Effect of different bedding materials on the reproductive performance of mice

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ABSTRACT

Vermiculite, pine shavings and unbleached eucalyptus pulp contact-bedding were compared using the number of litters and individuals born and weaned, mortality rates at different stages of the lactation period, and the weight increase of pups as evaluation indices for bedding quality. These bedding materials exerted different effects on the reproductive performance of the same mouse strain. The same is true for the effect of a specific bedding material on different mouse strains. These effects are most pronounced during the first 4 days of life. As a whole, the results demonstrated that eucalyptus pulp was the better bedding type, followed by pine shavings and vermiculite. The latter material had a detrimental effect on the mating success of AKR mice.

Key words: AKR, bedding, C57BL6, gestation, growth rate, hybrid, lactation, mice, pine shavings, survival success, unbleached eucalyptus pulp, vermiculite.

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INTRODUCTION

The regular provision of sterile bedding materials to laboratory animals is standard animal husbandry procedure. The main purpose of this is to improve hygiene as well as the animals' micro-environment, thus reducing stress.

Bedding materials can bring about changes in litter size, lactation, and number of offspring weaned[4,5,8,9]. Burkhart and Robinson[3] attributed the increased mortality rate observed with cedar shavings to a constituent of cedar, possibly one of the volatile hydrocarbons, which acts as a toxin via the dam. Foetal environment, *i.e.* maternal blood levels of hormones, oxygen, and the presence and levels of toxins, drugs, certain food elements and waste products, could be changed by bedding-related variables. By breeding mice on vermiculite, Hastings[4] noted a decrease in the number of litters born and their growth rate when compared to sawdust. This was attributed to vermiculite.

Very little, apart from the abovementioned findings with regard to vermiculite[4], the enzyme induction and cytotoxic properties[11], and dust production and content of bedding materials[12], is known about bedding types currently used in South Africa. In an effort to eliminate some of these inadequacies, vermiculite and pine shavings, with a users' frequency of 27 % and 21 % respectively[10], were evaluated for their influence on reproduction and litter survival. These materials were compared to a promising new bedding type consisting of unbleached pulp[11,12] derived from *Eucalyptus* spp.

MATERIALS AND METHODS

Caging

Type M2 polypropylene mouse breeding cages (330 × 150 × 130 mm; floor area 390 cm^2) with an angled recess for a slanted stainless steel grill with a food hopper and water bottle (Labotec) were used. To prevent cross-contamination of bedding-related variable constituents, 18 cages were assigned to each of 3 flexible-film breeding-isolators (187 × 120 × 85 cm; Labotec), *i.e.* 1 isolator for each bedding type investigated.

Bedding materials

(a) Vicafil Vermiculite (Mandoval Vermiculite) (exfoliated).
(b) Pine shavings from *Pinus elliottii* (Byproduct Development Services).
(c) Unbleached pulp from *Eucalyptus* spp. (Sappi Kraft).

All 3 types of bedding were steam-sterilised at 130 °C for 20 minutes and applied at a depth of 20 mm in cages. Cages and contact bedding were changed weekly.

Environmental parameters

A personal computer and EXP-16/16A multiplexer/amplifier system (Keithley Metrabyte Corporation) with appropriate software were used in conjunction with custom-built electronic probes for measuring temperature, relative humidity and air flow. Air-flow probes were calibrated against a hotpoint air-flow meter (Wilh. Lambrecht, Type 641 N). Environmental parameters were monitored half-hourly.

Room temperature and relative humidity (RH) were regulated using steam pre-heat and heat exchange coils and steam humidifiers in the fresh-air duct system entering the animal room. Room parameters (25.2 ± 0.5 °C and 54.4 ± 6.3 % RH) were measured 2.7 m above the floor at the centre of the room, while the same parameters were monitored inside the isolators (22.4 ± 0.3 °C and 48 ± 4 % RH).

Fresh air was supplied to the room at a rate of 8 air changes per hour, while isolator air flow, measured at the outlet, equalled 894 ± 0.6 cm/min. Spent air from the isolator was fed directly into the exhaust air-ducts of the room.

Light intensity in the room, measured 1 m above the floor, was 200–260 lux and inside the isolators it varied between 124 and 152 lux. A 12 h light (06:00–18:00) / dark cycle was maintained during the study.

Food (Rat and Mouse Breeder Feed, Specialist Animal Feeds) and chlorinated (0.1–0.3 mg/ℓ) tap water were supplied *ad libitum.*

Animals

The offspring originating from 2 inbred mouse strains of conventional health status, AKR and C57BL6, and their F$_1$ hybrid (B6AKF$_1$), were used. The hybrids were included to reduce the effect of inbreeding on reproduction to a minimum.

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Six nulliparous pairs, 60 days of age, of each strain were assigned to each bedding type (6 pairs × 3 strains × 3 bedding types). These monogamous paired mice were bred for 110 days after the first mating, *i.e.* one 5-day oestrous cycle plus 5 consecutive gestation periods [5 + (5 × 21) days], permitting a total of 30 litters per strain per bedding type. Breeding males were removed at this stage and the females allowed to produce and rear any ensuing litters. The breeding period was limited to 5 consecutive litters because fecundity studies can be affected by a decline in reproduction, with less frequent and smaller litters, after the first 5 litters[6]. As length of gestation varies between strains[14], offspring were weaned on day 18 *post partum.* Cages were examined at 12-hour intervals and all offspring found were recorded as newly born.

