

Absorbency and Other Physical Properties of Three Different Rodent Beddings in Thailand

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ABSTRACT

Absorbency and other physical properties of three different rodent bedding materials in Thailand corncob, woodchips, and para-rubber were tested to find the most appropriate rodent bedding for the NLAC-MU colony. Corncob had the maximum volumetric absorbency after 48 to 72 h soaking in saline. The volumetric absorbency, mass, and density of corncob were significantly higher ($p < 0.05$) than for woodchips and para-rubber (1.5 to 2.5 times). In contrast corncob had the lowest ($p < 0.05$) mass absorbency when compared to woodchips and para-rubber, due to its mass. Autoclaving influenced some properties of corncob bedding due to a reduction in the mass, density, and absorbency. However similar change were not found in woodchips or para-rubber. Woodchips generated significantly more ($p < 0.05$) dust particles than para-rubber and corncob respectively. Perimeter (mm) /area (mm²), and wood fiber space (μm) were used to measure the altered shape after soaking in saline. The results showed that all the bedding used in this study did not significantly change ($p > 0.05$) in shape and preserved its hardness after soaking. The study concluded that corncob and para-rubber were more appropriate for use as rodent bedding than woodchips. Further studies were needed to carry out toxicity, gas production and preference testing.

Key words: absorbency, physical properties, rodent beddings

INTRODUCTION

The microenvironment of a laboratory animal is the physical environment immediately surrounding it, with its own temperatures, humidity, gaseous and particulate composition of the air. Microenvironmental conditions can induce changes in the metabolic and physiological processes or alter disease susceptibility (Broderson *et al.*, 1976).

One of the most important factors affecting their microenvironment is bedding. Chips, shavings, shreds or grains made of wood or other material of plant origin are most frequently used as contact beddings for experimental animals. Substances in beddings may bias results and increase variations in pharmacological and toxicological studies (Pelkonen and Hanninen, 1997).

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Another important function of rodent bedding is to absorb moisture from urine and feces. Bedding slows bacterial growth, which reduces the production of gases such as ammonia and carbon dioxide and the build-up of harmful bacterial toxins (Perkins and Lipman, 1995). Bedding material may have an effect on temperature regulation which in turn can affect rodent health and well being (Gordon, 2004). Bedding type specifically wood bedding with a Payer's patch number higher than that for cotton bedding, has influenced the intestinal immune system (Sanford *et al.*, 2002). Apart from absorbing moisture, bedding should provide animals with a comfortable substrate, insulate them from temperature fluctuations and provide a form of enrichment, allowing animals to nest, dig and rest comfortably, or even to forage if food is scattered onto it (Kuhnen, 2002).

Several studies have reported the adverse effects of using the wrong bedding including: microbial contamination of untreated bedding (Hogan *et al.*, 1990); wood dust induced several respiratory diseases such as allergic rhinitis, chronic bronchitis, asthma, and sino-nasal adenocarcinoma (Kaliste *et al.*, 2004, Maatta *et al.*, 2006); pine shavings bedding commonly used all over the world, appeared in general to be highly cytotoxic and also a potent inducer of cytochrome P4501A1 (Davey *et al.*, 2003); high fungal spore counts may occur in untreated corncob/Beta chip bedding and this may be associated with opportunistic diseases in healthy, non-immunosuppressed animals (Royals *et al.*, 1999, Mayeux *et al.*, 1995).

Several new bedding materials (such as alpha cellulose, corncob, hardwood; maple, beech, poplar, recycle newspaper, long-fiber, high grade bleached pulp, pine, Bed-O cobs, ALPHA-dri, ALPHA-dri plus Bed-O cobs, Beta chip) and several new processing methods have been introduced to the international market in recent

years (Smith *et al.*, 2004). Woodchips, para-rubber and corncob are in widespread use in laboratory-animal facilities in Thailand. There are few reports of their performance (Smith *et al.*, 2004), with both respect to their toxicity and physical properties. One study (Pelkonen and Hanninen, 1997) found that extracts from corn cobs, rice hulls and straw, that were used in a few laboratories, were practically non-toxic).

