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Feasibility of *Miscanthus* as alternative bedding for dairy cows

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ABSTRACT: Despite dairy farmers' awareness of the importance of correct bedding for the health and comfort of their cows, they are often frugal with respect to these bedding materials in order to reduce costs. In addition, farmers are currently dependent on the availability and price fluctuations of traditional bedding materials. For these reasons, the scientific literature as well as the trade press point to an intensifying search for affordable alternative bedding materials. The aim of this study was to investigate whether *Miscanthus*, a woody grass that requires low input but generates high yield, could replace straw in deep little dairy cow cubicles. The cows' cubicles were lined for two consecutive 14-day periods with straw/chalk/water bedding, followed by two consecutive 14-day periods with ground-*Miscanthus*/chalk bedding. No significant differences were found in the following parameters: loss/waste of bedding material, bacterial growth in cubicles, cow skin lesions (except for carpus lesions), cow cleanliness or cow comfort. Dust concentrations measured as PM_{10} were higher when cubicles were filled with straw-based bedding, but never exceeded workspace quality safety limits. Based on these results, one can conclude that *Miscanthus* has potential as a viable alternative to straw when used as a bedding material. On-farm cultivation of *Miscanthus* may increase dairy farmer self-sufficiency and could reduce bedding costs.

Keywords: bedding material; cow comfort; bacterial load

List of abbreviations

ADF = acid detergent fibre, **ADL** = acid detergent lignin, **CCI** = cow comfort index, **DM** = dry matter, **NDF** = neutral detergent fibre, **PM** = particulate matter, **SUI** = stall use index

Increased evidence for the key role of dairy cattle bedding in maintaining and promoting cow health and cow comfort has raised the interest of both farmers and researchers in the type and amount of bedding material used on dairy farms. Bedding material can be an important source of bacterial exposure, with a potential negative impact on teat end colonisation, udder health and milk quality (Hogan and Smith 1997; Zdanowicz et al. 2004; Sampimon et al. 2006; Verbist et al. 2011). In addition, cows prefer dry and soft bedding material that gives them sufficient friction to lie down and rise without slipping (Chaplin et al. 2000; Fregonesi et al. 2007). Increased lying times and better cow comfort are associated with higher feed intake (Metz 1985), increased rumination activity, better claw health (Vokey et al. 2001), less stress (Munksgaard et al. 1999; Fisher et al. 2002) and higher milk production (Calamari et al. 2009; Cook and Nordlund 2009). Indeed, Bruijnis et al. (2013) revealed that a better lying surface (mattress and bedding) was one of the most cost-efficient measures for optimising animal health and welfare. Factors that can influence lying time include the dry matter content, water holding capacity and particle size of the bedding material, but also the presence of a sufficient amount of bedding. Tucker et al. (2009) found that cows spent three more minutes lying down per each additional kilogram of shavings (Tucker et al. 2009). The amount of bedding available depends on bed-

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ding management (amount provided and frequency of removal), as well as the capacity of the bedding material to remain in the cubicles. Both quality and amount of bedding also influence cow cleanliness and the presence of lesions. Lesions at carpus and hock are generally associated with hard bedding materials (Weary and Taszkun 2000; Wechsler et al. 2000; Vokey et al. 2001). Lesions can develop into more severe injuries due to continuous pressure and friction related to the lying surface (Schulze et al. 2007).

In addition, bedding is known to have a great impact on dust concentration in dairy barns (Samadi et al. 2012). Straw and sawdust, the most commonly used types of bedding, can be very dusty (Breum et al. 1999). A layer of chalk powder (lime) on straw or sawdust, which is used to absorb moisture, also adds to the dust concentration (Samadi et al. 2012). Dust, especially the concentration of the dust fraction up to 10 µm (PM_{10}), can have an adverse effect on animal health and productivity and can also negatively impact worker health (Takai et al. 1998; Dolejs et al. 2006; Cambra-Lopez et al. 2010).

Despite dairy farmers' growing awareness of the importance of good quality bedding material, they do not always apply this knowledge in practice because of the cost associated with good bedding. They either use lower amounts of bedding material or choose lower quality materials. Cost is therefore one reason to search for alternatives; another is the restricted and fluctuating availability of traditional bedding material (mainly sawdust). In this study we explore the possibility of using *Miscanthus* as bedding material for dairy cows.

Miscanthus is a C4 perennial woody grass that can be cultivated on-farm over a period of 15–20 years (Jezowski 2008). It has low nutritional requirements and high yields in a wide range of soils (Heo et al. 2010). The annual dry production rate is on average 20–25 t/ha (Scurlock 1999), but can rise to 28–38 t/ha depending on the conditions (Danalatos et al. 2007). Its high biomass potential and other characteristics have led researchers to study the potential of *Miscanthus* as a bio-energy crop. Its low input, high yield and the fact that it can be cultivated on swampy land with low value for crop production or livestock also make it worth studying as an alternative to straw for use as bedding material. On-farm cultivation of *Miscanthus* may lead to a greater self-sufficiency of farmers and could mean that they will be less dependent on the availability and price fluctuations of traditional bedding materials. The aim of this pilot study was to explore the potential of *Miscanthus* × *giganteus* as alternative bedding in deep litter cubicles for dairy cattle.

