, recovery, and calibration values for each calorimeter were obtained by comparison of respiratory quotient [RQ = (CO_2 Out – CO_2 In)/(O_2 In – O_2 Out)] and the ratio of gravimetric to volumetric measurements of the ethanol burning rate. Calibration values for RQ were 0.67, 0.66, and 0.63 for Calorimeters 1, 2, and 3, respectively. Burning pure ethanol should give an RQ of 0.67, so the values were very close. The gravimetric/volumetric O_2 use recoveries were 1.09, 1.00, and 0.97 for calorimeters 1, 2, and 3, respectively.

Calorimeter Tests. On calorimeter test days, data were collected for the following calculations: mouse and bedding weight, water and feed disappearance, ammonia mass production rate, water mass production rate, and oxygen consumption and carbon dioxide production rates. Mouse, bedding, water, and feed weights were measured on a precision balance at around 4 p.m. the night before the calorimeter test day and again at around 3 p.m. at the time of removal from the calorimeters and their return to the home environmental chamber. The various weights were determined by first measuring the entire cage with everything in it, then weighing after removing the water and bottle, after removing the feed and wire top, and after removing the mice. The weights of mice, bedding, feed disappearance, and water disappearance could then be determined by subtraction.

Calculations and Assumptions. All gas production and utilization rates were corrected for temperature, (0°C [273 K]) and pressure (760 mm Hg, sea level), which was calculated as

$$STP = (Pcal \times 273) / (760 + [273 + Tcal]) , \quad (1)$$

where

STP = standard temperature and pressure correction value;

Pcal = air pressure in the calorimeter (local barometric pressure – [calorimeter vacuum + vapor pressure]), mm Hg; and

Tcal = dry-bulb temperature of air in the calorimeter, (°C).

Ammonia mass production rates were calculated from the following equation:

$$NH_3 \text{ Production, mg/h} = (NH_3 \text{ Out} – NH_3 \text{ In}) \times (1E6) – 1 \times (759.8 \text{ mg } NH_3/L) \times (MCAF) \times (STP) \quad (2)$$

where

NH_3 Out = ammonia concentration of air exiting calorimeter, ppm;

NH_3 In = ammonia concentration of air entering calorimeter, ppm;

MCAF = measured calorimeter air exchange rate, L/h.

Water vapor mass production rate calculations did not correct STP for vapor pressure since they were based on the relative humidity levels of air entering and exiting the calorimeter.

Air moisture content and specific volume were based on standard psychrometric properties of air. The psychrometric properties of the air were based on the average measured temperature and relative humidity of the air during the last five hours of each calorimeter test period. Calculations used the following equation:

$$H_2O \text{ Vapor Production, g/h} = (MC \text{ Out} – MC \text{ In}) \times (SV) – 1 \times (MCAF) \times (STP) \quad (3)$$

where

MC Out = moisture content of air exiting calorimeter, g H_2O /g dry air;

MC In = moisture content of air entering calorimeter, g H_2O /g dry air;

SV = specific volume of air in calorimeter, L/g dry air;

MCAF = measured calorimeter air exchange rate, L/h.

Metabolic gas exchange rates (oxygen consumption and carbon dioxide production) rates were calculated based on the following equation:

$$\text{Metabolic gas exchange, L } CO_2/h = (CO_2 \text{ Out} - CO_2 \text{ In}) \times (1E2) - 1 \times (MCAF) \times (STP) \quad (4)$$

or

$$\text{L } O_2/h = (O_2 \text{ In} - O_2 \text{ Out}) \times (1E2) - 1 \times (MCAF) \times (STP) \quad (5)$$

where

CO_2 or O_2 Out = concentration in air exiting calorimeter, %;

CO_2 or O_2 In = concentration of air entering calorimeter, %;

MCAF = measured calorimeter air exchange rate, L/h.

In addition to the above data, a water balance for the mice was calculated to determine how well the system was accounting for all water. Water was being added to the mice by metabolism and by drinking. Water was being lost from the mice by urine, feces, and other secretions, which added to the bedding weight, and by evaporation from the lungs and other body surfaces, which was added to the air that was exhausted from the calorimeter. If we were measuring all values accurately and our assumptions were accurate, then the water input into the mice should equal the water output. Metabolic water production was estimated by assuming that 0.60 g H_2O was produced for each liter of oxygen consumed. This value was based on the assumption that a mixed respiratory quotient (RQ = 0.85) should reflect an energy production of around 4.86 kcal/L O_2 consumed; also, a mixed RQ should produce approximately 0.007 g of H_2O per each kcal of energy metabolism.

