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Effect of Bedding Conditioners on Bacteria Counts and pH in Shavings, Digested Manure Solids and Recycled Sand Bedding

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Abstract

A controlled trial was conducted in 16 commercial dairy herds to describe the effect of an alkalinizing (A) and an acidifying (B) bedding conditioner on bedding pH and bacteria counts when applied twice per week to stalls containing shavings (SH), digested manure solids (DS) or recycled sand (RS) bedding materials. Bedding pH and bacteria counts were measured in samples collected one day, and again in three to four days, after new conditioner was applied to stalls.

Conditioners A and B had an alkalinizing and acidifying effect, respectively, on bedding pH. Although conditioner A reduced coliform bacteria counts by between 0.7 and 1.6 logs in RS and DS bedding for one day, it had no effect on *Klebsiella* spp or *Streptococcus* spp bacteria counts in these bedding materials, and activity against coliform bacteria counts did not persist for three to four days. Conditioner A reduced bacteria counts in SH by between 0.3 to 1.3 logs for at least one day, but had no persistent activity for three to four days when stalls were rebedded.

Conditioner B did not reduce bacteria counts in either DS or RS bedding, and actually increased *Klebsiella* spp bacteria counts in DS. However, conditioner B did reduce bacteria counts in SH for one day (0.4 to 1.8 log reduction, depending on the bacteria group), with antibacterial activity persisting for three to four days post-application (0.4 to 0.6 log reduction), even though stalls had been rebedded in the interval between conditioner application and collection of the day 3 to 4 bedding samples.

A very interesting observational finding was that, for all bedding materials studied, bacteria counts were significantly lower in stalls for which fresh bedding had been added within the previous 24-48 hours. The magnitude of reduction in bacteria counts associated with having recently bedded the stalls (approximately 1.6, 0.6, and 1.6 log reduction in coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria, respectively) was, on average, greater than the magnitude of reduction in bacteria counts attributed to the bedding conditioner treatments being studied.

These findings suggest that the alkalinizing conditioner A will not be useful on commercial dairies, regardless of bedding type in use, if applied only twice per week in accordance with manufacturer recommendations. The acidifying conditioner B will not be useful on commercial dairies using DS or RS bedding, but may be useful to reduce bacteria counts in SH bedding. Finally, findings suggest that producers can significantly reduce bacterial exposure to teat ends simply by applying fresh bedding to stalls on a more frequent basis.

Keywords: bovine, dairy, bedding, free-stalls

Résumé

Un essai clinique a été mené dans 16 fermes laitières commerciales afin de décrire les effets d'un conditionneur de litière alcalinisant (A) ou acidifiant (B) sur le pH de la litière et sur les comptages bactériens lorsque le conditionneur est administré deux fois par semaine à des stalles contenant des copeaux (SH), des solides digérés de fumier (DS) ou du sable recyclé (RS). Le pH de la litière et les comptages bactériens ont été mesurés dans des échantillons prélevés soit une journée ou soit trois à quatre jours suivant l'ajout du conditionneur aux stalles.

Le conditionneur A avait un effet alcalinisant et le conditionneur B un effet acidifiant sur le pH de la litière. Le conditionneur A réduisait le nombre de bactéries coliformes de 0.7 à 1.6 unités en log dans les litières RS et DS lors de la première journée mais n'a pas eu d'effet sur le nombre de bactéries *Streptococcus* spp. et *Klebsiella* spp. dans ces deux types de litières. L'effet sur le nombre de bactéries coliformes n'était plus présent après trois à quatre jours. Le conditionneur A réduisait

pendant au moins une journée le nombre de bactéries de 0.3 à 1.3 unités en log dans la litière SH mais n'a pas eu d'effet trois à quatre jours plus tard suite au changement de la litière dans les stalles. Le conditionneur B n'a pas eu d'effet sur le nombre de bactéries dans les litières DS et RS et a en fait produit une augmentation du nombre de bactéries *Klebsiella* spp. dans la litière DS. Toutefois, le conditionneur B réduisait le nombre de bactéries pendant au moins une journée dans la litière SH (de 0.4 à 1.8 unités en log dépendant du type de bactérie) et l'activité antimicrobienne persistait de trois à quatre jours suivant l'application (réduction de 0.4 à 0.6 unités en log) bien que ces échantillons provenaient de la nouvelle litière rajouté après l'application.

Il est intéressant de noter incidemment que pour tous les types de litières, le nombre de bactéries était significativement moins élevé dans les stalles où on avait rajouté de la litière fraîche dans les dernières 24 à 48 heures. La degré de réduction du nombre de bactéries accompagnant le rajout de litière fraîche dans les stalles (réduction de 1.6, 0.6, et 1.6 unités en log pour les bactéries coliformes, *Klebsiella* spp et *Streptococcus* spp, respectivement) était en moyenne plus élevé que le degré de réduction dans le nombre de bactéries que l'on pouvait attribuer à l'ajout de l'un ou l'autre des conditionneurs dans cette étude.

Ces résultats suggèrent que le conditionneur alcalinisant ne serait pas utile dans les fermes laitières commerciales peu importe de type de litière utilisé si on ne l'applique que deux fois par semaine selon le mode d'emploi du manufacturier. Le conditionneur acidifiant B ne sera pas utile dans le fermes avec des litières de type DS ou RS mais pourrait réduire le nombre de bactéries dans les litières de type SH. Finalement, les résultats suggèrent que les producteurs pourraient réduire l'exposition du bout des trayons aux bactéries simplement en rajoutant de la litière fraîche aux stalles sur une base régulière.

