



Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in freestalls

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ABSTRACT

An experiment was conducted to compare bacterial counts of mastitis pathogens in deep-packed manure solids bedding with those in manure solids bedding replaced daily from mattresses. Eighteen Holstein cows were housed in 1 pen with 18 stalls. One row of 9 stalls was equipped with mattresses topped with bedding. The back one-third of these stalls toward the alleyway was covered in 25 mm of recycled manure solids, which was removed daily for the next 6 d and replaced with bedding from the brisket board and lunge space areas of stalls. The second row of 9 stalls was bedded for 3 wk with 100 to 150 mm of deep-pack recycled manure bedding from which only fecal matter was removed daily. After 3 wk, bedding treatments were changed between rows in a switchback design. Mean total gram-negative bacterial counts did not differ between treatments throughout the experiment. Coliform and *Klebsiella* spp. bacterial counts were lower in daily replaced bedding compared with deep pack across the experiment and on each of d 0, 1, 2, and 6. Streptococcal counts were reduced in daily replacement stalls compared with deep-pack stalls on d 0 and greater in daily replacement stalls compared with deep-pack stalls on d 1, 2, and 6. Daily replacement of recycled manure bedding from the back one-third of the stalls appeared to be an effective approach to reducing exposure to coliforms, specifically *Klebsiella*, but not streptococci. However, bacterial counts in bedding from both treatments were elevated throughout the trial and resulted in considerable risk for exposure to teats and development of intramammary infections.

Key words: recycled manure solids, bacterial counts, bedding

Short Communication

Increased cost and decreased availability of common bedding materials have led to a growing interest in using

recycled manure solids as bedding (Husfeldt et al., 2012). Two common ways of using recycled manure bedding are in the forms of deep-bedded packs or surface application on top of mattresses (Husfeldt and Endres, 2012). The use of deep-bedded manure solids in freestalls was associated with reduced lameness and hock abrasions compared with use of recycled manure solids as bedding on mattresses (Husfeldt and Endres, 2012). In addition to cow comfort and welfare, the choice of bedding for freestalls has an effect on mammary health. Bacterial populations in bedding were positively correlated with teat skin bacterial populations (Zdanowicz et al., 2004) and rates of clinical mastitis in lactating cows (Hogan et al., 1989). Use of manure solids as bedding has a large potential effect on pathogen load in stalls because it provides pathogens with a nutrient-rich environment (Gooch et al., 2006). In an observational study, the incidence of clinical mastitis was not different between bedding management practices, although deep-bedded stalls had reduced streptococcal counts compared with bedding on mattresses (Husfeldt et al., 2012).

The ability of bacteria to multiply in bedding can be influenced by physical, biochemical, or nutritional properties of the material chosen and the management of bedding in stalls (Godden et al., 2008). Management strategies to reduce mastitis pathogen populations in organic bedding have been minimally successful in controlled trials (reviewed by Hogan and Smith, 2012). An exception was the report by Bramley (1985) of daily replacement of sawdust bedding from the back one-third of concrete stalls that reduced bacterial contamination of bedding and was related to a reduced incidence of coliform mastitis. The purpose of the current controlled trial was to compare bacterial counts of mastitis pathogens in deep-packed manure solids bedding with those in manure solids bedding replaced daily from mattresses.

An experiment was conducted at the Ohio Agricultural Research and Development Center Krauss Dairy Center (Wooster, OH) during May and June 2013. Eighteen lactating Holsteins cows were housed in 1 pen with unlimited access to 18 freestalls. Each stall was a 203 × 127 cm concrete base with rubber-filled, vinyl-surfaced, cushioned mats. The pen consisted of 2 rows of freestalls separated by a 3.05-m alley. Fans

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(1.2-m diagonal propeller length) were every 6.1 m and suspended 2.7 m above both rows of stalls. Stalls were separated from feed and water by a 2.13-m-wide alley and a solid board partition measuring 1.22 m in height. Stalls were separated from the exterior side-open walls of the barn by a 1.22-m tall board partition and a 1.5-m cow transport alley. The pen and feed alleys were cleaned 8 times per day during the study by manual control of a mechanical scraper. Recycled manure was recovered solids (FAN PSS; Fan Separator Inc., Michigan City, IN) from the trial herd. Recycled manure solids were stored in a covered shed and were used as bedding within 3 d after separation.

