Evaluation of Free-Stall Mattress Bedding Treatments to Reduce Mastitis Bacterial Growth

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ABSTRACT

Bacterial counts were compared in free-stall mattresses and teat ends exposed to 5 treatments in a factorial study design on 1 dairy farm. Mattresses in five 30-cow groups were subjected to 1 of 5 bedding treatments every other day: 0.5 kg of hydrated limestone, 120 mL of commercial acidic conditioner, 1 kg of coal fly ash, 1 kg of kiln-dried wood shavings, and control (no bedding). Counts of coliforms, *Klebsiella* spp., *Escherichia coli*, and *Streptococcus* spp. were lowest on mattresses bedded with lime. Mattresses bedded with the commercial acidic conditioner had the next lowest counts for coliforms, *Klebsiella* spp., and *Streptococcus* spp. Wood shavings and the no-bedding control had the highest counts for coliform and *Klebsiella* spp. Compared with wood shavings or control, fly ash reduced the counts of coliforms, whereas for the other 3 bacterial groups, the reduction was not always significant. *Streptococcus* spp. counts were greatest in the control group and did not differ among the shavings and fly ash groups. Teat swab results indicated that hydrated lime was the only bedding treatment that significantly decreased the counts of both coliforms and *Klebsiella* spp. There were no differences in *Streptococcus* spp. numbers on the teats between any of the bedding treatments. Bacterial populations grew steadily on mattresses and were generally higher at 36 to 48 h than at 12 to 24 h, whereas bacterial populations on teats grew rapidly by 12 h and then remained constant. Hydrated lime was the only treatment that significantly reduced bacterial counts on both mattresses and teat ends, but it caused some skin irritation.

Key words: bedding, bacterial count, free-stall

INTRODUCTION

Mastitis is a major health problem for dairy cows, the control and treatment of which can be costly (De-

Graves and Fetrow, 1993). The pathogens causing mastitis are generally characterized as either contagious or environmental in origin (Smith and Hogan, 2001). Although substantial progress has been made in controlling contagious mastitis by implementation of teat dipping and dry cow therapy programs, there has been less progress made on the control of mastitis caused by environmental pathogens (Smith and Hogan, 2001).

The primary reservoir of environmental pathogens is the environment in which the cow is living. For example, the rate of intramammary coliform infections has been correlated to the number of bacteria on the teat end (Hogan et al., 1989); and the number of bacteria at the teat end has been correlated to the number of bacteria in the bedding (Natzke and LeClair, 1976; Hogan et al., 1989). Therefore, designing housing and management systems that reduce exposure of environmental pathogens to the teat end should result in less environmental mastitis (Smith and Hogan, 2001).

Various bedding treatments have been tested for suppressing bacterial growth and reducing mastitis occurrence, most of which involve alteration of pH with acidic or alkaline conditioners. For example, hydrated lime reportedly reduced bacterial counts when used with sawdust (Hogan and Smith, 1997) or with recycled manure as bedding in tie-stalls (Hogan et al., 1999). Previous research also suggested that adding fly ash to manure solids used for bedding was beneficial in reducing bacterial loads (Brazil et al., 1999). Fly ash is a by-product of fluidized bed combustion at coal-burning power plants; many of the by-products have a high pH (Dou et al., 2003). When mixed with animal manures, fly ash has been shown to reduce phosphorus solubility, due primarily to its high pH (Dou et al., 2003). If proved to be effective in suppressing bacterial growth, fly ash would be a good choice for a bedding treatment because it is available in large quantities in many regions at low or no cost for producers. Recently, commercial acidic bedding conditioners have become available. Hogan et al. (2007) found a commercial acidic bedding conditioner to be effective in suppressing gram-negative bacteria and *Streptococcus* spp. over the short term in recycled organic bedding.