Introduction

Environmental exposure to coliform bacteria (e.g. *Escherichia coli*, *Klebsiella* spp) and environmental *Streptococcus* species (e.g. *S. uberis*) presents a significant risk factor for intramammary infection (IMI) in dairy cows. Bedding materials support significant bacterial growth.[1,2,10,12] In one study, the mean population of common environmental bacterial species (coliform bacteria, *Klebsiella* spp, *Streptococcus* spp) in digested manure exceeded 10^5 or 10^6 cfu/ml prior to use as bedding material.[4] Because bacteria counts in bedding materials correlate with bacteria populations and counts on teat ends,[4,8] management practices to control or reduce bacteria populations in bedding should reduce teat end exposure and the incidence of IMI caused by environmental mastitis pathogens. Bedding conditioners have the potential to act as chemical disinfectants to reduce bacteria counts in some bedding materials. The antibacterial activity of bedding conditioners is related to the pH of bedding materials.[4,5]

Despite the potential for bedding conditioners to reduce bacteria counts, studies describing the efficacy of bedding conditioners are limited in number, and have not typically evaluated the products in commercial dairy herds or in accordance with manufacturer's recommendations. For example, Hogan *et al*[4,5] evaluated antibacterial activity of bedding conditioners applied only once per week to stalls in a single university herd, even though commercial dairy producers frequently apply new bedding material to stalls on a more frequent basis. At least one manufacturer of bedding conditioner[b] recommends application to stalls twice per week. Kristula *et al*[7] reported application of hydrated lime as the sole bedding source on free-stall mattresses three times per week significantly reduced bacterial counts of coliform bacteria (*Klebsiella* spp, *E. coli*) and *Streptococcus* spp. However, due to irritation (mild ulceration and scaling) occurring on cows' legs and udders, the authors suggested that routine long-term use of more than 1.1 lb (0.5 kg) of lime alone on mattresses (i.e., without bedding) may not be recommended. The objective of this study was to describe the relationship between treatment with two bedding conditioners, one acidic and one alkaline, and environmental bacteria counts and pH in bedding materials, when using the same application rates and frequencies recommended by the manufacturer.

Materials and Methods

Farm Selection

The study was conducted in June and July, 2007, on 16 commercial free-stall dairy herds in Minnesota and western Wisconsin. Of these, five farms used shavings (SH) on mattresses, six farms used deep-bedded digested manure solids (DS), and five farms used deep-bedded recycled sand (RS) as bedding material for lactating cows. These herds represented a convenience sample, based on the type of bedding material used in the herd and their willingness to cooperate and comply with study protocols.

Stall Treatment Allocation and Treatment Procedures

The study was conducted for a period of 14 days in each of the study herds. Within each herd a series of six sections of free-stalls, with five adjacent stalls per section, were selected for assignment to a treatment group. The treatment sections of stalls were located in the central region of the high- or mid-lactation milking group, with three of the six sections occupying the middle row of stalls and the remaining three sections

occupying the outside row of stalls. One 'non-enrolled' stall separated each treated section from the adjacent treated sections within each row of stalls to serve as a washout area between treatment groups. Two of the six sections, one section on the inside row and one section on the outside row of stalls, were then randomly assigned to one of three bedding conditioner treatment groups:

Treatment A. Proprietary Alkaline Conditioner[a]
Treatment B. ZorbiSan™ Conditioner[b]
Treatment C. Negative control

Bedding conditioner application to stalls was completed in accordance with manufacturer recommendations. Herd owners and farm staff were blinded to treatment groups. For herds using SH bedding, after brushing the shavings forward on the mattress, 480 mL (16 oz or 454 g) of the treatment article was sprinkled by hand as evenly as possible over the back third of the mattress. For herds using RS or DS bedding, after pushing the top three inches (7.6 cm) of RS or DS forward, 480 mL of the treatment article was sprinkled by hand, as evenly as possible, over the back third of the stall. Next, the bedding (SH, RS or DS) was brushed back evenly over the back third of the stall, then 150 mL (5 oz or 142 g) of the treatment article was sprinkled on top of the bedding in the back third of the stall. On subsequent biweekly visits to the herd, bedding samples were first collected, the bedding was leveled, and then 150 mL of the treatment article was again sprinkled over the back third of the stall on top of SH bedding.

Schedule and Procedure for Collecting Bedding Samples

Figure 1 describes the schedule for bedding sample collection and application of bedding conditioners to stalls. The study technician collected bedding samples on day 0 (baseline sample) prior to initial application of the bedding conditioner. Additional bedding samples were then collected one day after the bedding conditioner was applied (day 1 sample) and again three to four days after the bedding conditioner was applied (day 3-4 sample), immediately prior to reapplication of the bedding conditioner. On any given sample collection day and for each treatment section of five stalls, two stalls were randomly selected for sampling. Thus, a total of four stalls were sampled for each of the three treatment groups on each sample collection day. For each section of stalls a new pair of latex gloves was applied and three separate samples of bedding (approx. 1/2 cup or 63 mL per sample) were collected from the top half-inch (1.27 cm) of bedding at different points in the back third of the stall being sampled. Bedding samples were mixed into a new plastic resealable bag (one bag per stall sampled) and placed on ice packs in a cooler. Upon completion of sample collection, samples were transported on ice to the University of Minnesota Laboratory for Udder Health and then frozen at -4.0°F (-20.0°C).

Because the objective of the study was to assess how the twice/week application schedule (as recommended by the manufacturer) would work under commercial conditions, no attempt was made to alter the bedding application schedule within any of the herds, relative to the new application of bedding conditioner. However, for all farms and all sample collection days, records were kept as to whether new bedding had been added to stalls between the time of the most recent application of the treatment article and the time of all bedding sample collection events.

Laboratory Testing of Bedding Samples

Frozen bedding samples were thawed at room temperature. Bedding material was tightly packed into a sterile 50-mL beaker using the thumb and index finger to pack and exclude as much air as possible. This

Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon
Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
*	*		*	*			*	*		*	*			*
454 g[1] + 150 g[2]			150 g[2]				150 g[2]			150 g[2]				end

*Collection of bedding samples immediately prior to application (day 0) or reapplication of bedding conditioner.
[1]Application of 454 g of bedding conditioner to stall base.
[2]Application of 150 g of bedding conditioner to stall surface.