One row of 9 stalls was covered with vinyl-surfaced mattresses. All bedding was removed once per week on Mondays at 0700 h; approximately 30 kg of fresh recycled manure bedding was added to the brisket and lunge space areas of stalls and the back one-third of stalls was covered in 25-mm recycled manure solids. For the next 6 d, all bedding was completely removed daily from the back one-third of mattresses and bedding from the brisket area and lunge space of stalls was pulled to the back one-third of stalls to maintain a bedding depth of 25 mm. The remaining row of 9 stalls was bedded with 100 to 150 mm of deep-packed, recycled manure solids. Minimal bedding was added to deep-pack stalls only to replenish bedding removed due to fecal contamination. Stalls received the same treatment for 3 consecutive weeks. After 3 wk with a bedding treatment, bedding treatments were changed between rows. The trial was 6 wk long so that stalls were exposed to both bedding treatments in a switchback design to account for differences among stalls. Stalls directly across the walk alley between rows were paired for statistical analysis. The Ohio Agricultural Research and Development Center climatological station, located 1.9 km from the research dairy, reported that the average daily high temperature was 27.2°C (range: 23.8 to 31.1°C) and the average daily low temperature as 15.5°C (range 12.7 to 16.1°C) during the experiment.

Bedding samples in 4 stalls per treatment group were collected from the surface 25 mm of bedding in the back one-third of stalls. Samples were collected from both experimental groups immediately after fresh bedding was added to daily replacement stalls (d 0) and immediately before daily removal of bedding on d 1, 2, and 6 of each week. The samples were tested for DM composition and bacteriological counts. Two grams of sample were placed in a gravity-convection oven at 100°C for 24 h and the DM content was determined. Ten grams of bedding was added to 90 mL of sterile saline and mixed for 30 s in a stomacher (Stomacher Lab Blender 400; Tekmar Co., Cincinnati, OH). The solution was diluted in 225 mL of sterile saline and

plated on the surface of MacConkey agar (Becton Dickinson Microbiology Systems, Cockeysville, MD), MacConkey-inositol-carbenicillin agar with inositol (10 mg/L; Sigma Chemical Co., St. Louis, MO) and carbenicillin (75 mg/L; Pfizer Co., New York, NY), and Edwards modified media agar containing 5 mg/L of colistin sulfate and 2.5 mg/L of oxolinic acid (Becton Dickinson Microbiology Systems) with 50 mL/L of bovine plasma added. Dilutions plated on all media were 1:10² to 1:10⁶. Agar plates were incubated aerobically at 37°C for 24 h. Colony-forming units per gram were determined for gram-negative bacteria (total growth on MacConkey agar), coliforms (lactose-positive colonies on MacConkey agar), *Klebsiella* spp. (pink to red colonies on MacConkey-inositol-carbenicillin agar), and streptococci (total growth on modified Edwards agar). Counts were expressed as the logarithm of colony-forming units per gram of bedding DM. Bacterial counts and moisture content were analyzed by least squares ANOVA using the mixed procedure of SAS (SAS Institute, 2003). The main effects for each variable were treatment, pair, sample day, and week of period. Main effects and possible 2-way interactions were tested. Significance was defined as $P < 0.05$.

Mean total gram-negative bacterial (Figure 1A) counts did not differ ($P > 0.05$) between bedding samples taken from daily replacement stalls and those samples from deep-pack stalls across the entire trial ($P > 0.05$). Coliform (Figure 1B) and *Klebsiella* spp. (Figure 1C) counts were reduced ($P < 0.05$) in recycled manure solids bedding from daily replacement stalls compared with deep-pack stalls on each of d 0, 1, 2, and 6. Streptococcal counts (Figure 1D) also differed between treatment groups within each sample day. Streptococcal counts were reduced in bedding from daily replacement stalls compared with deep-pack stalls on d 0 ($P < 0.05$). In contrast, streptococcal counts were greater ($P < 0.05$) in bedding samples from daily replacement stalls compared with deep-pack stalls on d 1, 2, and 6. Dry matter percentage (Figure 1E) of recycled manure solids bedding in daily placement stalls was reduced ($P < 0.05$) on d 0, did not differ ($P > 0.05$) on d 1 and 2, and was enhanced on d 6 ($P < 0.05$) compared with DM percentage in deep-pack stalls.

Several approaches have been reported to decrease bacterial populations in recycled manure during the preparation of bedding before use (Gooch et al., 2006; Husfeldt et al., 2012). However, regardless of procedures used to prepare bedding, mastitis pathogen populations were similar in recycled manure bedding after use in stalls (Gooch et al., 2006; Husfeldt et al., 2012). An emphasis on managing bedding after it is placed into stalls appears to have a greater effect on bacterial populations compared with preparatory measures dur-

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