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The relationship between compost bedded pack performance, management, and bacterial counts

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ABSTRACT

The objective of this study was to assess the relationships among temperature, moisture, carbon-to-nitrogen (C:N) ratio, space per cow, and bacterial counts from bedding material collected from compost bedded pack (CBP) barns. A field survey of 42 routinely aerated CBP barns was conducted in Kentucky between October 2010 and March 2011. Two bedding material samples of 1,064.7 cm^3 each were collected during a single site visit from 9 evenly distributed locations throughout each barn and thoroughly mixed to create a composite sample representative of the entire CBP. Bacterial counts were determined for coliforms, *Escherichia coli*, streptococci, staphylococci, and *Bacillus* spp. University of Kentucky Regulatory Services (Lexington) laboratory personnel performed nutrient analyses to determine moisture, carbon, and nitrogen contents. Surface and 10.2-cm pack depth temperatures were collected for each of the 9 evenly distributed locations and the mean calculated to produce a composite temperature. Space per cow was calculated as the total CBP area divided by number of cows housed on the CBP. The GLM procedure of SAS (SAS Institute Inc., Cary, NC) generated models to describe factors affecting bacterial counts. Bacterial counts were 6.3 ± 0.6, 6.0 ± 0.6, 7.2 ± 0.7, 7.9 ± 0.5, and 7.6 ± 0.5 $\log_{10}$ cfu/g of dry matter for coliform, *Escherichia coli*, streptococci, staphylococci, and *Bacillus* spp., respectively. Composite temperature, CBP moisture, C:N ratio, and space per cow had no effect on coliform counts. *Escherichia coli* reached a peak concentration when the C:N ratio was between 30:1 and 35:1. Staphylococci counts increased as ambient temperature increased. Streptococci counts decreased with increased space per cow and composite temperature and increased with increasing ambient temperature and moisture. Streptococci counts peaked at a C:N ratio ranging from 16:1 to 18:1. *Bacillus* spp. counts were reduced with increasing moisture, C:N ratio, and ambient temperature. Mastitis-causing bacteria thrive in similar conditions to that of composting bacteria and microbes, making elimination of these at higher temperatures (55 to 65°C) difficult in an active composting environment. Producers must use recommended milking procedures and other preventative practices to maintain low somatic cell count in herds with a CBP barn.
Key words: compost bedded pack barn, bacterial analysis, somatic cell count

INTRODUCTION

Virginia dairy farmers developed the compost bedded pack (**CBP**) barn concept to improve cow comfort, increase cow longevity, and reduce initial barn costs (Barberg et al., 2007b) while potentially reducing the mastitis risks associated with the conventional bedded pack. Producers used the bedded pack system layout and incorporated composting methods. Compost bedded pack barns provide an open resting area free of stalls or partitions (Janni et al., 2007). Producers use fine wood shavings or sawdust as bedding (Janni et al., 2007). A cultivator or rototiller incorporates manure, urine, and air into the CBP typically during milking 2 or 3 times per day (Barberg et al., 2007a; Janni et al., 2007; Shane et al., 2010). Aeration increases metabolic heat production by aerobic microbes and bacteria (Suler and Finstein, 1977). Higher temperatures (55 to 65°C) promote pathogen destruction (Stentiford, 1996), which may be advantageous for mastitis-causing bacteria destruction. However, temperatures observed by Barberg et al. (2007a), Klaas et al. (2010), and Black et al. (2013) did not reach the level necessary for bedding sanitization. The lack of material sanitization during the microbial processes in the CBP indicates that the system is more of a semi-composting system that does not fully cycle through the entire composting process. Higher temperatures also increase moisture evaporation (NRAES, 1992). Manure, urine, and microbial activity

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moisture act as moisture sources in a CBP (Janni et al., 2007). The CBP should remain between 50 to 60% moisture for efficient composting (Gray et al., 1971; Suler and Finstein, 1977; NRAES, 1992).

