

## Abstract

There is a new IVC design from Lenderking which employs a different method for ventilating a cage. Bedding is placed on a perforated false bottom and air passes through the bedding. This design has important ramifications for researchers, animals, operation costs, and the safety of technicians who interact with these systems daily. Functionally, the addition of a perforated floor piece means that not all beddings will perform in this caging system. If the bedding is too soft, the metal floor will act like a sieve. If the bedding size starts out too small, it will immediately fall through the false bottom. Seven different beddings were evaluated in this alternative cage design. Parameters gathered include ammonia, temperature, relative humidity, moisture content of the bedding and starting and ending mass of the bedding. Cellulose based beddings performed the best in terms of ammonia, relative humidity, and allowance of the airflow to dry the latrine spots. Combination corn cobb and cellulose bedding performed well although not as well as cellulose only bedding. Pelletized corn cobb bedding proved to be too soft for the metal floor. Wood based bedding did not perform well in terms of ammonia, however this material did not hold onto water as measured by the moisture content and relative humidity parameters. One type of bedding proved to be too small of a particle size and immediately fell through the perforated floor piece of the cage assembly.



**Figure 1:** Schematic of the Lenderking IVC design, brand named Hygieia



**Figure 2:** Front of the cage with out of the cage water bottle supports (blue) and the sampling port (grey).

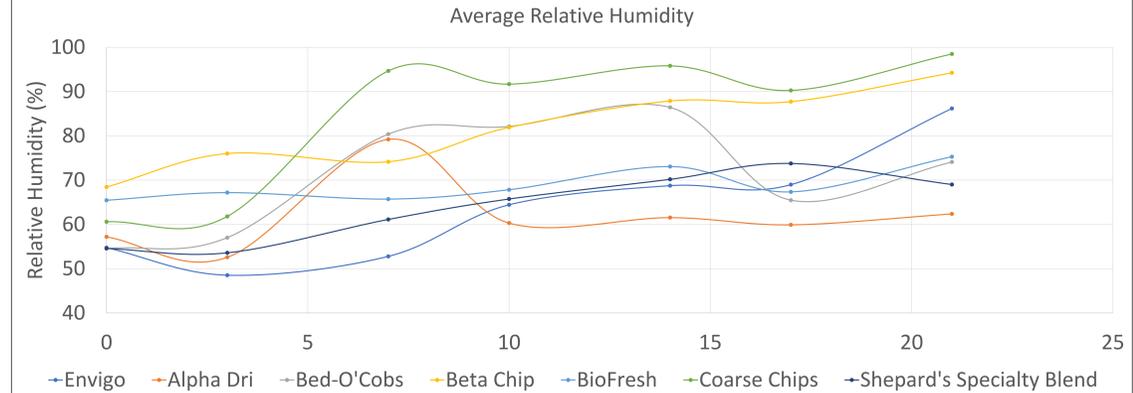
## Materials/Methods

### Materials

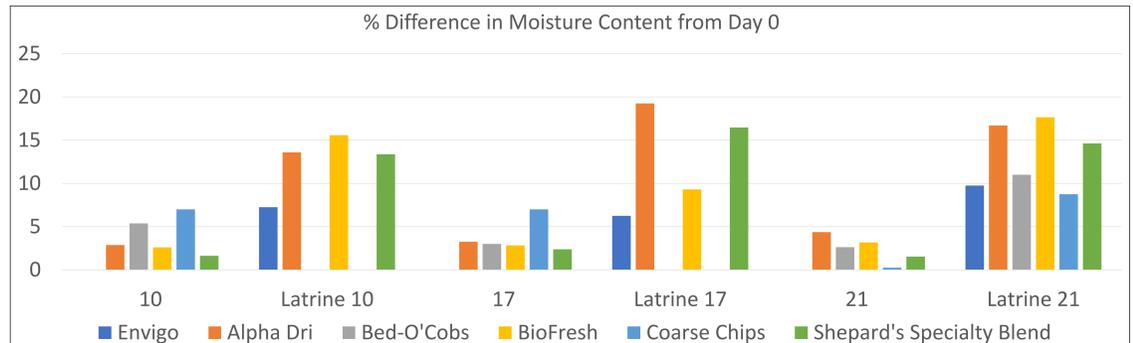
- Hygieia rack and individually ventilated cage set up (Figure 1)
- Sampling port: ½" Edstrom grommet press fit into cage wall 1.5" above floor with silicone seal on door flap (Figure 2)
- Hygieia Air Sentry Unit: 30 ACH, positive pressure
- Mice: Female, various strains, 12 weeks old
  - 5 mice/cage
- Food: NIH-07 standard diet, Zeigler
- Water: RO water in 300mL bottles, out of the cage assembly, changed weekly
- Bedding
  - 7086G, Envigo (pelletized hardwood)
  - Alpha Dri, Shepard's Specialty Papers (cellulose)
  - Bed-o-cobs, The Anderson's Lab Bedding (pelletized corn cob)
  - Beta Chip, Northeastern Products (virgin hardwood)
  - BioFresh, Healthy Pet (pelletized cellulose)
  - Coarse Sani Chips, PJ Murphy's (virgin hardwood)
  - Shepard's Specialty Blend, Shepard's Specialty Papers (corn cob and cellulose)
- Sensors
  - GX6000, RKI Instruments: Ammonia
  - Digital Hygrometer/Thermometer, VWR International LLC (35519-050): Temperature and relative humidity
  - BD-10 Moisture meter, Delmhorst Instrument Co.: Moisture Content Sensor