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Table 1. Treatments¹ by stall area

Week	Stall area				
	I	II	III	IV	V
1	Control	CAC	Shavings	Fly ash	Lime
2	CAC	Control	Lime	Shavings	Fly ash
3	Shavings	Lime	Fly ash	CAC	Control
4	Lime	Fly ash	CAC	Control	Shavings
5	Fly ash	Shavings	Control	Lime	CAC

¹Mattress treatments: control = no bedding; CAC = commercial acidic conditioner (300 mL for first and 120 mL for subsequent applications in each cycle); shavings = 1 kg of kiln-dried wood shavings; fly ash = 1 kg of coal fly ash; lime = 0.5 kg of hydrated lime.

Despite the growing interest in using various bedding conditioners for controlling mastitis bacteria, data are lacking regarding the effect of hydrated lime or other bedding treatments used as the sole bedding material on free-stall mattresses. The objective of the present study was to evaluate hydrated lime, a commercial acidic bedding conditioner (CAC), fly ash, and kiln-dried wood shavings compared with a no-bedding control, as sole free-stall mattress treatments for suppressing mastitis-causing bacterial populations.

MATERIALS AND METHODS

The experiment was conducted in June and July 2005 at the University of Pennsylvania's Marshak Dairy, a 200-cow teaching and research facility located at New Bolton Center (Kennett Square, PA). The study featured a factorial design with 5 treatments (Table 1) including hydrated lime, CAC, coal fly ash, kiln-dried wood shavings, and a no-bedding control. Treatments were applied to a group of 30 stalls in each of the 5 stall blocks (I to V) in the dairy. In subsequent sampling periods (weeks), treatments were allocated at random over the remaining 4 stall blocks, giving 5 replications of the treatments over time. On d 1 (Monday) of each of the sampling periods, the mattresses in each of the 5 stall blocks (I to V) were swept clean with a broom, and treatments (0.5 kg of hydrated lime, 300 mL of CAC, 1 kg of coal fly ash, 1 kg of kiln-dried wood shavings, or a no-bedding control) were applied to each of the 5 stall blocks according to the schedule outlined in Table 1; on d 3 (Wednesday) and 5 (Friday), subsequent applications were made after ordinary mattress cleaning and grooming was done by the dairy staff before milking. The CAC was applied according to manufacturer recommendations at 300 mL per stall on d 1 and a 120-mL maintenance rate on d 3 and 5, respectively. Each application was made by hand-applying the material to the rear 61 cm of the 1.83- × 1.07-m mattresses (Mayo mattresses; www.mayomats.com) as uniformly as possible.

Sample Collection

Sample collection was initiated on d 5 (Friday) in each of the 5 treatment cycles, with the first sampling accommodating the actual milking times beginning on Friday afternoon (0 h), followed by sampling at 12, 24, 36, and 48 h thereafter. For each sampling and from each treatment group, mattress samples were obtained from 5 stalls and teat swab samples were taken from 5 cows. Sample collection was repeated following the same procedures in the subsequent 4 wk of the study.

To obtain mattress samples, a group of 5 representative mattresses were selected for sampling from each treatment. Stalls that were wet or had gross manure contamination were not chosen to be sampled. For the selected stalls, a 15- × 15-cm Plexiglas quadrant was placed in the rear 61 cm of the mattress and all material within the quadrant was collected. Samples at 0 h were obtained after the treatments had been applied to the mattresses before the cows lying on the bedding. Subsequent samples were taken from the mattresses before the cows were taken to the milking parlor and before grooming of the stalls. The samples were refrigerated at 4°C and plated for bacterial counts the day after all the samples were taken. Ordinary mattress cleaning and grooming was performed at the normal times by the dairy staff before milking.

To obtain teat swab samples, 5 cows were randomly selected from each treatment group during the milking process at each sampling. The external teat orifices of both the right front and left hind teat of the selected cows were sampled by swabbing the teat end in a circular pattern 3 times with a cotton swab moistened in PBS, using 1 swab per teat. Together, the 10 swabs per treatment group were placed in 40 mL of PBS, refrigerated at 4°C, and plated for bacteria counts the day after the final sample collection on Sunday. Samples at 0 h were obtained after the postmilking teat dip had been applied for 30 s and teats dried with a paper towel. Subsequent samples were taken when cows entered the parlor before milking and application of the teat predip.