The following variables were measured:

(a) Litter size, number of animals born alive.
(b) Number of pups alive at 12 hours, and on days 4, 9, 12, 15 and 18.
(c) Number of animals weaned per litter.
(d) Mean weight of litters on days 4, 9, 12, 15 and 18.
(e) Number of breeding females producing litters.
(f) Number of litters born per female.

Mirone *et al.*[7] demonstrated that 91 % of all litter deaths occur during the first 4 days *post partum,* whereas only 9 % of deaths occur during the lactation period. The number of young alive on day 4 was therefore chosen as an indicator of pup viability.

Since young mice start to consume solid food by day 12[5] and milk yield reaches maximum levels on days 12–13 *post partum*[14], the day 12 data were used to determine the lactation index.

To reveal any antenatal effects of bedding on gestation and labour, expected litter size was determined by counting the number of foetuses in 10 pregnant females of each strain (day 17 of pregnancy). These animals were born and bred on pine shavings.

Statistical analysis

An estimation of the effect of different bedding materials on antenatal and postnatal litter sizes and postnatal weights at various ages was calculated using the indices of Iturrian and Fink[5]:

$$\text{Gestation \& birth} = \frac{\text{Average litter alive at birth} \times 100}{\text{Expected litter size}}$$

$$\text{Viability} = \frac{\text{Average litter alive on day } 4 \times 100}{\text{Average litter alive after birth}}$$

$$\text{Lactation} = \frac{\text{Average litter alive on day } 12 \times 100}{\text{Average litter alive on day } 4}$$

In order to avoid misinterpretation of results, Iturrian and Finks' reproduction index (average litter weaned expressed as a percentage of the average litter born alive), which described offspring survival rather than pup production, was renamed survival success:

$$\text{Survival success} = \frac{\text{Average litter weaned} \times 100}{\text{Average litter born alive}}$$

As the abovementioned indices did not incorporate all the possible bedding effects on fecundity (for example, the gestation and birth index addressed litter size, whereas bedding effects on the conception/fertility of breeding pairs were ignored), two more indicators for bedding-related influences were calculated. These were:

(a) mating success, *i.e.* index of parental conception/fertility given as the number of litters born expressed as a percentage of the number of potential litters (6 breeding pairs × 5 possible litters/pair), and
(b) production, *i.e.* the ratio between the number of animals weaned and the potential production (expected litter size × 30 possible litters):

$$\text{Mating success} = \frac{\text{Number of litters born} \times 100}{\text{Expected number of litters}}$$

$$\text{Production} = \frac{\text{Number of animals weaned}}{\text{Number of animals expected}}$$

To illustrate the overall effect of the materials investigated on the number of litters born and the number of animals weaned, data obtained on a specific bedding type, irrespective of strain, were pooled and expressed using the mating success and production indices respectively.

Two data sets, one including zero values and the other excluding these values, were used for interpretation. The reasons for this are as follows:

(i) Zero values were included to capture the effect of these materials on: (a) mating success, *i.e.* to ascertain whether bedding material could alter the number of litters born, and (b) the viability, lactation and survival success indices, *i.e.* to illustrate the effect of bedding on survival. In other words, if all the pups of a specific litter died during any one of the periods described by these indices, omission of that specific zero value will result in biased conclusions. See the production indices section under results for further explanation.
(ii) Zero values were excluded from the data set used to calculate the gestation and birth index (the influence of bedding material on the number of foetuses born, *i.e.* from conception until birth), in order to eliminate effects originating from the influence of bedding on conception/fertility of parents.

In order to secure all possible bedding-related effects, the data relating to the number of animals alive at the different stages of the study were also subjected to an analysis of covariance (using the number born as covariate), while an analysis of variance was performed on the data obtained for growth rate (SAS/STAT, Version 6, SAS Institute). Differences observed between bedding types were described as statistically significant if $p < 0.1$, *i.e.* at a confidence level of 90 %.

RESULTS

Analysis of covariance and variance

Results of the analysis of covariance, with regard to the number of animals alive at different stages during the study, showed statistically significant differences between the different bedding materials for AKR offspring only (Table 1). According to these results, vermiculite and eucalyptus pulp had fewer effects on AKR pup survival during the first 4 days of life than pine shavings, while vermiculite was the better material from day 9–18. This was also reflected by the viability, lactation, survival success and production indices obtained for AKR litter survival (Figs 1, 2).

The influence of different bedding materials on the growth rate of pups, evaluated with an analysis of variance, showed statistically significant differences for B6AKF1 pups during all the stages (at the 95 % confidence level) between eucalyptus pulp (high) and vermiculite (low). On day 18 a statistically significant difference occurred at the 90 % confidence level between eucalyptus pulp (high) and pine shavings (low). Day 4 recorded the highest growth rate for C57BL6 young on pine shavings (Fig. 4).