Values in Results, Tables, and Figures. Gas exchange data obtained from each calorimeter were used as the experimental unit (4 cages of 5 mice each, i.e., 20 mice) for testing experimental results. A 95% level of significance using Fisher's Protected Least Square Difference and Analysis of Variance was used for inference levels between unit values. Since body weight, bedding weight, and bedding age variables were all time dependent, values were reported on a calorimeter basis for comparisons at a given point in time (animal-environment relationship). Bedding weight and body weight data are shown in order to evaluate their relative influence on the experimental variables reported; however, inference evaluation was not related to either of these units. Both main effects and interactions between experimental variables are reported. The interactions between experimental variables are listed as follows, but the tables only show the interactions that were statistically significant ($P < 0.05$):

- Relative humidity × bedding type
- Relative humidity × mouse strain
- Relative humidity × bedding age
- Bedding type × mouse strain
- Bedding type × bedding age
- Mouse strain × bedding age
- Relative humidity × bedding type × bedding age
- Relative humidity × mouse strain × bedding age
- Bedding type × mouse strain × bedding age
- Relative humidity × bedding type × mouse strain × bedding age

RESULTS AND DISCUSSION

Effects of each environmental treatment on mouse body weights are shown in Table 1. If any of the mean values within the tabular columns and rows differ by 16 g (0.035 lb), they are statistically significant ($P < 0.05$). An analysis of treatment differences and their interactions is shown below the tabular data (Table 1). Treatments that occurred over time (humidity and bedding age) resulted in an increased body weight; however, this was more related to the normal growth that is associated with age. Mouse strain difference in body weight is genetically regulated. There was an interaction between the humidity and mouse strain treatments that may have been related to the younger age at which the BALB/c mice have a plateau in their normal growth curve.

Bedding weights are presented in Table 2 and have the same presentation format as Table 1. Bedding type, mouse strain, and bedding age all had an effect on bedding weight. The increase in bedding weight associated with mouse strain and bedding age reflects the accumulation of urine, feces, and spillage of water and feed. The wood pulp bedding had a lower bulk density and gave lower bedding weights than the wood chip bedding at all bedding ages. Between bedding aged two days and ten days, mean bedding weight increased 240 and 220 g (0.53 and 0.49 lb) for each experimental unit of 20 mice in four cages in the chip and pulp bedding treatments, respectively. Relative humidity treatment had no influence on bedding weight. The bedding weight responses of bedding age and humidity treatments indicate that accumulated litter moisture was dissipated into the air equally by both bedding types.

The mass generation rates for ammonia for the combined treatments are presented in Table 3. If any of the mean ammonia values shown in the tabular columns or rows differ by 9.72 (mg/h)/20 mice (2.14E-5 lb/h/20 mice) (Note: 20 mice = 4 cages of 5 mice each = experimental unit), they are statistically different ($P < 0.05$). This relatively large critical difference reflects the large differences in the response to the various treatments (a range of 0 to 57.3 (mg/h)/20 mice [0 to 1.26E-4 lb/h/20 mice]). All main effects of experimental treatments had a significant effect ($P < 0.05$) on ammonia generation rate.

The very low ammonia generation rate that occurred throughout the 35% humidity trial tends to mask the other treatment relationships at this low humidity; however, bedding age and bedding type (material) caused a difference in ammo-

Table 1. Effects of Experimental Treatments on Body Weight, g/20 mice (lb/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	551±4 (1.22±0.009)	399±2 (0.880±0.004)	554±4 (1.22±0.009)	395±3 (0.871±0.007)	509±2 (1.12±0.004)	373±3 (0.823±0.007)	516±4 (1.14±0.009)	373±3 (0.823±0.007)
BA†= 6 d	565±4 (1.25±0.009)	383±18 (0.845±0.040)	569±7 (1.25±0.015)	396±4 (0.873±0.009)	516±9 (1.14±0.020)	383±4 (0.845±0.009)	528±5 (1.16±0.011)	382±1 (0.842±0.002)
BA†= 10 d	575±7 (1.27±0.015)	413±3 (0.911±0.007)	573±7 (1.26±0.015)	405±2 (0.893±0.004)	534±5 (1.18±0.011)	394±3 (0.869±0.007)	539±4 (1.19±0.009)	395±2 (0.871±0.004)

* Table values are the mean and standard error of the mean (SEM) of mice in individual cages recorded on their designated calorimeter day. The experimental unit was a calorimeter, and each calorimeter contained 4 cages with 5 mice each for a total of 20 mice. Means that differ by at least 16 (0.035) are significant; $n = 3$ (three calorimeter test days for each treatment combination); $P < 0.05$.

† BA = bedding age; number of days since the last bedding change in the cage.

Main effects of experimental variables (g/20 mice [lb/20 mice]): (Values are calculated from individual daily measurements across all other variables.)

