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Evaluation of micro-environment and microbiological monitoring of various bedding materials for laboratory rodents

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Abstract

In biomedical research, the use of laboratory animals is very vital and a critical part of effort to prevent, cure and treat a vast range of ailments. Globally around 50-100 million laboratory animals are used annually for experimentation. Rat, mouse, Guinea pig and rabbit are the most commonly used laboratory animals and among these, laboratory mouse is an important species. Variety of environmental factors can affect the outcomes of studies using laboratory rodents. One such factor is bedding. Physiological changes may occur after exposure to some types of bedding and could affect experimental results. Some bedding materials generate dust and particulates that might cause respiratory or ocular changes. Several new bedding materials have been introduced for laboratory rodents in the recent past, but there are only a few evaluation reports about their performance. In this study, we have compared the performance of different bedding materials like saw dust, paddy husk, corncob and paper shredding. We measured the micro-environment parameters like ammonia, sulfate, temperature, biomass changes, pH, moisture content, microbial load viz., total plate count, yeast and mold count, when housed on various types of bedding materials. We observed that the bedding materials have no significant effect on cage temperature, humidity and pH. The ammonia level in cages using corncob bedding (242 ± 3.65 mg/100g) was less when compared to all other beddings (Saw dust, 454 ± 2.4 mg/100g) and so prolong the interval between cage changing. The microbial monitoring also revealed less microbial load when corncob was used as bedding material. Hence, the present study suggests that corncob is more suitable as bedding material for housing laboratory mice.

Key words: bedding materials, microbial load, ammonia, moisture, laboratory mice

Introduction

Laboratory rodents account for the majority of animals used in scientific procedures worldwide. In general, laboratory caging for rodents provides a confined and barren environment. Since the animals spend the greatest proportion of their lives in their home cage, improving or enriching this environment affords a significant opportunity to improve their overall well-being. There are many important factors involved in conducting animal studies, including the choice of bedding used in the cages and the addition of enrichment items. Bedding and enrichment compose the majority of the animal's environment and can play an influential role in its development; thus, an educated selection is critical in optimizing both the welfare of the mouse and the output of the study. Bedding is one of the most important items within

the micro-environment of laboratory animals in captivity. It provides warmth, maintains the environment of the cage, and adds to the overall welfare of the animals in care. The type of bedding may interact with experimental treatments and affect the outcome of certain experiments such as those on enzyme-induction, cytotoxic and carcinogenic compounds and anaesthetics (Torronen *et al*., 1989; Potgieter and Wilke, 1992). Cage bedding must be able to absorb liquid discharge and prevent ammonia build up. Moisture absorbency is one of the most important characteristics of rodent beddings for controlling bacterial growth which reduces the ammonia production, and the build-up of harmful bacterial toxins (Raynor *et al*., 1983, Perkins & Lipman, 1995; Hawkins *et al*., 2003). Levels of ammonia commonly encountered in animal boxes and cages have been shown to cause histopathological changes in the tracheal epithelium of rats, and it is suggested that 'abnormal respiratory histology' could be a reflection of the standards of husbandry employed before and during an experiment (Gamble and Clough, 1976). It should be comfortable for the animal, simulating a natural environment in which the mouse can burrow and nest contentedly. To avoid accidentally introducing unwanted variables, it should be dust-free and standardized across cages. The objective of the present study was to study the microbiological profile in different bedding materials such as corn cob, saw dust, paper shredding, and paddy husk and to study the effect of ammonia and sulfate on laboratory rodents and micro-environmental parameters under various housing conditions.

Materials and methods

Bedding samples

The bedding materials such as saw dust, paddy husk, paper shredding were used in the present study. Corncob was supplied as complimentary samples by a commercial company Bangalore, India. Saw dust and paddy husk were obtained from the regular suppliers to the animal house, CFTRI, Mysore. Paper shredding was collected from the CFTRI Press, Mysore.

Experimental Designs

All experiments were conducted in Animal house where the temperature was maintained at 30-35^{0}C. Animal cages was of 11"x 9"x 5.5" (LxBxH) size and each contains four animals/mice of 60 days old age.

Environmental parameters (Physico- chemical properties)

Bedding materials were analyzed for free amino nitrogen, sulfate level, pH, biomass, temperature, moisture level and microbial load of bedding materials.