The aim of this study was to measure and compare the absorbency and other physical properties of three different rodent beddings: (1) woodchips that were commonly used in laboratory animal units in Thailand; (2) corncobs; and (3) para-rubber shavings that are a more recent choice because they have fewer dust particles than woodchips. The data was also considered to be useful for toxicological studies and also for determining animal preferences.

MATERIALS AND METHODS

Bedding materials

The bedding materials used were woodchips (from a local sawmill, Thailand), para-rubber (from exotic-pet market, Thailand) and corncobs that were supplied as complimentary samples by the Betagro company, Thailand.

Woodchips and para-rubber were chosen because they are very commonly used in Thailand. Corncob was chosen because it is known to produce relatively low levels of ammonia, and very low levels of dust particles.

Absorbency testing

The method used to measure absorbency was modified from Burn and Mason 2005. Fourteen 50 cm³ samples of each bedding material were placed into plastic cups. Each sample was then weighed, and 100 cm³ of 1% saline was added. Two samples of each bedding material were left to soak for 1, 2, 3, 6, 24, 48 and 72 hour respectively. The cups were covered with plastic

bags and shaken gently at the beginning of the soaking period to release any air bubbles trapped between the bedding particles. After soaking, the excess water was poured away and a small sieve was used to catch the wet bedding. The sieve was tapped lightly against the beaker a few times to dislodge any remaining water droplets, and the bedding was weighed in the sieve. The volume of water absorbed was calculated by subtracting the dry mass from the wet mass of each bedding sample.

Bedding size measuring

Corncob size was measured based on the perimeter (μm) and area (μm^2) using an image analysis program. The fiber space length (μm) in woodchips and para-rubber was measured to calculate the engorgement after soaking for different times. From each specimen, colour images of 640×480 pixel resolution (at 10x and 40x magnification) were acquired with a Stereoscope (Olympus®) and digital camera (Moticam 1000, Moticam®) using an imaging analysis program (ImageJ, NIH).

Measuring dust content

Bedding dusts were characterized by a modified process based on Thigpen *et al.* (1989). The dusted weight after sieving was determined from four g of each bedding after being shaken for one minute in a portable sieve (size 1×1 mm).

Data analysis

Data were presented as mean values \pm SEM for: corncob size perimeter (μm) and area (μm^2); woodchips and para-rubber fiber space (μm); the density/mass of each bedding; and the volumetric/mass absorbency of each bedding. The normality of the data was analyzed using the Kolmogorov-Smirnov test. Statistical comparisons among groups were made using Student's *t* test or Analysis of Variance (ANOVA) for normally distributed data. The Mann-Whitney test or the Kruskal Wallis test were applied to non-normally distributed data. Regression analysis was used to define the curve for the prediction of absorbency based on the soaking time. The level of statistical significance was $p < 0.05$.

RESULTS

The mass and density

The 50 cm^3 mass (g) and density (g/cm^3) (Figure 1) differed significantly ($p < 0.05$) between unautoclaved rodent beddings. Corncob had the highest ($19.09 \pm 0.36 \text{ g}$, $0.38 \pm 0.0067 \text{ g}/\text{cm}^3$) difference from woodchips ($2.24 \pm 0.11 \text{ g}$, $0.045 \pm 0.0025 \text{ g}/\text{cm}^3$) and para-rubber ($1.35 \pm 0.05 \text{ g}$, $0.027 \pm 0.0011 \text{ g}/\text{cm}^3$). The mass and density of corncob ($13.58 \pm 0.16 \text{ g}$, $0.27 \pm 0.01 \text{ g}/\text{cm}^3$) were significantly reduced ($p < 0.05$) after autoclaving but there was no change in woodchips ($2.25 \pm 0.06 \text{ g}$, $0.05 \pm 0.004 \text{ g}/\text{cm}^3$) or para-rubber ($1.35 \pm 0.04 \text{ g}$, $0.02 \pm 0.004 \text{ g}/\text{cm}^3$) (Figure 2).