Production indices

Zero values were included in the data set used to calculate the mating success, viability, lactation and survival success indices, as their omission would have biased some of the conclusions drawn. For example, the viability of the AKR strain kept on eucalyptus pulp was 57.88 % with zero values included versus 84.4 % when excluded. The same values obtained with AKRs on vermiculite were

Table 1: **A comparison of the statistically significant effect of bedding type on the number of AKR, B6AKF1 and C57BL6 pups alive at different stages during the lactation period. The 2 bedding types compared per column are listed according to their LS mean (mean of the model) values, *i.e.* the bedding contributing to the better rate is listed first.**

Variable	**AKR**	**B6A**	**C57**	**90 % confidence level**	**95 % confidence level**
12 Hour	υ				Pulp and pine (p <0.014)
12 Hour	υ				Vermiculite and pine (p <0.010)
Day 4	υ				Pulp and pine (p <0.029)
Day 4	υ				Vermiculite and pine (p <0.002)
Day 9	υ			Vermiculite and pulp (p <0.098)	Vermiculite and pine (p <0.003)
Day 12	υ			Vermiculite and pulp (p <0.088)	Vermiculite and pine (p <0.003)
Day 15	υ			Vermiculite and pulp (p <0.088)	Vermiculite and pine (p <0.003)
Day 18	υ			Vermiculite and pulp (p <0.087)	Vermiculite and pine (p <0.003)

Table 2: **A comparison of the effect of different bedding materials on the number of AKR, C57BL6 and B6AKF1 pups born and bred on 3 different bedding materials, namely vermiculite (V), pine shavings (P) and eucalyptus pulp (E). Data from breeding pairs not producing offspring during the study were omitted from the calculation of mean litter size and standard deviation. Viable pups equals the number of pups alive 12 hours after parturition. The number of litters born per bedding type illustrates bedding influences on parental conception/fertility. Expected litter size was obtained using pine shavings as bedding material.**

	Born			**Viable**			**Day 4**			**Day 9**			**Day 12**			**Day 15**			**Day 18**		
AKR	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**
Number of pups	87	138	132	82	87	117	69	42	76	67	38	60	67	38	59	67	38	59	66	37	58
Mean litter size	7.91	6.00	6.00	8.20	5.44	5.32	6.90	5.25	5.07	7.44	5.43	5.00	7.44	5.43	4.92	7.44	5.43	4.92	7.33	5.29	4.83
(SD)	(2.21)	(2.66)	(2.60)	(0.92)	(2.50)	(2.77)	(2.02)	(2.12)	(2.22)	(1.13)	(1.81)	(2.09)	(1.13)	(1.81)	(2.19)	(1.13)	(1.81)	(2.19)	(1.12)	(1.80)	(2.17)
Number of litters	11	23	22	10	16	22	10	8	15	9	7	12	9	7	12	9	7	12	9	7	12
Expected litter size		6.00																			
(SD)		(2.00)																			
Number of litters		10																			
C57BL6	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**
Number of pups	164	126	143	150	114	127	135	106	111	133	105	107	132	95	105	130	94	105	130	92	104
Mean litter size	8.63	7.41	7.53	7.89	7.13	6.68	7.11	6.63	5.84	7.00	6.56	5.63	6.95	5.94	5.53	6.84	5.88	5.53	6.84	5.75	5.47
(SD)	(2.73)	(2.45)	(1.90)	(2.85)	(2.25)	(1.67)	(2.56)	(2.22)	(1.83)	(2.45)	(2.22)	(1.77)	(2.41)	(2.32)	(1.74)	(2.39)	(2.33)	(1.74)	(2.39)	(2.35)	(1.84)
Number of litters	19	17	19	19	16	19	19	16	19	19	16	19	19	16	19	19	16	19	19	16	19
Expected litter size		8.50																			
(SD)		(1.72)																			
Number of litters		10																			
B6AKF1	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**	**V**	**P**	**E**
Number of pups	265	307	301	263	299	298	257	290	291	250	286	289	250	286	288	250	285	288	250	284	288
Mean litter size	12.05	11.37	10.38	11.95	11.07	10.28	11.68	10.74	10.03	11.36	10.59	9.97	11.36	10.59	9.93	11.36	10.56	9.93	11.36	10.52	9.93
(SD)	(3.48)	(2.66)	(2.51)	(3.57)	(2.44)	(2.40)	(3.67)	(2.41)	(2.40)	(3.29)	(2.48)	(2.43)	(3.29)	(2.48)	(2.42)	(3.29)	(2.48)	(2.42)	(3.29)	(2.48)	(2.42)
Number of litters	22	27	29	22	27	29	22	27	29	22	27	29	22	27	29	22	27	29	22	27	29
Expected litter size		9.92																			
(SD)		(2.91)																			
Number of litters		10																			

79.31 % and 87.24 % respectively. Thus, with zero values excluded, a nearly negligible difference of 2.84 % was obtained for viability between eucalyptus pulp and vermiculite. However, more than 3 times as many AKR pups died on eucalyptus pulp compared to vermiculite, the mortality rates being 42.42 % and 20.69 % respectively. Lactation and survival success indices proved to be similarly misleading. Thus, although the inclusion of zero values had a negative effect on the means and standard deviations, it provided a more precise account of the effect of the different bedding materials on the variable tested.

Zero values were excluded from the data set used to calculate the gestation and birth index (average litter size expressed as a percentage of the expected litter size). Their inclusion led to distortions similar to those described above, namely that the AKR gestation and birth index indicated that vermiculite had a more adverse effect on litter size, only 48.33 % of the animals expected being born. This was not true, as the zero values reflected the effect of bedding on the conception/fertility of the parents rather than its influence on the foetus. If excluded, an AKR mean litter size of 7.91 ± 2.21 was obtained. This was even better than the expected litter size of 6.0 ± 2.0 for this strain (Table 2).