### Methods

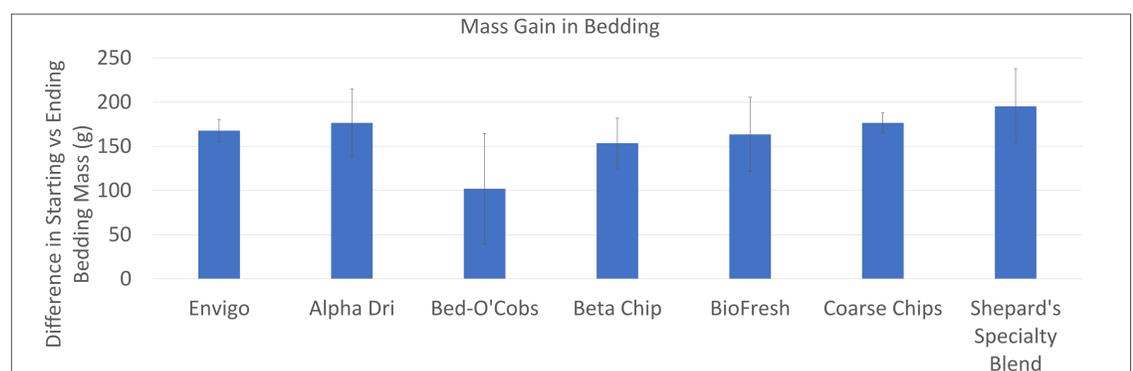
Standard husbandry protocols as determined by the National Institute for Neurological Disorder and Stroke's IACUC were followed, with an exception allowed for the 21-day cage change interval. Sensors were inserted into the cage through a sampling port in the front of the cage. Sampling for most parameters (ammonia, temperature, and relative humidity) was conducted twice per week, creating a consistent schedule as follows: 0, 3, 7, 10, 14, 17, 21 days after cage change. The moisture content of the bedding was analyzed in bulk bedding and in latrine spots on day 0, 10, 17, and day 21. Bedding was weighed at the beginning and end of every cage change cycle.



**Figure 5:** Differences in relative humidity by bedding types. Wood based bedding (coarse chips and beta chip) performed the worst with relative humidity values approaching 100%, while Alpha Dri and Shepard's Specialty Blend performed the best.



**Figure 6:** Moisture content difference between day 0 and days 10, 17, and 21 as measured in bulk bedding and latrine spots. Interestingly, cellulose based beddings would retain moisture in the latrine more than other types of beddings.



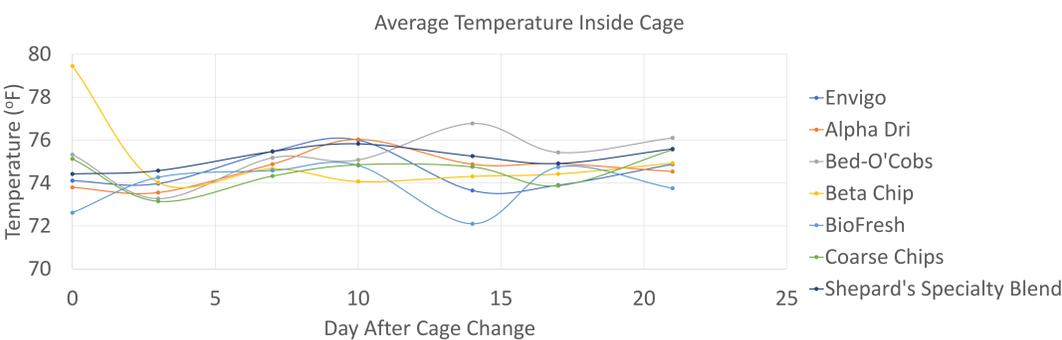
**Figure 7:** Difference in bedding mass from day 0 and day 21. No significant differences.