MATERIAL AND METHODS

Experimental setup. Fifty lactating Holstein dairy cows were housed in a stable with 59 deep litter cubicles. The cubicles, which were arranged in two rows, measured 1.15 × 1.85 m and had open fronts. The cows were subjected to a longitudinal study on a well-managed, privately-owned Belgian dairy farm from October 2012 to November 2012. They were milked by a milking robot and forced cow traffic (feed to milk) was used. Cubicles were normally filled with a combination of 400 kg straw (8.89 m^3), 400 kg water and 2000 kg chalk powder (lime). Each day, dirt and faeces were manually removed from the cubicles and the remaining bedding was equally distributed over the cubicle by raking. After 14 days, the bedding in the cubicles was refreshed.

To test the effect of replacing straw by *Miscanthus*, an experiment was set up in which samples and measurements were carried out in two consecutive periods of 14 days with cubicles filled with the traditional combination of straw/chalk/water as bedding material. Afterwards, straw was replaced by 400 kg (3.10 m^3) *Miscanthus* for two consecutive periods of 14 days. After harvest for use in the bio-energy industry, *Miscanthus* is typically chopped. But for use as a bedding material, the *Miscanthus* must be ground due to the presence of large woody pieces. Before use as bedding, a base sample was taken and analysed for dry matter (DM) (EN 13040), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) (adopted from Van Soest et al. 1991) and particle size. Particle size was determined by applying 50 g of material (straw or *Miscanthus*) on a shaker with six sieves (diameters 31.5, 16, 8, 4, 2 and 1 mm) during 5 min. Base sample analyses revealed a relatively low DM content of the ground *Miscanthus*. For this reason, no additional water was added when using *Miscanthus* as bedding. Instead, a combination of 400 kg ground *Miscanthus* (3.10 m^3) and 2000 kg lime was applied to 59 cubicles for two consecutive periods of 14 days.

Measurements of bedding. Five cubicles (8.5%) distributed at equal distances throughout the stable were selected. On Day 0 (immediately after adding fresh bedding), Day 9 and Day 14 (before

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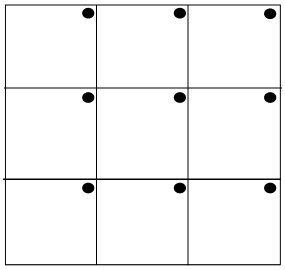

Figure 1. Diagram of the grid used to measure bedding depth of 5 selected cubicles equally distributed throughout the stable on day 0 and day 14. The grid measured 1.15 × 1.2 cm and was placed on the cubicle partition. Measuring points are indicated as black circles

refreshing the bedding material), nine equal size subsamples were taken from standardised places from the back 2/3 of the cubicles and mixed as one sample. Mixed samples from each of the five selected cubicles were cooled (1–5 °C) and on the same day transported to the lab for analysis of DM content (EN 13040) and microbiological analyses. Microbiological analyses were performed on the day of sampling. The total count of aerobic bacteria, Enterobacteriaceae, *Escherichia coli* (*E. coli*) and *Enterococcus* spp. was determined on Plate Count Agar (PCA, Bio-Rad 355-4457, Hercules, CA, USA), Violet Red Bile Glucose Agar (VRBGA, Bio-Rad 356-4584), Rapid *E. coli*2 (Bio-Rad 355 5299), and in Slanetz and Bartley medium (S&B, Oxoid CM0377, Basingstroke, Hampshire, UK), respectively. The incubation time/temperature conditions were 72 ± 3 h at 30 ± 1 °C for total aerobic bacteria, 24 ± 2 h at 37 ± 1 °C for Enterobacteriaceae, 21 ± 3 h at 44 ± 1 °C for *E. coli* and 48 ± 3 h at 37 ± 1 °C for *Enterococcus* spp. Another five cubicles, also equally distributed throughout the stable, were selected for the purpose of estimating the bedding waste over 14 days. A grid (1.15 m width and 1.20 m length) was used to measure the distance between nine fixed points and the bedding surface (Figure 1). The grid was placed on the cubicle partition and the distance was measured on Day 0 (immediately after adding new bedding) and Day 14 (before bedding refreshment) using a laser distance meter (Leica Disto^{TM} A5, leica Geosystems AG, Heerbrugg, Switzerland). For each of the five cubicles, the mean depth of bedding was calculated as the mean depth measured at the nine locations.

Measurements on dairy cattle. From the 50 cows, 20 cows were randomly selected (40%) and marked. On Day 0 (new bedding material), 4, 9, 14 (before refreshing the same bedding material), 18 (Day 4 of the second period of 14 days), 23 (Day 9 of the second period of 14 days) and 28 (Day 14 of the second period of 14 days), the cows were scored for the presence of skin lesions and cleanliness. Scoring was done by four trained observers.

Skin lesions were scored on a 5-point scale (1 = no swelling, no lesions; 2 = hairless patches; 3 = swelling; 4 = damaged skin in form of a scab; 5 = damaged skin in form of a wound, adapted from Regula et al. (2004)) and assessed at 6 places on the cow's body: carpal joint, tarsal joint (medial and lateral), knee, tuber ischium and tuber coxae. All locations were scored for the left and right side, and the highest score of both sides was registered as the final score. Because of non-normality, results are reported as median (minimum – maximum). For further statistical analyses, the 5-point scale was reduced to a binary scale with 0 = cows with score 1 and 1 = cows with score > 1.