Relative humidity: 35% = 481±14 (1.06±0.031); 75% = 454±12 (1.00±0.027); $n = 36$; $P < 0.05$

Bedding type: Chip = 466±13 (1.03±0.029); Pulp = 469±14 (1.03±0.031); $n = 36$; $P > 0.05$

Mouse strain: CD-1 = 544±4 (1.20±0.009); BALB/c = 391±2 (0.862±0.004); $n = 36$; $P < 0.05$

Bedding age: Day 2 = 459±16 (1.01±0.035); Day 6 = 465±17 (1.03±0.038); Day 10 = 478±16 (1.05±0.035); $n = 24$; $P < 0.05$

Significant ($P < 0.05$) interactions between experimental variables:

Relative humidity × mouse strain

Table 2. Effects of Experimental Treatments on Cage Bedding Weight, g/20 mice (lb/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	725±13 (1.60±0.029)	711±14 (1.57±0.031	279±7 (0.615±0.015)	267±1 (0.589±0.002)	727±20 (1.60±0.044)	700±4 (1.54±0.009)	277±4 (0.611±0.009)	248±2 (0.547±0.004)
BA†= 6 d	872±3 (1.92±0.007)	803±15 (1.77±0.033)	402±8 (0.886±0.018)	363±0 (0.800±0)	884±2 (1.95±0.044)	800±5 (1.76±0.011)	406±7 (0.895±0.015)	341±1 (0.752±0.002)
BA†= 10 d	997±1 (2.20±0.024)	907±2 (2.00±0.060)	516±10 (1.14±0.022)	443±1 (0.977±0.002)	1023±24 (2.26±0.053)	895±12 (1.97±0.027)	532±5 (1.17±0.011)	463±9 (1.02±0.020)

* Table values are the mean and standard error of the mean (SEM) of bedding as measured in individual cages recorded on their designated calorimeter day. The experimental unit was a calorimeter, and each calorimeter contained 4 cages with 5 mice each for a total of 20 mice. Means that differ by at least 34 (0.075) are significant; $n = 3$ (three calorimeter test days for each treatment combination); $P < 0.05$. Note: Data are reported on a calorimeter basis because body weight was different for experimental variables. Refer to body weight data in Table 1 to convert to a mouse weight basis.

† BA = bedding age; number of days since the last bedding change in the cage.

Main effects of experimental variables (g/20 mice [lb/20 mice]): (Values are calculated from individual daily measurements across all other variables.)

Relative humidity: 35% = 607±42 (1.34±0.093); 75% = 608±43 (1.34±0.095); $n = 36$; $P > 0.05$

Bedding type: Chip = 837±18 (1.85±0.040); Pulp = 378±16 (0.843±0.035); $n = 36$; $P < 0.05$

Mouse strain: CD-1 = 637±44 (1.40±0.097); BALB/c = 578±40 (1.27±0.088); $n = 36$; $P < 0.05$

Bedding age: Day 2 = 492±47 (1.08±0.104); Day 6 = 609±49 (1.34±0.108); Day 10 = 722±50 (1.59±0.110); $n = 24$; $P < 0.05$

Significant ($P < 0.05$) interactions between experimental variables:

Bedding type × mouse strain

Mouse strain × bedding age

nia production. There were no interactions between treatments within the 35% relative humidity.

In contrast, once ammonia generation was initiated in the 75% relative humidity treatment (around six days for the wood pulp bedding), ammonia levels were obviously higher than at the two-day levels and all the ammonia levels measured in the 35% relative humidity treatment. Within the 75% relative humidity treatment, all variables (mouse strain, bedding type, and bedding age) significantly influenced ammonia generation. For mouse strain, CD-1 mice generated significantly more ammonia than BALB/c mice. For bedding type, wood pulp bedding generated significantly more ammonia than wood chip bedding. All combinations of two-way comparisons showed significant interaction. A four-treatment interac-

Table 3. Effects of Experimental Treatments on Mass Generation Rates of Ammonia, (mg/h)/20 mice (lb/h/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	0.058±0.032 (1.28E-7 ±7.06E-8)	0.101±0.042 (2.23E-7 ±9.26E-8)	0.212±0.064 (4.67E-7 ±1.41E-8)	0.103±0.012 (2.27E-7 ±2.65E-8)	0.014±0.007 (3.09E-8 ±1.54E-8)	0.000± 0.000	0.010±0.010 (2.21E-8 ±2.21E-8)	0.280±0.003 (6.17E-7 ±6.62E-9)
BA†= 6 d	0.085±0.019 (1.87E-7 ±4.19E-8)	0.071±0.004 (1.57E-7 ±8.82E-9)	0.199±0.041 (4.39E-7 ±9.04E-8)	0.142±0.028 (3.13E-7 ±6.17E-8)	0.303±0.065 (6.68E-7 ±1.43E-7	0.418±0.370 (9.22E-7 ±8.16E-7)	28.2±9.75 (6.22E-5 ±2.15E-5)	0.675±0.172 (1.49E-6 ±3.79E-7)
BA†=10 d	0.156±0.012 (3.44E-7 ±2.65E-8)	0.153±0.005 (3.37E-7 ±1.10E-8)	0.298±0.048 (6.57E-7 ±1.06E-7)	0.271±0.033 (5.98E-7 ±7.28E-8)	32.8±6.56 (7.23E-5 ±1.45E-5)	9.72±9.24 (2.14E-5 ±2.04E-5)	57.3±4.24 (1.26E-4 ±9.35E-6)	19.1±6.22 (4.21E-5 ±1.37E-5)