Compost bedded pack barns do not have stalls or partitions and cows are allotted a given amount of space per cow. Wagner (2002) originally recommended 9.4 m^2/cow for CBP barns. However, to accommodate cow manure and urine output, Janni et al. (2007) recommended 7.4 m^2/cow for a 540-kg Holstein cow or 6.0 m^2/cow for a 410-kg Jersey cow. Overstocking the CBP barn may result in increased bedding needs or dirty cows. Proper cow hygiene management can reduce mastitis risk (Neave et al., 1969; Philpot, 1979; Schreiner and Ruegg, 2003; Reneau et al., 2005). Barberg et al. (2007b) observed a mean hygiene score of 2.66, where 1 = clean and 5 = very dirty (Reneau et al., 2005), for the 12 CBP barns visited, whereas Shane et al. (2010) observed a mean hygiene score of 3.1 for 6 CBP barns. A study comparing CBP barns, cross-ventilated (**C-V**) barns, and naturally ventilated (**N-V**) barns noted that cows housed in CBP barns had increased ($P < 0.05$) hygiene scores (3.18) compared with the C-V (2.83) and N-V (2.77) barns (Lobeck et al., 2011). Udder health, indicated by SCC, improved after moving cows into the CBP barn in a study by Barberg et al. (2007b), where the mastitis infection rate (cows with SCC ≥200,000 cells/mL) decreased from 35.4 to 27.7%. Klaas et al. (2010) observed SCC of 133,000, 214,000, and 229,000 cells/mL for the 3 barns in Israel operating CBP barns without additional bedding added.

A direct correlation exists between the bacteria load at the teat end and mastitis incidence (Neave et al., 1966). Bedding contributes to teat end bacterial load (Hogan et al., 1989; Hogan and Smith, 1997; Zdanowicz et al., 2004) and minimizing bedding bacterial counts is an important management strategy. Janni et al. (2007) recommended avoiding green or wet (from uncured wood) sawdust or shavings because of possible increased teat-end exposure to *Klebsiella* spp. (Newman and Kowalski, 1973; Bagley et al., 1978; Fairchild et al., 1982). Inorganic bedding, such as sand or crushed limestone, typically hinders bacterial growth within bedding material through a lack of nutrients compared with organic bedding materials (Fairchild et al., 1982; Hogan et al., 1989; Zdanowicz et al., 2004; LeJeune and Kauffman, 2005). However, composting microbes and bacteria require a carbon source to proliferate, making inorganic bedding an impractical choice for use in CBP barns. Reported ranges for bacterial counts in dairy sawdust bedding are highly variable [15.8 $\log_{10}$ cfu/g (Fairchild et al., 1982); 6.2 $\log_{10}$ cfu/g (Hogan et al., 1989); 17.8 $\log_{10}$ cfu/g (Rendos et al., 1975)] and *Klebsiella* spp. [15.0 $\log_{10}$ cfu/g (Fairchild et al., 1982); 4.8 $\log_{10}$ cfu/g (Hogan et al. 1989); 15.3 $\log_{10}$ cfu/g (Rendos et al., 1975)] and streptococci [7.1 $\log_{10}$ cfu/g (Hogan et al., 1989); 16.2 $\log_{10}$ cfu/g (Rendos et al., 1975)] in sawdust bedding have been reported in bedding used in dairy barns. Chopped straw contained similar coliform counts (7.1 $\log_{10}$ cfu/g), *Klebsiella* spp. (6.3 $\log_{10}$ cfu/g), and streptococci (7.8 $\log_{10}$ cfu/g) compared with sawdust (Hogan et al., 1989). The bacterial concentration in organic bedding makes it imperative to manage teat-end cleanliness.