Mating success

AKRs maintained on vermiculite, compared to their counterparts kept on eucalyptus pulp and pine shavings, demonstrated a marked reduction in the number of litters produced, namely 36.7 % (n = 11) in contrast to 73.3 % on

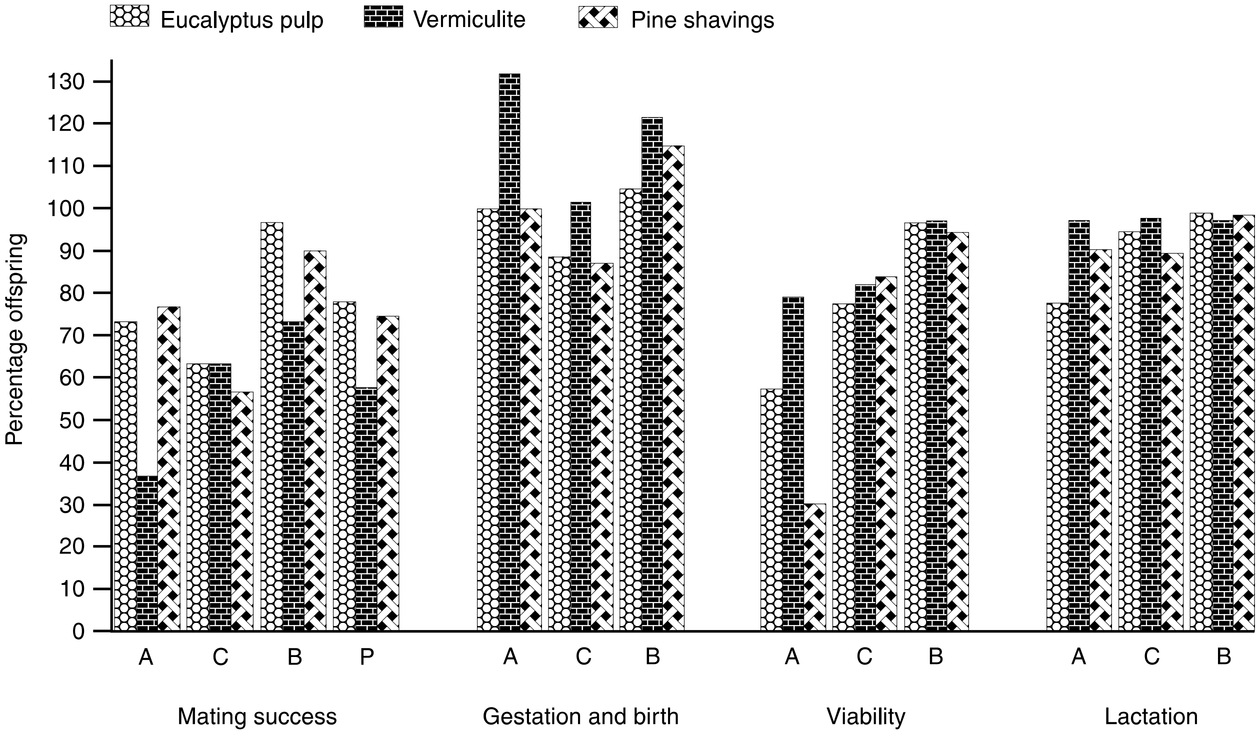

Fig. 1: **The effect of 3 different bedding materials on the mating success, gestation period, viability and lactation indices of AKR (A), C57BL6 (C) and B6AKF1 (B) mice. The pooled litter size (P) (*i.e.* the number of litters born on a specific bedding material, irrespective of strain, and expressed as a percentage of the number of possible litters) is used as an indicator of the overall bedding effect on mating success.**

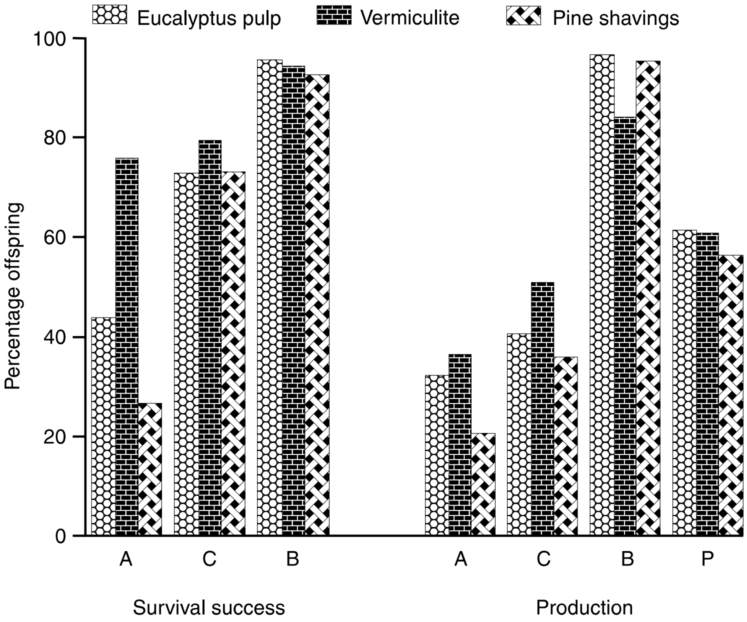

Fig. 2: **The effect of 3 different bedding materials on the survival success and production indices of AKR (A), C57BL6 (C) and B6AKF1 (B) mice. The total number of animals weaned on a specific bedding material – irrespective of strain – were pooled and expressed as a percentage of the expected litter size × the possible number litters (P). The latter is used as an indicator of the overall bedding effect on the number of animals produced.**

eucalyptus pulp (n = 22) and 76.7 % on pine shavings (n = 23). Slightly fewer (6.6 %) C57BL6 litters were born on pine shavings compared to the other bedding materials used. The lowest number of B6AKF1 litters was born on vermiculite, *i.e.* 73.33 % compared to 96.67 % on eucalyptus pulp and 90.0 % on pine shavings (Fig. 1).