Cleanliness of the udder (both front and back side), lower legs and flank/upper legs were scored on a 5-point scale: 0 = no dirt; 0.5 = minor splashing, between 25–50% of the total surface covered with dirt; 1 = 50% of the total surface covered with dirt; 1.5 = between 50 and 75% of the surface covered with dirt; 2 = total surface covered with dirt. Similarly as for the scoring of skin lesions, left and right legs and both flanks were scored and the highest score of both sides was registered as the final score. Because of non-normality, results are reported as median (minimum–maximum). For further statistical analyses, the 5-point scale was reduced to a binary scale: 0 = cows with score 0 or 0.5; 1 = cows with score ≥ 1 for udder and flank. For the lower legs, the binary scale consisted of 0 for cows with score < 1.5 and 1 for cows with score ≥ 1.5.

Two indices of cow comfort. Cow comfort was determined using two indices: the cow comfort index (CCI) and the stall use index (SUI). To calculate these two indices, five types of behaviour were registered as defined in Van Gastelen et al. (2011): (1) active in alley (i.e. a cow in the alley and engaged in activity), (2) waiting in the waiting room to be milked by the robot or being milked, (3) standing idle in the alley, (4) standing with at least one foot in a cubicle, (5) lying in the cubicle. At three time points per day (8:30, 13:00 and 17:00) the number of cows expressing each type of behaviour was counted. These time points were chosen based on roughage feeding time in the stable (9:00 and 16:00) and

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on literature data reporting an average number of cows lying down at these time points (Munksgaard et al. 2011). CCI and SUI were calculated as the proportion of cows touching a cubicle that are actually lying down (CCI) and as the proportion of cows not involved in feeding or milking that are lying down (SUI) (Cook et al. 2005). Behavioural observations were made at Day 0, 4, 9 and 14 (just before refreshing the bedding material).

Dust, temperature and relative humidity. To measure dust, temperature and relative humidity, a Grimm 1.109 aerosol spectrometer (Grimm Aerosol Technik GmbH & Co. KG, Ainring, Germany) was used. It was mounted in weatherproof housing and equipped with a temperature and relative humidity sensor. This direct-reading portable PM monitor operates on the principle of scattered light beams. It counts the number of particles in 32 size fractions. The counts are converted into PM mass density. The instrument provided a continuous data output with measurement intervals of one minute for each of the 32 channels which measure particle sizes ranging from 0.25 μm to 32 μm. The spectrometer was protected from the cows by being placed in an iron housing which obstructed the cows' entry into the cubicle containing the spectrometer as well as the adjacent cubicle. The spectrometer was put in the first cubicle next to the selection fence. For further statistical analyses, mean daily PM_{10}, $PM_{2.5}$ and PM_1 concentrations and mean daily temperature and relative humidity were also measured.

Statistical analyses. Prior to statistical analysis, all data were checked for unlikely values (2% of the dust concentration measured at a minute level was excluded for that reason). To approximate normality, a logarithmic transformation of all bacterial counts was used.

To determine the effect of type of bedding, days after adding fresh bedding (time) and their interaction (independent variables), linear mixed regression models were performed with day as random effect to correct for repeated measurements within one cubicle (in case of bacterial counts or depth measurements as dependent variables) or within one measuring period (in case of CCI, SUI, PM_1, $PM_{2.5}$, PM_{10} as dependent variables). The covariance between the repeated measurements was modelled using the AR (1) structure. Next, logistic regression models were built with the binary lesion or cleanliness scores as dependent variables. Again, day was added as a random effect to correct for repeated measurement from one cow. The fit of the final models was evaluated by examination of the normal probability plots of the residuals. All statistical analyses were performed using SAS 9.3 (SAS Institute Inc, NC, USA). Statistical significance was assumed when $P < 0.05$.

Table 1. Analysis of the dry matter content and the fibre fraction of chopped straw and chopped

	Chopped straw	Chopped *Miscanthus*
DM (%)	91.3	91.9
NDF (% on DM basis)	82.5	91.7
ADF (% on DM basis)	50.6	64.6
ADL (% on DM basis)	6.6	11.2

DM = dry matter, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin

RESULTS

Base samples

The analysis of chopped straw and chopped *Miscanthus* pointed to a higher fibre concentration and a lower mineral content in *Miscanthus* than in straw (Table 1). Dry matter content was high and comparable between the two bedding types (91.3% and 91.9% for chopped straw and chopped *Miscanthus*, respectively). Grinding *Miscanthus* to make it usable as bedding resulted in a lower DM content (69%). As expected, a higher proportion of

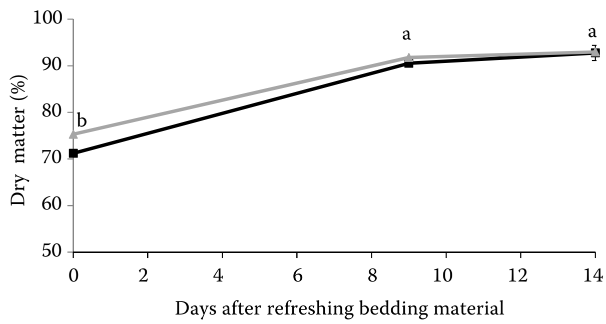

Figure 2. Mean dry matter content ± standard deviation (%) in five marked cubicles filled with either a combination of straw/chalk/water (black), or a combination of *Miscanthus*/chalk/water (grey), measured on Day 0 (immediately after bedding was refreshed) and Day 9 and 14 after bedding was refreshed. Significant differences in time are indicated with different letters

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Table 2. Particle size distribution of chopped straw and ground

	< 31.5 mm	< 16 mm	< 8 mm	< 4 mm	< 2 mm	< 1 mm
Chopped straw (%)	3	9	37.7	35.5	12.2	3
Ground *Miscanthus* (%)	0	1	21	43	18	17

small particles were present in ground *Miscanthus* compared to chopped straw (Table 2).