Determination of pH and temperature

The pH of the bedding material was measured using a control dynamics digital pH meter, followed by the hand book of laboratory analysis (Mani *et al*., 2007). The temperature of various bedding materials were measured using thermometer at regular intervals (Misselbrook and Powell, 2005).

Determination of bedding weight

Bedding materials were weighed for biomass changes and body weight of individual mice in each cage was checked for three weeks at an interval of one week. Each cage was filled with 236.30, 188.70, 100, 315g (standard weight) of saw dust, paddy husk, paper shredding, and corncob bedding materials respectively. Cage with different bedding materials were arranged in a randomized manner. For each bedding material there were three replicates each containing four number of cages.

Estimation of moisture content

The moisture content of bedding materials was determined by heating a known weight of the sample in an oven at 105 to 110°C for eight hours. The samples were weighed after drying by using the electronic balance (Model: DS- 852 series) Essae- Teraoka Ltd., The loss in weight was reported as moisture content of the sample. (Mani *et al*. 2007).

Determination of free amino nitrogen (Titration method)

The ammonia level in various bedding materials were analyzed using the standard method (BIS – 3839:1989). The amino nitrogen content was calculated using the following formula

$$\text{Amino nitrogen, mg/100g} = \frac{1400\ (A - B)}{M}$$

Where
A - volume of barium hydroxide in sample titration
B - volume of barium hydroxide in blank titration
C - mass in g of the material taken for the test

Determination of sulfate

The sulfate level in various bedding materials were analyzed using UV S5pectrophotometer by the standard procedure (Singh *et al*. (2011).

Microbiological monitoring in bedding materials

The samples were analyzed for the microbial load of bacteria, yeast and moulds using the following method.

Total plate count, yeast and mould count

The samples were prepared by soaking 3g of bedding materials in 100ml of water for one hour. The mixture was filtered through Whatmann filter paper. The filtrate was serially diluted (the dilutions 10^{-6}, 10^{-8} were used for bacterial isolation and 10^{-2}, 10^{-4} dilutions were used for yeast and mold isolation) and spread plate onto Nutrient Agar and Czapek Dox Agar medium with chloramphenicol (HiMedia Laboratories Pvt Ltd, Bombay) respectively using standard procedures. The plates were incubated at 37ºC for 36 h and the total no of bacteria, yeast and mould were counted using colony counter and tabulated.

Microbiological surveillance in experimental animals. Detection of *Salmonella* and beta-hemolytic *Streptococci*

Blood samples

Blood samples and throat swabs were collected from the animals using sterile tubes. Added 1/5 volume of EDTA as anticoagulant. One ml of blood was mixed with RV broth for enrichment and it was incubated for overnight and then streaked to XLD, HEA and BSA specific for *Salmonella*. One loopful of blood was streaked on to KF Streptococcus agar for beta- hemolysis *Streptococci* and confirmation with Blood agar plate. All the plates were incubated for 48-72 h at 37ºC.

Throat samples

One ml of swab sample was added to RV broth and BHI broth for enrichment, it was incubated for overnight and then streaked onto XLD, HEA and BSA for *Salmonella*. RV broth and BHI broth was incubated for overnight at 43 ºC. Spread plate was done on KF Streptococcus agar medium with the wet swab for beta- hemolysis *Streptococci*. All the Plates were incubated for 48 – 72 h at 37 °C.

Statistical analysis

All the results were subjected to statistical analysis and one way analysis of variance was used to determine the statistical significance.

Results

Evaluation of cage micro-environment for mice

The moisture level was observed on different bedding materials consecutively for three weeks are presented in Table-1. Towards the end of the experiments in corncob the moisture level was found to be highest (87.85%)when compared to other bedding materials where as at the same period a test moisture content of 28.2% was recorded in paddy husk.

Table 1. Moisture percentage in different bedding materials (Mean ± Standard error)

Bedding samples	0th week* (%)	1st week (%)	2nd Week (%)	3rd Week (%)
Saw dust	15.561±1.16	19.677±0.46	28.975±0.79	40.033±0.99
Paddy husk	12.797±1.91	15.832±1.61	23.250±1.39	28.205±0.77
Paper shreds	14.625±0.56	31.925±2.14	53.775±1.07	63.150±1.81
Corncob	17.425±1.18	36.625±1.85	61.500±1.11	87.850±2.57

* 0th week - control boxes containing bedding but no mice. Values were based on 4 samples per group

Change in bedding weight in different bedding materials

Variations in weight of different bedding materials are presented in Table 2. The increase in bedding weight and bedding age reflects the accumulation of urine, feces and spillage of water and feed. Towards the end of experiment period (3rd week), an increased weight was observed with corncob (499.92g) where as least increase weight was observed with paper shredding (293.02g).

