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Short communication: Environmental mastitis pathogen counts in freestalls bedded with composted and fresh recycled manure solids

K. J. Cole and J. S. Hogan[1]
Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

An experiment was conducted to compare bacterial counts of environmental mastitis pathogens in composted recycled manure solids bedding with those in fresh recycled manure solids. Eighteen Holstein cows were housed in 1 pen with 18 stalls. One row of 9 freestalls included mattresses and was bedded weekly with composted recycled manure solids. The second row of 9 freestalls included mattresses and was bedded weekly with fresh recycled manure solids. The back one-third of stalls toward the alleyway was covered in 25 to 50 mm of bedding. Samples were taken from the back one-third of 4 stalls for both treatments on d 0, 1, 2, and 6 of each week. After 3 wk, bedding treatments were switched between rows, making the total duration 6 wk. Mean total gram-negative bacterial counts were approximately 0.5 $\log_{10}$ cfu/g of dry matter lower in the composted recycled manure solids on d 0 compared with fresh recycled manure solids. *Klebsiella* species, coliform, and *Streptococcus* species counts were at least 1.0 $\log_{10}$ cfu/g of dry matter lower in composted compared with fresh recycled manure solids on d 0. Only gram-negative bacterial counts on d 1 were reduced in composted recycled manure solids compared with fresh recycled manure solids. Differences were not observed between treatments in gram-negative bacterial, coliform, *Klebsiella* species, or *Streptococcus* species counts on d 2 and 6. Ash content was higher in composted recycled manure solids compared with fresh recycled manure solids on d 0, 1, 2, and 6. Despite the increase in ash after composting, bacterial counts of mastitis pathogens in composted recycled manure solids were comparable with those in fresh recycled manure when used as freestall bedding.

Key words: recycled manure solids, compost, bacterial count

Short Communication

Recycled manure solids (**RMS**) used as bedding are prepared by separation of the solids from the liquids of cow manure (Carroll and Jasper, 1978). Unused RMS initially have low bacterial counts of environmental mastitis pathogens, but counts rise exponentially within a few hours after being exposed to the cows and introducing bacterial contamination (Sorter et al., 2014). Composting can be a beneficial method in decreasing bacterial load in organic materials such as RMS (Carroll and Jasper, 1978). Composting is the process of breaking down organic material by bacteria, which helps decrease the populations of potential pathogens in materials coming in contact with plants and animals (NRAES, 1992). Compost piles can reach thermophilic temperatures of 50 to 60°C, with temperatures upwards of 55°C inhibiting coliforms and other bacteria commonly associated with bovine mastitis (Keener, 2011). Studies on the effects of composting bedding for dairy cows have compared composted RMS and fresh RMS by survey of different farms (Husfeldt et al., 2012). Reports are limited comparing fresh RMS and composted RMS under similar environmental conditions using the same cows within one farm. The purpose of the current study was to investigate if composted RMS decreases bacterial counts of mastitis pathogens compared with fresh RMS used as freestall bedding for dairy cows.

A 6-wk research trial was conducted July 8 to August 18, 2014, at the Krauss Dairy, Ohio Agricultural Research and Development Center, Wooster, Ohio. This experiment took place in an 18-freestall pen, housing 18 multiparous mid- to late-lactation Holstein cows. Each stall consisted of a concrete base 203 × 127 cm with rubber filled, vinyl-surfaced, cushioned mats. The pen consisted of 2 rows of 9 freestalls separated toward the back of stalls by a 3.05-m alleyway. Fans (1.2 m diagonal propeller length) were placed in the barn every 6.1 m and suspended 2.7 m above both rows of freestalls. Stalls were separated from feed and water by a 2.13-m-wide alley and a solid wall 1.22 m in height. Stalls were separated from the exterior side-open walls of the barn by 1.22-m-high wall and a 1.5-m transport alley (Sorter

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[1]Corresponding author: hogan.4@osu.edu

et al., 2014). The pen and feed alleys were cleaned by manual scrapers that ran 8 cycles every 24 h. Climatological data were recorded at the Ohio Agricultural Research and Development Weather Station 1.7 km from the Krauss Dairy and 1.4 km from the compost pad. During the experimental days of July 8 to August 18, 2014, average daily temperature ranged from 15.8 to 25.6°C and average daily relative humidity ranged from 23.3 to 35%.