Measurements of bedding

DM content significantly increased between day 0 (fresh bedding material) and Day 9 after refreshing of the bedding material (Figure 2). No significant increase was seen afterwards for both types of bedding. Dry matter content did not significantly differ between the two bedding treatments.

Bacterial counts were generally low at Day 0 and significantly increased with the time that the bedding was used, except for *Enterobacteriaceae* (Figure 3). For Enterobacteriaceae, *Enterococcus* spp. and total aerobic bacteria, no significant difference between straw and *Miscanthu*s was found ($P > 0.05$). A significant interaction time × bedding type was found for *E. coli* ($P < 0.001$). *E. coli* concentrations in unused *Miscanthus* (Day 0) were significantly higher than in unused straw (Day 0).

The loss of bedding material over the course of 14 days, measured as the mean depth between nine reference points and the bedding surface, was comparable between the two bedding types ($P > 0.05$) (Figure 4).

Measurements on dairy cattle

Due to two cows drying off, only 18 cows remained in the cohort group to monitor skin lesions and cleanliness throughout the study. Median (minimum–maximum) skin lesions scores for straw and *Miscanthus,* respectively, were 1 (1–3) and

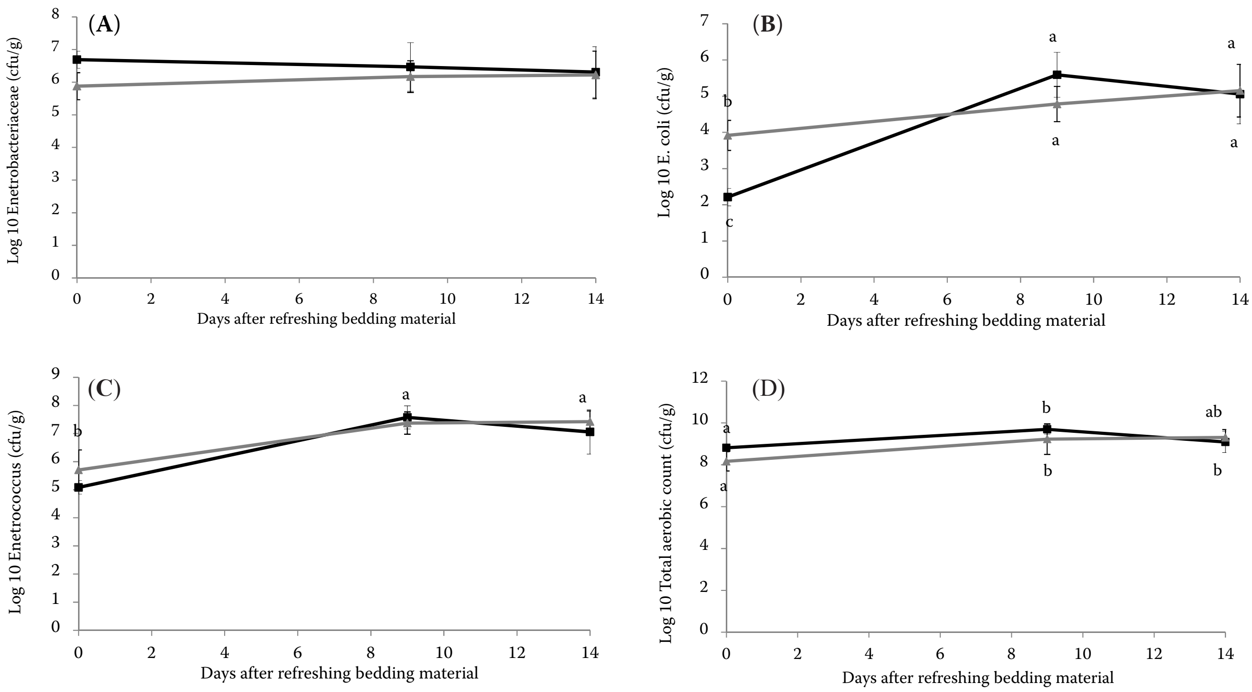

Figure 3. Mean bacterial counts ± standard deviation (log10 cgf/g) in five marked cubicles filled with either a combination of straw/chalk/water (black), or a combination of *Miscanthus*/chalk/water (grey), measured on Day 0 (immediately after bedding was refreshed) and Day 9 and 14 after bedding was refreshed. Significant differences in time × bedding (for *E. coli* figure 3B) or in time (for *Enterobacteriaceae*, *Enterococcus* and total aerobic bacteria) are indicated with different letters

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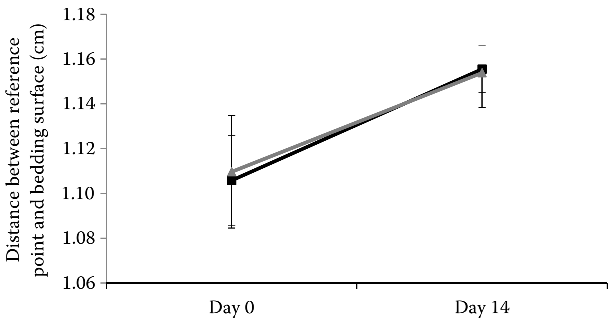