Table 2. Change in bedding weight in different bedding materials (Mean ± Standard error).

Bedding material	0th week weight (g)	1st week weight (g)	2nd week weight (g)	3rd week weight (g)
Saw dust	236.30*	228.55±2.93	290.08±4.87	416.35±6.32
Paddy husk	188.70*	213.38±2.79	279.10±4.21	416.35±6.02
Paper shreds	100.00*	171.98±2.84	194.00±5.22	293.02±4.99
Corncob	315.00*	354.58±2.89	399.50±4.60	499.92±5.46

Bedding weight = Initial weight – Final weight (Difference)

*The amount of bedding used in cages initially based on the standard procedure (in grams or depth in inches).

Change in pH of different bedding materials

The pH was recorded for different bedding materials for three weeks and presented in Fig 1. It was in the range between 7 and 8.5 for all the bedding. Initially, it was low and first two weeks it increased and then reduced to 7.2 in the final week.

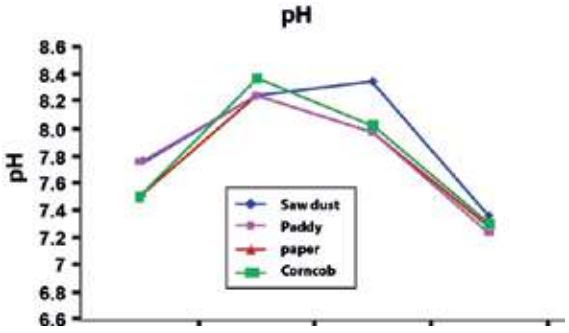

Fig 1. Change in the pH in different bedding material.

Temperature in different bedding materials

The temperature of all the four bedding materials was similar from the initial stage to the final stage. The temperature range was 28.1- 33.8°C and did not differ significantly among the bedding types (Fig 2).

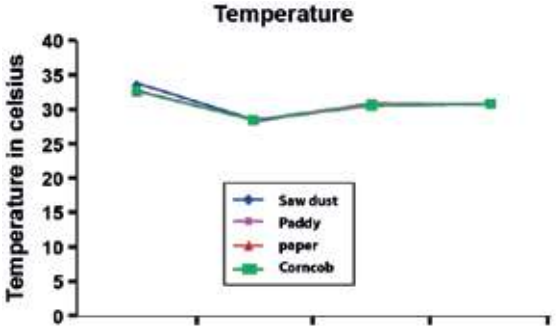

Fig 2. Change in temperature of different bedding materials

Change in ammonia concentration in different bedding materials

The ammonia level was measured by titrating method and the values were calculated by using the formula. The level of ammonia in bedding materials are shown in the Table 3. The ammonia concentration in cages with mice housed on saw dust showed highest level and the lowest level in the corncob. The paper shredding and paddy husk bedding materials was not significantly different when compared with each other, but paddy husk significantly varied with saw dust. The range of ammonia levels were around 242 – 454 mg/100g during 3rd week.

Table 3. Change in ammonia concentration in different bedding material (Mean ± Standard error).

Bedding material	0th Week* (mg/100g)	1st Week (mg/100g)	2nd Week (mg/100g)	3rd Week (mg/100g)
Saw dust	45.80±0.92	131.13±2.70	216.60±1.63	454.18±2.49
Paddy husk	45.78±0.68	89.50±1.65	226.18±1.61	267.00±4.51
Paper shreds	46.18±0.75	85.62±2.33	205.00±3.27	255.78±0.82
Corncob	46.33±0.95	80.83±2.78	184.88±2.72	242.40±3.65

Values are based on 4 samples per group
* 0th week is considered to be control where the cages contain only bedding material and not mice.

Change in sulfate level in different bedding materials

The sulfate level was measured in all bedding materials once in a week, consecutively for three weeks and calculated the value, which is given in Table 4. The sulfate level ranges from 44.48 - 109.68 mg/L. During 3rd week, It shows high amount of sulfate in corncob bedding and least amount in paper shredding.

Table 4. Change in sulfate level in different bedding materials (Mean ± Standard error).