Figure 1. Example schedule for collection of bedding samples and application of bedding to stalls in a study herd.

0.2 cup (50 mL) of packed bedding material was then transferred into a new whirl-pak bag, 250 ml of sterile distilled water was added, the contents were mixed and left to stand for 10 minutes, and then remixed.

The pH of the bedding-water mixture was determined using a Corning 320 pH meter.[c] A 200 µl sample was aseptically removed prior to pH determination and placed in sterile Brain Heart Infusion (BHI) broth. Serial 10-fold dilutions of the samples were immediately made in BHI broth. Sample dilutions were immediately plated in triplicate onto MacConkey agar (for gram-negative bacteria selection), MacConkey agar containing carbenicillin and inositol (selective for *Klebsiella* spp[5]), and colistin naladixic acid (CNA) (selective for gram-positive bacteria[6]) media plates and incubated at 98.6°F (37°C) for 24 hours. For the MacConkey plates, lactose fermenting (pink) colonies were counted as coliform bacteria. For the MacConkey agar plates containing carbenicillin and inositol, all colonies with morphology typical of *Klebsiella* spp were counted as *Klebsiella* spp (colony identity was confirmed for five to six colonies using the API 20E test[d]) and for CNA plates, colony morphology and the catalase test were used to differentiate staphylococci from streptococci. Five to six representative colonies of each were then selected for confirmatory identification using the API Staph test,[e] and API Strep test.[f] For each sample the total coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria counts per mL of bedding material were calculated and recorded.

Statistical Methods

Analysis was performed separately for each of the three bedding types studied. Descriptive statistics were generated describing bacteria counts and pH measures for the samples collected on day 0 (baseline samples) and on subsequent sampling days, by treatment group, and by day of sample collection (day 1 or day 3-4 samples). Baseline measures were first compared using ANOVA to investigate if initial bedding counts were similar across the three treatment groups. Measures from samples collected on all visits after day 0 were then analyzed using multiple linear regression analysis.[g] Dependent variables in these models included 1) $\log_{10}$(total coliform count, cfu per mL of bedding); 2) $\log_{10}$(total *Streptococcus* spp, cfu per mL of bedding); 3) $\log_{10}$(total *Klebsiella* spp count, cfu per mL of bedding); and 4) pH of bedding. Explanatory variables offered into these models included 1) treatment group (forced); 2) time interval between the most recent previous application of treatment article and sample collection (day1 or day 3-4); 3) whether or not the stalls had been rebedded in the interval between the most recent application of the treatment article and sample collection (Rebedded? Yes/No); and 4) baseline bacteria counts. Furthermore, a variable describing whether samples were collected in week 1 or week 2 of the study was offered to all models to investigate whether estimates of treatment effect would vary over the two-week period that the study was conducted in each herd. All possible two-way interaction terms between treatment group and other covariates were also offered into the models. With the exception of the variable describing treatment group, which was forced, all other covariates were then subjected to a backwards elimination procedure. Final significance was declared at $P<0.05$.

A variable describing 'herd' was included as a random effect in the model to control for the herd-level clustering created by repeated sampling of stalls within herd over the two-week period. The total number of samples collected was expected to provide for 95% confidence and 85% power to detect a predicted difference of approximately a 1 $\log_{10}$ reduction in total coliform or total *Streptococcus* spp bacteria counts in bedding treated with either of the two bedding conditioners, as compared to the control group (assume std. dev. = 1.5).

Results

A total of 1,655 bedding samples were collected from the 16 study herds. Results describing pH measures for treated and control stalls are presented in Table 1. Bacteria counts in treated and control stalls are presented in Table 2 (Conditioner A) and Table 3 (Conditioner B). Baseline measures of bacteria counts and pH on day 0 were not different among treatment and control groups, with a couple of exceptions in the SH group: baseline *Klebsiella* spp counts in SH for assessment of Conditioner A, and baseline coliform and *Klebsiella* spp counts in SH for assessment of Conditioner B were higher in control vs treated stalls. As a consequence, these models offered to control for baseline bacteria counts as a covariate in the model. However, baseline bacteria counts were found not to be significant in the final models and did not affect (confound) the estimates or statistical inferences for the effect of treatment on the outcomes of interest. The authors expect that these differences did not significantly affect study findings because the magnitude of difference that originally existed between treated and control stalls for baseline samples was smaller than the magnitude of difference measured between treated and control stalls after the conditioner was added to the stalls.

A variable describing whether samples were collected in week 1 or week 2 of the study was not significant, suggesting that estimates of treatment effect were constant over the two-week period that the study was conducted in each herd.

Due to the detection of interactions, all analyses were stratified for time of sample collection (day 1 vs day 3-4) as well as for whether or not stalls had been

Table 1. Effect of bedding conditioners on pH in bedding materials.