Figure 4. Mean depth ± standard deviation (cm) of the bedding of five marked cubicles measured as mean perpendicular distance between nine reference points and bedding surface of either straw/chalk/water (black), or *Miscanthus*/chalk/water (grey)

1 (1–3) for carpus, 1 (1–4) 1and 1 (1–2) for medial tarsus and 1 (1–4) and 1 (1–3) for lateral tarsus, 1 (1–2) and 1 (1–2) for knee, 1 (1–2) and 1 (1–4) for tuber ischium, 1 (1–2) and 1 (1–2) for tuber coxae. No significant effects were found for time × treatment ($P > 0.05$), over time ($P > 0.05$), nor between treatments ($P > 0.05$) for tarsal lesions, knee lesions, tuber ischium lesions and tuber coxae lesions (Figure 5). However, the risk of having a lesion with score > 1 at the carpal joint was higher when cubicles were filled with straw compared to *Miscanthus* (odds ratio = 3.22, $P = 0.04$).

Median (minimum–maximum) cleanliness scores for straw and *Miscanthus* were, respectively, 0 (0–2) and 0.5 (0–1.5) for the front udder, 0.5 (0–2) and 0.5 (0–1.5) for the back udder, 0.5 (0–2) and 0.5 (0–1.5) for the flank/upper legs and 1 (0–2) and 0 (0–2) for the lower legs. No significant effects were found for time × treatment ($P > 0.05$), over time ($P > 0.05$), nor between treatments ($P > 0.05$) for cleanliness of front udder, back udder and flank/upper legs (Figure 6). However, the odds of having a cow with > 50% of her lower legs covered with dirt was 2.63 times higher when cubicles were filled with straw compared to *Miscanthus* ($P = 0.02$).

Two Indices of cow comfort

CCI was on average 88 ± 6% and 86 ± 6% and SUI was on average 71 ± 10% and 64 ± 7% for straw and *Miscanthus*, respectively. No significant influence was found between days after adding fresh bedding ($P = 0.93$ and $P = 0.37$ for CCI and SUI, respectively),

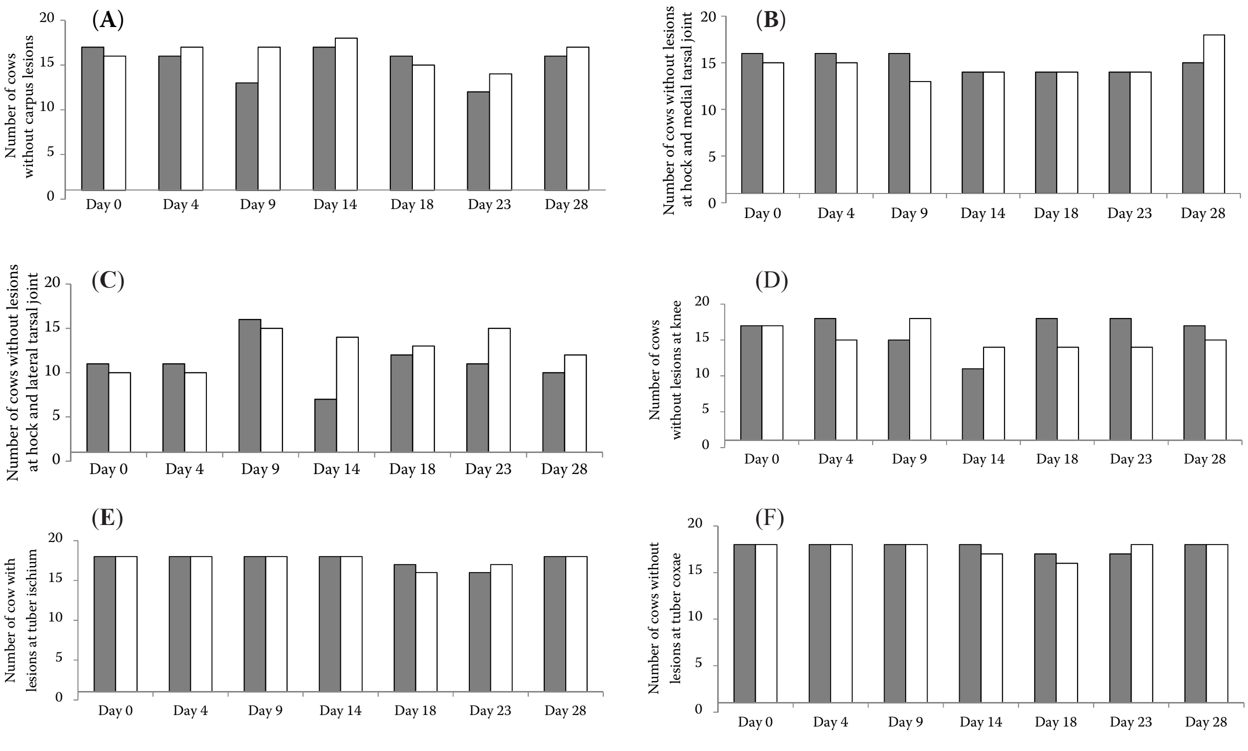

Figure 5. Number of cows without skin lesions at six different locations from a total number of scored cows of 20. Cubicles were filled for 28 days with either a combination of straw/chalk/water (grey), or a combination of *Miscanthus*/chalk/water (white). On Day 14 new bedding material was added

