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

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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 particular 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™ and Omega-Dri™), reclaimed wood pulp (Tek-Fresh™), 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 as seen 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 details of bedding absorbencies in terms of volume, in order to help predict the relative absorbencies of the beddings in practical situations.

Keywords Bedding; ammonia; rodents; hygiene

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 (Raynor *et al.* 1983, Perkins & Lipman 1995, Hawkins *et al.* 2003). If ammonia is allowed to rise in animal cages, it can cause respiratory damage (Serrano 1971, Broderson *et al.* 1976, Gamble & Clough 1976) 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).

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Ammonia can build up if cages are not cleaned frequently enough, if there is a high density of animals, if ventilation rates are low, or if ambient temperature and/or humidity is favourable for bacterial growth (Gamble & Clough 1976, Eveleigh 1993, Ishii *et al.* 1998). In addition, it rises dramatically if a water spillage occurs in the cage and causes the bedding to become waterlogged (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. Sokol & Valtin 1982, Homma *et al.* 2002). As a result, an animal unit at Oxford University maintains C57BL/ksOlaHsd-L*epr* 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, personal communication).

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 levels 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 cage-mates than did those 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 (Wirth 1983, Odynets *et al.* 1991, Ewaldsson *et al.* 2002, Burn *et al.* in preparation). Highly absorbent beddings should also not be used for animals at risk of ringtail or other dry skin conditions (Clough 1976).

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 gram or kilogram), 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 animal technicians filled cages with different beddings to the same depth, or to depths appropriate for their absorbencies by mass (Experiment 2).

Methods

Experiment 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 (Perkins & Lipman 1995, Hawkins *et al.* 2003), 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.

Absorbency testing Four 50 cm^3 samples of each bedding were placed into glass beakers. Each sample was then weighed, and 100 cm^3 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 and 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 and Wilke's study (1996).

Analyses Data were checked for normality and were 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).

Experiment 2

Three technicians in different animal units were each asked to fill six rat cages with aspen woodchips and six 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-type and technician, plus their interaction, as factors.

Results

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 Fig 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).

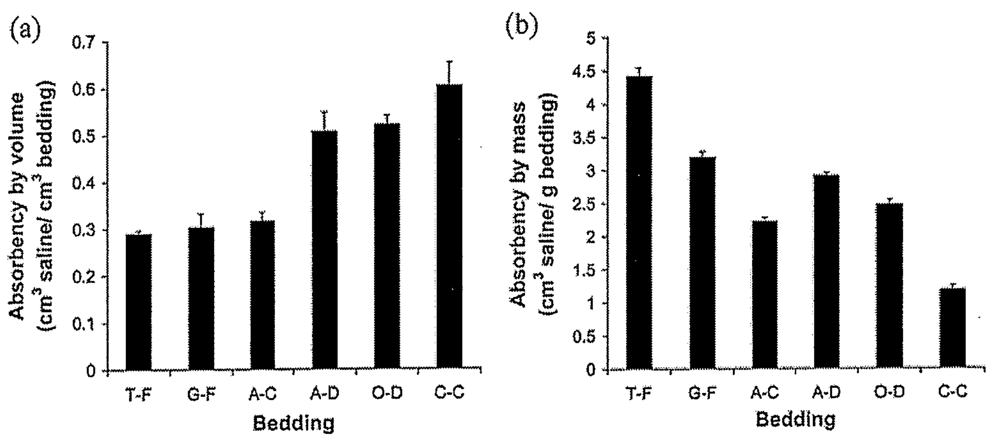

Fig 1 The saline absorbency (mean ± SD) of six different rodent beddings ($n = 4$) calculated (a) by volume, and (b) by mass of bedding. Depending on how the absorbency is calculated, the relative ranking of the beddings is almost completely reversed. T-F = Tek-Fresh; G-F = Gold Flake; A-C = aspen chips;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

When the absorbency values were calculated per gram, however, the pattern was almost completely reversed (Fig 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 (Fig 2) correlated positively with absorbency by volume (Pearson coefficient $= 0.835$; $n = 6$; $P = 0.039$), but negatively with absorbency by mass (Pearson coefficient $= -0.845$; $n = 6$; $P = 0.034$) (Fig 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 $= -0.701$; $n = 6$; $P = 0.121$).