* Table values are the mean and standard error of the mean (SEM) of daily measurements recorded at hourly intervals from each calorimeter. The experimental unit was a calorimeter, and each calorimeter contained 4 cages with 5 mice each for a total of 20 mice. Means that differ by at least 9.72 (2.14E-5) are significant; $n = 3$ (three calorimeter test days for each treatment combination); $P < 0.05$. Note: Data are reported on a calorimeter basis because body weight was different for experimental variables. Refer to body weight data in Table 1 to convert to a mouse weight basis.

† BA = bedding age; number of days since the last bedding change in the cage.

Main effects of experimental variables (mg/h/20 mice [lb/h/20 mice]): (Values are calculated from individual daily measurements across all other variables.)

Relative humidity: 35% = 0.154±0.015 (3.40E-7±3.31E-8); 75% = 12.4±3.21 (2.73E-5±7.08E-6); $n = 36$; $P < 0.05$

Bedding type: Chip = 3.66±1.74 (8.07E-6±3.84E-6); Pulp = 8.88±3.01 (1.96E-5±6.64E-6); $n = 36$; $P < 0.05$

Mouse strain: CD-1 = 9.98±3.19 (2.20E-5±7.03E-6); BALB/c = 2.56±1.22 (5.64E-6±2.69E-6); $n = 36$; $P < 0.05$

Bedding age: Day 2 = 0.066±0.016 (1.46E-7±3.53E-8); Day 6 = 3.77±2.18 (8.31E-6±4.81E-6); Day 10 = 15.0±4.30 (3.31E-5±9.48E-6); $n = 24$; $P < 0.05$

Significant ($P < 0.05$) interactions between experimental variables:

- Relative humidity × bedding type
- Relative humidity × mouse strain
- Relative humidity × bedding age
- Relative humidity × bedding type × bedding age
- Bedding type × mouse strain
- Bedding type × bedding age
- Mouse strain × bedding age
- Relative humidity × mouse strain × bedding age

tion did not occur; however, all three-way interactions had a significant effect on ammonia production except for the bedding type × mouse strain × bedding age interaction. The most obvious treatment effect combination that influenced an increase in ammonia generation rate was the CD-1 strain of mice that was housed on the wood pulp bedding.

Figure 1 presents ammonia mass generation rates on a body weight (BW) basis (mg NH_3/h per kg BW [lb NH_3/h/lb BW]) for the 35% and 75% relative humidity treatments. Again, the general trends are that ammonia generation was very low for all other treatments at 35% relative humidity. At 75% relative humidity, ammonia mass generation rate was much higher once the ammonia generation initiated (after bedding age = two days). The CD-1 mice had much higher ammonia generation rates on a unit body weight basis than the BALB/c mice. All ammonia is produced in the bedding, and the greater mass of urine in the same amount of bedding across treatments may account for some of this difference. The mice had a higher ammonia generation rate on the wood pulp bedding than on the wood chip bedding. Note that ammonia generation levels will also vary with mouse age and sex and ambient temperature, so the values presented in this paper are only for the cases studied.

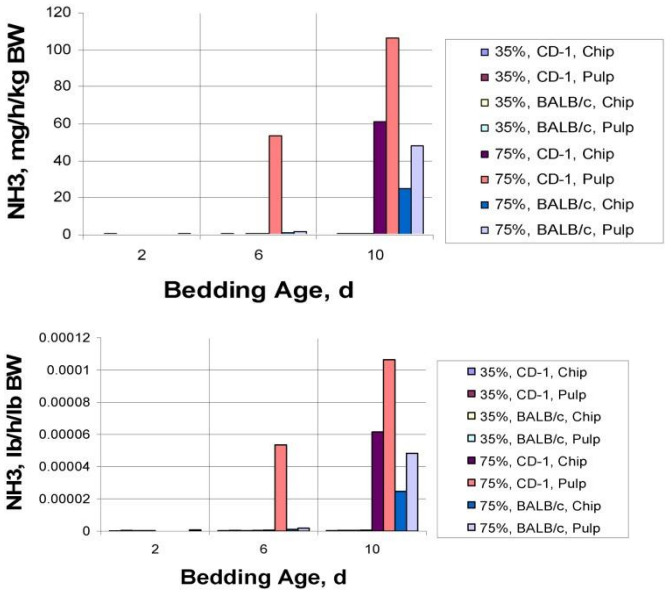

Figure 1 *Ammonia mass generation rates on a body weight (BW) basis (mg NH_3/h per kg BW [lb NH_3/h/lb BW]) for the 35% and 75% relative humidity treatments.*

