Bacteria Counts in Sawdust Bedding[1]

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ABSTRACT

Bacteria counts in untreated sawdust bedding were compared with those in sawdust bedding after the addition of lime and after daily replacement of bedding in the back one-third of the stalls. Addition of 1 kg of lime to 10 kg of sawdust reduced Gram-negative bacteria, coliforms, *Klebsiella* spp., and streptococci prior to use as bedding. Sawdust treated with lime also showed decreased bacteria counts compared with bacteria counts for sawdust that was replaced daily and compared with bacteria counts for control bedding after 1 d in the stall. The decrease in bacterial populations was related to an increase in bedding pH. Mean pH in the sawdust treated with lime was greater prior to use and after 1 d in the stall than the pH of other treatments. After 2 and 6 d in stalls, however, bacteria counts and pH were similar among treatments. Dry matter content of bedding did not differ among bedding treatments. Bacteria counts in bedding were positively correlated with teat skin swabs. Gram-negative bacteria and *Klebsiella* spp. counts on teat swabs were lower for cows housed on bedding treated with lime on d 2 compared with those for cows housed on control bedding and bedding that was replaced daily. Addition of lime to sawdust in the back one-third of tie stalls caused a decrease in exposure of teats to environmental mastitis pathogens in bedding for 1 d. Daily replacement of bedding had a minimal effect on bacteria counts in bedding and on teat skin.
(**Key words**: sawdust bedding, bacteria counts, lime)

Abbreviation key: **MCIC** = MacConkey-inositol-carbenicillin.

INTRODUCTION

The number and type of bacteria in bedding are related to the microbial load on teat ends and rates of clinical mastitis in lactating dairy cows (6). Organic materials such as straw, corn fodder, and sawdust often contain $>10^6$ cfu/g of coliform bacteria when used as bedding (3). Bacteria counts also differ within organic beddings; wood products often contain the greatest number of coliform bacteria (9). Wood products, such as sawdust and shavings, have been found to be heavily contaminated with *Klebsiella* spp. (8). Sawdust and wood shavings continue to be popular choices as bedding despite evidence that outbreaks of coliform mastitis within a herd are commonly attributed to contaminated bedding (2).

The control of bacterial populations in organic materials often involves intensive management of stalls and bedding. A common practice on many farms that use wood products for bedding is to add hydrated lime to the stalls to control bacterial populations (4) because lime elevates the pH and reduces the moisture content of the bedding to inhibit bacterial growth. Bramley (2) reported that daily replacement of sawdust in the back one-third of stalls also reduced bacterial contamination of stalls. Reduced coliform populations in the sawdust from daily replacement of bedding was associated with reduced incidence of clinical mastitis (2). Despite a number of studies that have investigated the merits of these bedding management practices, data are limited comparing the effectiveness of lime and daily replacement of bedding within a similar environment to control teat end exposure to mastitis pathogens. The purpose of the current study was to compare the effects of lime and daily replacement of bedding on bacterial populations in sawdust and the bacterial flora of teat ends.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at the Ohio Agricultural Research and Development Center (Wooster). Cows were housed in a tie-stall barn. Each stall had a 170- × 150-cm concrete base with inlaid rubber mats. Three rows of tie stalls were bedded with 10 kg of kiln-dried sawdust (particle size ≤10 mm × 10 mm), and each row of stalls received one of three treatments: 1) daily replacement of sawdust in the back

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one-third of the stall, 2) addition of 1 kg of hydrated lime (Genlime Group Inc., Genoa, OH) spread evenly over the sawdust in the back one-third of the stall on the days that stalls were cleaned and covered with fresh bedding, or 3) control sawdust bedding with no treatment. Three groups of 4 lactating cows were assigned to treatment groups. Cows were bedded on the same material for 3 consecutive wk. Within each 3-wk period, all bedding was removed, stalls were allowed to dry, and fresh sawdust was applied every 7 d. After 3 wk on a bedding treatment, cows remained in the same stalls, and bedding treatments were changed. The trial lasted 9 wk so that cows were exposed to all bedding treatments in a 3 × 3 Latin square design.

