Absorbencies of six different rodent beddings:
commercially advertised absorbencies are potentially misleading

Short title: Rodent bedding absorbencies

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1 Summary

Moisture absorbency is one of the most important characteristics of rodent beddings for controlling bacterial growth and ammonia production. However, bedding manufacturers rarely provide information on the absorbencies of available materials, and even when they do, absorption values are usually expressed per unit mass of bedding. Since beddings are usually placed into cages to reach a required depth rather than a mass, their volumetric absorbencies are far more relevant. This study therefore compared the saline absorbencies of sawdust, aspen woodchips, two virgin loose pulp beddings (Alpha-dri$^\text{TM}$ and Omega-dri$^\text{TM}$), reclaimed wood pulp (Tek-Fresh$^\text{TM}$), and corncob, calculated both by volume and by mass. Absorbency per unit volume correlated positively with bedding density, while absorbency per unit mass correlated negatively. Therefore, the relative absorbencies of the beddings were almost completely reversed depending on how absorbency was calculated. By volume, corncob was the most absorbent bedding, absorbing about twice as much saline as Tek-Fresh, the least absorbent bedding. Conversely, when calculated by mass, Tek-Fresh appeared to absorb almost three times as much saline as the corncob. Thus, in practical terms the most absorbent bedding here was corncob, followed by the loose pulp beddings, and this is generally supported by their relatively low ammonia production in previous studies. Many factors other than absorbency determine whether a material is suitable as a rodent bedding, and they are briefly mentioned here. However, manufacturers should provide bedding absorbencies in terms of volume, in order to help predict the relative absorbencies of the beddings in practical situations.
2 Introduction

An important function of rodent beddings is to absorb moisture from urine and faeces. By doing so, beddings slow bacterial growth, which reduces the production of gases such as ammonia and carbon dioxide, and the build-up of harmful bacterial toxins (Hawkins et al. 2003, Perkins & Lipman 1995, Raynor et al. 1983). If ammonia is allowed to rise in animal cages, it can cause respiratory damage (Broderson et al. 1976, Gamble & Clough 1976, Serrano 1971) and eye problems (Van Winkle & Balk 1986). Potentially it could also cause burns on skin that is in prolonged contact with soiled material (in chickens: Weaver & Meijerhof 1991), which might be a particular problem in neonates and hairless strains of rodents (Berg et al. 1986). Concentrations of 100 and 300 ppm have also caused lethargy in mice and rats (Tepper et al. 1985).

Ammonia can build up if cages are not cleaned frequently enough, there is a high density of animals, ventilation rates are low, or ambient temperature and/or humidity is favourable for bacterial growth (Eveleigh 1993, Gamble & Clough 1976, Ishii et al. 1998). In addition, it rises dramatically if a water spillage occurs in the cage and causes the bedding to become soaked (Gamble & Clough 1976). The strain of rodents used can also influence the rate at which ammonia and other pollutants are produced. For example, some diabetic strains of mice and rats produce more urine than their non-diabetic counterparts (e.g. Homma et al. 2002, Sokol & Valtin 1982). As a result, an animal unit at Oxford University maintains C57BL/ksOlaHsd-Lepr diabetic mice on an absorbent paper bedding (Alpha-dri™, Lillico Biotechnology, Surrey, UK), and cleans their cages twice-weekly rather than once-weekly (Denise Jelfs, pers. comm.).
Apart from absorbing moisture, a 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, Lawlor 2002, Sherwin 2002, Waiblinger 2002, Wolfensohn & Lloyd 2002). This is clearly important for welfare. Rats reared with bedding on solid floors had lower anxiety and higher weight gain than those reared on wire floors (Satinder 1967). They also carried, manipulated and dug in bedding, and had greater interaction with cagemates than on wire (Holland & Griffin 2000). Preference tests have generally shown that rodents prefer solid floors with bedding to wire floors, particularly for resting (Arnold & Estep 1994, Blom et al. 1996). However, beddings must be non-toxic and dust-free if they are truly to benefit the animals (Burn et al. in preparation, Ewaldsson et al. 2002, Odynets et al. 1991, Wirth 1983).