A preliminary trial was conducted to determine if refrigerated storage would affect bacteria populations from teat swabs. Three teat-end swab samples obtained as described above were placed in PBS individually and stored in a refrigerator (4°C). Aliquots of the samples were then plated in duplicate at 0, 24, 48 and 72 h on MacConkey's agar (gram-negative bacteria; Difco Laboratories, Detroit, MI) and tryptic soy agar (total aerobic bacteria; Becton Dickinson, Franklin Lakes, NJ); colony-forming units were counted after 24 h of incubation at 37°C. Changes in bacteria populations over time were tested by repeated-measures ANOVA using PROC GLM in SAS (SAS Institute Inc., Cary, NC) with a repeated statement and time as a dependent variable. Refrigeration time did not significantly affect bacteria populations plated on MacConkey (unadjusted $P > F = 0.1039$) or tryptic soy agars ($P > F = 0.1814$).

Bacteriological Methods

Bedding Samples. Twenty grams of bedding was added to 180 mL of sterile buffered peptone water (pH 7.0) in a 500-mL plastic bag and placed on a shaker at room temperature for 30 min. After contents had settled (2 to 3 min), appropriate dilutions were plated on the surface of each of 3 media: MacConkey's agar (Difco Laboratories), cystine-lactose-electrolyte deficient (CLED) agar with Andrade indicator (Oxoid Ltd., Basingstoke, UK), and Edward's modified medium agar (Remel Products, Lenexa, KS) with 5% plasma (Hemostat Laboratories, Dixon, CA). Six 10- μ L samples of each dilution were plated on one half plate containing each agar medium and incubated for 18 h at 37°C. Bacterial groups were identified as coliforms (lactose-positive pink-red colonies on MacConkey's agar), *Klebsiella* spp. (extremely mucoid yellow colonies on CLED agar), *Escherichia coli* (bright pink semitranslucent colonies with surrounding pink halo on CLED agar), or *Streptococcus* spp. (total growth on Edward's modified medium agar).

Teat Samples. Containers with the teat swabs and 40 mL of PBS were placed on a shaker for 30 min at room temperature. The same bacteriologic methods described previously for the bedding samples were used for the teat samples.

Data Analysis

The original bacterial counts (x) were transformed using the formula $\ln(x + 1)$ before data analysis; results were reported as the natural log-transformed data. An ANOVA was performed using JMP version 4.0 (SAS Institute Inc.). Comparisons of treatment effects were tested with least squares means contrasts, where each

level of a fixed effect was compared with all other levels. For levels to be considered significantly different, the probability of a type-1 error was less than the α -level of rejection ($P < 0.05$) for both the t -statistic comparison between each pair of levels and the F -test of all comparisons considered in the contrast.

RESULTS

Mattress Samples

There was a significant treatment effect ($P < 0.0001$) on the mean bacterial counts for all 4 bacterial groups. Hydrated lime was the most effective treatment in suppressing bacterial growth on the mattresses, with the lowest bacterial counts for all 4 types of bacteria (Table 2). The commercial product CAC provided the next lowest bacterial counts and was more effective than fly ash, shavings, and the no-bedding control for coliforms, *Klebsiella* spp., and *Streptococcus* spp. Fly ash effectively reduced coliform populations compared with the no-bedding control or shavings. However, populations of *Klebsiella* spp. and *E. coli* with the fly ash treatment were similar to that of the control. The shavings and control treatments had the highest *Klebsiella* spp. counts. Shavings had no effect on the coliform and *E. coli* counts compared with the no-bedding control. *Streptococcus* spp. counts were highest for the no-bedding control (Table 2).

There were also significant differences ($P < 0.0001$) in the mean natural log-transformed bacterial populations (cfu/g of DM) over the different sampling times. As shown in Table 3, bacterial counts at 0 h were lowest for all 4 types of bacteria although *E. coli* and *Streptococcus* spp. counts at 0 h were not different from those at 12 h. For all bacterial types, there was a clear trend of increasing bacterial populations as sampling time progressed to 12, 24, 36, and 48 h (Table 3).