A Minnesota study by Barberg et al. (2007a) reported total bacterial counts of 7.0 $\log_{10}$ cfu/g in 12 CBP barns, a content less than the 13.8 $\log_{10}$ cfu/g expected to increase risk for clinical mastitis (Jasper, 1980). Lobeck et al. (2012) determined that bedding in CBP, C-V, and N-V barns exhibited no difference ($P > 0.05$) in coliform, *Klebsiella* spp., environmental *Streptococcus*, or *Staphylococcus* species counts. However, CBP barns contained greater ($P < 0.05$) *Bacillus* levels (798,000 cfu/g) in the summer than N-V (366,000 cfu/g) and C-V barns (59,000 cfu/g) and lesser *Bacillus* spp. (800 cfu/g) in the winter than N-V barns (9,881,000 cfu/g). Bulk tank milk contained similar levels of *Staphylococcus aureus*, non-*agalactiae Streptococcus* spp., *Staphylococcus* spp., and coliforms for the 3 housing systems. The objectives of the current study were to define total bacteria populations of streptococci, staphylococci, *Bacillus* spp., coliforms, and *Escherichia coli* within the CBP barn system and evaluate management strategies for reducing CBP bacteria levels.

MATERIALS AND METHODS

A field survey of 42 aerated CBP barns was conducted in Kentucky between October 2010 and March 2011. Each farm was visited once during the study period, with 2 to 3 different site visits per collection day. Of the 42 barns, 32 barns were used as the primary housing facility for lactating cows. The remaining 13 barns were used as supplemental housing for special needs cows (i.e., lame, old, and sick cows). A companion paper describes herd characteristics; management practices; producer perception of the CBP system; compost characteristics, including CBP temperature, moisture, and nutrient values; and herd performance, including lameness, hygiene, and production and reproductive performance (Black et al., 2013). Damasceno (2012) described structure characteristics for these barns, including building material, dimensions, and layout. Compost characteristics, including physical, bacterial, chemical, and thermal properties, observed in this study were also described previously (Damasceno, 2012).

Bedding Material Bacterial Count Analysis

Samples of bedding material were collected during a single site visit from 9 evenly distributed locations throughout each barn. Researchers collected 2 samples, each of 118.3 cm^3 of surface layer bedding material from each location (total of 1,064.7 cm^3) using a 59.1-cm^3 measuring cup in a 3.8-L plastic bag and thoroughly mixed the material to create 2 composite samples representative of the entire CBP. One sample was used for bacterial analysis, whereas the second sample was used for nutrient composition analysis. Samples were stored in a −40°C freezer until at least 20 composite samples were collected and available for analysis. Sample preparation consisted of diluting material by mixing 25 g of bedding material with 225 g of 0.1% nonsterile peptone solution in a 1:10 dilution. The mixture was hand mixed until the bedding material was well suspended within the peptone solution. To determine total coliform and *E. coli* counts, further serial dilutions out to 1:10^4 were generated and plated to ensure countable plates, or plates with fewer than 200 cfu. Researchers then added 1 mL of the appropriate dilution, using a pipette, to 2 separate 3M Petrifilm *E. coli*/Coliform Count Plates (3M Microbiology Products, St. Paul, MN), and incubated the plates at 35°C for 24 h. Colony-forming units were counted manually, obtaining both a coliform and *E. coli* count. Streptococci count was determined by creating further serial dilutions out to 1:10^5 to ensure countable plates. Then, 50 μL of each dilution was added to 2 separate TKT agars (1 g of aesculin, 13 g of agar, 0.0013 g of crystal violet, 5 g of meat extract, 10 g of meat peptone, 5 g of sodium chloride, and 0.333 g of thallium sulfate, brought to 1,000 mL; with bovine blood added at 5% before pouring plates; pH adjusted to 7.5 ± 0.2) prepared in the laboratory and spiral plated (Eddy Jet; IUL Instruments I.K.S., Leerdam, the Netherlands) the diluted material onto the plate. Plates were incubated 48 h at 35°C, with colony-forming units counted automatically using a colony counter (Flash & Go; IUL Instruments I.K.S.). For staphylococci, further serial dilutions out to 1:10^6 were created and plated to ensure countable plates. One milliliter of each dilution was added to 2 separate BBL Columbia CNA agars (colistin and nalidixic acid agar; Becton, Dickinson and Co., Franklin Lakes, NJ) prepared according to manufacturer directions. The diluted material was spread across the plate surface using a smooth sterilized spreader. Plates were incubated 48 h at 35°C and then flooded with peroxide. Catalase-positive colonies were counted as staphylococci using a colony counter (Flash & Go; IUL Instruments I.K.S.). Counts of *Bacillus* spp. were ascertained by creating further serial dilutions out to 1:10^6 to ensure countable plates. Researchers added 1 mL of each dilution to 2 separate Difco MYP (mannitol-egg yolk-polymyxin B) agars (Becton, Dickinson and Co.) prepared according to the manufacturer directions, and spiral plated (Eddy Jet; IUL Instruments I.K.S.) the diluted material onto the plate. Incubation of MYP plates occurred at 35°C for 48 h, with colony-forming units counted automatically using a colony counter (Flash & Go, IUL Instruments I.K.S.). All bacterial counts were converted to a DM basis by dividing the wet matter basis count (cfu/g) by the moisture percentage of the sample, resulting in colony-forming units per grams of DM.