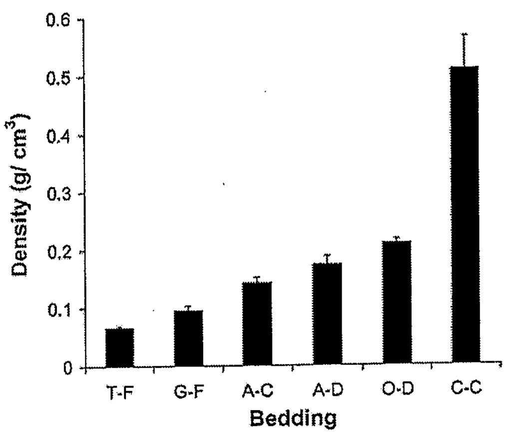

Fig 2 The density (mean ± SD) of six different rodent beddings ($n = 4$). T-F = Tek-Fresh; G-F = Gold Flake; A-C = aspen chips; A-D = Alpha-Dri; O-D = Omega-Dri; and C-C = corncob. The rank order of bedding density is the same as that for absorbency calculated per unit volume, but not per unit mass,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

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 $= 2.29 \pm 0.4$ cm), while the other two simply shook bedding from its bag into the cages to reach a desired depth (mean depths $= 1.75 \pm 0.3$ 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 ($F_{1,30} = 0.00$; $P > 0.995$) (Fig 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 ($F_{1,30} = 11.59$; $P = 0.002$) (Fig 5). In turn, this means that the amount of Alpha-Dri added to each cage would be predicted

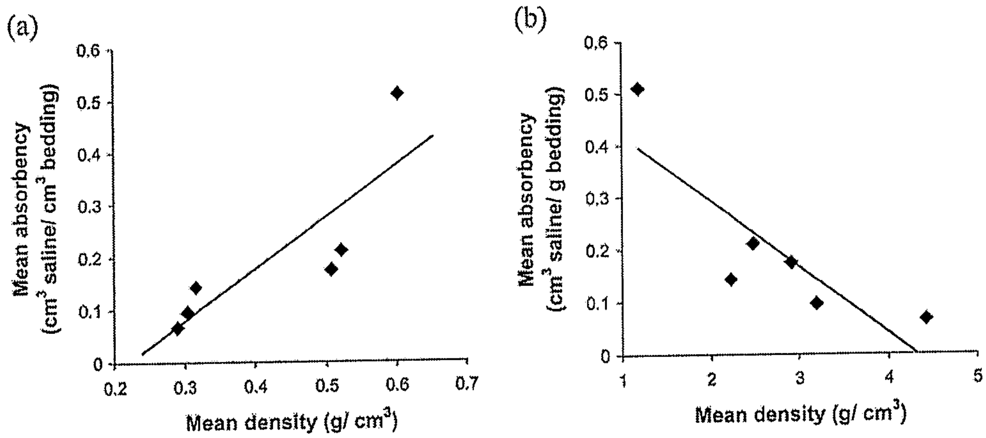

Fig 3 (a) The absorbency calculated by volume of bedding shows a positive relationship with bedding density, while (b) the absorbency calculated by mass shows a negative relationship with bedding density. From left to right, the points represent Tek-Fresh, Gold Flake, aspen chips, Alpha-Dri, Omega-Dri, and corncob 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 (Fig 6).

