NEW BIOLOGICAL MATERIALS FOR IMPROVING ANIMAL WELFARE: AN IDEAL BEDDING?

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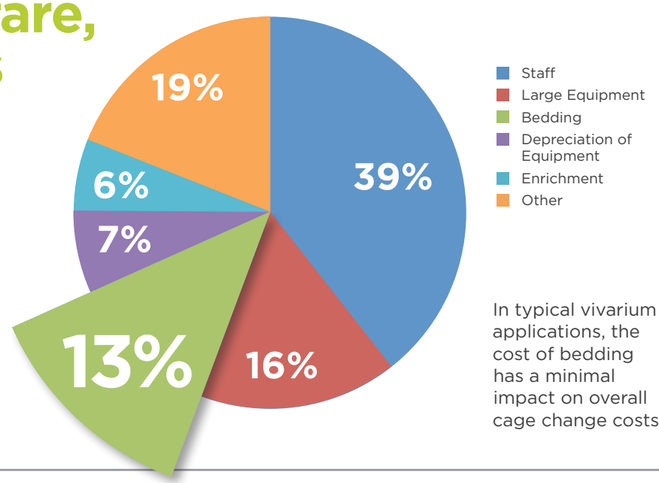
Objective: Improving Welfare, Reducing Costs

Finding out how costs can be reduced while animal welfare and quality of services can also be improved. To this end, we propose increasing cage change intervals for the following reasons:

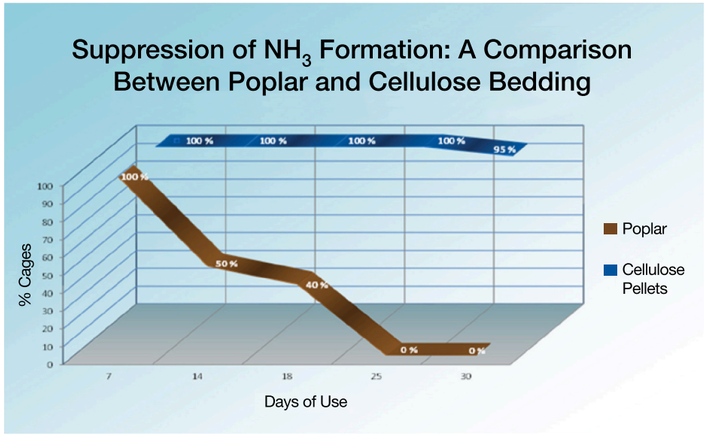
- Improvement of animal welfare, limiting stress of a "new environment"
- Decrease in financial costs
- Environmental sustainability
- More time for technicians to complete daily animal welfare

Cost of Basic Elements Involved in Cage Changes



In typical vivarium applications, the cost of bedding has a minimal impact on overall cage change costs.

Results



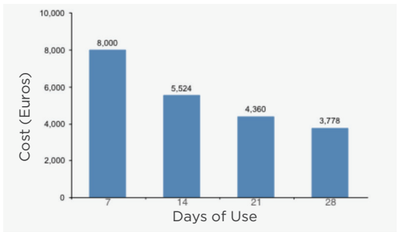
Longevity of Various Bedding Types

Bedding	Cage Type	Nº Cages	Cages < 25 ppm NH3				
			<14 days	14 days	18 days	22 days	30 days
Cellulose	Ventilated	61	100%	100%	100%	95%	52%
Cellulose	Conventional	12	100%	100%	67%	33%	0%
Wood	Ventilated	48	54%	46%	13%	0%	0%

Additional factors causing an increase of NH_3 levels were observed:

- Bedding type
- Increasing relative humidity of the room (>60%) even for a short time
- Higher animal weight (>40g)
- Insufficient bedding in the cage (<0.3g/cm^2)
- Water leaking from bottles

Cost of 1000 Cages in One Month



Cost per Change Interval

Interval (days)	Cages per week	Working days	Cost per cage/month	Cost **1000** cages month	Cost **2000** cages month	Cost **3000** cages month	Cost **4000** cages month
7	1000	5	8€	8.000€	16.000€	24.000€	32.000€
9	715	7	6€	6.000€	12.000€	18.000€	24.000€
14	500	10	4€	4.000€	8.000€	12.000€	16.000€
21	333	15	3€	3.000€	6.000€	9.000€	12.000€
28	250	20	2€	2.000€	4.000€	6.000€	8.000€

For organizing purpose, the most suitable interval for changing the cages is set up in weekly periods of 7 days. On that basis, 8 Euros is the calculated cost for the monthly maintenance of one cage of mice with one weekly change. This calculation also took into account other items not related directly to animal housing, such as health controls, feed consumption and energy supply costs that are not affected by the reduction of changes.

Acknowledgements

Centro de Experimentación Animal de la UAH y su Personal
Depto. de Biomedicina y Biotecnología (Área Microbiología) de la UAH Rettenmaier Ibérica S.L.
Centro Nacional de Investigaciones Oncológicas (CNIO)
Janvier Labs
L. Monje. Fotografía Científica (UAH)

Introduction

The Animal Welfare Unit from the AWSHEL-IAS Research Group of the University of Alcalá (Spain) directed by Professor Dr Jorge Pérez Serrano, Director of the Centre for the supporting of Medical and Biological Research, to which the Animal Research Experimentation Centre is attached, was created in order to study and improve animal welfare by objective quality control testing.

Research with animals requires a high level of standards, but unlike other elements related to animal welfare, (e.g. feed or cages), there is insufficient information in regards to bedding and animal preference, the impact on their welfare and its efficiency, even though bedding is the only element in direct and permanent physical contact with the animal.