Compost Bed Conditions

The same 9 evenly distributed locations throughout the barn were used to collect bed temperatures for all farms. Compost bedded pack temperatures were collected 10.2 and 20.3 cm deep using a thermocouple-based thermometer (0.22-m length, accuracy of ± 2.2°C; model 87; Fluke Inc., Everett, WA). The mean of the surface and 10.2-cm depth CBP temperatures was calculated to produce a composite temperature (**CT**). Compost bedded pack surface temperatures were collected using an infrared thermometer (accuracy of ± 1°C; model 62; Fluke Inc.). Ambient temperature was collected using a weather meter (accuracy of ± 1°C; model 4000; Kestrel Meters, Sylvan Lake, MI). Bedding material nutrient analyses were performed by University of Kentucky Regulatory Services (Lexington) laboratory personnel on the second bedding material sample collected on each farm to determine moisture, C, and N concentrations by methods specified by Peters et al. (2003). The carbon-to-nitrogen (C:N) ratio was calculated for all barns. Space per cow was calculated by dividing the total pack area (not including feeding space) by the total number of lactating cows housed on the CBP.

Statistical Analysis

Variable selection criteria to describe bacterial counts included CBP and management characteristics with a correlation ($r > 0.3$; $P < 0.05$) with at least 1 bacterial species, using the CORR procedure of SAS (SAS Institute Inc., Cary, NC; Table 1). Variables tested included space per cow, CT, moisture, C:N ratio, ambient temperature, stirring frequency, stirring depth, bedding addition amount, and time spent on pasture. Explanatory variables used to describe each bacterial count included moisture, CT, ambient temperature, C:N ratio, and space per cow. Bacterial counts were transformed using a logarithmic transformation to produce normally distributed values. The GLM procedure of SAS

Table 1. Pearson correlations between bacterial species and management or compost parameters considered to affect bacterial counts within the compost bedded pack barn

Variable	Bacterial species[1]				
	Coliforms	*Escherichia coli*	Staphylococci	Streptococci	*Bacillus* spp.
Space per cow,[2] m^2/cow	−0.08	−0.03	0.05	−0.38*	0.07
Composite temperature,[3] °C	0.42*	0.54*	0.27	−0.01	0.00
Moisture, %	−0.34*	−0.45*	−0.44*	0.03	−0.07
C:N	0.01	−0.17	−0.52*	−0.03	−0.29
Ambient temperature, °C	0.29	0.46*	0.53*	0.08	0.08
Tilling frequency, times/d	0.03	−0.05	−0.28	−0.18	−0.30
Tilling depth, cm	0.19	0.17	0.06	0.03	0.09
Amount of bedding added,[4] m^3/d	0.13	−0.05	−0.15	−0.29	0.05
Percentage of day spent on pasture	0.10	0.07	0.15	−0.06	0.13

[1]All bacterial species tested using logarithmic transformation.