Bedding type Sample type	No. farms (No. samples)	Treatment A	Treatment B	Control
Digested Solids				
Day 0 – Baseline	6 (22)	9.092 (0.077)[a]	8.978 (0.077)[1]	8.962 (0.078)[a,1]
Day 1 – All	6 (254)	9.198 (0.097)[a]	8.681 (0.097)[1]	9.025 (0.097)[b,2]
Day 1 – Not rebedded	5 (85)	9.222 (0.093)[a]	8.255 (0.093)[1]	8.967 (0.092)[b,2]
Day 1 – Rebedded	5 (169)	9.168 (0.105)[a]	8.874 (0.105)[1]	9.036 (0.105)[b,2]
Day 3 – All	6 (251)	8.951 (0.086)[a]	8.793 (0.086)[1]	8.903 (0.086)[a,2]
Day 3 – Not rebedded	1 (12)	8.790 (0.094)[a]	8.40 (0.094)[1]	8.683 (0.094)[a,1]
Day 3 – Rebedded	5 (239)	8.993 (0.085)[a]	8.846 (0.085)[1]	8.948 (0.085)[a,2]
Recycled Sand				
Day 0 – Baseline	5 (20)	8.319 (0.253)[a]	8.268 (0.253)[1]	8.400 (0.252)[a,1]
Day 1 – All	5 (241)	9.426 (0.141)[a]	8.075 (0.141)[1]	8.607 (0.141)[b,2]
Day 1 – Not rebedded	2 (96)	9.515 (0.223)[a]	7.80 (0.223)[1]	8.403 (0.223)[b,2]
Day 1 – Rebedded	3 (145)	9.364 (0.199)[a]	8.252 (0.199)[1]	8.744 (0.199)[b,2]
Day 3 – All	5 (240)	8.931 (0.207)[a]	8.251 (0.207)[1]	8.452 (0.207)[b,2]
Day 3 – Not rebedded	1 (25)	9.398 (0.148)[a]	8.421 (0.148)[1]	8.651 (0.140)[b,1]
Day 3 – Rebedded	5 (215)	8.894 (0.204)[a]	8.247 (0.204)[1]	8.444 (0.204)[b,2]
Shavings				
Day 0 – Baseline	5 (20)	7.381 (0.528)[a]	7.346 (0.527)[1]	7.100 (0.528)[a,1]
Day 1 – All	5 (240)	8.398 (0.327)[a]	6.067 (0.327)[1]	7.242 (0.327)[b,2]
Day 1 – Not rebedded	4 (119)	8.515 (0.508)[a]	5.960 (0.508)[1]	7.180 (0.508)[b,2]
Day 1 – Rebedded	4 (121)	8.313 (0.356)[a]	6.219 (0.354)[1]	7.337 (0.354)[b,2]
Day 3 – All	5 (242)	7.410 (0.348)[a]	7.204 (0.348)[1]	7.255 (0.348)[a,1]
Day 3 – Not rebedded	0 (0)	NA	NA	NA
Day 3 – Rebedded	5 (242)	7.410 (0.348)[a]	7.204 (0.348)[1]	7.255 (0.348)[a,1]

Report LS Means (SE) of pH; NA = Not assessed (no stalls fit this category).
[a,b]Differences between Treatment A and Controls, within row, significant at $P \leq 0.05$.
[1,2]Differences between Treatment B and Controls, within row, significant at $P \leq 0.05$.

rebedded in the interval between the most recent application of the conditioner article and collection of the bedding sample. Because all herds using SH had routinely rebedded stalls before any 3-4 day samples could be collected, no estimates could be made for effectiveness of treatment at 3-4 days post-application if the stalls were not rebedded.

An extremely interesting finding: when controlling for conditioner treatment group, bedding type, and sampling interval (day 1 vs day 3-4), bacteria counts in all bedding materials were consistently and significantly lower for stalls that had been rebedded in the interval between the most recent application of treatment and collecting the bedding sample (usually within the past 24-48 hrs), as compared to stalls that had not been rebedded in this interval. Adjusted LS means (SE) counts for coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria were 3.12 (0.21), 0.98 (0.14), and 5.21 (0.26) in stalls that had not been rebedded, as compared to 1.56 (0.19), 0.37 (0.13), and 3.65 (0.25) for stalls that had been rebedded in this interval.

Relationship between Treatment with an Alkaline Conditioner A and pH and Bacteria Counts in Bedding

Digested Solids

When examining day 1 samples, treatment with conditioner A was associated with increased pH (vs controls), whether or not the stall had been rebedded in the interval between the most recent conditioner application and the time of sample collection (Table 1). Treatment was associated with reduced coliform bacteria counts on day 1 when the producer had not rebedded the stall in the interval between the most recent bedding application and the time of sample collection, but not when stalls had been rebedded in this interval (Table 2). There was no effect of treatment on *Klebsiella* spp or *Streptococcus* spp

Table 2. Effect of an alkalinizing bedding conditioner (A) on bacteria counts in bedding materials.