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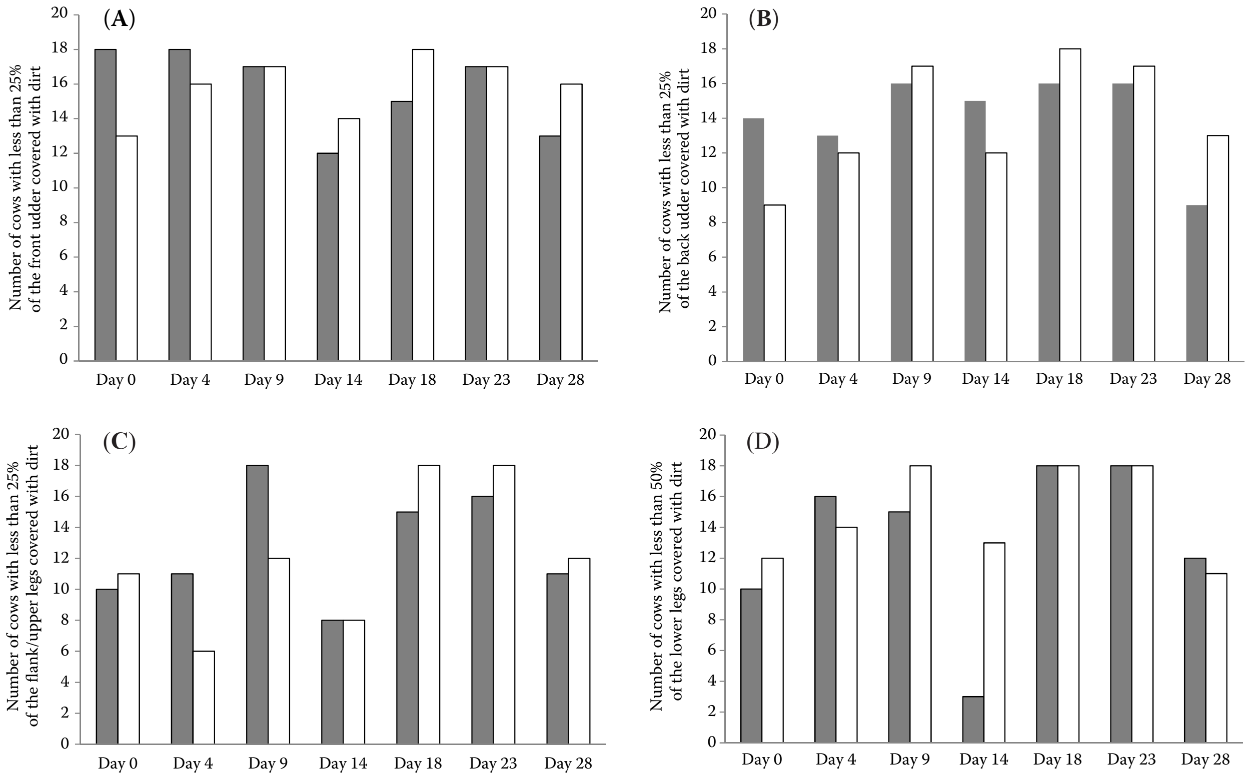

Figure 6. Number of cows in which less than 25% of the total surface of the udder (front and back side) and flank/upper legs was covered with dirt (A, B and C) or less than 50% of the total surface of the lower legs was covered with dirt (D). Cubicles were filled for 28 days with either a combination of straw/chalk/water (grey), or a combination of *Miscanthus*/chalk/water (white). On Day 14 new bedding material was added

nor between the two bedding treatments (P = 0.40 and P = 0.80 for CCI and SUI, respectively). Despite the relatively large variation between measurements (up to 10% for SUI), no effect of measurement times (8:30, 12:30 and 17:00) was found (P = 0.96 and P = 0.99 for CCI and SUI, respectively).

Dust, temperature and relative humidity

Figure 7 illustrates the mean daily PM_{10} concentrations measured during the 14 days after refreshing the bedding material. The PM_{10} concentration was significantly influenced by the interaction time × bedding type (β = –8.95; P = 0.01). When cubicles were filled with a combination of straw/chalk/water, the PM_{10} concentrations were higher. These concentrations increased with increased use of the bedding material. In addition, PM_{10} concentrations also tended to be positively associated with the stable temperature (β = –5.91; P = 0.06). Although they were not significant, the PM_{10} concentrations were also slightly higher on the day the cubicles were filled with fresh bedding (β = 53.9; P = 0.10).