In addition, the stall block areas to which the various treatments in the factorial design were applied seemed to differ in bacterial counts of coliforms, *Klebsiella* spp., and *Streptococcus* spp. ($P < 0.05$), but not for *E. coli*. Stall areas III and V were associated with lower counts of coliforms and *Klebsiella* spp. (Table 4).

There were significant interactions of treatment $\times$ hour for coliforms, *Klebsiella* spp., and *Streptococcus* spp. ($P < 0.0002$) and treatment $\times$ stall area for all 4 types of bacteria ($P < 0.005$). Despite the treatment $\times$ stall area interactions, the effect of treatment was still significant for all 4 bacterial populations when the variation from stall area was accounted for.

Teat-End Swabs

Hydrated lime significantly reduced coliform and *Klebsiella* spp. counts but not *E. coli* or *Streptococcus*

Table 2. Sample mean (ln-transformed cfu/g of DM) and post-ANOVA groups for bacterial counts from bedding samples by treatment group over all samplings

Treatment ¹	Coliforms		<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Streptococcus</i> spp.	
	Mean (SE)	Contrast ² (1.03)	Mean (SE)	Contrast (1.26)	Mean (SE)	Contrast (1.95)	Mean (SE)	Contrast (1.20)
Lime	8.07 (1.77)	a	6.39 (1.65)	a	3.96 (1.48)	a	7.23 (2.01)	a
Shavings	20.73 (0.40)	b	19.32 (0.38)	b	14.93 (1.76)	c	20.69 (0.38)	b
Fly ash	17.56 (1.20)	d	16.28 (1.12)	d	13.44 (1.74)	bc	19.64 (0.92)	b
CAC	14.81 (1.96)	c	11.94 (1.90)	c	9.83 (2.12)	b	15.51 (2.20)	c
Control	21.56 (0.48)	b	17.91 (1.17)	bd	13.65 (2.14)	bc	23.53 (0.44)	d

^{a-d}Means within a column with different letters differ significantly ($P < 0.05$).

¹Treatments: lime = 0.5 kg of hydrated lime; shavings = 1 kg of kiln-dried wood shavings; fly ash = 1 kg of coal fly ash; CAC = commercial acidic conditioner (300 mL for first and 120 mL for subsequent applications in each cycle); control = no bedding.

²Standard errors for post-ANOVA contrasts.

Table 3. Sample mean (ln-transformed cfu/g of DM) and post-ANOVA groups for bacterial counts from all bedding samples by sampling time

Sample time, ¹ h	Coliforms		<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Streptococcus</i> spp.	
	Mean (SE)	Contrast ² (1.03)	Mean (SE)	Contrast (1.26)	Mean (SE)	Contrast (1.95)	Mean (SE)	Contrast (1.20)
0	10.05 (2.02)	a	9.28 (1.87)	a	6.83 (1.90)	a	11.37 (2.10)	a
12	15.84 (1.58)	b	13.07 (1.63)	b	8.67 (2.06)	ab	16.28 (1.92)	ab
24	16.88 (1.60)	b	14.98 (1.61)	bc	11.42 (1.96)	bc	16.86 (1.78)	b
36	19.95 (0.97)	c	17.04 (1.34)	c	15.26 (1.80)	c	21.17 (1.05)	c
48	19.99 (0.94)	c	17.46 (1.15)	c	13.62 (1.94)	c	20.93 (1.00)	c

^{a-c}Means within a column with different letters differ significantly ($P < 0.05$).

¹Bedding samples taken at 0 h, and 12, 24, 36, and 48 h after treatments were applied.

²Standard errors for post-ANOVA contrasts.