As the experimental design varied between the different groups in bedding material only, since the same environmental conditions, feeding regime and health and genetic status applied, the observed decline in the number of litters born between bedding types could only be bedding-related. If the numbers of litters born on a specific bedding type, irrespective of strain, were pooled and expressed as a percentage of the expected number of litters, vermiculite also had the lowest percentage of litters born, 57.8 % versus 74.4 % for pine shavings and 77.8 % for eucalyptus pulp (Fig. 1).

The decline in the mating success of AKRs on vermiculite seems to be a bedding-initiated conception/fertility problem in the parents. Four of the original AKR females from the pairs held on vermiculite that produced only 1 litter, were replaced with young F_2 females at the conclusion of this study. Three of these pairs produced litters within 30 days of mating, eliminating male infertility as a possible cause of this decline in reproduction.

The cause of the decline in female reproduction was not investigated, but might be dust-related. Venter[13] stated that talc, used for personal hygiene by women, can enter the vagina during coitus. This might also occur in mice. The fact that one of the AKR pairs on vermiculite produced their only litter towards the end (day 129) of this study could be an indication that dust can interfere with embryonic growth or that foreign bodies might impede implantation. This might also be the reason for the reproduction failure observed in one of the vigorous B6AKF1 pairs kept on vermiculite. This, however, requires further investigation.

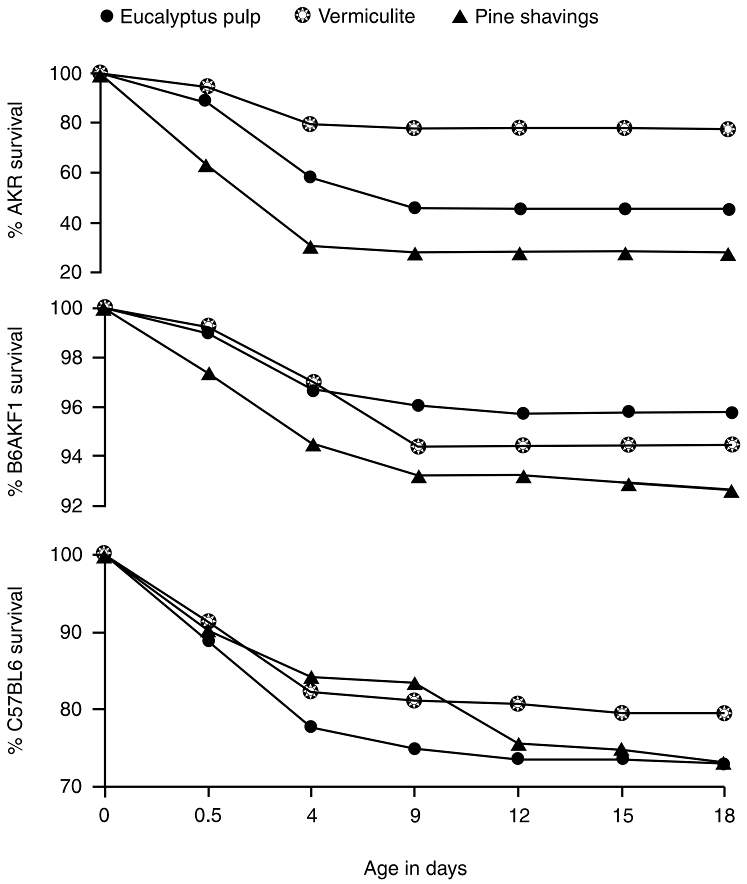

Fig. 3: **The survival rate of AKR (A), B6AKF1 (B) and C57BL6 (C) pups, expressed as the ratio between the number born alive and those alive at different stages up to weaning, on 3 different bedding materials.**

Gestation and birth

Using the expected litter size as indicator to estimate the antenatal effects of bedding on litter size, more AKR, C57BL6 and B6AKF1 pups were born per litter on vermiculite than on eucalyptus pulp and pine shavings (Table 2; Fig. 1). Litter sizes obtained for these strains on vermiculite were in all instances better than the expected litter sizes obtained for these strains (Table 2).

Compared to vermiculite, smaller litter sizes were also recorded for the C57BL6 and B6AKF1 strains bred on eucalyptus pulp and pine shavings. Nearly 10 % more B6AKF1 pups were born per litter on pine shavings compared to eucalyptus pulp (Table 2; Fig. 1).

Vermiculite thus had fewer or no post-conception effects on litter size. This is substantiated by the *in vitro* cytotoxic properties of these bedding materials[11]. Vermiculite, a chemically inert material, had the smallest effect, followed by unbleached eucalyptus pulp and pine shavings.

Burkhart and Robinson[3] attributed a higher pup mortality rate observed with aromatic cedar shavings to a toxic compound in the shavings. According to these authors this compound is ingested or inhaled by rat pups or even transferred via the dams' milk. The effect of bedding materials on the number of pups born per litter in the present study thus verifies an *in utero* involvement. Vermiculite, an inert material, enhanced litter size, while litter size decreased on eucalyptus pulp and pine shavings. A change in foetal environment, *i.e.* maternal blood levels of toxins, thus occurred on eucalyptus pulp and pine shavings which could be the result of constituents in these wood-derived bedding materials. This is further substantiated by the variance observed in C57BL6 litter size (vermiculite > eucalyptus pulp > pine shavings) between these materials that followed the same sequence reported for the cytotoxicity of these materials (vermiculite < eucalyptus pulp < pine shavings)[11].