Bedding type Sample type	No. farms (No. samples)	Coliforms Treatment A	 Control	*Klebsiella* spp Treatment A	 Control	*Streptococcus* spp Treatment A	 Control
Digested Solids							
Day 0 – Baseline	6 (22)	2.79 (0.80)[a]	3.05 (0.80)[a]	0.67 (0.59)[a]	0.84 (0.59)[a]	4.53 (0.86)[a]	4.57 (0.87)[a]
Day 1 – All	6 (254)	1.82 (0.55)[a]	2.12 (0.55)[a]	0.20 (0.19)[a]	0.42 (0.19)[a]	3.71 (0.47)[a]	3.86 (0.47)[a]
Day 1 – Not rebedded	5 (85)	3.03 (0.33)[a]	3.69 (0.33)[b]	0.23 (0.33)[a]	0.78 (0.33)[a]	4.81 (0.50)[a]	4.70 (0.50)[a]
Day 1 – Rebedded	5 (169)	0.98 (0.36)[a]	1.07 (0.36)[a]	0.04 (0.04)[a]	0.09 (0.04)[a]	3.07 (0.41)[a]	3.33 (0.41)[a]
Day 3 – All	6 (251	1.55 (0.56)[a]	1.80 (0.56)[a]	0.085 (0.15)[a]	0.14 (0.15)[a]	3.49 (0.63)[a]	3.71 (0.63)[a]
Day 3 – Not rebedded	1 (12)	3.75 (0.28)[a]	3.71 (0.28)[a]	0 (0.20)[a]	0.35 (0.20)[a]	4.21 (0.19)[a]	4.47 (0.19)[a]
Day 3 – Rebedded	5 (239)	1.17 (0.51)[a]	1.44 (0.51)[a]	0.10 (0.17)[a]	0.14 (0.17)[a]	3.35 (0.74)[a]	3.57 (0.74)[a]
Recycled Sand							
Day 0 – Baseline	5 (20)	2.53 (0.80)[a]	3.45 (0.80)[a]	0.62 (0.34)[a]	0.88 (0.33)[a]	4.47 (0.64)[a]	4.68 (0.63)[a]
Day 1 – All	5 (241)	1.22 (0.55)[a]	2.34 (0.55)[b]	0.47 (0.44)[a]	0.74 (0.44)[a]	4.30 (0.55)[a]	4.64 (0.55)[a]
Day 1 – Not rebedded	2 (96)	1.87 (0.68)[a]	3.44 (0.68)[b]	1.17 (1.12)[a]	1.27 (1.12)[a]	5.90 (0.11)[a]	5.78 (0.11)[a]
Day 1 – Rebedded	3 (145)	0.79 (0.56)[a]	1.60 (0.56)[b]	0.003 (0.18)[a]	0.39 (0.18)[b]	3.23 (0.38)[a]	3.87 (0.38)[a]
Day 3 – All	5 (240)	0.81 (0.31)[a]	1.19 (0.31)[a]	0.13 (0.12)[a]	0.25 (0.13)[a]	3.54 (0.44)[a]	3.58 (0.44)[a]
Day 3 – Not rebedded	1 (25)	1.85 (0.48)[a]	3.00 (0.45)[a]	0.0 (0)[a]	0.21 (0.18)[a]	6.29 (0.17)[a]	6.19 (0.16)[a]
Day 3 – Rebedded	5 (215)	0.61 (0.35)[a]	0.89 (0.35)[a]	0.12 (0.13)[a]	0.23 (0.13)[a]	3.24 (0.31)[a]	3.28 (0.31)[a]
Shavings							
Day 0 – Baseline	5 (20)	3.04 (0.46)[a]	3.41 (0.46)[a]	1.10 (0.64)[a]	1.73 (0.64)[b]	4.76 (0.40)[a]	5.18 (0.40)[a]
Day 1 – All	5 (240)	2.05 (0.36)[a]	3.27 (0.36)[b]	0.51 (0.37)[a]	0.96 (0.37)[b]	4.48 (0.58)[a]	5.05 (0.58)[b]
Day 1 – Not rebedded	4 (119)	2.59 (0.27)[a]	3.77 (0.27)[b]	0.70 (0.46)[a]	1.35 (0.46)[b]	5.36 (0.58)[a]	5.60 (0.58)[a]
Day 1 – Rebedded	4 (121)	1.52 (0.46)[a]	2.79 (0.46)[b]	0.30 (0.25)[a]	0.57 (0.25)[b]	3.45 (0.74)[a]	4.38 (0.74)[b]
Day 3 – All	5 (242)	2.38 (0.36)[a]	2.61 (0.36)[a]	0.83 (0.39)[a]	1.16 (0.39)[a]	4.56 (0.46)[a]	4.57 (0.46)[a]
Day 3 – Not rebedded	0 (0)	NA	.	.	.	.	.
Day 3 – Rebedded	5 (242)	2.38 (0.36)[a]	2.61 (0.36)[a]	0.83 (0.39)[a]	1.16 (0.39)[a]	4.56 (0.46)[a]	4.57 (0.46)[a]

Report LS Means (SE) of $\log_{10}$(total bacteria count cfu/cc); NA = Not assessed (no stalls fit this category).
[a,b]Differences between Treatment A and Controls, within bedding type, row and bacteria group significant at $P \leq 0.05$.

bacteria counts (vs controls) on day 1, regardless of the bedding schedule. When examining day 3 samples, there was no effect of treatment on pH or any bacteria counts, regardless of whether or not stalls had been rebedded within the interval between bedding article application and collecting the day 3 samples (Tables 1 and 2).

Recycled Sand

Treated stalls had higher bedding pH (vs controls) in both day 1 and day 3-4 samples, regardless of the rebedding schedule (Table 1). For day 1 samples, treatment was associated with lower coliform counts, regardless of the rebedding schedule (Table 2). However, this relationship did not persist: there was no effect of treatment on coliform counts in day 3-4 samples, regardless of the rebedding schedule (Table 2). Treatment was associated with reduced *Klebsiella* spp counts in stalls that had been rebedded in the interval between treatment application and sample collection (Table 2). However, treatment was not associated with *Klebsiella* spp counts in stalls that had not been rebedded in this interval. Since the authors would expect to more likely observe a treatment effect in 'non-rebedded' stalls (should a treatment effect exist at all), then an explanation for these counter-intuitive results is not immediately apparent. There was no relationship between treatment and *Streptococcus* spp counts (vs controls) in day 1 or day 3 samples, regardless of the rebedding schedule (Table 2).

Shavings

When examining the day 1 samples, treatment with the alkaline bedding conditioner was associated with significantly higher bedding pH and lower coliform and *Klebsiella* spp bacteria counts (versus controls) in SH, regardless of whether the stalls had been rebedded in the interval between the most recent bedding application and the time of sample collection (Tables 1 and 2). Treatment was associated with lower *Streptococcus* spp counts (versus controls) if the producer had rebed-

Table 3. Effect of an acidifying bedding conditioner (B) on bacteria counts in bedding materials.