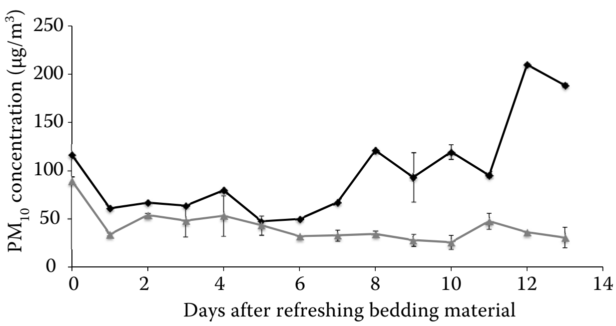

Figure 7. Mean daily PM_{10} concentration ± standard deviation (μg/m^3) in cubicles filled with either a combination of straw/chalk/water (black), or a combination of *Miscanthus*/chalk/water (grey)

In contrast to PM_{10}, no significant effects of bedding type, day after bedding refreshing, or stable temperature were found for $PM_{2.5}$ or PM_1 (P > 0.05). Over the two 14-day periods, mean $PM_{2.5}$ concentra-

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tions were 21.8 ± 10.65 μg/m^3 and 20.4 ± 3.33 μg/m^3 and mean PM_1 concentrations were 14.7 ± 10.23 μg/m^3 and 16.6 ± 4.25 μg/m^3 for straw and *Miscanthus*, respectively. Both concentrations were negatively associated with relative humidity (β = 0.49; P = 0.01 and β = 0.50; P = 0.005 for $PM_{2.5}$ and PM_1, respectively).

DISCUSSION

Analytical differences of DM content and fibre fractions between straw and *Miscanthus* were as expected, considering the plant versus wood origin and comparable with results found in the literature (Qin et al. 2012; Ververis et al. 2004). Even after being chopped, *Miscanthus* still contains a high proportion of relatively thick and woody stalks, which makes chopped *Miscanthus* unsuitable for application as bedding material. The lignin content of *Miscanthus* was almost twice as high as that of straw. Grinding *Miscanthus* makes it appropriate as bedding and seems to decrease the DM content (91.9% chopped versus 74.7% ground *Miscanthus*). After grinding, *Miscanthus* was stored outside covered with plastic for four weeks. Increased water content was probably caused by this storage under the following environmental conditions: mean temperature of 11.7 °C and mean relative humidity of 89%. Huisman and Kortleve (1994) reported an increased water content of *Miscanthus* due to storage under higher relative humidity.

The smaller particle size of ground *Miscanthus* results in an increased surface area. Enlarged water holding capacity as a result of increased surface area may favour bacterial growth (Rendos et al. 1975). On the other hand, as all woody plants, *Miscanthus* contains fewer free nutrients, such as simple sugars, pentoses and amino acids and more cellulose and lignin than straw, all of which might impede bacterial growth (Rendos et al. 1975). No differences in bacterial content between the two combinations of bedding materials were found in this study, except for *E. coli* on Day 0. Higher *E. coli* concentrations were found at the start in *Miscanthus* compared to straw. However, these differences disappeared the longer the bedding material was used. Total aerobic bacteria and *E. coli* counts during use (Day 9 and 14) were lower than those found by Kudi et al. (2009). In their study average total aerobic bacteria and *E. coli* on different bedding materials (sand, sawdust and straw) for dairy cattle were 10^{11} cfu/g and 10^6 cfu/g, respectively. The daily manual removal of dirt and faeces from the cubicles in this study performed on a well-managed herd might have prevented the high bacterial growth in both types of bedding reported by Kudi et al. (2009) and Dodd et al. (1984).

No difference was found between the mean depth between nine reference points and the bedding surface; thus, one can conclude that the loss of the two bedding materials during a 14-day period was similar. After 14 days of use without adding extra bedding material, the amount of bedding was dramatically reduced. However, the remaining amount was still acceptable and did not result in an increase in skin lesions at the carpus or the tarsus, for example, nor in a reduction in the cleanliness of the cows over time.

The likelihood of having skin lesions was generally low in this study. Also, the number of cows with skin lesions and the severity of the lesions (maximum skin lesion score) was low, again demonstrating that this was a well-managed herd. No differences were found between the two treatments, except for carpus lesions. Although it is questionable whether the duration of the trial (four weeks) was long enough to properly evaluate the incidence of skin lesions, the probability of carpus lesions was higher when straw was used instead of *Miscanthus*. Besides the amount of bedding material, which was comparable between straw and *Miscanthus*, also the physical properties of the surface (softness, thermal comfort, coefficient of friction, compressibility, etc.) can also contribute to the development of skin lesions at carpus and hock (Tucker et al. 2009; Tucker and Weary 2004). Although none of these physical characteristics were measured during this trial, subjective observations during daily raking of the cubicles revealed that *Miscanthus* was much looser in consistency. Together with urine, the straw/chalk/water combination tended to form clots that did not degrade, whereas dry straw normally degrades during use. More effort was therefore needed to loosen straw clots and to equally distribute the material over the cubicle by raking. The formation of hard clots might be responsible for the increase in carpus lesions. However, these subjective results need to be confirmed and the practical relevance of this small difference in carpus lesions is debatable.

The probability of a high cleanliness score (meaning a soiled cow as indicated by more than 25%

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dirt for udder and flank and more than 50% dirt for lower legs) was low in this study. No differences were found between the use of straw and *Miscanthus* except for on the lower legs. Fifteen cows with more than 50% dirt coverage on their lower legs were found in straw-filled cubicles just before new straw was added to the cubicle (Day 14). The odds for a dirty cow were 3.63 when straw was used compared to *Miscanthus*. It should be noted, however, that these higher odds were more likely to have been caused by a dirty walking area as result of a defective cleaning robot on Day 13 and 14 during the trial with straw than by the type of bedding material. Significant differences were no longer present if those two days were excluded from the analysis.