[2]Total compost bedded pack area divided by the total number of lactating cows housed on the pack.

[3]Mean of surface and 10.2-cm depth temperature.

[4]Amount of bedding (m^3) added during addition of new bedding divided by days between new bedding additions.

*$P < 0.05$.

generated models to describe factors affecting bacterial counts, using the explanatory variables selected using the CORR procedure described above. All models tested the same explanatory variables for each bacterial species to produce consistent models. Explanatory variable quadratic and cubic transformations were tested for all explanatory variables ($P < 0.05$) and all 2- and 3-way interactions between explanatory variables and significant transformations were tested ($P < 0.05$) using backward elimination and type I sums of squares.

RESULTS AND DISCUSSION

Herd and CBP Characteristics

Compost bedded pack barns housed 90.1 ± 41.8 cows (n = 47) at the time of the visit. Producers subjectively reported a daily milk production and SCC of 27.3 ± 4.0 kg (n = 39) and 246,500 ± 84,422 cells/mL (n = 38), respectively. Producers provided 9.0 ± 2.2 m^2 of CBP space per cow (n = 44). In summer, 28 producers stirred the CBP 2 times per day, whereas 18 producers stirred the CBP 1 time per day and 1 producer stirred the CBP 3 times per day. In winter, 33 producers stirred the CBP 2 times per day, 13 producers stirred 1 time per day, and 1 producer stirred 3 times per day. Mean hygiene and locomotion scores were 1.5 ± 0.9 (n = 34), where 1 = clean and 4 = filthy (Cook and Reinemann, 2007) and 2.2 ± 0.7 (n = 34), where 1 = normal and 5 = severely lame (Sprecher et al., 1997), respectively. A mean C:N ratio of 26.7 ± 7.8 was determined, ranging from 11.3 to 43.2. Mean collection day ambient temperature, CBP surface temperature, and CBP temperatures at depths of 20.3 and 10.2 cm were 9.9 ± 9.4, 10.5 ± 8.0, 36.1 ± 11.0, and 32.3 ± 10.6°C, respectively. Mean CBP moisture content was 56.1 ± 12.4%.

Bedding Material Bacterial Counts

Bacterial counts are reported in Table 2. Of the total bacteria sampled (8.2 ± 0.4 $\log_{10}$ cfu/g of DM), coliform, streptococci, staphylococci, and *Bacillus* spp. comprised 1.86, 20.61, 52.28, and 25.25% of all bacteria, respectively. Barberg et al. (2007a) observed lesser bacteria levels compared with the present study, with total bacterial count equaling 7.0 ± 6.8 $\log_{10}$ cfu/g. Additionally, Barberg et al. (2007a) noted different bacterial count proportions of 10.7% for coliforms, 39.4% for environmental streptococci, 17.4% for environmental staphylococci, and 32.5% for *Bacillus* spp. Lobeck et al. (2012) also observed lesser counts of 4.1 $\log_{10}$ cfu/g for coliforms, 6.5 $\log_{10}$ cfu/g for streptococci, 4.0 $\log_{10}$ cfu/g for staphylococci, and 4.4 $\log_{10}$ cfu/g for *Bacillus* spp. These differences may be due to differences in environment between Kentucky and Minnesota, management practices, or bedding materials. Time relative to pack stirring may also have also influenced differences due to reintegration of surface layer material into the warmer, deep layers of the CBP and deep, warmer layers exposed on the surface after stirring. In the current study, producers typically stirred the CBP before milking 2 times per day. Site visits were conducted during the morning, evening, and night and did not account for this variable. Additionally, the study by Barberg et al. (2007a) did not indicate time relative to stirring when taking bedding samples.