Bedding Samples

Samples were collected immediately after fresh bedding was added to the stalls (d 0) and on d 1, 2, and 6 after each of the weekly changes in stalls with sawdust treated with lime and control stalls. Bedding samples were collected immediately after fresh bedding was added to stalls on d 0 and prior to removal of bedding on d 1, 2, and 6 from stalls in which bedding was replaced daily. Samples were a composite of bedding from the back one-third of each stall. Twenty-five grams of composite sample were placed in a convection oven at 100°C for 24 h to determine DM. A total of 10 g of bedding was suspended in 90 ml of sterile distilled water, and pH was measured. Bacterial populations in bedding were enumerated by adding 10 g of sample to 90 ml of sterile PBS and mixing the solution for 40 s in a stomacher (Stomacher Lab-Blender 400; Tekmar Co., Cincinnati, OH). Serial dilutions of the liquid phase in sterile PBS were plated on the surface of MacConkey agar (Beckman Dickinson Microbiology Systems, Cockeysville, MD), MacConkey-inositol-carbenicillin agar (**MCIC**), and TKT/FC agar (Beckman Dickinson Microbiology Systems). Inositol (10 mg/L; Sigma Chemical Co., St. Louis, MO) and carbenicillin (75 mg/L; Pfizer Co., New York, NY) were added to MacConkey agar for MCIC (1). Bovine plasma (50 ml/L) was substituted for whole blood to prepare TKT/FC as described by Hogan et al. (6). Serial dilutions plated on all media were 1:10^2 to 1:10^6. Inoculated plates were incubated for 24 h at 37°C. Colony-forming units per gram were identified as Gram-negative bacteria (total growth on MacConkey agar), coliforms (lactose-positive colonies on MacConkey agar), *Klebsiella* spp. (pink to red colonies to MCIC), and streptococci (total growth on TKT/FC agar).

Teat Swabs

Teat swabs were collected from the right front teat of each cow within 1 h prior to the collection of bedding treatments on d 0, 1, 2, and 6. Swabs were obtained as described by Rendos et al. (9). Briefly, sterile cotton swabs were moistened in a swab solution of PBS containing 0.1% sodium thiosulfate before swabbing. Teat ends were sampled by rotating a moistened swab five times around the exterior teat orifice. Swabs were placed into a test tube containing 4.0 ml of the swab solution. Test tubes containing swabs and swab solution were maintained on ice until bacteriological analyses were initiated within 1 h. Test tubes were shaken vigorously for 20 s, and appropriate dilutions of rinse solution were plated on agar media. Bacterial populations on teat skin were enumerated on the same media that were used to determine bacteria counts in bedding. Dilutions plated on each agar medium were 1:10, 1:10^2, and 1:10^3 for TKT/FC and 1:3 and 1:10 for MacConkey and MCIC.

Statistical Analyses

Bacteria counts were analyzed by ANOVA (10). Main effects for bedding counts were treatment, stalls, experimental period, and week of period. Main effects for teat end counts were cow plus those for bedding counts. Treatment differences were tested by Tukey's multiple comparison test. Correlations among bacteria counts in bedding, bacterial populations on teat ends, DM, and pH were determined by Pearson's correlation coefficients.

RESULTS AND DISCUSSION

Treatment Effects on Bedding Counts

Bacteria counts (Figure 1) and pH (Figure 2) differed among bedding treatments prior to use and after 1 d in the stalls. Addition of lime to sawdust reduced each bacteria count compared with the bacteria counts in untreated sawdust prior to use as bedding on d 0 ($P < 0.05$). The reduction in bacteria counts coincided with a difference in the pH of the bedding but not with DM (Figure 3). Mean (±SE) pH of the sawdust treated with lime on d 0 was 12.1 (±0.2) compared with 4.5 (±0.2) for untreated sawdust ($P < 0.05$). Mean DM prior to use as bedding was similar between untreated sawdust (89.9%) and sawdust treated with lime (91.3%). These results concur with those reported by Grier (5) in which lime had a bactericidal effect on Gram-negative bacteria in recycled manure and pelleted corn cobs. In the present study, coliform and *Klebsiella* spp. counts on d 0 were below the sensitivity of the plating procedures (<10^2 cfu/g) in each of the nine samples of sawdust treated with lime. Prior to use, streptococcal counts in sawdust treated with lime were <10^2 cfu/g in 78% of the samples. In contrast, prior to use as bedding, only 50 and 6% of untreated sawdust samples had coliform and streptococcal counts <10^2 cfu/g, respectively.