Bedding samples	0th Week* (mg/L)	1st Week (mg/L)	2nd Week (mg/L)	3rd Week (mg/L)
Saw dust	0.645±0.05	11.708±0.55	37.905±1.17	98.75±1.59
Paddy husk	0.744±0.11	11.667±0.95	38.172±1.31	101.30±4.92
Paper shreds	0.692±0.05	11.675±1.61	28.082±1.31	44.48±1.74
Corncob	0.262±0.04	11.730±0.81	48.455±1.17	109.68±3.98

Values were based on 4 samples per group
* 0th week is considered to be control where the cages contain only bedding material and not mice.

Total plate count (TPC) among different bedding materials

The total plate count was done for different bedding consecutively for three weeks on weekly basis and given in Table 5. The highest colony count was observed in paddy husk while the lowest colony count was observed in corncob (Fig. 3).

Table 5. Total plate count of bacteria among different bedding materials

Bedding samples	0th Week* (X 10^9cfu/g)	1st Week (X 10^9cfu/g)	2nd Week (X 10^9cfu/g)	3rd Week (X 10^9cfu/g)
Saw dust	0	1.747	4.245	9.830
Paddy husk	0	2.912	6.247	13.91
Paper shreds	0	2.997	4.332	3.162
Corncob	0	0.450	1.450	1.912

Values were based on 4 samples per group
* 0th week is considered to be control where the cages contain only bedding material and not mice.

Fig. 3. The bacterial colonies in nutrient agar for TPC

Yeast and mould count in different bedding materials

The yeast and mould counts were increased along with incubation period. A highest of 2.8 x 10^5 cfu/g was observed in paddy husk at the end of incubation period (3rd week). Similarly least of 0.931 x 10^5 cfu/g was recorded in corncob and presented in the Table 6. The Yeast and mould colonies in agar plates given in fig 4 and 5

Table 6. Yeast and mould count in different bedding materials

Bedding samples	0th Week* (x10^5 cfu/g)	1st Week (x10^5 cfu/g)	2nd Week (x10^5 cfu/g)	3rd Week (x10^5 cfu/g)
Saw dust	0	0.162	1.080	2.0803
Paddy husk	0	1.830	1.327	2.830
Paper shreds	0	0.745	1.577	1.662
Corncob	0	0.611	0.750	0.931

Values were based on 4 samples per group
* 0th week is considered to be control where the cages contain only bedding material and not mice.

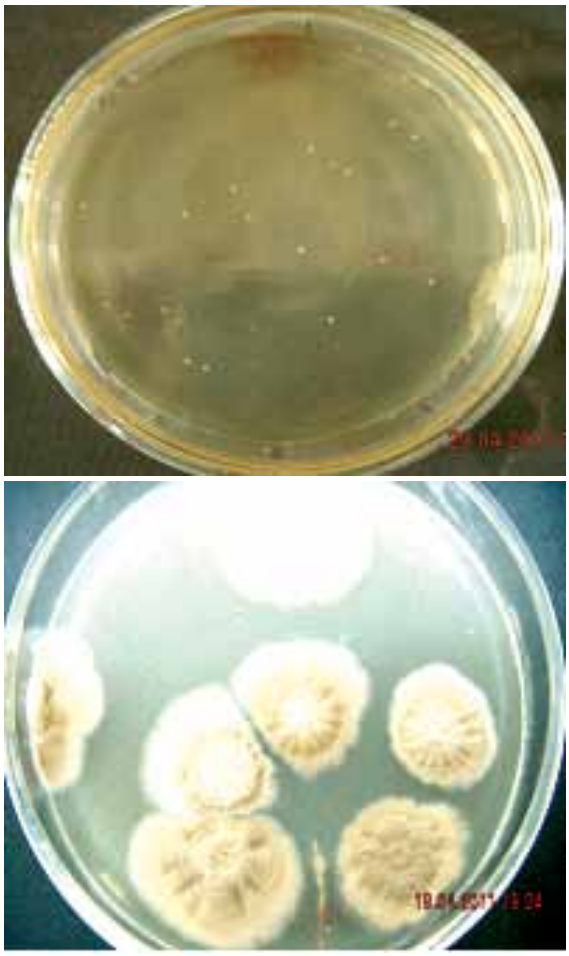

Fig. 4 and 5.Red Yeast and mould colonies in agar plates.

Microbiological surveillance in laboratory animals

Screening for *Salmonella* sp

The blood and swab samples were used to confirm the presence or absence of *Salmonella* and beta hemolytic *Streptococci* on specific media and some differential media. After incubation period, the results showed negative for *Salmonella* (fig 6).