The water vapor production rates are presented in Table 4. Values within the tabular columns and rows that differ by 0.14 (g/h)/20 mice (3.09E-4 lb/h/20 mice) are significantly different ($P < 0.05$). Relative humidity and mouse strain treatments both showed an effect on water vapor production. The water vapor production rate averaged 2.86 and 3.44 (g/h)/20 mice (0.0063 and 0.0076 lb/h/20 mice) for the BALB/c and CD-1 strains, respectively. When vapor production is converted to a body weight basis, the BALB/c mice are greater than the CD-1 (7.3 vs. 6.3 (g/h)/kg BW [0.0073 vs. 0.0063 lb/h/lb BW]). The water vapor production rate over all variables for 75% relative humidity was significantly higher than for 35% relative humidity (3.36 (g/h)/20 mice [0.0074 lb/h/20 mice] at 75% vs. 2.94 [0.0065] at 35%; or, on a body weight basis, 7.4 (g/h)/kg BW [0.0074 lb/h/lb BW] at 75% vs. 6.1 [0.0061] at 35%). One possible explanation for the difference in water vapor production in the humidity treatments was that the high humidity treatment was evaporating more water from the bedding material (Table 7). This is counter to what one would expect but probably occurred because the cages were moved

Table 4. Effects of Experimental Treatments on Water Vapor Production, (g/h)/20 mice (lb/h/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	2.93±0.078 (0.0065 ±1.7E-4)	2.34±0.029 (0.0052 ±6.4E-5)	3.33±0.141 (0.0073 ±3.1E-4)	2.78±0.081 (0.0061 ±1.8E-4)	3.43±0.159 (0.0076 ±3.5E-4)	2.85±0.212 (0.0063 ±4.7E-4)	3.28±0.275 (0.0072 ±6.1E-4)	3.01±0.224 (0.0066 ±4.9E-4)
BA†= 6 d	3.39±0.084 (0.0075 ±1.9E-4)	2.40±0.196 (0.0053 ±4.3E-4)	3.21±0.107 (0.0071 ±2.4E-4)	2.82±0.117 (0.0062 ±2.6E-4)	3.58±0.302 (0.0079 ±6.7E-4)	3.23±0.092 (0.0071 ±2.0E-4)	3.61±0.079 (0.0080 ±1.7E-4)	3.09±0.274 (0.0068 ±6.0E-4)
BA†=10 d	3.34±0.231 (0.074 ±5.1E-4)	2.59±0.018 (0.0057 ±4.0E-5)	3.37±0.036 (0.0074 ±7.9E-5)	2.76±0.083 (0.0061 ±1.8E-4)	3.84±0.141 (0.0085 ±3.1E-4)	3.21±0.218 (0.0071 ±4.8E-4)	3.95±0.194 (0.0087 ±4.3E-4)	3.28±0.290 (0.0072 ±6.4E-4)

* Table values are the mean and standard error of the mean (SEM) of daily measurements recorded at hourly intervals from each calorimeter. The experimental unit was a calorimeter, and each calorimeter contained 4 cages with 5 mice each for a total of 20 mice. Means that differ by at least 0.14 (3.09E-4) are significant; $n = 3$ (3 calorimeter test days for each treatment combination); $P < 0.05$. Note: Data are reported on a calorimeter basis because body weight was different for experimental variables. Refer to body weight data in Table 1 to convert to a mouse weight basis.

† BA = bedding age; number of days since the last bedding change in the cage.

Main effects of experimental variables (g/h/20 mice [LB/H/20 MICE]): (Values are calculated from individual daily measurements across all other variables.)