Table 4. Sample mean (ln-transformed cfu/g of DM) and post-ANOVA groups for bacterial counts from bedding by stall area over all samplings

Stall area ¹	Coliforms		<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Streptococcus</i> spp.	
	Mean (SE)	Contrast ² (1.03)	Mean (SE)	Contrast (1.26)	Mean (SE)	Contrast (NA)	Mean (SE)	Contrast (1.20)
I	17.45 (1.68)	a	16.00 (1.55)	a	13.32 (2.08)	a	17.98 (1.64)	ab
II	17.75 (1.47)	a	15.71 (1.48)	a	12.34 (1.97)	a	17.51 (1.60)	ab
III	15.09 (1.79)	b	11.74 (1.84)	b	10.37 (1.92)	a	16.26 (1.98)	a
IV	18.37 (1.23)	a	16.40 (1.34)	a	11.00 (2.02)	a	19.24 (1.52)	b
V	14.06 (1.86)	b	12.00 (1.734)	b	8.77 (2.05)	a	15.61 (2.06)	a

^{a,b}Means within a column with different letters differ ($P < 0.05$).

¹Five groups of 30-cow free-stall areas.

²Standard errors for post-ANOVA contrasts.

Table 5. Sample mean (ln-transformed cfu) and post-ANOVA groups for bacterial counts from teat end samples by treatment group over all samplings

Treatment ¹	Coliforms		<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Streptococcus</i> spp.	
	Mean (SE)	Contrast ² (0.87)	Mean (SE)	Contrast (1.04)	Mean (SE)	Contrast (1.18)	Mean (SE)	Contrast (1.03)
Lime	15.29 (1.01)	a	12.69 (1.38)	a	10.15 (1.33)	ab	13.36 (0.97)	a
Shavings	16.54 (0.97)	ab	15.17 (0.97)	b	9.22 (1.56)	a	13.31 (0.93)	a
Fly ash	17.61 (0.89)	b	15.71 (0.82)	b	13.65 (1.28)	c	15.42 (0.84)	a
CAC	17.73 (0.80)	b	16.19 (0.80)	b	12.09 (1.44)	bc	14.10 (1.03)	a
Control	17.92 (0.67)	b	16.29 (0.72)	b	11.57 (1.48)	abc	14.98 (0.83)	a

^{a-c}Means within a column with different letters differ significantly ($P < 0.05$).

¹Treatments: lime = 0.5 kg of hydrated lime; shavings = 1 kg of kiln-dried wood shavings; fly ash = 1 kg of coal fly ash; CAC = commercial acidic conditioner (300 mL for first and 120 mL for subsequent applications in each cycle); control = no bedding.

²Standard errors for post-ANOVA contrasts.

spp. compared with the control (Table 5). The other treatments (CAC, fly ash, and shavings) did not differ from the control or from each other in terms of bacterial counts for coliforms, *Klebsiella* spp., or *Streptococcus* spp. The counts of *E. coli* appeared to be erratic (Table 5).

Similar to the mattress sample results, there was a significant effect of sampling time on bacterial counts of teat swab samples ($P < 0.0006$). As indicated in Table 6, bacterial populations were lowest at 0 h for all 4 types of bacteria. However, unlike the mattress samples, which showed a trend of increase from 12 to 48 h for all bacterial types (Table 3), there was no difference among the 4 sampling times (12, 24, 36, and 48 h) in coliform or *Streptococcus* spp. counts associated with the teat swabs. For *Klebsiella* spp. and *E. coli*, there

were small but sometimes significant differences during 12, 24, 36, and 48 h (Table 6).

Stall area appeared to have significant impact on the mean natural log-transformed populations of coliforms ($P < 0.028$), but not *Klebsiella* spp., *E. coli*, or *Streptococcus* spp. Coliform counts were lower in stall area V compared with stall areas III and IV (Table 7).