Various commercially available beddings exist, which differ in many respects. However, although one of the most important qualities of a bedding is its capacity to absorb moisture, information on this feature is rarely available to the user. Furthermore, when it is available, it is usually expressed as a function of the bedding’s mass (absorbency per g or kg), rather than its volume (absorbency per cm$^3$). In fact, absorbency by volume would be a more relevant measure as beddings tend to be placed in rodent cages to fill a certain volume or depth, not to reach a given mass (e.g. Gamble & Clough 1976, Ras et al. 2002). Therefore, this study aimed to distinguish the absorbencies of six beddings marketed for use in rodent cages, and to compare their volumetric absorbencies with their equivalent absorbencies by mass (Experiment 1). We also verified whether or not animal technicians filled cages with different beddings to the
same depth, or instead to depths appropriate for their absorbencies by mass (Experiment 2).

3 Methods

3.1 Experiment 1

3.1.1 Bedding materials

The bedding materials used were Aspen chips (grade 8), Gold Flake sawdust, Corncob (grade 12) and Alpha-dri™ (all from Lillico Biotechnology, Surrey, UK), and Omega-dri™, and white Tek-Fresh™ (from Harlan Teklad, Bicester, UK). All the beddings were supplied as complimentary samples from their respective companies. Sawdust and aspen woodchips were chosen because they are very commonly used. Corncob was chosen because it is known to produce relatively low levels of ammonia (Hawkins et al. 2003, Perkins & Lipman 1995), and Tek-Fresh, Alpha-Dri and Omega-Dri are all marketed as being highly absorbent. Alpha-Dri and Omega-Dri are both virgin loose pulp beddings, the former having square particles and the latter having polygonal ones, while Tek-Fresh consists of reclaimed wood pulp.

3.1.2 Absorbency testing

Four 50 cm³ samples of each bedding were placed into glass beakers. Each sample was then weighed, and 100 cm³ of 1% saline was added (as used for the absorbency values quoted in data sheets supplied with the beddings from Lillico Biotechnology). The beddings were left to soak for one hour, as in a similar study by...
Potgieter & Wilke (1996). The beakers were 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, as in Potgieter & Wilke's study (1996).

3.1.3 Analyses

Data were checked for normality and square-root or log transformed where necessary. One-way analyses of variance were used to compare values between beddings, and Pearson correlations were used to test for relationships between the mean densities and absorbencies of the beddings. All the statistics were carried out using Minitab™ version 13.20 (Minitab Ltd, Pennsylvania, USA).

3.2 Experiment 2

Three technicians in different animal units were each asked to fill 6 rat cages with aspen woodchips and 6 with Alpha-Dri, for use in another study. They were not told that the amount of bedding used would be assessed. When they had filled the cages, the bedding depth was measured for each cage. The mass of bedding in the resulting 36 cages (12 cages for each technician), and their total predicted absorbencies, were calculated from the volume used. Values were compared using a general linear model with bedding and technician, plus their interaction, as factors.
4 Results

4.1 Experiment 1

As expected, absorbency differed significantly between beddings (absorption per cm$^3$: $F_{5, 18} = 88.49; P = <0.001$, and per gram: $F_{5, 18} = 753.90; P = <0.001$). Corncob had the highest absorbency per cm$^3$ (0.60+/−0.05 cm$^3$ saline/cm$^3$), as shown in Figure 1a. Aspen chips, Gold Flake and Tek-Fresh had relatively low volumetric absorbencies (0.32+/−0.02, 0.30+/−0.03, and 0.29+/−0.006 cm$^3$ saline/cm$^3$, respectively).

When the absorbency values were calculated per gram, however, the pattern was almost completely reversed (Figure 1b). Tek-Fresh was the most absorbent bedding by mass (4.42+/−0.13 cm$^3$ saline/g), while Corncob was the least absorbent (1.19+/−0.07 cm$^3$ saline/g).