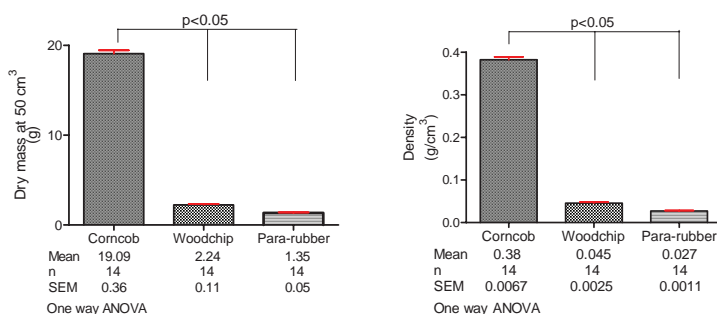


Figure 1 The mass at 50 cm^3 and density (mean \pm SEM) of three different unautoclaved rodent beddings.

Absorbency

The volumetric absorbency of three unautoclaved rodent beddings for different soaking times (Figure 3) for corncob was higher than for both woodchips and para-rubber. The relationship between volumetric absorbency (Y) and soaking time (X) of corncob was describe by an S-curve (Figure 4) as shown in Equation 1, however woodchips and para-rubber were best described by a Cubic-curve (Figure 5 and 6) as shown in Equation 2 and 3 respectively.

$$\ln(Y) = -0.271 - \frac{0.372}{X}$$

: $R^2 = 0.81$ Equation 1

$$Y = 0.359 + 0.115X - 0.041X^2 + 0.003X^3$$

: $R^2 = 0.62$ Equation 2

$$Y = 0.21 + 0.13X - 0.03X^2 + 0.002X^3$$

: $R^2 = 0.48$ Equation 3

With respect to the mass absorbency for different soaking times (Figure 7), although there was no different between woodchips and para-rubber, corncob was significantly lower ($p < 0.05$). The relationship between mass absorbency (Y) and soaking time (X) of corncob, woodchips, and para-rubber were described by Cubic-curves (Figures 4, 5, and 6) as shown in Equations 4 and 5 respectively. The mass absorbency of para-rubber could not be predicted (Figure 6).

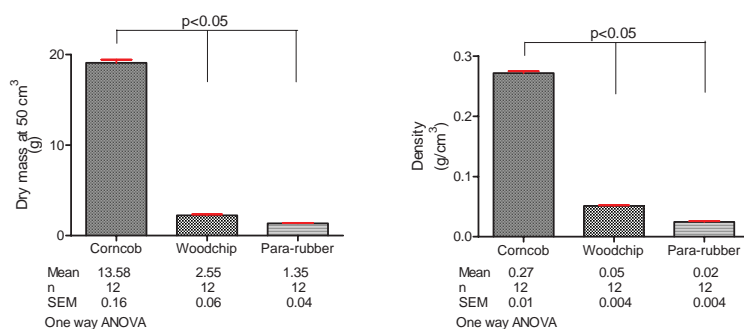


Figure 2 The mass at 50 cm³ and density (mean \pm SEM) of three different autoclaved rodent beddings.

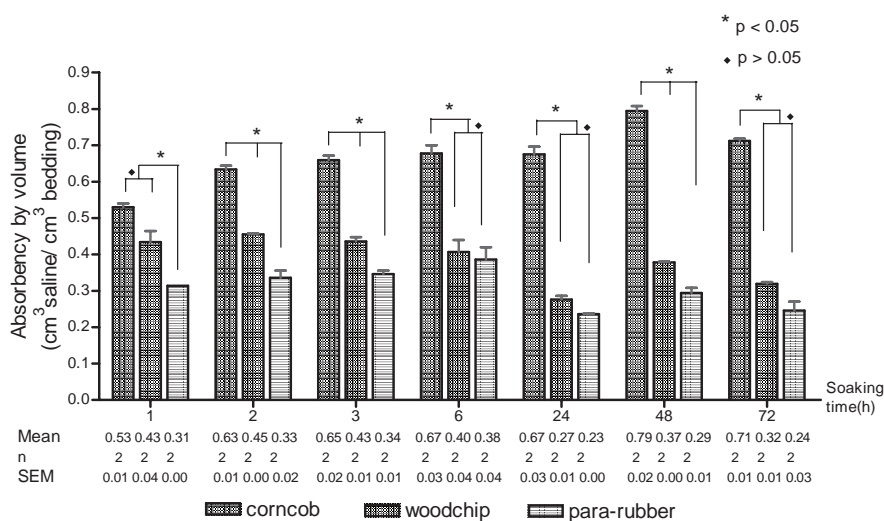


Figure 3 The saline absorbency (mean \pm SEM) of three unautoclaved rodent beddings calculated by volume of beddings.