DISCUSSION

Mattresses

Hydrated lime has been shown to effectively lower bacterial counts when added to organic bedding materials. For example, the addition of hydrated lime to sawdust significantly reduced bacterial counts for 1 d in a

Table 6. Sample mean (ln-transformed cfu) and post-ANOVA groups for bacterial counts from all teat-end samples by sampling time

Sample time, ¹ h	Coliforms		<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Streptococcus</i> spp.	
	Mean (SE)	Contrast ² (0.87)	Mean (SE)	Contrast (1.04)	Mean (SE)	Contrast (1.18)	Mean (SE)	Contrast (1.03)
0	12.91 (0.85)	a	10.37 (1.02)	a	8.26 (1.21)	a	7.84 (1.23)	a
12	18.64 (1.13)	b	17.09 (1.06)	bc	12.01 (1.73)	bc	16.37 (0.37)	b
24	17.41 (0.61)	b	16.11 (0.59)	bc	11.58 (1.31)	bc	14.54 (0.36)	b
36	18.74 (0.58)	b	17.46 (0.61)	b	13.74 (1.35)	b	16.37 (0.42)	b
48	17.39 (0.61)	b	15.03 (0.89)	c	11.08 (1.39)	c	16.04 (0.45)	b

^{a-c}Means within a column with different letters differ significantly ($P < 0.05$).

¹Teat-end bacterial samples taken at 0 h, and 12, 24, 36, and 48 h after treatments were applied.

²Standard errors for post-ANOVA contrasts.

Table 7. Sample mean (ln-transformed cfu) and post-ANOVA groups for bacterial counts from teat ends by stall area over all samplings

Stall area ¹	Coliforms	
	Mean (SE)	Contrast ² (0.87)
I	17.02 (0.81)	ab
II	16.80 (0.84)	ab
III	17.67 (0.67)	a
IV	18.19 (0.87)	a
V	15.42 (1.13)	b

^{a,b}Means within a column with different letters differ significantly ($P < 0.05$).

¹Five groups of 30-cow free-stall areas.

²Standard error for post-ANOVA contrast.

tie-stall study (Hogan and Smith, 1997), whereas in a second study (also tie-stalls), no statistical differences were detected although bacterial counts were numerically lower in lime-treated sawdust than in the untreated sawdust control (Hogan et al., 1999).

For the first time, the present study showed that hydrated lime can effectively reduce bacterial growth on free-stall mattresses when used as the sole bedding treatment. Compared with the other treatments, the hydrated lime treatment produced the lowest bacterial counts of all bacterial types. However, the amount of hydrated lime used in the present study (0.5 kg per stall every 48 h) did cause some irritation to the skin of the udder and legs. Approximately one-third of the cows in the hydrated lime treatment group had evidence of mild ulceration and scaling on both the legs and udder. The lesions were localized to areas where the lime had caked on the skin caused by the mixture of the hydrated lime with manure, milk, or urine. Lesions were evident approximately 3 d after exposure to the hydrated lime. This suggests that routine long-term use of more than 0.5 kg of lime alone on mattresses may be associated with undesirable side effects despite the benefit in bacteria suppression. The extent of irritation caused by hydrated lime when used as the sole bedding on mattresses could be exacerbated by stall designs that allow more manure to be deposited on the back of the mattress. Further studies may be warranted to test the efficacy of lower application rates to avoid the skin irritation problem.

The CAC was applied according to the manufacturer's recommendation and did not cause skin irritation or any other visible problems. The commercial product contains 92.5% calcium carbonate, 5% sodium dichloroisocyanurate, and 2.5% aluminum sulfate and has a pH

of 3.7. The ingredient sodium dichloroisocyanurate has been used as part of a teat dip formulation to control mastitis in dairy cows (Boddie and Nickerson, 1996) and as a hospital disinfectant (Bloomfield and Uso, 1985). In the present study, CAC was effective in suppressing the populations of coliforms, *Klebsiella* spp., and *Streptococcus* spp. although its efficacy was less than that of hydrated lime. It is not clear whether the antibacterial feature was mainly due to chlorine generated by the sodium dichloroisocyanurate or a combined effect with the low pH.