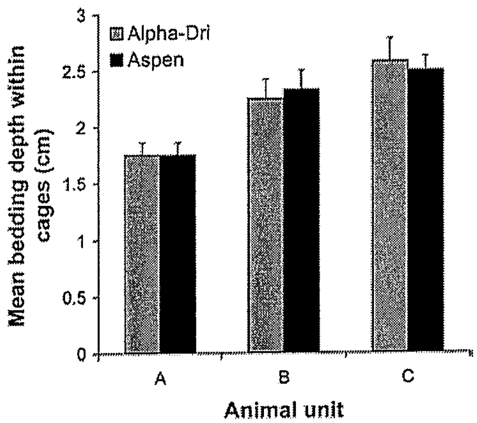

Fig 4 The mean (±SD) depths of two beddings (Alpha-Dri and aspen chips) within rat cages ($n = 6$ for each group) in three different animal units. The depths do not differ between the beddings, showing that in practice, bedding is added on the basis of volume, regardless of bedding type. However, the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

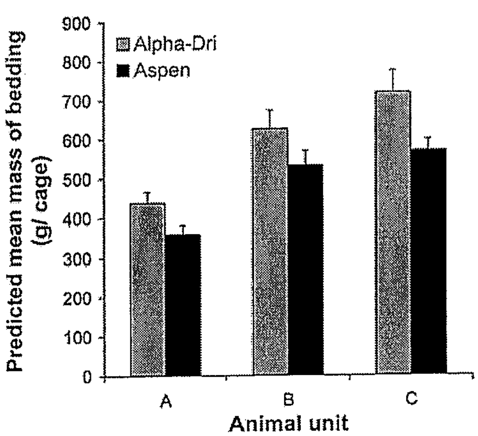

Fig 5 The mean (±SD) masses of two beddings (Alpha-Dri and aspen chips) within rat cages ($n = 6$ for each group) in three different animal units. Mass values were predicted using the volumes used and the known densities of the two beddings. In each animal unit, the total mass per cage of the denser 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

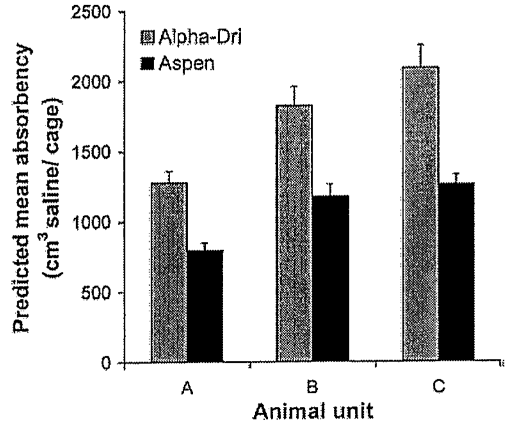

Fig 6 The mean (±SD) saline absorbencies of two beddings (Alpha-Dri and aspen chips) within rat cages ($n = 6$ for each group) in three different animal units. The absorbency values per cage were predicted using the volumes added by working technicians, and the known saline absorbencies of the two beddings.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

Discussion

Experiment 2 showed that technicians fill cages to reach a desired depth of bedding, rather than to provide a desired 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 impairs the animals' 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 for illustrating how the reported absorbency values of the beddings can be misleading in practice, because Alpha-Dri is more absorbent than aspen chips regardless of whether absorbency is calculated per unit volume or by mass (Figs 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 Figs 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 would.

Therefore, we show that under applied conditions in rodent cages, corncob and, to a lesser extent, Omega-Dri and Alpha-Dri, were 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 (Perkins & Lipman 1995, Hawkins *et al.* 2003). However, a cross-laboratory study (Burn *et al.* in preparation) found no difference in the ammonia levels produced after one week by aspen chips and by 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 techniques used to purify it, will affect the amounts and types of pollutants produced (Gale & Smith 1981, Potgieter & Wilke 1992, Ewaldsson *et al.* 2002, Hawkins *et al.* 2003). Potgieter and 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 (Wirth 1983, Odynets *et al.* 1991, Potgieter & Wilke 1992, Arnold & Estep 1994, Blom *et al.* 1996, Ewaldsson *et al.* 2002). 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 to be relatively non-toxic (Odynets *et al.* 1991; but *see* Burn *et al.* in preparation). To our knowledge, not all the beddings here have been tested with respect to 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 practice.

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