The two indices of cow comfort were high: for straw and *Miscanthus,* CCI was on average 88% and 86%, respectively and SUI was on average 71% and 64%, respectively. A value greater than 85% for CCI is proposed as a realistic goal (Cook et al. 2005). Considerable variation in both CCI and SUI was found between and within days, which indicates that one measurement may not reflect the average of the indices over time, as previously noted by Cook et al. (2005) and van Gastelen et al. (2011). Nevertheless, no significant effect of measurement time (8:30, 12:30 and 17:00) on CCI and SUI was found. CCI for both straw and *Miscanthus* was comparable or slightly higher than results found in the literature for mattresses (76–78%), sand (86%) and box compost (81%), whereas the SUI was comparable or slightly lower (68–70% for mattresses, 72–76% for sand and 68% for box compost) (Cook et al. 2005; van Gastelen et al. 2011). In addition, both indices did not significantly differ between straw and *Miscanthus*. Although lying time and duration of lying was not recorded in our study, a sufficient and comparable cow comfort when using both types of bedding material can be assumed. No significant differences in CCI and SUI were found in time, indicating an immediate acceptance of *Miscanthus* by the cows without prior familiarity at the start of the experiment and a sufficient amount of soft bedding surface remaining after 14 days of use.

Cow comfort is also known to be influenced by the dryness of the bedding material. According to Fregonesi et al. (2007) lying time is reduced by more than 11 h a day when DM content decreases from 86.4% to 26.5%. More relevant DM contents for practice (89.8–34.7%) were tested by Reich et al. (2010), revealing more moderate decreases in lying time (maximum 1.1 h/day). Moreover, it was concluded that the decrease in lying time was only relevant below 60% DM and that cows may not have a strong preference for drier bedding when options between 60 and 90% are available (Reich et al. 2010). In this study, DM content at the start was 71.2% and 75.3% for straw and *Miscanthus* combinations, respectively. A significant increase towards 92.8% and 93% was seen within 14 days. Although lying time was not measured, we did not expect either negative influences of this high DM content or differences in lying time between the two types of bedding material caused by DM content.

In general, mean daily PM_{10} concentrations were low (25–209 μg/m^3) and comparable with data found for dairy barns (42–132 μg/m^3 (Dolejs et al. 2006); 60–370 μg/m^3 (Schmidt et al. 2002); 100–170 μg/m^3 (Joo et al. 2013); 40–80 μg/m^3 (Takai et al. 1998)). PM_{10} concentrations remained far below the workplace air quality safety limits of 3000 and 10 000 μg/m^3, as prescribed by the Belgian government (Royal Decree of 30 June 2011). Both fresh bedding combinations were quite wet (DM content of the combination 71.2% and 75.3% for straw and *Miscanthus*, respectively). Wet bedding material can capture dust (Takai et al. 1998). However, during the use of the bedding, the material becomes more and more dry (DM content of 92.8% and 93.0% for the combination straw/chalk/water and *Miscanthus*/chalk on Day 14, respectively) due to ventilation and water evaporation. But PM_{10} concentrations only increased towards the end of the measuring period when the combination straw/chalk/water was used. No such increase was seen in the two 14-day periods when the cubicles were filled with *Miscanthus*. The degradation of straw during use and formation of small particles might be responsible for the increased release of dust particles. The plant versus wood origin of the bedding material might also contribute to the greater dust concentration in straw-based versus *Miscanthus*-based bedding (Samadi et al. 2012).

Dust generated by bedding materials may also be influenced by animal activity or human handling (Takai et al. 1998), explaining the tendency of PM_{10} to be higher on day 0 due to the cleaning of cubicles and the refreshing of bedding material.

Besides bedding, environmental factors such as relative humidity and temperature can affect dust

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concentrations. At high relative humidity, dust particles will contain condensed water and aggregate, which might explain the negative relationship between PM_1 and $PM_{2.5}$ and the relative humidity (Takai et al. 1998). In contrast, higher temperatures potentially result in more dispersed and drier particles and may therefore contribute to the increase in PM_{10} concentrations (Joo et al. 2013).

To our knowledge, this is the first study that investigates the potential use of *Miscanthus* × *giganteus* as bedding material in deep litter cubicles for dairy cattle. As no significant differences could be found in bacterial growth, the capacity of the material to remain in the cubicles, skin lesions (except for carpus lesions) and cleanliness score of the cows, and two cow indices for cow comfort (CCI and SUI), it can be concluded that straw can indeed be replaced by *Miscanthus*. In addition, dust concentrations were significantly lower after *Miscanthus* was used for 14 days as bedding. The dust concentrations never exceeded workspace air quality safety limits when either straw or *Miscanthus* were used. Consequently, dairy farms may increase their self-sufficiency and become less dependent on the availability and price fluctuations of the traditional bedding materials by cultivating *Miscanthus* on-farm. Total cultivation costs of *Miscanthus* vary from 27 euro/t to 70 euro/t, depending on the length of the production period (15 to 21 years), the average yield (10 t/ha to 20 t/ha), dry matter percentage, harvest method and post-harvest costs, herbicide application and local production circumstances (Bullard 1999; Bullard 2001; DEFRA 2001; Khanna et al.2008; Styles et al. 2008; Muylle and Snauwaert 2012). These calculated costs from literature are much cheaper than the prices for straw (from 90 euro/t up to 165 euro/t). However, the extra costs of the grinding process of *Miscanthus* are not included in the total production costs and must be taken into account. Future studies might include a total cost/benefit calculation.

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