The fly ash treatment resulted in lower coliform counts than shavings or the control and in lower *Klebsiella* spp. counts than shavings and could serve as an alternative to shavings on mattresses. In a previous study, fly ash obtained from a different source showed antibacterial effects on the first day when mixed with sawdust and used on tie-stall mattresses (Z. Dou, unpublished data). University of California researchers claimed that adding fly ash to manure solids used for bedding was beneficial in reducing the bacterial load (Brazil et al., 1999), but they cautioned that fly ash characteristics should be evaluated before using it as a bedding source because fly ash from different sources had different pH values and its effectiveness in suppressing bacterial growth is related to pH. The fly ash material used in the present study had a pH of 11.4, whereas other fly ash materials could have pH values as low as 7.9 or as high as 13 (Dou et al., 2003). It is not clear if there is a pH threshold regarding fly ash's efficacy in suppressing bacterial growth. On the other hand, dust, which may affect the cows' breathing, appeared to be a nuisance when using fly ash as the sole bedding material in this study. Fly ash did not appear to cause any skin irritation.

Notably, the use of shavings resulted in the highest *Klebsiella* spp. counts compared with all bedding materials, although the difference between shavings and control was not statistically significant (Table 2). Outbreaks of *Klebsiella* spp. mastitis have been attributed to sawdust and shavings contaminated with *Klebsiella* spp. (Newman and Kowalski, 1973). In the present study, shavings had lower *Streptococcus* spp. counts but comparable counts for the other bacterial groups compared with the no-bedding control.

Time zero was defined as the beginning of the sampling period when cows had not had access to freshly bedded stalls. The lowest bacterial counts were found at 0 h for coliforms and *Klebsiella* spp. For *Streptococcus* spp. and *E. coli*, counts at 0 h were lower than at 24, 36, and 48 h. Also, coliform and *Streptococcus* spp. counts were higher at 36 and 48 h than at 12 and 24 h, whereas *Klebsiella* spp. and *E. coli* counts were higher at 36 and 48 h than at 12 h. A general trend

was that bacterial numbers on the mattresses increased over time and were highest at 36 and 48 h, suggesting that daily bedding would be superior to alternate-day bedding for controlling bacteria on mattresses at this dairy. Presumably cows would be exposed to fewer bacteria for a longer period when new bedding was added daily.

Teats

The only notable treatment effect on teat-end bacteria was associated with the hydrated lime treatment, which reduced both coliform and *Klebsiella* spp. counts. For *E. coli* and *Streptococcus* spp., the teat-end samples with the lime treatment were similar to the other treatments in most cases. Comparing bacterial counts of the mattress samples (Table 2) with those of the teat swabs (Table 5), it seemed that much of the treatment effect on suppressing bacterial growth on the mattresses diminished in the teat samples. In other words, suppressed bacterial growth on the mattresses did not always transfer to lower bacterial numbers on the teat ends. Teat ends can be in contact with manure or other surfaces in addition to mattresses. As pointed out by Rendos et al. (1975), bacterial populations on teat ends are greatly influenced by uncontrolled environmental factors.

It is not surprising that all bacterial counts for teat-end samples were consistently the lowest at 0 h compared with other times, because the 0-h samples were taken after the cows were milked and their teats were postdipped, whereas all other samples were taken before the cow's teats were predipped upon entrance into the parlor. For the subsequent sampling times, the small differences in bacterial counts between the first day (12 to 24 h) and second day (36 to 48 h) shown in mattress samples diminished with the teat-end samples (Tables 3 and 6), because bacterial counts were similarly high from 12 to 48 h. We presume that the lack of a treatment effect over time on teat-end bacterial counts was a result of bacteria growth prevailing over the weak treatment effect. It is unlikely that differences in storage time for samples collected at various times (0, 12, 24, 36, and 48 h) would have had considerable impact on the results considering the findings from the preliminary trial. We do not know why *Klebsiella* spp. and *E. coli* counts on teat ends were lower at 48 h than at 36 h. Previous studies have found a modest correlation between bacterial counts on the teat ends and in sawdust bedding (Rendos et al., 1975; Hogan et al., 1999; Zdanowicz et al., 2004), whereas Zdanowicz et al. (2004) reported a weak correlation between bacterial counts in sand bedding and on teat ends.