Gram-negative bacteria, coliforms, *Klebsiella* spp., and streptococci each continued to be lower in bedding treated with lime than in the bedding that was replaced daily or in the untreated control bedding after 1 d in the stall ($P < 0.05$). bacteria counts did not differ between bedding replaced daily and untreated control bedding. Mean pH on d 1 was greater ($P < 0.05$) in sawdust treated with lime (pH = 9.8) than in bedding replaced daily (pH = 7.1) and control sawdust (pH = 7.0). Dry matter did not differ among bedding treatments after 1 d in the stall.

Treatment effects on bedding variables were limited after 1 d in the stall. The ability of lime to alter bacteria counts and pH apparently was diminished within 48 h after application. The only difference among treatments was that *Klebsiella* spp. counts on d 2 were lower in bedding that was treated with lime than in bedding that was replaced daily ($P < 0.05$). *Klebsiella* spp. counts did not differ between bedding treated with lime and control bedding on d 2. Gram-negative bacteria, coliforms, and streptococci

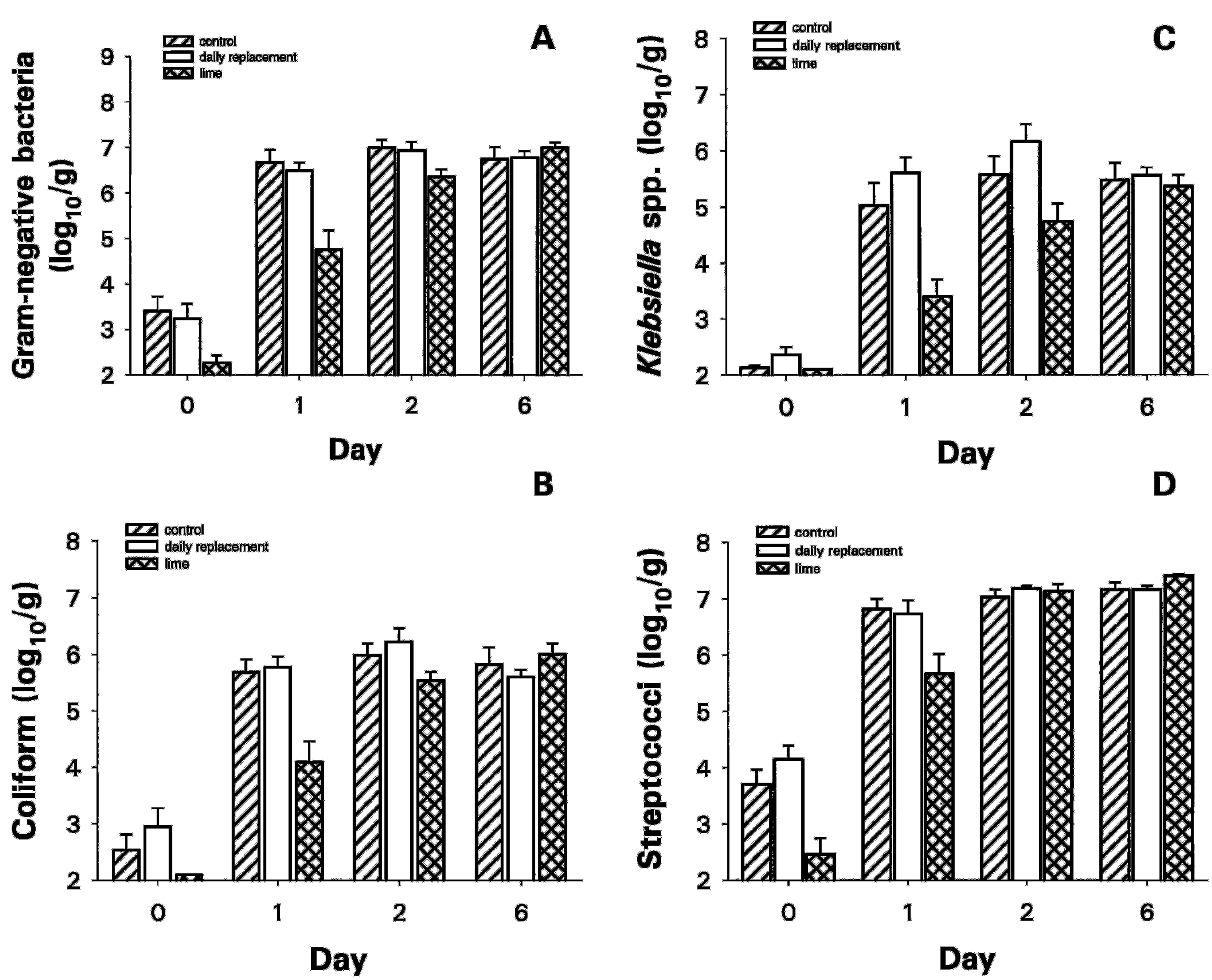

Figure 1. Counts of Gram-negative bacteria (A), coliforms (B), *Klebsiella* spp. (C), and streptococci (D) in untreated sawdust, sawdust treated with lime, and sawdust replaced daily on d 0, 1, 2, and 6 after use as bedding in tie stalls.

did not differ among treatments on d 2. Mean pH and DM also were similar among treatments on d 2. bacteria counts, pH, and DM did not differ among treatment groups on d 6.

Dynamics of Bedding Counts Within Treatments