All RMS used in the trial were the result of manure collected from alleyways of freestalls and tie-stalls housing lactating cows at the Krauss Dairy. Manure was scraped into a central liquid retention pit and pumped through an extractor (FAN PSS, Fan Separator Inc., Michigan City, IN) to separate the solids from the liquids. The fresh RMS were solids from the separator stored under a covered shed for 48 h before use as bedding in the trial. Composted RMS were produced from 2 rows of 25 to 30 m^3 of the fresh RMS composted sequentially for 4 wk each on a 2,023 m^2, uncovered, concrete composting pad with a 2% slope and a leachate collection system. The dimensions of windrows were approximately 23 m long, 3 m wide, and 1 m high. Windrows were turned weekly with a tractor-assisted Aeromaster 120 windrow turner (Midwest Bio-Systems, Tampico, IL; Michel et al., 2004). Four equidistant, representative samples of RMS were taken from the windrows before the composting periods and at the completion of composting periods to monitor bacteriological counts and DM. Windrow 1 was on the pad from June 2 to July 7, 2014 (total rainfall 16 cm, average daily temperature ranged from 15.9 to 27.3°C, and average daily humidity ranged from 18.8 to 32.8%). Windrow 2 was on the pad from June 23 to July 28, 2014 (total rainfall 11.15 cm, average daily temperature ranged from 16.3 to 27.8°C, and average daily humidity ranged from 22.2 to 34.5%).

Both rows of 9 freestalls were bedded with 30 kg of RMS per stall weekly. The back one-third of stalls toward the alleyway in one row was bedded to a depth of 25 to 50 mm of composted RMS. The back one-third of stalls toward the alleyway of the other row was bedded to a depth of 25 to 50 mm of fresh RMS. Bedding treatments remained in their assigned rows for 3 consecutive weeks. After the first 3 wk, the treatments were switched between rows. The trial lasted 6 wk, allowing both rows to be exposed to the treatments for 3 wk each.

Bedding samples from 4 stalls per treatment group were collected from the surface 25 mm of bedding in the back one-third of stalls. Stalls located directly across the walk alley between rows (tail to tail) were paired for statistical analysis. Stalls selected were the second, fourth, sixth, and eighth stall in the sequence of the 9 stall rows. Each sample from each stall consisted of 3 equidistant subsamples that were combined to form one sample weighing approximately 200 g. Samples were collected at the same time each week from both experimental groups immediately after fresh bedding was added to stalls (d 0) and on d 1, 2, and 6 after use as bedding. The samples were tested for DM, ash, and bacteriological counts.

Dry matter was determined by placing 2 g of sample in 5.7 × 1.6 cm aluminum pans. The samples were weighed for wet weight and then placed in a gravity-convection oven at 100°C for 24 h. The samples were removed from the oven and re-weighed to determine the DM content. Ash was determined by placing 2 g from each sample, in a 43 × 37 mm high form porcelain crucible (VWR International, Radnor, PA), in a gravity convection oven at 100°C for 24 h. Following DM determination, crucibles containing dried bedding were then placed in an oven and heated to 600°C for at least 12 h to determine ash. Bacteriological counts were determined by serial dilution of samples in PBS using the procedures and materials detailed by Sorter et al. (2014).

Differences in bacterial counts and DM in RMS windrows before composting and after composting were compared using Student's *t*-test. Data analysis for bacterial counts, DM, and ash content in freestall samples consisted of multivariate ANOVA. Factors included in the model were treatment, pair (i.e., location), sample day, period, and week (nested within period). The analysis included main effects and possible sample day × treatment interactions. Analysis was performed using the GLM and GLIMMIX procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC). Significance level was set at $P < 0.05$ for main effects. To adjust for multiple comparisons within each model, Tukey's adjusted *P*-values ($P < 0.05$ for each pair-wise difference) were calculated for each variable.

Gram-negative bacteria, coliform, and streptococcal counts in RMS windrows were reduced ($P < 0.05$) after 4 wk composting compared with counts in windrows before composting (Table 1). *Klebsiella* counts and DM did not differ ($P > 0.05$) in samples taken from windrows before composting and samples taken from the windrows after 4 wk of composting RMS.

Composting RMS reduced bacterial counts before use as bedding, but had little effect on bacterial counts after use as bedding in freestalls. Gram-negative bacterial, coliform, *Klebsiella* species, and *Streptococcus* species counts were each reduced ($P < 0.05$) in the composted RMS compared with fresh RMS at the time bedding was placed in the freestalls on d 0 (Table 2). Gram-negative bacterial counts were the only counts reduced ($P < 0.05$) in composted RMS compared with