Relative humidity: 35% = 2.94±0.068 (0.0065±1.5E-4); 75% = 3.36±0.740 (0.0074±1.6E-3); $n = 36$; $P < 0.05$

Bedding type: Chip = 3.09±0.087 (0.0068±1.9E-4); Pulp = 3.21±0.071 (0.0071±1.6E-4); $n = 36$; $P > 0.05$

Mouse strain: CD-1 = 3.44±0.061 (0.0076±1.3E-4); BALB/c = 2.86±0.066 (0.0063±1.5E-4); $n = 36$; $P < 0.05$

Bedding age: Day 2 = 3.00±0.085 (0.0066±1.9E-4); Day 6 = 3.17±0.094 (0.0070±2.1E-4); Day 10 = 3.29±0.105 (0.0073±2.3E-4); $n = 24$; $P < 0.05$

Significant ($P < 0.05$) interactions between experimental variables:

None

Table 5. Effects of Experimental Treatments on Oxygen Consumption, (L/h)/20 mice (in.^{3}/h/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	1.78±0.132 (109±8.05)	1.10±0.080 (61.6±4.88)	1.52±0.032 (92.8±1.95)	1.11±0.100 (67.7±6.10)	1.92±0.084 (117.2±5.13)	1.19±0.034 (72.6±2.07)	1.94±0.110 (118.4±6.71)	1.38±0.232 (84.2±14.2)
BA†= 6 d	1.83±0.230 (112±14.0)	1.14±0.108 (69.6±6.59)	1.48±0.066 (90.3±4.03)	0.897±0.011 (54.7±0.671)	1.99±0.044 (121.4±2.68)	1.01±0.073 (61.6±4.45)	1.65±0.110 (100.7±6.71)	1.08±0.036 (65.9±2.20)
BA†=10 d	1.59±0.134 (97.0±8.18)	1.16±0.087 (70.8±5.31)	1.63±0.118 (99.5±7.20)	105±0.015 (64.1±0.915)	1.75±0.025 (106.8±1.53)	1.07±0.059 (65.3±3.60)	1.63±0.036 (99.5±2.20)	0.973±0.017 (59.4±1.04)

* Table values were calculated from oxygen consumption values in Table 5. O_2 (L/h)/20 mice × 4.86 / 0.86 = W/20 mice (O_2 [L/h]/20 mice × 4.86 × 3.97 = Btu/h/20 mice). Kcal/h = W × 0.86.

† BA = bedding age; number of days since the last bedding change in the cage.

from the home chamber where humidity was controlled at 75% to the calorimeters where humidity had less control and operated at lower levels. Relative humidity was not controlled in the calorimeters because it would have led to erroneous ammonia measurements.

The oxygen consumption rates are presented in Table 5. All experimental variables had a significant influence ($P < 0.05$) on oxygen consumption. Bedding aged two days and bedding aged ten days were also significantly different and, in general, there was a linear decrease with age. Oxygen use was higher for the 75% relative humidity treatment than for the 35% relative humidity treatment (Table 5). In both the bedding age and humidity treatments, the oxygen consumption rate was inversely related to the number of exposures to the calorimeter and may have been due to decreased activity level that occurs when the mice become accustomed to being handled. The BALB/c mice had an average oxygen consumption rate lower than the CD-1 strain (Table 5). The same strain difference in oxygen use was shown when based on a body mass basis (2.8 vs. 3.2 LO_2/h per kg BW [77.1 vs. 88.0 $in.^3$ O_2/h/lb BW] for BALB/c vs. CD-1, respectively), which indicates a strain-related difference in metabolic rate under these environmental conditions.

Table 6 gives heat production values, which were calculated based on the mean oxygen consumption values presented in Table 5. Heat production in kcal/h per 20 mice was calculated by multiplying oxygen consumption (L/h/20 mice) by 4.86, then converted to W/20 mice (Btu/h/20 mice) by dividing by 0.86 (multiplying by 3.969). The 4.86 value was based on the assumption that a mixed respiratory quotient applies (RQ = 0.85 with 4.86 kcal/L O_2). Heat production on a body mass basis (Figure 2) averaged 17.9 W/kg BW (27.7 Btu/h/lb BW) for CD-1 mice and 15.7 W/kg BW (24.3 Btu/h/lb BW) for BALB/c mice across bedding ages. These values are bracketed by those found in the literature (ASHRAE 2001; Gordon 1993). ASHRAE (2001) publishes values of 21.5 W/kg BW (33.3 Btu/h/lb BW) for CD-1-sized mice (27.2 g/mouse [0.06 lb/mouse]) and 23.4 W/kg BW (36.2 Btu/h/lb BW) for BALB/c-sized mice (19.6 g/mouse [0.043 lb/mouse]). Gordon (1993) cites values that vary from 9.3 to 15.1 W/kg BW (14.4 to 23.4 Btu/lb BW). Mouse heat production has been shown to be highly sensitive to ambient temperature (Gordon 1993) and light conditions (Memarzadeh 1998). Also note that heat production will vary with mouse age, sex, nutrition level, and genetics, so values presented in this paper are only for the cases studied.