In summary, results from the present study suggest that hydrated lime was the only treatment that significantly reduced bacterial counts of coliforms, *Klebsiella* spp., *E. coli*, and *Streptococcus* spp. on mattress samples. Hydrated lime also reduced bacterial counts of both coliforms and *Klebsiella* spp. in teat-end samples. Although fly ash and CAC effectively suppressed some bacterial groups on the mattresses compared with the no-bedding control or shavings, they had little effect on reducing bacterial counts at the teat ends. None of the treatments was effective in reducing *Streptococcus* spp. counts on the teats. Additional work is needed regarding the quantity and frequency of application of hydrated lime used as the sole bedding material on free-stall mattresses to minimize skin irritation and maximize its antibacterial efficacy. Further research is needed to develop economical, non-dust-producing, and nonirritating products with antibacterial effects that effectively reduce the numbers of bacteria on mattresses and teat ends in free-stall conditions, where cows are repeatedly contaminating and removing bedding with their feet as they enter and exit the free-stalls. In addition, identification of current and novel management practices that reduce the contamination of manure on teats between milking times in free-stalls is needed.

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REFERENCES

- Bloomfield, S. F., and E. E. Uso. 1985. The antibacterial properties of sodium hypochlorite and sodium dichloroisocyanurate as hospital disinfectants. *J. Hosp. Infect.* 6:20–30.
- Boddie, R. L., and S. C. Nickerson. 1996. Efficacy of teat dips containing a hypochlorous acid germicide against experimental challenge with *Staphylococcus aureus* and *Streptococcus agalactiae*. *J. Dairy Sci.* 79:1683–1688.
- Brazil, L., J. Kirk, R. Finger, C. Holmberg, W. Sischo, and G. Higginbotham. 1999. Studies on the potential use of fly ash in bedding on dairies. http://www.vetmed.ucdavis.edu/vetext/INF-DA/INF-DA_Flyash99.html Accessed June 27, 2007.
- DeGraves, F. J., and J. Petrow. 1993. Economics of mastitis and mastitis control. *Vet. Clin. North Am. Food Anim. Pract.* 9:421–434.
- Dou, Z., G. Y. Zhang, W. L. Stout, J. D. Toth, and J. D. Ferguson. 2003. Efficacy of alum and coal combustion by-products in stabilizing manure phosphorus. *J. Environ. Qual.* 32:1490–1497.
- Hogan, J. S., V. L. Bogacz, L. M. Thompson, S. Romig, P. S. Schoenberger, W. P. Weiss, and K. L. Smith. 1999. Bacterial counts associated with sawdust and recycled manure bedding treated with commercial conditioners. *J. Dairy Sci.* 82:1690–1695.
- Hogan, J. S., and K. L. Smith. 1997. Bacteria counts in sawdust bedding. *J. Dairy Sci.* 80:1600–1605.
- Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman,

- L. E. Heider, B. L. Brockett, and H. R. Conrad. 1989. Bacterial counts in bedding materials used on nine commercial dairies. *J. Dairy Sci.* 72:250–258.
- Hogan, J. S., S. L. Wolf, and C. S. Petersson-Wolfe. 2007. Bacterial counts in organic materials used as free-stall bedding following treatment with commercial conditioner. *J. Dairy Sci.* 90:1058–1062.
- Natzke, R. P., and B. J. LeClair. 1976. Coliform contaminated bedding and new infections. *J. Dairy Sci.* 59:2152–2154.
- Newman, L. E., and J. J. Kowalski. 1973. Fresh sawdust bedding—A possible source of *Klebsiella* organisms. *Am. J. Vet. Res.* 34:979–980.
- Rendos, J. J., R. J. Eberhart, and E. M. Kesler. 1975. Microbial populations on teat ends of dairy cows and bedding materials. *J. Dairy Sci.* 58:1492–1500.
- Smith, K. L., and J. S. Hogan. 2001. The World of Mastitis. Pages 1–12 in *Proc. 2nd Int. Symp. Mastitis and Milk Quality*, Vancouver, Canada. Natl. Mastitis Counc., Madison, WI, and Am. Assoc. Bovine Pract., Rome, GA.
- Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. G. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *J. Dairy Sci.* 87:1694–1701.