Bedding type Sample type	No. farms (No. samples)	Coliforms Treatment B	Control	*Klebsiella* spp Treatment B	Control	*Streptococcus* spp Treatment B	Control
Digested Solids							
Day 0 – Baseline	6 (22)	2.76 (0.80)[a]	3.05 (0.80)[a]	0.52 (0.59)[a]	0.84 (0.59)[a]	4.69 (0.86)[a]	4.57 (0.87)[a]
Day 1 – All	6 (254)	2.39 (0.55)[a]	2.12 (0.55)[a]	0.53 (0.19)[a]	0.42 (0.19)[a]	3.85 (0.47)[a]	3.86 (0.47)[a]
Day 1 – Not rebedded	5 (85)	4.00 (0.33)[a]	3.69 (0.33)[a]	1.25 (0.33)[a]	0.78 (0.33)[a]	4.96 (0.50)[a]	4.70 (0.50)[a]
Day 1 – Rebedded	5 (169)	1.36 (0.36)[a]	1.07 (0.36)[a]	0.03 (0.04)[a]	0.09 (0.04)[a]	3.22 (0.41)[a]	3.33 (0.41)[a]
Day 3 – All	6 (251)	2.01 (0.56)[a]	1.80 (0.56)[a]	0.38 (0.15)[a]	0.14 (0.15)[b]	3.75 (0.63)[a]	3.71 (0.63)[a]
Day 3 – Not rebedded	1 (12)	3.75 (0.28)[a]	3.71 (0.28)[a]	0 (0.20)[a]	0.35 (0.20)[a]	4.39 (0.19)[a]	4.47 (0.19)[a]
Day 3 – Rebedded	5 (239)	1.65 (0.51)[a]	1.44 (0.51)[a]	0.40 (0.18)[a]	0.14 (0.17)[b]	3.61 (0.74)[a]	3.57 (0.74)[a]
Recycled Sand							
Day 0 – Baseline	5 (20)	2.53 (0.80)[a]	3.45 (0.80)[a]	0.53 (0.34)[a]	0.88 (0.33)[a]	4.30 (0.64)[a]	4.68 (0.63)[a]
Day 1 – All	5 (241)	2.58 (0.54)[a]	2.34 (0.55)[a]	0.91 (0.44)[a]	0.74 (0.44)[a]	4.67 (0.55)[a]	4.64 (0.55)[a]
Day 1 – Not rebedded	2 (96)	3.73 (0.68)[a]	3.44 (0.68)[a]	1.45 (1.12)[a]	1.27 (1.12)[a]	5.65 (0.11)[a]	5.78 (0.11)[a]
Day 1 – Rebedded	3 (145)	1.83 (0.55)[a]	1.60 (0.56)[a]	0.55 (0.18)[a]	0.39 (0.18)[a]	4.02 (0.38)[a]	3.87 (0.38)[a]
Day 3 – All	5 (240)	1.28 (0.32)[a]	1.19 (0.31)[a]	0.35 (0.12)[a]	0.25 (0.13)[a]	3.68 (0.44) [a]	3.58 (0.44)[a]
Day 3 – Not rebedded	1 (25)	4.04 (0.48)[a]	3.00 (0.45)[a]	0.25 (0.19)[a]	0.21 (0.18)[a]	6.25 (0.17)[a]	6.19 (0.16)[a]
Day 3 – Rebedded	5 (215)	0.89 (0.35)[a]	0.89 (0.35)[a]	0.34 (0.13)[a]	0.23 (0.13)[a]	3.41 (0.31)[a]	3.28 (0.31)[a]
Shavings							
Day 0 – Baseline	5 (20)	2.47 (0.46)[a]	3.41 (0.46)[b]	0.95 (0.64)[a]	1.73 (0.64)[b]	5.03 (0.40)[a]	5.18 (0.40)[a]
Day 1 – All	5 (240)	2.02 (0.36)[a]	3.27 (0.36)[b]	0.72 (0.37)[a]	0.96 (0.37)[a]	3.29 (0.58)[a]	5.05 (0.58)[b]
Day 1 – Not rebedded	4 (119)	2.26 (0.27)[a]	3.77 (0.27)[b]	1.23 (0.46)[a]	1.35 (0.46)[a]	3.63 (0.58)[a]	5.60 (0.58)[b]
Day 1 – Rebedded	4 (121)	1.79 (0.46)[a]	2.79 (0.46)[b]	0.21 (0.25)[a]	0.57 (0.25)[b]	2.84 (0.74)[a]	4.38 (0.74)[b]
Day 3 – All	5 (242)	2.02 (0.36)[a]	2.61 (0.36)[b]	0.70 (0.39)[a]	1.16 (0.39)[b]	4.19 (0.46)[a]	4.57 (0.46)[b]
Day 3 – Not rebedded	0 (0)	NA	.	.	.	.	.
Day 3 – Rebedded	5 (242)	2.02 (0.36)[a]	2.61 (0.36)[b]	0.70 (0.39)[a]	1.16 (0.39)[b]	4.19 (0.46)[a]	4.57 (0.46)[b]

Report LS Means (SE) of $\log_{10}$(total bacteria count cfu/cc); NA = Not assessed (no stalls fit this category).
[a,b]Differences between Treatment B and Controls, within row and bedding type, significant at $P \leq 0.05$.

ded the stall in the interval between the most recent bedding conditioner application and the time of sample application. However, there was no relationship between treatment and *Streptococcus* spp counts if the stalls had not been rebedded in this interval (Table 2). When examining the day 3 samples, there was no relationship between treatment and either bedding pH or counts of coliform, *Klebsiella* spp or *Streptococcus* spp bacteria (versus control stalls) when stalls had been rebedded within the interval between bedding article application and collecting the day 3 samples (Tables 1 and 2). However, because all SH stalls had been rebedded in this interval and because treatment was associated with pH and bacteria counts in day 1 samples, regardless of the rebedding schedule, it could not be extrapolated whether or not a treatment would continue to be associated with either increased pH or reduced bacteria counts at 3-4 days if stalls had not been rebedded.

Relationship between Treatment with an Acidifying Conditioner B and pH and Bacteria Counts in Bedding

Digested Solids

When examining day 1 samples, treatment with conditioner B was associated with lower bedding pH (vs controls), regardless of the stall rebedding schedule. This relationship was still present in day 3 samples in stalls that had been rebedded in the interval between the most recent bedding application and collection of the day 3 bedding samples (Table 1). A numeric reduction in pH was also observed on day 3 in stalls that had not been rebedded. However, due to relatively few observations (only one herd), the study probably lacked sufficient power to detect a significant effect of conditioner B on pH in this category. When examining day 1 and day 3 samples, treatment with conditioner B was not associated

with reduced counts of coliform, *Klebsiella* spp, or *Streptococcus* spp bacteria (vs controls), regardless of the stall rebedding schedule. In fact, *Klebsiella* spp counts were significantly higher in treated stalls on day 3 (Table 3).