Bedding density (Figure 2) correlated positively with absorbency by volume (Pearson coefficient = 0.835; $n = 6; P = 0.039$), but negatively with absorbency by mass (Pearson coefficient = −0.845; $n = 6; P = 0.034$) (Figure 3). To illustrate, corncob had the highest density and the highest absorbency by volume, but the lowest absorbency by mass, while the pattern was reversed for Tek-Fresh. The two absorbency calculations did not correlate directly with each other, but unsurprisingly showed a negative trend (Pearson coefficient = −0.701; $n = 6; P = 0.121$).
4.2 Experiment 2

There were considerable differences between the amounts of bedding that each technician used (F$_{2,30}$ = 14.15; $P < 0.001$). The technician in animal unit B used a metal scoop to measure out volumes of bedding (mean depth = 2.29 +/- 0.4 cm), while the other two simply shook bedding from its bag into the cages to reach a desired depth (mean depths = 1.75 +/- 0.3 and 2.54 +/- 0.4 cm, for units A and C respectively). However, in all cases, the depth of bedding in the cages did not depend on the type of bedding used (F$_{1,30}$ = 0.00; $P > 0.995$) (Figure 4).

With the depths of the two beddings being so similar, the total mass of the aspen chips per cage would be significantly less than the Alpha-Dri (F$_{1,30}$ = 11.59; $P = 0.002$) (Figure 5). In turn, this means that the amount of Alpha-Dri added to each cage would be predicted to absorb approximately 160% of that absorbed by the aspen, a mean of 1732 +/- 109 cm$^3$ of saline compared with only 1081 +/- 62 cm$^3$ respectively (Figure 6).

5 Discussion

Experiment 2 showed that technicians fill cages to reach a certain depth of bedding desired, rather than a certain mass of it (see also Gamble & Clough 1976, Ras et al. 2002). Indeed, bedding depth (and therefore volume) is probably the more relevant aspect for the animals concerned, since it must be deep enough to lie on and dig in for
example, but not so deep that it contacts the water spout and floods the cage, or fails to support the animals’ weight adequately and impairs their movement.

Since cages are filled by volume, absorbency per unit volume is the most relevant descriptor of a bedding’s moisture absorbing properties. Experiment 1 showed that the relative volumetric absorbencies of the six beddings were almost the exact reverse of their absorbencies per mass, which are usually reported. Experiment 2 was an opportunistic one, and the Alpha-Dri and aspen chips used were not particularly suitable beddings to illustrate how these reported absorbency values can be misleading in practice, because Alpha-Dri is more absorbent than aspen chips regardless of whether absorbency is calculated per unit volume or mass (Figures 1 and 2). However, to give the most extreme examples, if Tek-fresh and corncob had been similarly compared, the Tek-Fresh would have only absorbed about half the liquid absorbed by the corncob (see Figures 1 and 2). In contrast, reported absorbency values calculated per unit mass would give the misleading impression that Tek-Fresh would absorb around three times more liquid than corncob.

Therefore, we show that under applied conditions in rodent cages, corncob and to a lesser extent Omega-dri and Alpha-Dri, are the most absorbent beddings tested here. By implication then, these should be the most effective of the beddings for reducing the build-up of ammonia and other cage pollutants, assuming that absorption of saline adequately predicts the absorption of moisture from urine and faeces. Indeed, in studies that have directly measured the ammonia production of these beddings, corncob has produced the least ammonia, Alpha-dri produced relatively little, aspen chips produced a lot, and CareFresh® (which is very similar to Tek-Fresh) produced the most (Hawkins et
al. 2003, Perkins & Lipman 1995). However, a cross-laboratory study (Burn et al. in prep.) found no difference in the ammonia levels produced after 1 week by aspen chips and Alpha-Dri. This may be because absorbency is not the only quality that affects bacterial growth and ammonia production (Perkins & Lipman 1995). For example, the urease content, toxins and bacterial flora inherent within the source material of the bedding, as well as the technique used to purify it, will affect the amounts and types of pollutants produced (Ewaldsson et al. 2002, Gale & Smith 1981, Hawkins et al. 2003, Potgieter & Wilke 1992). Potgieter & Wilke (1996) also suggested that the particle size of the bedding might be important, since beddings that provide a larger surface area will facilitate the evaporation of water and volatiles.