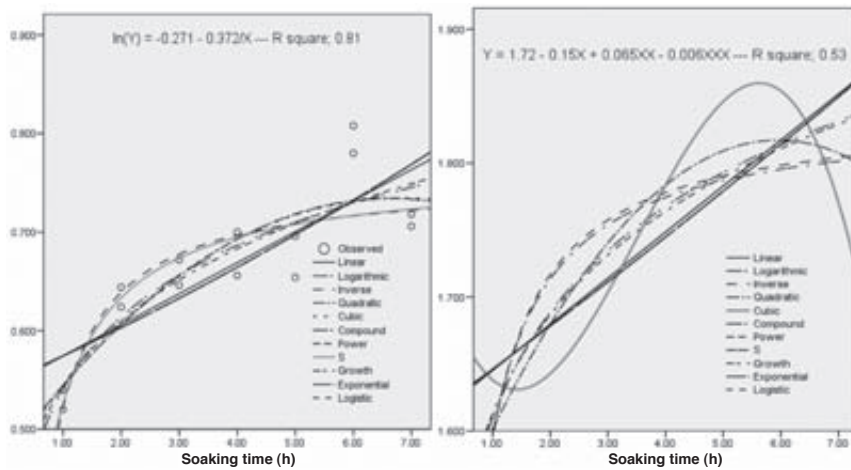


Figure 4 Unautoclaved corncob bedding volumetric absorbency S-curve (left) and mass absorbency Cubic-curve (right) with the predicted line.

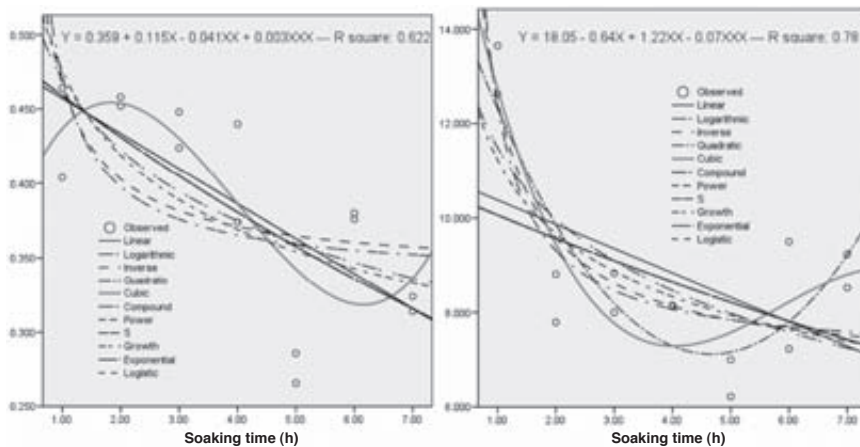


Figure 5 Unautoclaved woodchip bedding volumetric absorbency Cubic-curve (left) and mass absorbency Cubic-curve (right) with the predicted line.

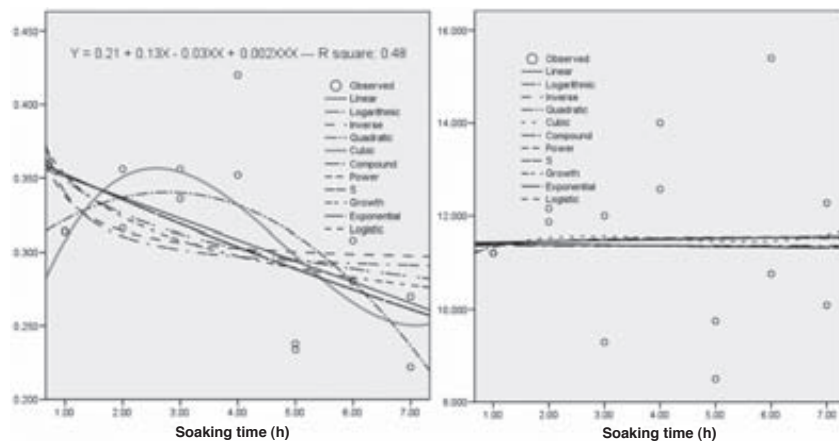


Figure 6 Unautoclaved para-rubber bedding volumetric absorbency Cubic-curve (left) and mass absorbency (right) with the predicted line.