Recycled Sand

When examining day 1 samples, treatment with conditioner B was associated with reduced bedding pH (vs controls), whether or not the stall had been rebedded in the interval between the most recent conditioner application and the time of sample collection (Table 1). When examining day 3 samples, treated stalls continued to have lower pH values, but these differences were only significant in stalls that had been rebedded in the interval between the most recent conditioner application and the time of sample collection. When examining day 1 and day 3 bacteria counts, treatment did not reduce counts of coliform, *Klebsiella* spp, or *Streptococcus* spp bacteria (vs controls), regardless of the rebedding schedule (Table 3).

Shavings

When examining the day 1 samples, treated stalls had significantly lower bedding pH and bedding coliform and *Streptococcus* spp counts (versus controls), whether or not the stall had been rebedded in the interval between the most recent conditioner application and the time of sample collection (Tables 1 and 3). Lower *Klebsiella* spp counts were recorded for treated stalls that had been rebedded in the interval between the most recent conditioner application and the time of sample collection. However *Klebsiella* spp counts were not different for treated stalls that had not been rebedded in this interval (Table 3). When examining day 3 samples, treatment was not associated with pH in bedding (Table 1). However, treated stalls had significantly lower coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria counts in SH (vs controls), even though all stalls had been rebedded within the interval between bedding article application and collecting the day 3 sample (Table 3).

Discussion

The objective of this study was to describe the relationship between using two bedding conditioners, one acidic and one alkaline, when used in wood shavings, digested manure solids, and recycled sand bedding materials in commercial dairy herds, and environmental bacteria counts and pH in bedding materials, when using the same application rates and frequencies as recommended by the manufacturer. A strength of the study is that the design allowed investigators to describe the relationship between treatment and outcome measures when the bedding conditioners were used according to manufacturer's directions in multiple commercial herds, and with a variety of commonly used bedding materials. The study design was controlled in the sense that side-by-side measures were collected from randomly assigned treated and control stalls.

It is both a strength and a weakness that the study did not seek to control the rebedding schedule on study farms. This is a strength of most multi-herd field studies because results should predict what might be expected on commercial farms with irregular bedding schedules, should they adopt the twice-weekly conditioner application schedule that is recommended by the manufacturer of the bedding conditioners being evaluated. Conversely, this introduced a weakness in that the effect of the rebedding schedule within any one herd was essentially observational in nature, introducing a new variable that needed to be controlled for in the regression models. When both 'rebedded' and 'non-rebedded' stalls were well represented across multiple farms and in the analysis of both day 1 and day 3-4 sample results, as was the case for herds using RS and SH, then this was not a major concern, albeit relatively few observations were available for day 3 non-rebedded stalls (only one herd represented in this category). However, for farms using SH, a limitation was introduced because all farms had routinely rebedded all stalls before day 3-4 bedding samples could be collected for testing. This limited the authors' ability to make inferences about the persistence of treatment effect for as long as 3-4 days for some outcomes.

One criticism that might be leveled at this study is that the basis for reporting culture results was cfu/mL of bedding material, and not cfu/g of bedding material, as has been reported by some other laboratories.[3,4,5] The authors are unaware of any official publications of laboratory standards (e.g. the International Dairy Federation or the National Mastitis Council) describing how bedding culture results should be reported. Perhaps this needs to be developed in future. Furthermore, because the current study is seeking to make 'within-bedding' comparisons (e.g. between treated and control stalls within a given bedding type), and is not trying to make 'between-bedding' comparisons (e.g. is not comparing bacteria counts in SH vs RS bedding), then the results reported in the current study should be valid.

Relationship between Treatment with an Alkaline Conditioner A and pH and Bacteria Counts in Bedding

For herds using RS bedding, treatment with conditioner A was associated with increased pH for as long as three to four days, regardless of the rebedding schedule. Treatment was associated with reduced coliform counts for only one day, and treatment was not associated with reduced counts of *Streptococcus* spp or *Klebsiella* spp

bacteria for either one, three or four days, regardless of the rebedding schedule. For herds using DS bedding, even though treated stalls had reduced coliform counts for one day if the stall was not rebedded, there was no effect of conditioner A on counts of *Klebsiella* spp or *Streptococcus* spp bacteria for even one day, regardless of the rebedding schedule. Future research will need to investigate the persistency of conditioner A (for up to three or four days) in reducing coliform counts in DS if stalls are not rebedded after the twice-weekly application of this conditioner. However, when taken as a whole, these results would seem to suggest that the alkaline conditioner A will be ineffective for most bacteria if applied to RS or DS-bedded stalls according to manufacturer's directions.

For herds using SH bedding, treatment with conditioner A reduced counts of coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria in SH for at least one day, but had no persistent antibacterial activity for three or four days when stalls had been rebedded. Future research will need to investigate the three or four-day persistency of conditioner A in reducing bacteria counts in SH if stalls are not rebedded after the twice-weekly application of this conditioner. These results suggest that conditioner A could be effective if used in SH-bedded stalls. However, producers considering using conditioner A in SH would be advised to apply the treatment more frequently than twice per week and each time after new bedding is applied to stalls.

With respect to the alkaline conditioner A, the results of this study agree with previous studies of alkaline conditioners. Hogan *et al*[4] reported that both proprietary alkaline conditioner and hydrated lime effectively inhibited bacteria in recycled manure for one day. However antibacterial activity deteriorated between day 2 and 6. Kristula *et al*[7] reported that hydrated lime applied to unbedded mattresses every 48 hours reduced bacterial growth on mattresses. However, because it caused irritation to teat skin and legs of approximately one-third of cows in this treatment group, the authors discouraged the use of hydrated lime alone on mattresses. The cumulative results from this and previous studies suggest that alkaline conditioners, such as hydrated lime or the proprietary conditioner used in this study, have limited duration of activity in bedding, and so would need to be reapplied more frequently than twice per week, and after each event of putting new bedding into stalls.