Bacteria counts in organic material often are extremely low prior to use, but increase rapidly within hours after being placed in stalls as bedding (5). Gram-negative bacteria and streptococci in the environment can contaminate the bedding and use organic materials as a source of nutrients and moisture (11). In the present study, bacteria counts in control bedding were lower prior to use on d 0 than after 1, 2, or 6 d in the stalls ($P < 0.05$). The physical characteristics of bedding changed to coincide with the increase in bacteria populations during the first 24 h in stalls. As evidence of this change, for control bedding, DM was higher, and pH was lower, on d 0 than on d 1, 2, or 6 in the stall ($P < 0.05$). Each bacteria count reached a stationary growth phase by d 1. Streptococcal counts, *Klebsiella* spp. counts, DM, and pH did not differ among d 1, 2, and 6 in control bedding. However, Gram-negative bacteria and coliform counts in control bedding were greater on d 1 than on d 6. These decreases in bacteria counts implied that the populations had entered the death phase of the growth cycle. Fairchild et al. (4) previously reported that bacteria counts in organic bedding tended to be lower after extended use than populations within the 1st wk after placement in stalls.

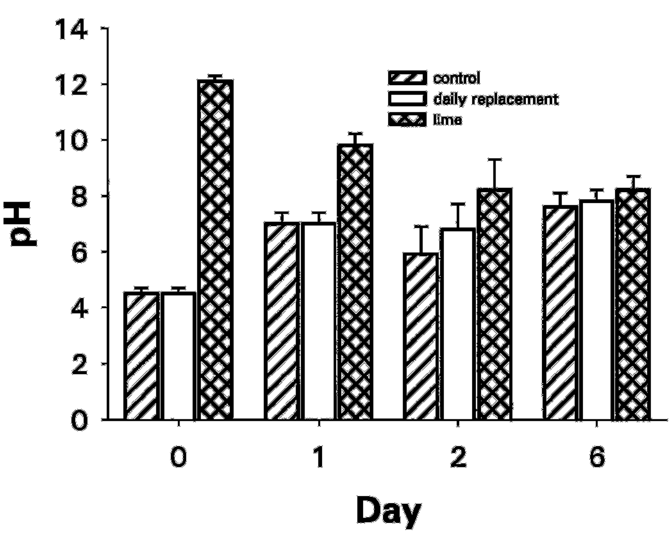

Figure 2. Mean pH in untreated sawdust, sawdust treated with lime, and sawdust replaced daily on d 0, 1, 2, and 6 after use as bedding in tie stalls.

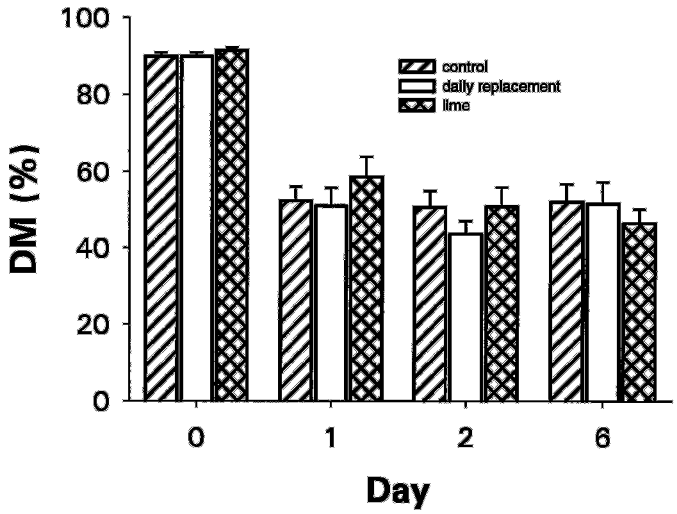

Figure 3. Dry matter in untreated sawdust, sawdust treated with lime, and sawdust replaced daily on d 0, 1, 2, and 6 after use as bedding in tie stalls.