A direct correlation exists between bacterial counts in bedding and bacterial counts on the teat ends (Hogan and Smith, 1997; Zdanowicz et al., 2004) and clinical

Table 2. Descriptive statistics for bacterial species sampled on 42 compost bedded pack barns in Kentucky

	Mean		SD		Minimum		Maximum	
Bacterial species	cfu/g	$\log_{10}$ cfu/g	cfu/g	$\log_{10}$ cfu/g	cfu/g	$\log_{10}$ cfu/g	cfu/g	$\log_{10}$ cfu/g
Coliforms	5.1×10^6	6.3	8.8×10^6	0.6	1.2×10^5	5.1	4.4×10^7	7.6
Escherichia coli	2.8×10^6	6.0	5.3×10^6	0.6	5.4×10^4	4.7	3.1×10^7	7.5
Streptococci	5.6×10^7	7.2	1.1×10^8	0.7	4.2×10^5	5.6	6.4×10^8	8.8
Staphylococci	1.4×10^8	7.9	2.5×10^8	0.5	6.3×10^6	6.8	1.6×10^9	9.2
Bacillus spp.	6.6×10^7	7.6	7.0×10^7	0.5	1.3×10^6	6.1	3.2×10^8	8.5

mastitis rates (Hogan et al., 1989). Bedding containing greater than 10^6 cfu of total bacteria/g increased IMI risk (Jasper, 1980). Bacteria proliferate more easily in organic bedding (gram-negative: 7.1 $\log_{10}$ cfu/g; coliform: 6.2 $\log_{10}$ cfu/g; *Klebsiella* spp.: 4.3 $\log_{10}$ cfu/g; streptococci: 7.5 $\log_{10}$ cfu/g) compared with inorganic bedding (gram-negative: 6.41 $\log_{10}$ cfu/g; coliform: 5.7 $\log_{10}$ cfu/g; *Klebsiella* spp.: 3.4 $\log_{10}$ cfu/g; streptococci: 6.8 $\log_{10}$ cfu/g) because organic bedding can supply nutrients, temperature, and moisture for bacteria sustenance (Hogan et al., 1989).

Maintaining clean, dry udders reduces IMI risk (Neave et al., 1969). Drier CBP surface layers resulted in cleaner cow legs and udders (Black et al., 2013), accomplished through a high drying rate, deep CBP stirring, and adequate space per cow. In the current study, high bacteria levels were observed in the bedding material; however, SCC (252,860 cells/mL) remained under the state average for Kentucky (313,000 cells/mL; Norman et al., 2010). Producers did not report clinical mastitis rates within the herds and, although the SCC was less than the reported state average, clinical mastitis incidence may have increased or decreased by housing cows on the CBP. More research on this subject is necessary.

Coliforms

Tests of significance and estimated coefficients for the coliform model are given in Table 3. Coliforms showed a strong positive correlation with CT ($r = 0.42$; $P < 0.05$) and a moderate negative correlation with moisture ($r = -0.34$; $P < 0.05$). These relationships were maintained when accounting for additional variables. In low ambient temperature conditions (−3°C), coliform count increased above 9 $\log_{10}$ cfu/g in several different situations: when CT decreased below 4°C and space per cow decreased below 6 m^2/cow, CT decreased below 6°C and C:N decreased below17:1, CT decreased below 9°C and moisture decreased below 35%, space per cow decreased below 6 m^2/cow and moisture decreased below 30%, space per cow increased above 13 m^2/cow and moisture increased above 70%, C:N ratio decreased below 20:1 and moisture decreased below 35%, and C:N ratio increased above 42:1 and moisture increased above 69%. Conversely, coliform count decreased below 5 $\log_{10}$ cfu/g in several situations: when CT decreased below 3°C and space per cow increased above 12.5 m^2/cow, CT decreased below 7°C and C:N ratio increased above 37:1, CT increased above 35°C and moisture decreased below 35%, space per cow increased above 10.5 m^2/cow and moisture decreased below 32%, and C:N ratio increased above 31:1 and moisture decreased below 39%.