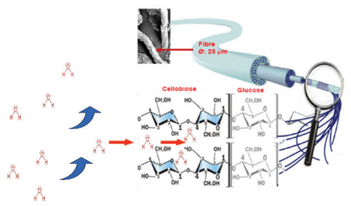
On the other hand, any attempt to improve quality often means increased economic costs due to the complexity of the equipment, health status, required animal welfare and quality standards. Therefore, experimental animals absorb a great deal of research resources.

Preliminary Study

Several commercial wood-based beddings were evaluated, 60% of the cages reached ammonia levels above 25 ppm, which is the maximum exposure threshold permitted for humans during an 8-hour work day according to the guidelines of the ACGIH (American Conference on Governmental Industrial Hygienists). Notice that animals are exposed 24 hours per day.

These ammonia levels are often at animal facilities, but they are usually unnoticed since the smell is perceptible but not penetrating in concentrations of around 30-40 ppm that can even be found in not particularly dirty bedding.

We also found that once a significant concentration of ammonia is acheived, this level is doubled in a relatively short time (24 hours).



The internal structure of cellulose only allows water to circulate through it; for simple reasons of space, no other organic or inorganic molecule can penetrate its structure.

Aspects Assessed in Study

Of thirty quality parameters studied, the key factors were the following:

- Degree of absorbency
- Dust level
- Capacity to maintain a balance between absorption and deabsorption
- Capacity to enable decantation or sedimentation of feces
- Lower density than fecal matter
- Bacteriostatic capacity
- Adequate thermal diffusion coefficient to protect animals from “cold” sensation
- Inclusion of environmental enrichment
- Ammonia reactions are kept under control
- Deodorant effect: preventing ammonia odor from permeating cage

Methodology

The study was performed on two types of wood-based bedding and another on cellulose pellets, distributed among 121 conventional cages and cages housed in IVCs.

In addition, there were two hundred “companion” cages with the same kind of bedding. In those cages, ammonia was only measured when a small damp patch caused by animal urine was observed.

The experimental conditions were as follows:

- An animal room of 6 m^2
- 20 air changes per hour, filtered with a 99.995% efficient HEPA filter
- Temperature: 21 to 2°C
- Relative Humidity: 30-50%
- Light/dark cycle: 12:12 hours
- Light intensity at a height of one meter at the cage center: 100 lux

Methodology (continued)

- Two ICV ventilated racks (Tecniplast SUM-LINE). One with 530 cm^2 cages, the other with 435 cm^2 cages. Both with 70 air changes per hour.
- The animal room was separated from the services. An experimental ante-room with a laminar flow cabinet was used for handling animals and changing cages.

The bedding was evaluated with these conditions:

- Amount of bedding per tray: 0.4 g/cm^2 of cage surface
- Minimum floor area per animal: between 70 and 100 cm^2 according their animal weight (European Directive 63/2010/EU and Spanish Royal Decree 53/2013). In the “stress” tests, that area was reduced in order to evaluate the conditions for both adult females with a litter and young animals in the first days post-weaning
- Weighing: the animals were weighed at the start and at the end of the evaluation

Every two days the following parameters were evaluated:

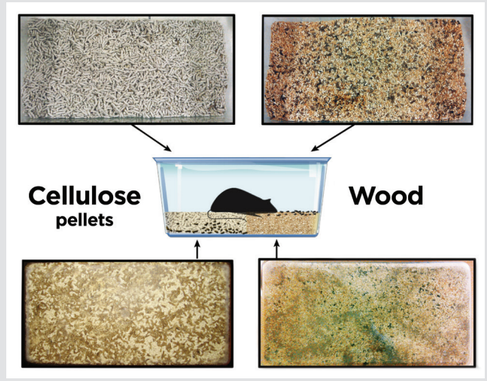
- Feed consumption
- Drink consumption
- Weight of cage with dirty bedding and with no animals
- Shape produced on bedding by animal activity
- Ammonia levels in ppm: measurement started at day 7, or whenever a damp patch was observed on floor of cage

Evaluations performed regularly:

- The volume of bedding was measured at the beginning and end of the evaluation, as well as its initial and final height in the cage
- Bedding was tested with different animal strains
- Bedding was evaluated in ventilated and conventional cages
- Evaluation was finished once the cages reached ammonia levels of 25 ppm or above. NH_3 was measured with a colorimetric pipette (Accuro) which sucked up air from the cage and colorimetric tubes (5/B 5A 100 ppm and 2A 30 ppm)
- Microbiological bedding controls by means of a plate count of bacterial colonies were conducted weekly after being processed, inoculated, and incubated in plates with Plate Count Agar and Sabouraud Chloramphenicol

Sedimentation of Feces Through Bedding to Cage Floor

Another novel property of cellulose pelleted bedding is the settling of fecal matter to the floor of the cage, which means it can be separated from the bedding even though the bedding density (D=1.17) is greater than that of the excrement (D=0.85) when water density is taken as D=1.



Microbiology: Dramatic Reduction in Bacterial Colonies

Monitoring cages over a period of three weeks showed a gradual increase in the number of bacteria, especially from the second to the third week. Nevertheless, bacteria counts for cellulose pelleted bedding were far below those of wood-based bedding and other kinds of bedding materials, as is shown in figure below. (Babu A. 2013. Evaluation of micro-environment and microbiological monitoring of various bedding materials for laboratory rodents).