Bramley (2) reported that the daily replacement of bedding in the back one-third of stalls markedly reduced the exposure of teats to coliform bacteria. Results of the current trial, in which bacteria counts were taken daily, implied that the advantage of this practice was minimal. Bacteria counts and pH were lower in bedding replaced daily on d 0 than on d 1, 2, and 6 ($P < 0.05$). The DM of bedding on d 0 was greater than that on d 1, 2, or 6. Bedding variables on d 1, 2, and 6 were expected to be similar because samples on each of these days represented 24 h of use as bedding. This assumption was realized because bacteria counts, DM, and pH did not differ among d 1, 2, and 6 in bedding that was removed daily. The rapid increase in bacteria populations to a stationary growth phase during the first 24 h diminished the impact of daily replacement of bedding in the present study.

Gram-negative bacteria, coliform, *Klebsiella* spp., and streptococcal counts were lower in bedding treated with lime on d 0 than those on d 1, 2, and 6 ($P < 0.05$). Each bacteria count also was lower on d 1 compared with those on d 2 and 6 ($P < 0.05$). Bacterial counts did not differ between d 2 and 6 in bedding treated with lime. The pH of bedding treated with lime followed a trend inverse to that of bacteria counts. Bedding treated with lime had a higher pH on d 0 than on d 1, 2, and 6 ($P < 0.05$). Comparison among d 1, 2, and 6 revealed that pH was greater on d 1 than on d 2 and 6 ($P < 0.05$). The pH of bedding treated with lime did not differ between d 2 and 6. Correlation coefficients between pH and Gram-negative bacteria, coliforms, *Klebsiella* spp., and streptococci were –0.81, –0.78, –0.68, and –0.83, respectively. Bedding treated with lime had higher DM prior to use than that on d 1, 2, and 6 ($P < 0.05$). Dry matter in bedding treated with lime did not differ among d 1, 2, and 6.

Teat End Swabs

Bacteria counts in bedding profoundly affect the microbial populations on teat skin (9). The prolonged contact of the udder with bedding provided a physical means of transferring bacteria onto teat skin. The adherence of bedding on teat skin increases the population of transient flora, including environmental mastitis pathogens (7). In the present study, the treatment effects of lime on bacterial populations in sawdust bedding had a similar impact on teat swab bacterial counts. On d 2, Gram-negative bacteria (Figure 4A) and *Klebsiella* spp. (Figure 4C) counts on teat swabs were lower for cows bedded on sawdust treated with lime compared with those for cows bedded on control sawdust and sawdust that was replaced daily ($P < 0.05$). Coliform (Figure 4B) and streptococcal (Figure 4D) counts on teat swabs did not differ among treatments on d 2. Gram-negative bacteria, coliform, *Klebsiella* spp., and streptococcal counts on teat swabs did not differ among treatments on d 0, 1, and 6. Comparing counts on teat swabs within treatments, cows on bedding treated with lime had lower *Klebsiella* spp. counts on d 1 than on d 0 and 6. Gram-negative bacterial, coliform, and strep-