Relationship between Treatment with an Acidifying Conditioner B and pH and Bacteria Counts in Bedding

Conditioner B had an acidifying effect on bedding pH for up to three to four days in DS and RS, and for at least one day in SH. Use of conditioner B was not associated with reduced bacteria counts in DS or RS, regardless of the rebedding schedule. In fact, DS stalls treated with conditioner B actually had increased counts of *Klebsiella* spp bacteria in day 3 samples. However, use of conditioner B was associated with reduced counts of coliforms, *Klebsiella* spp, and *Streptococcus* spp bacteria in SH for up to three to four days post-application, even when the stalls had been rebedded in the interval between conditioner application and collection of the bedding sample. These results suggest that using the acidifying conditioner B would be ineffective in reducing bacteria counts in DS or RS-bedded stalls, but effective in reducing bacteria counts in SH-bedded stalls when used according to the manufacturer's recommendations to apply this treatment to stalls twice per week.

With respect to the acidic conditioner B, results of this study generally agree with findings from a previous study of a different commercial acidic conditioner.[4] Those authors also reported that an acidic conditioner had little effect on bacteria counts in recycled manure bedding. However, the same acidic conditioner had a bacteriostatic effect in sawdust for at least two days after application. This antibacterial activity had diminished by six days after application, which was when the next bedding sample was collected for culture. However, it could not be determined from the published data for how long between the two and six day sampling points the conditioner remained effective.[4]

Despite the promising results of this study, particularly as they relate to the effectiveness of the acidic conditioner B in SH bedding, this study was not designed to investigate whether the use of bedding conditioners had an effect on udder health. Hogan *et al*[4] described a positive correlation between bacteria counts in bedding and from teat swabs. However, various studies have reported that suppressed bacterial growth on mattresses treated with conditioners did not consistently result in lower bacterial numbers on the teat ends.[4,7] Furthermore, studies are lacking that describe whether the use of bedding conditioners impacts measures of udder health, such as risk for clinical mastitis or somatic cell count measures. As such, the biological efficacy measured as improved udder health, and economic cost-benefit of using bedding conditioners on commercial dairy farms requires further study.

Apart from the major objective and findings of this study, there was another very interesting finding. Bedding bacteria counts were consistently and significantly lower across all bacteria groups, bedding material types, and conditioner treatment groups for stalls that had been rebedded in the interval between the previous and next sampling interval (usually within the last 24-48 hours), as compared to stalls that had not been rebedded in the same interval. Though observational in nature, these findings are extremely interesting because the

magnitude of reduction in bacteria counts associated with having recently bedded the stalls (approximately 1.6, 0.6, and 1.6 log reductions in coliform, *Klebsiella* spp, and *Streptococcus* spp bacteria, respectively) was, on average, greater than the magnitude of reduction in bacteria counts attributed to the bedding conditioner treatments being studied. Hogan *et al*[4] reported that bacteria counts in sawdust bedding increased over a six-day period in stalls. However, a similar pattern was not evident for bacteria counts in recycled manure. Kristula *et al*[7] reported that bacterial populations grew steadily on mattresses over a 48-hour period. Taken as a whole, the results of this and previous studies would support recommendations by mastitis experts, suggesting that more frequent application of new bedding into stalls may reduce bacteria counts in all bedding types.[9]

Conclusions

These findings suggest that the alkalinizing conditioner A will not be useful on commercial dairies, regardless of bedding type in use, if applied only twice per week in accordance with manufacturer recommendations. The acidifying conditioner B will not be useful on commercial dairies using DS or RS bedding, but may be useful to reduce bacteria counts in SH bedding. Finally, findings suggest that producers can significantly reduce bacterial exposure to teat ends simply by applying fresh bedding to stalls on a more frequent basis.

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Endnotes

[a]Proprietary Alkaline Conditioner, WestfaliaSurge, Inc., Naperville, IL

[b]ZorbiSan™, WestfaliaSurge Inc. Naperville, IL

[c]Corning Inc., Corning, NY

[d]API 20E test, BioMerieux, St. Louis, MO

[e]API Staph test, BioMerieux, St. Louis, MO

[f]API Strep test, BioMerieux, St. Louis, MO

[g]SAS, Cary, NC

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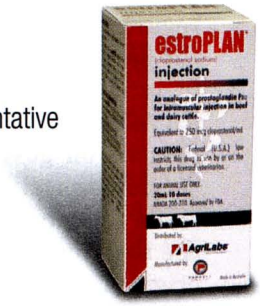

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For intramuscular use to induce luteolysis in beef and dairy cattle. The luteolytic action of **estroPLAN** injection can be utilized to manipulate the estrous cycle to better fit certain management practices, to terminate pregnancies resulting from mismatings and to treat certain conditions associated with prolonged luteal function.

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SAFETY AND TOXICITY:
At 50 and 100 times the recommended dose, mild side effects may be detected in some cattle. These include increased uneasiness, slight frothing, and milk let-down.

CONTRAINDICATIONS:
estroPLAN should not be administered to a pregnant animal whose calf is not to be aborted.

WARNINGS:
For animal use only. Women of child-bearing age, asthmatics, and persons with bronchial and other respiratory problems should exercise extreme caution when handling this product. In the early stages women may be unaware of their pregnancies.

estroPLAN injection is readily absorbed through the skin and may cause abortion and/or bronchospasms; direct contact with the skin should therefore be avoided. Accidental spillage on the skin should be washed off immediately with soap and water.

PRECAUTIONS:
There is no effect on fertility following the single or double dosage regimen when breeding occurs at induced estrus or at 72 and 96 hours post treatment. Conception rates may be lower than expected in those fixed time breeding programs which omit the second insemination (i.e., the insemination at or near 96 hours). This is especially true if a fixed time insemination is used following a single **estroPLAN** injection.

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