Water balance data were calculated from other directly measured parameters recorded during the conduct of this research and are presented in Table 7. Water balance (water

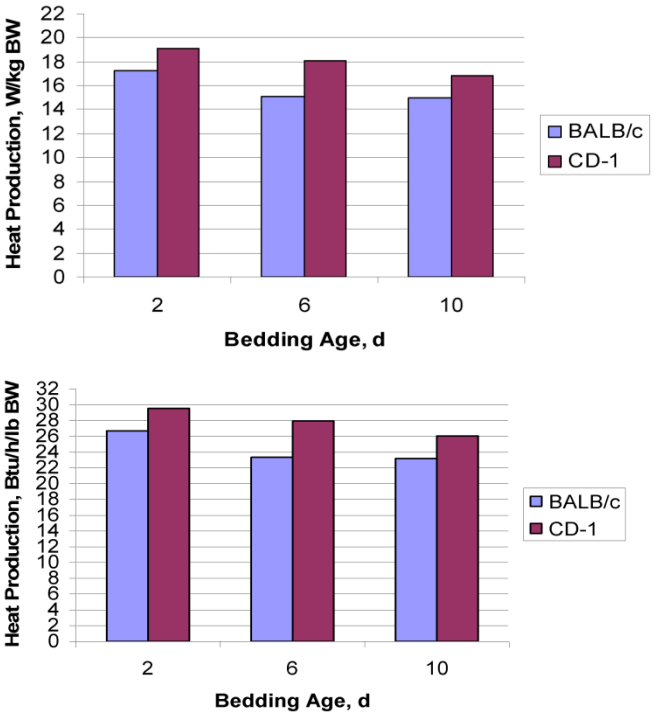

Figure 2 *Heat production on a body mass basis.*

Table 6. Effects of Experimental Treatments on Heat Production, W/20 mice (Btu/h/20 mice)*

Relative Humidity:	35%	35%	35%	35%	75%	75%	75%	75%
Bedding:	Chip	Chip	Pulp	Pulp	Chip	Chip	Pulp	Pulp
Mouse Strain:	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c	CD-1	BALB/c
BA†= 2 d	10.06 (34.34)	5.72 (19.52)	8.62 (29.42)	6.26 (21.37)	10.83 (36.97)	6.71 (22.90)	10.98 (37.48)	7.79 (26.59)
BA†= 6 d	10.34 (35.29)	6.43 (21.95)	8.23 (28.09)	5.07 (17.31)	11.27 (38.47)	5.67 (19.35)	9.31 (31.78)	6.10 (20.82)
BA†=10 d	8.99 (30.69)	6.58 (22.46)	9.21 (31.44)	5.95 (20.31)	9.90 (33.79)	6.06 (20.69)	9.22 (31.47)	5.50 (18.77)

* Table values were calculated from oxygen consumption values in Table 5. O_2 (L/h)/20 mice × 4.86 / 0.86 = W/20 mice (O_2 [L/h]/20 mice × 4.86 × 3.97 = Btu/h/20 mice). Kcal/h = W × 0.86.

† BA = bedding age; number of days since the last bedding change in the cage.

Table 7. Effects of Experimental Treatments on Water Balance, (g/h)/20 mice (lb/h/20 mice)*

	Water Added to the System		Water Lost from the System		
Treatment[1]	**Drinking**	**Metabolic**	**Water Vapor**	**Bedding wt**	**Balance**
HUMIDITY					
35%	4.77±0.16 (0.015±3.5E-4)	0.82±0.04 (0.0018±8.8E-5)	2.94±0.07 (0.0065±1.5E-4)	2.05±0.06 (0.0045±1.3E-4)	+0.60 (+1.3E-3)
75% BEDDING	3.65±0.14 (0.0080±3.1E-4)	0.89±0.04 (0.0020±8.8E-5)	3.36±0.07 (0.0074±1.5E-4)	1.68±0.09 (0.0037±2.0E-4)	–0.50 (–1.1E-3)
TYPE					
Chip	4.14±0.19 (0.0091±4.2E-4)	0.89±0.04 (0.0020±8.8E-5)	3.09±0.09 (0.0068±2.0E-4)	1.88±0.09 (0.0041±2.0E-4)	+0.05 (+1.1E-3)
Pulp	4.28±0.16 (0.0094±3.5E-4)	0.83±0.04 (0.0018±8.8E-5)	3.21±0.07 (0.0071±1.5E-4)	1.88±0.07 (0.0041±1.5E-4)	+0.02 (+4.4E-4)
MOUSE STRAIN					
CD-1	4.91±0.15 (0.0108±3.3E-4)	1.05±0.02 (0.0023±4.4E-5)	3.44±0.06 (0.0076±1.3E-4)	2.18±0.07 (0.0048±1.5E-4)	+0.35 (+7.7E-4)
BALB/c	3.51±0.12 (0.0077±2.6E-4)	0.66±0.02 (0.0015±4.4E-5)	2.86±0.07 (0.0063±1.5E-4)	1.55±0.06 (0.0034±1.3E-4)	–0.24 (–5.3E-4)
BEDDING AGE					
2 d	3.88±0.20 (0.0086±4.4E-4)	0.90±0.05 (0.0020±1.1E-4)	3.00±0.09 (0.0066±2.0E-4)	1.76±0.09 (0.0039±2.0E-4)	+0.03 (+6.6E-5)
6 d	4.26±0.22 (0.0094±4.9E-4)	0.84±0.05 (0.0019±1.1E-4)	3.17±0.09 (0.0070±2.0E-4)	1.94±0.11 (0.0043±2.4E-4)	–0.01 (–2.2E-5)
10 d	4.49±0.23 (0.0099±5.1E-4)	0.83±0.04 (0.0018±8.8E-5)	3.29±0.11 (0.0073±2.4E-4)	1.89±0.11 (0.0042±2.4E-4)	+0.13 (+2.9E-4)
MEAN[2]					
Added	+5.07±0.14 (+0.0112±3.1E-4)				
Lost			–5.02±0.09 (–0.0111±2.0E-4)		+0.05 (+1.1E-4)