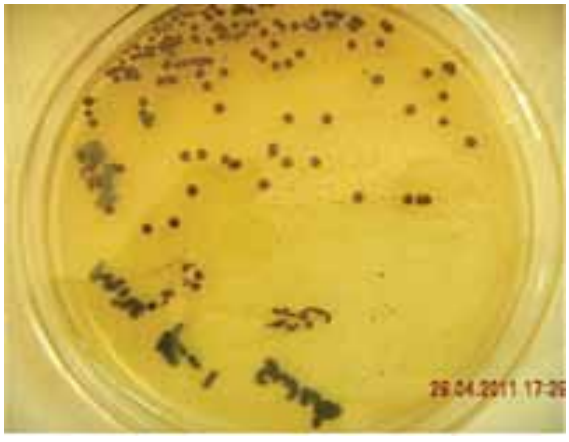

Fig. 6. *Salmonella* (Black centered colonies) in BSA agar

Screening for beta-hemolytic Streptococcus sp.

We observed for beta hemolytic Streptococcus on KF Streptococcus agar and blood agar base to confirm the beta hemolytic activity. The results showed that negative for the beta hemolytic *Streptococcus* (Fig 7).

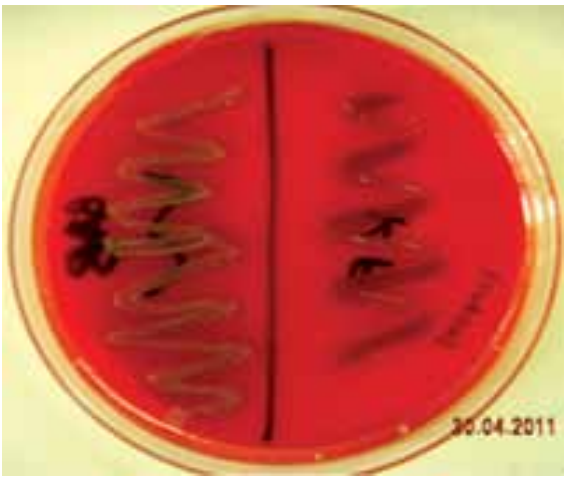

Fig. 7. Negative for β-hemolysis on Blood Agar Base

Discussion

The present study was conducted to evaluate the physio-chemical and microbiological profile of different bedding materials such as corn cob, saw dust, paper shredding and paddy husk. The moisture level was highest in corn cob and lowest in paddy husk. Krohn and Hansen (2008) reported the corn cob has lower water absorption when compared to paper bedding. The highest moisture level in corn cob might be attributed to accumulation of urine at the bottom.

The bedding weight was highest in the paddy husk and least in corn cob materials. This may be due to low amount of urine absorbed by corn cob and therefore the evaporation of urine from the bedding is higher, leading to lower weight of the corn cob material (Perkins and Lipman, 1995). The pH did not reveal any difference among the bedding materials used. Similarly the temperature of all the bedding materials were similar throughout the study. The body weight of mice housed on different bedding materials also did not differ significantly.

The ammonia concentration in different bedding materials revealed highest in saw dust and comparable among others. Smith *et al*. (2004) reported that lowest ammonia concentration occurred in cages housing mice on hard wood bedding or a mixture of corncob and alpha cellulose. Krohn and Hansen (2008) revealed reduced ammonia level in corn cob bedding when compared to paper bedding. Similarly in our studies corn cob along with paddy husk and paper shredding showed lesser ammonia concentrations. The reduced level of ammonia prolongs the interval between cage changing and it may therefore be beneficial for the facility to use corn cob.

The sulfate levels of the bedding materials shown that least in paper shredding and comparable among others. So far there are no reports on sulfate levels in the bedding material.

The microbiological surveillance of cage environment revealed highest total plate count in paddy husk and least with corn cob bedding. The yeast and mold count showed highest in paddy husk and least in corn cob material. Weisbroth (1979) reported that corn cob showed least number of yeast and mold count as well as Coliform and Aerobic plate count, when compared to hardwood, pine shavings, paper chip etc.

The outcome of the study supports corn cob as favourable bedding material for housing mice when compared to saw dust, paddy husk and paper shredding. The lowest unfavourable microbial load in corn cob bedding material further assures health and pathogen free environment.

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