$$Y = 1.72 - 0.15X + 0.065X^2 - 0.006X^3$$

$$: R^2 = 0.53 \quad \text{Equation 4}$$

$$Y = 18.05 - 0.64X + 1.22X^2 - 0.07X^3$$

$$: R^2 = 0.78 \quad \text{Equation 5}$$

For any soaking time (Table 1), the volumetric absorbency was of autoclaved corncob was significantly lower ($p < 0.05$) than for unautoclaved corncob. In contrast, with the mass absorbency results, there was no difference between autoclaved and unautoclaved bedding materials. There were no significant differences for either volumetric or mass absorbency between autoclaved and unautoclaved sample for woodchips or para-rubber.

Volumetric absorbency could be estimated for unautoclaved corncob from Equation 1 which indicated maximum absorbency occurred after 48 to 72 h following soaking with saline solution (Figure 8).

Dust particle mass

The percentage of dust particles by mass (Figure 9) was found to be significantly different ($p < 0.05$) among autoclaved rodent bedding with materials woodchips the highest (2.55 ± 0.52 %) compared to corncob (0 ± 0 %) and para-rubber (0.33 ± 0.16 %).

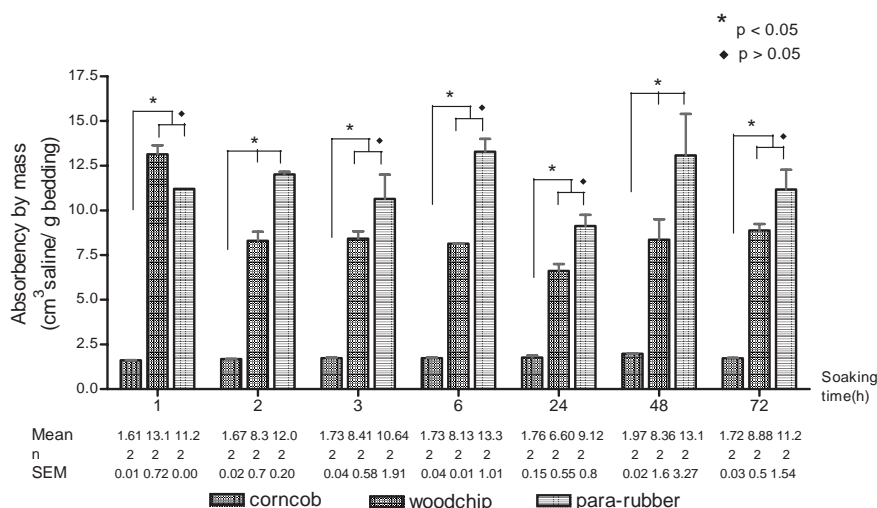


Figure 7 The saline absorbency (mean \pm SEM) of three different unautoclaved rodent beddings calculated by mass of bedding material.

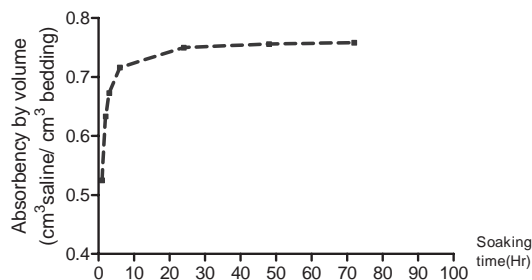


Figure 8 Corncob bedding prediction line to estimate the maximum volumetric saline absorbency.

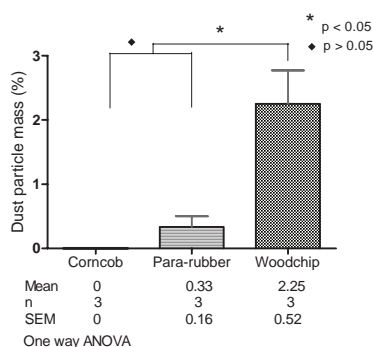


Figure 9 Dust particle by mass (mean \pm SEM) of three different autoclaved rodent beddings.

Table 1 The volumetric and mass absorbencies of three different bedding material compared with and without autoclaving.