Viability (Birth to day 4)

In this study the mortality rate was highest during the first 4 days of life, which corresponds with the findings of Mirone *et al.*[7]. It was most pronounced in the AKR strain (between 20.7 % and 69.6 % mortality), while considerably lower mortality rates were observed in the B6AKF1 (3.0–5.5 %) and C57BL6 (15.9–22.4 %) strains (Table 2; Figs 1, 3).

The lowest AKR pup viability figures were experienced with pine shavings (30.43 %) followed by eucalyptus pulp (57.58 %) and vermiculite (79.31 %) (Fig. 1). The analysis of covariance obtained for this time interval indicated that the difference in AKR litter size between eucalyptus pulp and pine shavings, and between vermiculite and pine shavings, was statistically significant (Table 1). Although, according to that procedure, the observed difference of 21.73 % in mortality rate between vermiculite (<) and eucalyptus pulp was not statistically significant, this must certainly be of clinical importance, especially from a production and/or ethical viewpoint.

Most of the dead AKR pups (born on pine shavings) recorded within 12 hours of birth ($n = 21$), *i.e.* those that were not completely cannibalised by parents, had milk in their stomachs ($n = 17$), whereas only 4 had no milk. This is similar to the findings of Mirone *et al.*[7], *i.e.* that most deaths are the result of something other than a lactation problem. As bedding material was the only variable, the difference in AKR pup mortality at 4 days of age can only be ascribed to this factor.

Comparison of the increase in mortality rate of both AKR and B6AKF1 (Fig. 3) mice on eucalyptus pulp and vermiculite, between birth and 12 hours and between 12 hours and day 4, reveal that mortality increased more rapidly during the latter period. This might indicate a postnatal effect rather than one exerted during gestation. If the effect was exerted during gestation one would expect that higher mortality would occur early and stabilise later. Such an initially high mortality rate that stabilised between 12 hours and 4 days *post partum* was observed for C57BL6 pups on pine shavings, *i.e.* pine shavings' postnatal effect on C57BL6 pups (age 12 hours to 4 days) was less than that experienced during the preceding period (*i.e.* antenatal effect > postnatal effect). A higher mortality rate during the birth to 12 hour interval was experienced with AKR and B6AKF1 pups on pine shavings.

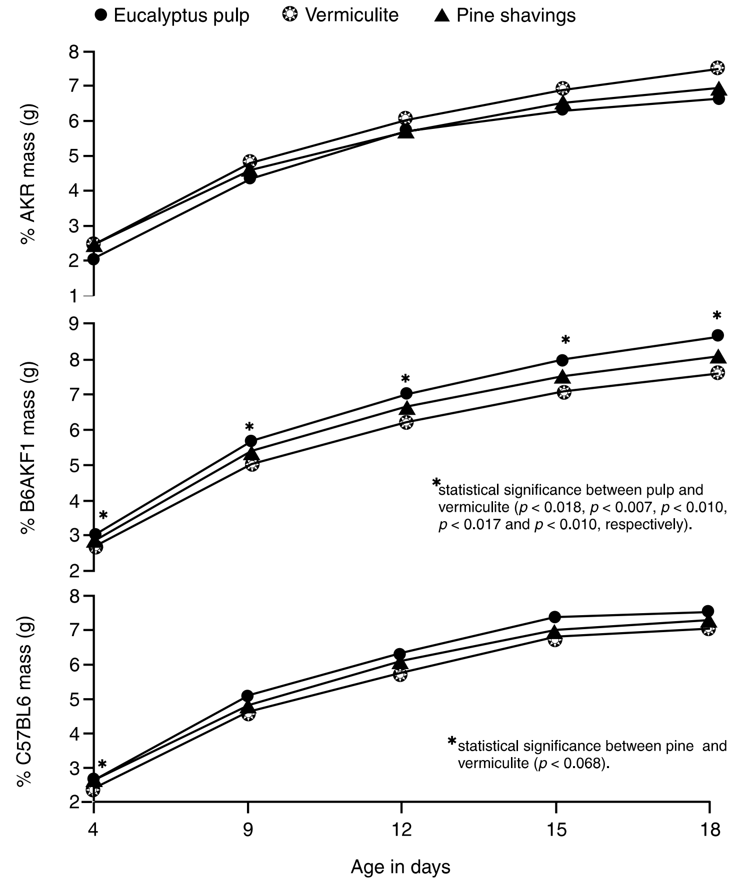

Fig. 4: **A comparison of the growth rate of AKR, B6AKF1 and C57BL6 pups on 3 different bedding materials. Incidents of statistical significance in B6AKF1 and C57BL6 pup growth rate between different bedding materials are identified by asterisks (*).**

This did not, however, decline as in the previous case, but continued at the same rate up to the 4-day interval, *i.e.* there was a linear increase in mortality rate from birth until day 4 (Fig. 3). This suggests that pine shavings can exert both antenatal and postnatal effects on AKR and B6AKF1 pups.

These results are similar to the findings of Burkhart and Robinson[3] with regard to the effect of volatiles in wood shavings on mortality rate, while the sequence in mortality rates between these materials also coincides with the cytotoxic properties reported for these materials[11] (vermiculite < eucalyptus pulp < pine shavings). This variation in pup mortality rates during the first 4 days of life between pine shavings and the other bedding materials could therefore be due to the volatile hydrocarbons in pine shavings.