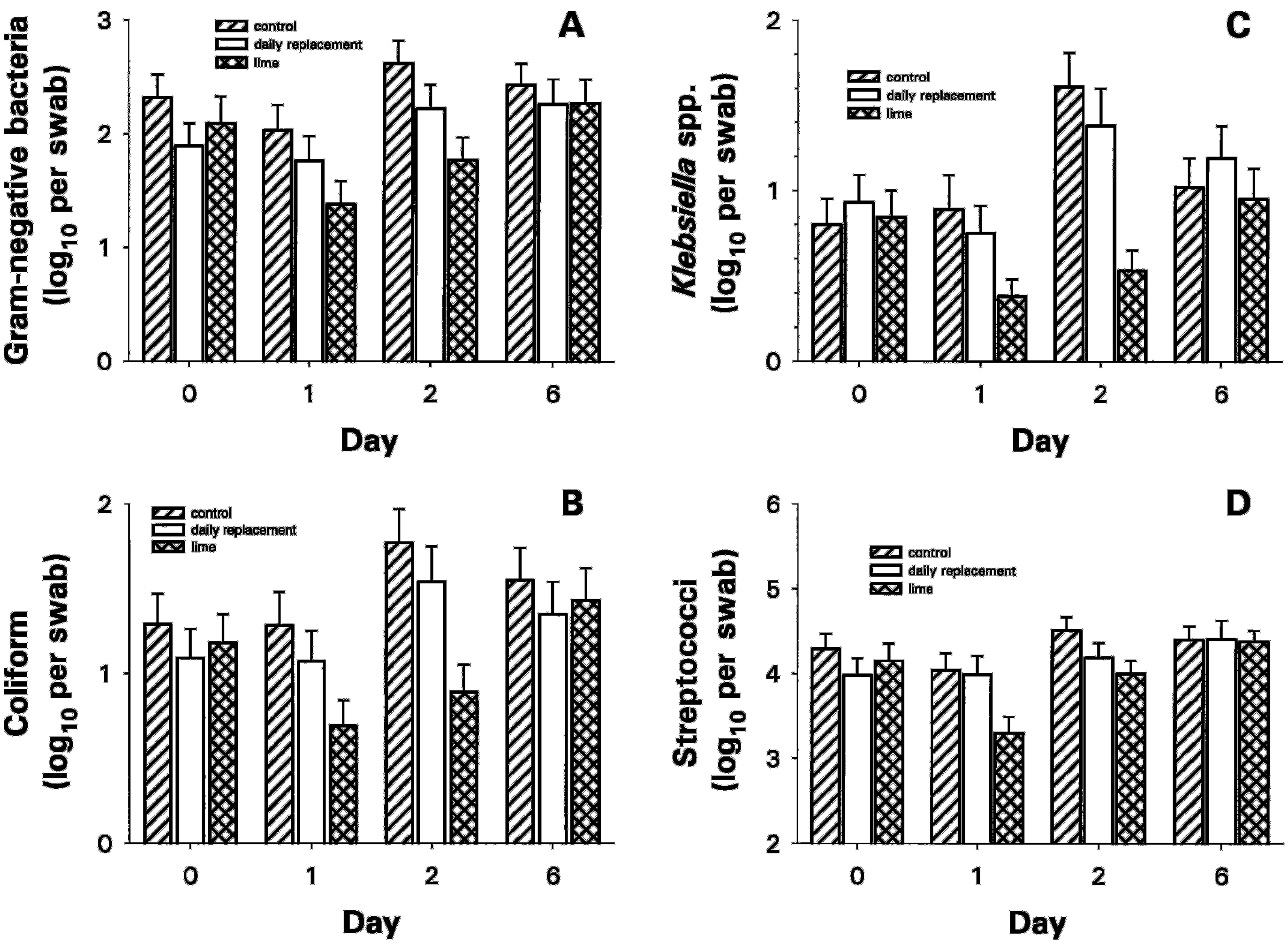

Figure 4. Counts of Gram-negative bacteria (A), coliforms (B), *Klebsiella* spp. (C), and streptococci (D) on teat end swabs for cows bedded in tie stalls on untreated sawdust, sawdust treated with lime, and sawdust replaced daily. Measurements were taken on d 0, 1, 2, and 6.

tococcal counts on teat swabs did not differ among d 0, 1, 2, and 6 for cows on bedding treated with lime. Teat swab counts did not differ among d 0, 1, 2, and 6 for cows on either control bedding or daily replacement of bedding. Correlation coefficients between bedding counts and mean teat swab counts by bacterial groups were 0.66 for Gram-negative bacteria, 0.62 for coliforms, 0.78 for *Klebsiella* spp., and 0.90 for streptococci.

CONCLUSIONS

The addition of lime to sawdust significantly reduced bacteria counts in the bedding for 1 d. The decrease in bacterial populations appeared to be related to an increase in bedding pH. Daily replacement of bedding had a minimal effect on bacteria counts in bedding and on teat skin.

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