Bedding	Soaking Time(Hr)	Volumetric absorbency			Mass absorbency		
		Autoclaved	Unautoclaved	p-value	Autoclaved	Unautoclaved	p-value
Corncob	1	0.437(0.015)	0.530(0.010)	0.036↓	1.60(0.073)	1.61(0.010)	0.848
	3	0.466(0.004)	0.659(0.013)	0.005↓	1.70(0.060)	1.73(0.03)	0.680
	6	0.452(0.01)	0.678(0.02)	0.01↓	1.72(0.076)	1.73(0.029)	0.909
	24	0.606(0.02)	0.675(0.02)	0.151	2.12(0.01)	1.76(0.11)	0.084
	48	0.538(0.004)	0.794(0.014)	0.003↓	1.992(0)	1.97(0.015)	0.376
	72	0.536(0.012)	0.712(0.006)	0.006↓	2.01(0.01)	1.72(0.02)	0.006↑
Woodchip	1	0.411(0.013)	0.434(0.03)	0.555	7.90(0.25)	13.13(0.511)	0.012↓
	3	0.408(0.012)	0.436(0.012)	0.241	7.41(0.083)	8.41(0.415)	0.142
	6	0.412(0.034)	0.407(0.033)	0.926	6.56(0.189)	8.13(0.009)	0.014↓
	24	0.447(0.041)	0.276(0.01)	0.056	9.91(0.25)	6.60(0.39)	0.019↑
	48	0.538(0.004)	0.794(0.014)	0.003↓	9.99(0)	9.97(0.015)	0.376
	72	0.437(0.027)	0.319(0.01)	0.05	8.93(0.733)	8.88(0.334)	0.954
Para-rubber	1	0.226(0)	0.313(0)	0.00↓	8.071(0)	11.21(0)	0.00↓
	3	0.209(0.021)	0.346(0.01)	0.028↓	7.46(0.75)	10.64(1.35)	0.176
	6	0.245(0.037)	0.386(0.034)	0.107	7.86(0.133)	13.28(0.714)	0.018↓
	24	0.331(0.065)	0.236(0.002)	0.282	11.71(1.485)	9.12(0.625)	0.249
	48	0.269(0.027)	0.294(0.014)	0.497	9.27(0.028)	13.08(2.31)	0.242
	72	0.203(0.009)	0.246(0.024)	0.235	9.22(0.409)	11.18(1.09)	0.236

↓ After autoclaved, absorbency of each bedding was reduced.

The size of bedding material materials

The perimeter/area for corncob and the wood fiber space of woodchips and para-rubber were used to measure the size of each unautoclaved bedding material after different soaking times. There was no difference among bedding materials after different soaking times (Table 2).

DISCUSSION

Rodent cages are filled with bedding to reach a desired depth of bedding, rather than to provide a desired mass because bedding depth (and therefore volume) was considered to be the more relevant consideration for the animals concerned, as the bedding was required to be deep enough to lie on and dig in for example, but not so deep that it could come in contact with the cage water supply

and flood the cage, or impair the animal's movement. Thus absorbency per unit volume was considered the most relevant descriptor of the bedding's moisture-absorbing properties (Burn and Mason, 2005). Other important factors were the beddings physical properties in terms of mass, density and shape.

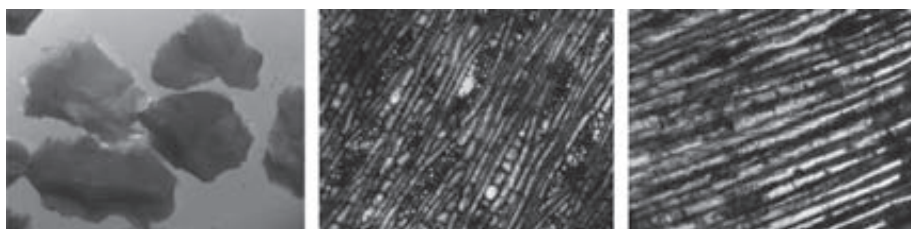
This study found that under the applied conditions corncob had the highest (1.5 to 2.5 times that of woodchips or para-rubber) volumetric absorbency level which agreed with previous studies (Burn and Mason, 2005). It generated a very low level of dust particle and because of its cuboid shape, it had a higher mass and density than either woodchips or para-rubber. The sterilization technique which involved autoclaving may have changed its properties due to shrink age, as it had a reduced volumetric absorbency, density,

Table 2 The size of three different bedding material after soaking with saline compared by perimeter, area, and wood fiber space.