## Conclusions

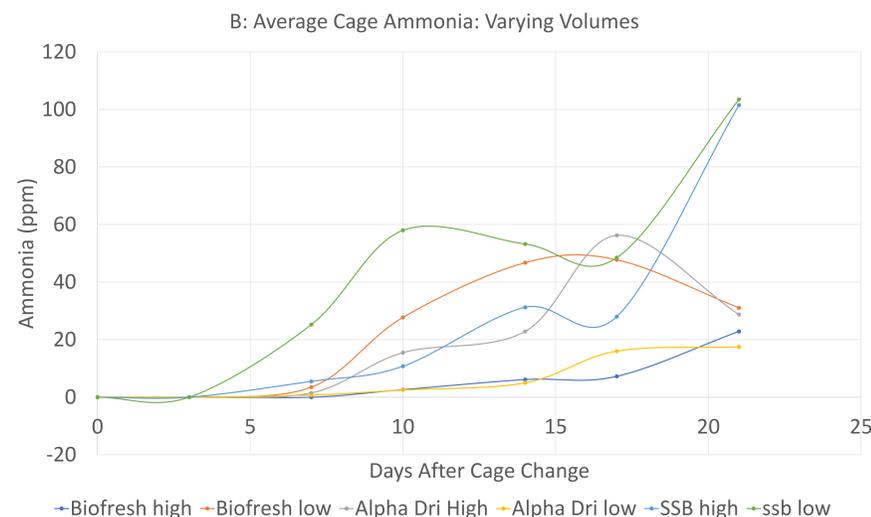
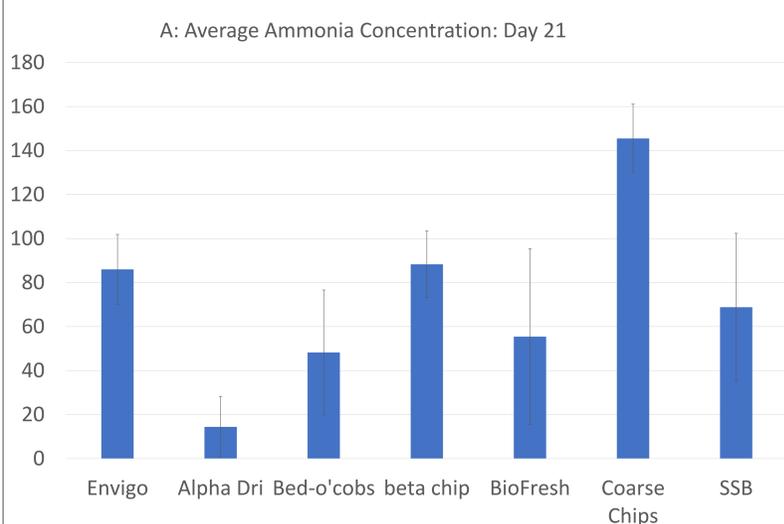
Temperature did not vary by bedding type (Figure 3). Cellulose based bedding (Alpha Dri and BioFresh) performed the best in terms of ammonia generation (Figure 4). Relative humidity did vary by bedding type, with both of the Shepard's beddings with best performance (Figure 5). Wood based bedding performed poorly in terms of relative humidity (Figure 5). Cellulose based bedding did hang on to moisture as evidenced by the latrine spots which held on to moisture in the moisture content measurements (Figure 6). Mass gains over 21 days between cage changes were not significant (Figure 7) between bedding types. Facilities have many different options when it comes to bedding selection, and many factors will go into the decision. From the perspective of ammonia, cellulose based bedding performs the best, with Alpha Dri a clear winner from the ammonia perspective.

## Acknowledgements

All animal work was conducted at the NIH with no direct financial support from Lenderking, other than equipment and in-kind labor where appropriate. Thanks to the following companies who donated their products for evaluation; Shepard's Specialty Papers, The Anderson's Lab Bedding, Healthy Pet, and PJ Murphy's.



**Figure 3:** Averaged daily temperatures inside the cage. Bedding did not affect temperature variations, and no significant differences emerged between bedding types.



**Figure 4:** Ammonia performance in Hygieia cage system by different bedding types. Figure A is the final concentration inside the cage on day 21. As beddings displayed favorable performance, they would progress into different phases of the study therefore n values are not consistent: Envigo n=4, Alpha Dri n=24, Bed-O'Cobs n=4, Beta Chip n=20, BioFresh n=44, Coarse Chips n=4, Shepard's Specialty Blend n=20. B shows a single cage change interval of 21 days in 3 different beddings with varying volumes (n=4). Both BioFresh and Alpha Dri are cellulose based beddings, but it was discovered that a low volume of Alpha Dri and a high volume of BioFresh work best in this system.