* Table values are the mean and standard error of the mean (SEM); $n = 36$

† Mean values; $n = 72$

Note: **Drinking** values were calculated from the change in weight of drinking water bottles during a calorimetry day. Values were not corrected for spillage.
Metabolic values were calculated from energy values estimated from metabolic gas exchange (Table 5).
Water vapor values were calculated from direct calorimeter readings (Table 4).
Bedding weight values were calculated from the change in bedding weight during a calorimetry day. Values were not corrected for fecal mass or moisture (Table 2).

added – water lost) averaged over all experimental conditions was +0.05 (g/h)/20 mice (+1.1E-4 lb/h/20 mice), which reflected a good accounting for water in the system. The largest disparity in water balance was associated with humidity treatments. Water balance was +0.60 (g/h)/20 mice (+1.3E-3 lb/h/20 mice) in the 35% relative humidity and –0.50 (–1.1E-3) in the 75% relative humidity. BALB/c mice also showed a negative average water balance (–0.24 (g/h)/20 mice [–5.3E-4 lb/h/20 mice]), and the CD-1 mice were positive (+0.35 (g/h)/20 mice [+7.7E-4 lb/h/20 mice]). When strain treatment water balance was compared on a body weight basis, drinking water intake and bedding weight water loss were not different. The difference in water balances for the different relative humidities could be explained by short-term water gain/loss equilibrium between the mouse cage bedding and the calorimeter air. Relative humidity in the calorimeter was higher than in the 35% home chamber, so the bedding may have held more urine, fecal, and spilled moisture as the bedding came into equilibrium with the new state of air. For the high relative humidity tests (75%), the opposite bedding equilibrium conditions would have the effect of producing a reduced retention of moisture in the litter.

CONCLUSIONS

Physical and biological variables used in this experiment caused significant changes in the gas exchange equilibrium between mice, their cages, and the surrounding environment.

- Relative humidity had a dramatic influence on the ammonia generation rate from the mouse cages, especially after bedding material had been in the cages for six days. Generation rates at 35% were essentially negli-

gible across all treatments. At 75% relative humidity, it took around six days bedding age for ammonia generation to start, but it increased rapidly by ten days bedding age. The ammonia mass generation rate was 12.4 and 0.154 (mg/h)/20 mice (2.73E-5 and 3.40E-7 LB/H/20 MICE) in the 75% and 35% relative humidity treatments, respectively. The highest generation rate measured in this study was 106 mg (10.6E-5 lb) ammonia/h per kg (lb) mouse body weight (75%, CD-1, Pulp, ten days).

- At treatments where significant levels of ammonia were generated, mouse strain and bedding type had a significant effect with CD-1 mice producing larger amounts of ammonia than BALB/c mice and pulp bedding producing larger amounts of ammonia than chip bedding. For pulp bedding, the values were 106 (mg/h)/kg BW (10.6E-5 lb/h/lb BW) (75%, CD-1, Pulp, ten days) vs. 48 (4.8E-5) (75%, BALB/c, Pulp, ten days) and, for chip bedding, the values were 61 (6.2E-5) (75%, CD-1, Chip, ten days) vs. 25 (2.5E-5) (75%, BALB/c, Chip, ten days).
- Relative humidity significantly influenced ammonia emission interactions between mouse strain, bedding type, and bedding age.
- Carbon dioxide and oxygen exchange was not affected by humidity.
- Heat production on a body mass basis was calculated to be 17.9 and 15.7 W/kg BW (27.7 and 24.3 Btu/h/lb BW) for CD-1 and BALB/c mice, respectively.

In general, there were interactions between experimental variables that clearly demonstrate that a single standard environmental management protocol is not feasible and the relationship between each environmental component will need to be evaluated to establish workable laboratory animal conditions.

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