Lactation (Days 1–12)

No statistically significant differences in the number of pups reaching 12 days of age were observed for C57BL6 and B6AKF1 between the 3 bedding types. In contrast, significant differences for AKR pups between vermiculite and eucalyptus pulp, and vermiculite and pine shavings, were observed for the same variable. The exact p-values are listed in Table 1. AKR pup mortality rate between birth and day 4, as stated above, was most pronounced on pine shavings (69.6 %), followed by eucalyptus pulp (42.4 %) and vermiculite (20.7 %). During days 4–9 of the lactation period, a more rapid increase in AKR pup mortalities was observed with eucalyptus pulp (21 %), compared to vermiculite (3 %) and pine shavings (9.5 %). As no statistically significant differences were recorded in AKR pup growth rate between bedding types (*i.e.* weight loss as a result of decreased milk production did not occur), these mortalities could not be the result of lactation failure, but appear to be a continued effect of eucalyptus pulp on AKR pup survival. Compared to eucalyptus pulp, the effect of both vermiculite and pine shavings on pup survival stabilised (Fig. 3). A similar decrease in numbers was also observed during days 4–9 for B6AKF1 pups on vermiculite (2.7 %) and pine shavings (1.4 %) (Fig. 3). A more pronounced decrease in the number of C57BL6 pups (7.9 %) was also observed from days 9–12 on pine shavings. Growth rate, compared to pups of the same strain but kept on other bedding materials, seemed normal, thus again implicating bedding material. This heightened effect of pine shavings on the mortality rate of C57BL6 pups at 12 days could again be the result of volatile hydrocarbons (Fig. 3). Why an effect of such a magnitude revealed itself at such a late stage, compared to the AKR and B6AKF1 animals, is uncertain.

Survival success (Days 1–18)

Compared to eucalyptus pulp and pine shavings, a statistically significantly larger number of AKR pups born on vermiculite reached weaning age. This corresponds with the survival success obtained for these animals on vermiculite. Eucalyptus pulp resulted in the second-best survival rate for this strain. No statistically significant differences in the number of C57BL6 and B6AKF1 animals weaned were observed between the different bedding materials (Table 1).

Further small increases in pup mortality rate during the last phase of the lactation period (days 15–18) were observed for AKRs on pine shavings (0.73 %), pulp (0.76 %) and vermiculite (1.24 %), for B6AKF1 on pine shavings (0.65 %), and C57BL6 pups on eucalyptus pulp (0.7 %) and pine shavings (2.38 %) (Table 2; Fig. 3).

Production

Although vermiculite had the most pronounced effect on AKR mating success, since approximately 50 % fewer AKR litters were born on vermiculite compared to eucalyptus pulp and pine shavings (Fig. 1; Table 2), more AKR pups bred on vermiculite reached weaning age, *i.e.* 36.67 % ($n = 66$) as opposed to 32.22 % ($n = 58$) on eucalyptus pulp and 20.56 % ($n = 37$) on pine shavings (Fig. 3). The reason for this was twofold:

(a) Firstly, litter size was unaffected, on the contrary it was even better than the expected AKR litter size of 6.0 ± 2.0 (obtained on pine shavings), namely 7.91 ± 2.21. This indicates less or no antenatal influence on the foetus. Compared with the latter figure, average AKR litter size decreased on eucalyptus pulp (6.0 ± 2.60) and pine shavings (6.0 ± 2.66) (Table 2), *i.e.* a notable post-conception effect on the number of foetuses was observed. This again follows the cytotoxicity sequence noted for these bedding materials[11].

(b) Secondly, more AKRs born on vermiculite reached weaning age. This is because vermiculite had the lowest number of postnatal effects (viability, lactation and survival success) (Figs 1, 2) on AKR litter size.

The number of C57BL6 mice produced on these materials followed the same order was followed for t, namely vermiculite (50.98 %, n = 130) > eucalyptus pulp (40.78 %, n = 104) > pine shavings (36.08 %, n = 92) (Fig. 2). Average litter size at birth was again better on vermiculite (8.63 ± 2.73), followed by eucalyptus pulp (7.53 ± 1.90) and pine shavings (7.41 ± 2.45). The lowest number of antenatal and postnatal effects were thus again experienced using vermiculite.

Owing to the lower mating success observed with vermiculite, fewer B6AKF1s were produced on vermiculite (84.01 %, n = 250) than on pine shavings (95.43 %, n = 284) or eucalyptus pulp (96.77 %, n = 288). The eucalyptus pulp was thus responsible for the best B6AKF1 production figures (Fig. 2).

If the numbers of animals weaned on a specific bedding material were pooled, irrespective of strain, eucalyptus pulp produced more animals (61.43 %, n = 450) than vermiculite (60.88 %, n = 446) and pine shavings (56.37 %, n = 413) (Fig. 2).

Growth rate

The only statistically significant difference in the mean body mass of pups was observed between the B6AKF1 on eucalyptus pulp and vermiculite (days 4, 9, 12, 15, and 18), eucalyptus pulp and pine shavings (day 18), and between pine shavings and vermiculite on day 4 for the C57BL6 strain (Fig. 4). That a statistically significant difference for growth rate could be indicated between bedding types using B6AKF1, was probably the result of the more vigorous growth of these animals since it was easier to demonstrate a significant difference than was the case in the already impaired inbred animals. Inbreeding was most probably also responsible for the fact that only 1 incident of statistical significance was observed in these strains. The differences observed in mean pup weight on day 18 for example, 0.5 g for C57BL6 between eucalyptus pulp and vermiculite, and 0.9 g for AKR between vermiculite and eucalyptus pulp, might, however, be relevant from the point of view of husbandry (Fig. 4).