Bedding	Parameters	Soaking time (Hr)							p-value ANOVA
		1	2	3	6	24	48	72	
Corncob	Perimeter (mm)	12.26	11.81	12.32	11.48	11.63	11.70	11.92	0.661
	n	23	20	24	20	22	18	25	
	SEM	0.44	0.39	0.43	0.35	0.35	0.35	0.33	
	Area (mm ²)	9.21	8.86	9.28	8.66	8.88	9.39	8.73	0.924
	n	23	20	24	20	22	18	25	
	SEM	0.64	0.52	0.55	0.49	0.46	0.65	0.39	
Woodchip	Wood fiber space(μm)	5.59	5.59	5.24	5.26	5.65	5.39	5.20	0.488
	n	18	19	23	26	28	20	32	
	SEM	0.24	0.22	0.22	0.15	0.18	0.22	0.18	
Para-rubber	Wood fiber space(μm)	5.48	5.00	5.16	5.59	5.68	5.69	5.66	0.62
	n	37	40	18	25	19	20	25	
	SEM	0.17	0.18	0.28	0.19	0.21	0.20	0.18	

and mass, following autoclaving. However it was considered the most appropriate method to eliminate microbial contamination and still provide an acceptable level of absorbency. Regardless of the soaking time, corncob did not change its shape and remained firm which was considered an important property in rodent bedding. It was also known to produce relatively low levels of ammonia. Volumetric absorbency was estimated for unautoclaved corncob. Using Figure 8 to predict the time to achieve maximum absorbency after soaking with saline solution. This indicated that the appropriate time for between changes in the corncob bedding should be not more than three days.

Woodchips and para-rubber had relatively low volumetric absorbency levels and generated some dust particles because their small rectangular shape which meant they were lower in mass and density than corncob. The sterilization technique involving autoclaving did not change their properties, as absorbency (at any soaking time), density, and mass were almost stable. During soaking, their shapes like for corncob were not changed. The low mass and density of woodchips and para-rubber resulted in a poor prediction of the maximum volumetric absorbency (Figure 6 and 7) with R^2 values of only 0.62 and 0.48, respectively. However data from this study suggested that the maximum volumetric

**Figure 10** Acquired colour images, 640x480 pixel resolution of corncob; magn. 10X (left), woodchip; magn. 40X (middle), and para-rubber; magn. 40X (right).

absorbency of woodchips and para-rubber occurred at 2 to 3 h after soaking with saline. This study recommended filtrating woodchips and para-rubber before use to reduce the dust particles in the micro-environmental system.

Corncob is now available from Thai commercial vendors. Although it is more costly either than woodchips or para-rubber, its high volumetric absorbency properties recommended its use for laboratory animal transportation.

This study provided baseline data on the physical properties of these options which can be used as a preliminary tool to select the appropriate rodent bedding. More work is necessary to fully quantify the relative health and welfare benefits of the various beddings materials available. Topics for study include: toxic chemical contamination, ammonia production, carbon dioxide production, toxicological studies of bedding materials with respect (to the immune system, the respiratory system, the reproductive system, the gastrointestinal system etc.) and a preference study for each laboratory animal.

CONCLUSION

The volumetric absorbency, mass and density of corn cob were significantly higher than for woodchips and para-rubber. In mass absorbency for corn cob was the lowest compared with woodchips and para-rubber. Autoclaving influenced some properties of corn cob bedding as the mass, density, and absorbency obviously declined, however these reductions did not occur with woodchips and para-rubber. Woodchips and para-rubber generated significantly more dust particles than corn cob. All rodent bedding material used in this study did not change in shape and preserve their hardness after soaking for anytime period. A prediction curve indicated that the duration between cage changes with corn cob bedding was not more than three days. Corn cob and para-rubber were considered appropriate for

rodent bedding but woodchip were considered to generate unacceptable levels of dust particles.

ACKNOWLEDGEMENTS

The authors express their deep appreciation and thanks to the Betagro Company who provided the corn cob bedding, to Ms. Aunchalee Sirimontaporn and to the Quality Control Division, NLAC-MU for the provision of the laboratory instruments.

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