It is also important to consider the other qualities of beddings that could affect the health and welfare of animals. These qualities include tactile characteristics, thermal properties, and the levels of dust, bacteria and toxins inherent within them (Arnold & Estep 1994, Blom et al. 1996, Ewaldsson et al. 2002, Odynets et al. 1991, Potgieter & Wilke 1992, Wirth 1983). Aspen chip beddings, although relatively un-absorbent, are preferred by rats and mice over most other beddings (Mulder 1975, Odynets et al. 1991, Ras et al. 2002; but see Blom et al. 1996), and have proven relatively non-toxic (Odynets et al. 1991; but see Burn et al. in prep.). To our knowledge, not all the beddings here have been tested regarding animal preference or bedding toxicity.

More work is thus necessary to fully quantify the relative health and welfare benefits of the various beddings available, if we are to be able to compare them on the basis of all their important characteristics. However, when considering absorbency, bedding manufacturers should publicise absorbency values and express them in terms of
volume, enabling users to have some measure of the likely polluting rates of different
beddings in applied situations.

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Figure 1

(a) Absorbency by volume (cm³ saline/cm³ bedding)

(b) Absorbency by mass (cm³ saline/g bedding)
Figure 2
Figure 3

(a) 

(b)
Figure 4

Mean bedding depth within cages (cm)

Animal unit

Alpha-Dri
Aspen
Figure 5

Predicted mean mass of bedding (g/cage)

Animal unit

Alpha-Dri
Aspen
Figure 6

![Graph showing predicted mean absorbency (cm³ saline/cage) across animal units A, B, and C for Alpha-Dri and Aspen products.](image)
Figure captions

Figure 1. The saline absorbency (Mean +/- SD) of six different rodent beddings (n = 4) calculated (a) by volume, and (b) by mass of bedding. Depending on how the absorbency is calculated, the relative ranking of the beddings is almost completely reversed. T-F = Tek-Fresh; G-F = Gold Flake; A-C = aspen chips; A-D = Alpha-Dri; O-D = Omega-Dri; and C-C = corncob.

Figure 2. The density (Mean +/- SD) of six different rodent beddings (n = 4). T-F = Tek-Fresh; G-F = Gold Flake; A-C = aspen chips; A-D = Alpha-Dri; O-D = Omega-Dri; and C-C = corncob. The rank order of bedding density is the same as that for absorbency calculated per unit volume, but not per unit mass, of bedding.

Figure 3. (a) The absorbency calculated by volume of bedding shows a positive relationship with bedding density, while (b) the absorbency calculated by mass shows a negative relationship with bedding density. From left to right, the points represent Tek-Fresh, Gold Flake, aspen chips, Alpha-Dri, Omega-Dri, and corncob.

Figure 4. The mean (+/- SD) depths of two beddings (Alpha-Dri and aspen chip) within rat cages (n = 6 for each group) in three different animal units. The depths do not differ between the beddings, showing that in practice bedding is added on the basis of volume,
regardless of bedding type. However, the depths used do differ significantly between animal units.

Figure 5. The mean (+/- SD) masses of two beddings (Alpha-Dri and aspen chip) within rat cages ($n = 6$ for each group) in three different animal units. Mass values were predicted using the volumes used and the known densities of the two beddings. In each animal unit, the total mass per cage of the denser Alpha-Dri bedding is higher than that of the less dense aspen chips, because the beddings are added to the same depth in each cage.

Figure 6. The mean (+/- SD) saline absorbencies of two beddings (Alpha-Dri and aspen chip) within rat cages ($n = 6$ for each group) in three different animal units. The absorbency values per cage were predicted using the volumes added by working technicians, and the known saline absorbencies of the two beddings. The actual volume of Alpha-Dri used in cages would absorb more saline than the equivalent volume of aspen chips.