The B6AKF1 and C57BL6 strain followed the same sequence in growth rate on the different materials (eucalyptus pulp > pine shavings > vermiculite), whereas the AKR strain showed exactly the reverse response.

DISCUSSION

It is uncertain why an inert material such as vermiculite retarded the growth rate of C57BL6 and B6AKF1 pups (vermiculite < pine shavings < eucalyptus pulp). Hastings[4], comparing growth rate of TO mice on sawdust and vermiculite, reported a statistically significantly lower rate at 18 weeks on vermiculite. During the current study statistically significant incidents were recorded from as early as day 4. Could this retardation in growth rate on vermiculite perhaps be the result of dust, especially as vermiculite produced more dust than the other materials investigated[12], and Hastings[4] related histological changes observed in the lungs of mice kept on vermiculite to this material? This could well be the case, as Brüssow[1,2], investigating the effect of adverse environments (simulated bronchoconstriction) on the lung development of growing rats, reported not only alterations in lung function and structure, but also a reduction in the growth rate of these animals, *i.e.* dust from vermiculite could have incapacitated the lung function of these animals, thus impeding growth rate.

These observations suggest that:

(i) different bedding materials can exert different effects on the same mouse strain,
(ii) this effect probably starts during the antenatal phase; an inter-dependency exists between the foetal and maternal environments,
(iii) this effect manifests during the first 9 days of life, and
(iv) contrasting effects could be evoked in different mouse strains by the same bedding type.

Although vermiculite resulted in the best figures for both the antenatal and postnatal indices of the AKR strain, *i.e.* gestation and birth, viability, lactation, survival success and production, it had a more detrimental effect on their mating success. Vermiculite also had a negative influence on B6AKF1 mating success. The reduction in the number of litters produced on vermiculite is similar to the findings of Hastings[4], who recommended that the use of vermiculite as bedding material should be discontinued.

Compared to the other materials, pine shavings were responsible for the lowest figures obtained for the mating success (C57BL6), gestation and birth (C57BL6), viability (AKR, B6AKF1), lactation (C57BL6), survival success (AKR, B6AKF1) and production (AKR, C57BL6) and total number produced (pooled production) indices. By contrast, eucalyptus pulp was responsible for the lowest pup production index in only 2 instances, namely C57BL6 viability and AKR lactation. The differences between these 2 materials are most probably due to volatile hydrocarbons retained in pine shavings. These chemicals are removed from pulp during the pulping process. Unbleached eucalyptus pulp consists mainly of cellulose, hemicellulose, lignins and chemical residues from the pulping process (black liquor), with the result that volatiles that could have an effect on antenatal and postnatal pup survival and growth are reduced. This is confirmed by *in vitro* cytotoxicity studies showing eucalyptus pulp to be less toxic than pine shavings from *Pinus elliottii*[11].

Although vermiculite demonstrated a retardation of B6AKF1 growth rate only, it is likely that the use of this material could have the same effect on other mouse strains. Despite the small difference in the growth rate of pups, except for B6AKF1, between eucalyptus pulp and pine shavings, the results indicated a better growth rate on eucalyptus pulp.

In conclusion, the reduction in both the number of litters born and growth rate on vermiculite confirms that this material is not suitable for bedding applications during animal husbandry, while the data obtained for the variables measured on the remaining bedding types favour the use of eucalyptus pulp as a bedding type rather than pine shavings derived from *P. elliottii.*

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Book review — Boekresensie

Veterinary reproduction & obstetrics (7th edn)

Edited by G H Arthur, D E Noakes, H Pearson and T J Parkinson

1996. W B Saunders Company, London, 726pp. Price: £ 65.00. ISBN 0-7020-1785-X.

The latest edition of *Veterinary reproduction and obstetrics* is an improvement on the previous book, which already provided an excellent text on the topic of reproduction and obstetrics of domestic animals. The improvements include additional information on reproductive physiology of both sexes, more emphasis on the use of ultrasound diagnostics for cyclical changes and pathology of the genital tract and newer and more complete information on a variety of topics. The book is well illustrated with numerous figures, photographs, tables and, new in this edition, 7 colour plates.

The book is divided into 8 parts, each with a number of chapters. Part 1 deals with normal oestrous cycles of domestic animals. This section would have been much more complete if it had been introduced with some information on basic endocrinology. Part 2 amply covers the field of pregnancy and parturition and is well supported by figures, photographs and tables. Dystocia and other disorders associated with parturition is dealt with in Part 3. As in previous editions, this is the best section of the book. Part 4 deals with operative interventions, with a practical approach to operative interventions such as Caesarean sections. Part 5 on infertility provides a sound background on the causes, treatment and control of infertility. This section is divided into 7 chapters 3 of which deal with cattle and the others with the ewe and doe, mare, sow, and bitch and queen, respectively. Functional infertility in the cow is particularly well addressed. Part 6 deals with the male animal. Here the step-by-step approach to examining the male and dealing with andrological problems is logical and far better than in the previous edition. Part of this section deals with artificial insemination. The text on cattle is good but for the other species somewhat disappointing. The section on exotic species (Part 7) is misleading as it deals rather superficially with reproduction in the camel and (water) buffalo only. In my opinion it does not serve a useful purpose in this book. Embryo transfer (Part 8) is a very important aspect of veterinary reproduction and warrants better attention than the limited information supplied in the new book.

All in all, this is a very good text on reproduction and obstetrics of domestic animals for the veterinary undergraduate and practitioner alike.

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