In high ambient temperature conditions (27°C), coliform count increased above 9 $\log_{10}$ cfu/g in several different situations: when CT increased above 36°C and moisture decreased below 34%, CT decreased below 8°C and C:N ratio increased above 36:1, CT decreased below 3°C and space per cow increased above 12.5 m^2/cow, space per cow increased above 11 m^2/cow and moisture decreased below 31%, and C:N ratio increased above 32:1 and moisture decreased below 38%. Alternatively, coliform count decreased below 5 $\log_{10}$ cfu/g in several different situations: when CT decreased below 3°C and moisture decreased below 29%, CT decreased below 11°C and C:N ratio decreased below 22:1, C:N ratio increased above 37:1 and CT increased above 37°C, CT decreased below 6°C and space per cow decreased below 7 m^2/cow, CT increased above 39°C and space per cow increased above 11.5 m^2/cow, space per cow increased above 10 m^2/cow and moisture increased above 64%, and C:N ratio increased above 35:1 and moisture increased above 62%. Many of the environments that would interrupt bacterial growth would also prohibit composting activity. This gives merit to the concept that the CBP does not reduce the population of coliform bacteria due to similar optimal conditions.

Coliforms are gram-negative bacteria and environmental mastitis pathogens (Hogan et al., 1999). Additionally, coliforms are associated with the intestinal tract, and are likely in high concentrations because the CBP system uses manure as a substrate for composting. Potential coliform pathogens causing mastitis include *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. (Eberhart, 1984). The composting process requires an available organic carbon source; however, organic

Table 3. Estimated coefficients for the model of coliform count ($R^2 = 0.71$)

Variable	Estimate	SE	*t*-value	*P*-value
Intercept	58.9463	12.53	4.71	<0.01
Ambient temperature, °C	−3.3369	0.72	−4.65	<0.01
Moisture, %	−0.7759	0.18	−4.22	<0.01
Space per cow,[1] m²/cow	−2.2566	0.71	−3.19	<0.01
C:N	−0.9068	0.29	−3.18	<0.01
Composite temperature,[2] °C	−0.5443	0.19	−2.88	<0.01
Ambient temperature × moisture	0.0448	0.01	4.62	<0.01
Ambient temperature × C:N	0.0661	0.02	3.44	<0.01
Ambient temperature × space per cow	0.1298	0.04	3.13	<0.01
Ambient temperature × composite temperature	0.0448	0.01	3.59	<0.01
Moisture × C:N	0.0134	0.00	3.19	<0.01
Moisture × space per cow	0.0316	0.01	3.19	<0.01
Space per cow × composite temperature	0.0158	0.01	2.48	0.02
Moisture × composite temperature	0.0055	0.00	2.14	0.04
C:N × composite temperature	0.0031	0.00	1.61	0.12
Ambient temperature × moisture × C:N	−0.0009	0.00	−3.48	<0.01
Ambient temperature × moisture × space per cow	−0.0016	0.00	−2.89	<0.01
Ambient temperature × space per cow × composite temperature	−0.0014	0.00	−2.61	0.02
Ambient temperature × C:N × composite temperature	−0.0005	0.00	−2.40	0.03
Ambient temperature × moisture × composite temperature	−0.0004	0.00	−2.21	0.04

[1]Total compost bedded pack area divided by number of cows housed on the pack.

[2]Mean of surface and 10.2-cm depth pack temperatures.

bedding materials expose cows to more gram-negative bacteria than cows that are exposed to an inorganic bedding material (Hogan et al., 1989). Additionally, using fresh or green sawdust (Newman and Kowalski, 1973; Bagley et al., 1978) can increase *Klebsiella pneumoniae* mastitis incidence. Current recommendations (Janni et al., 2007) suggest that bedding with sawdust or wood shavings possibly increases the likelihood of exposure to *Klebsiella* spp. pathogens. Other management practices should be used to help minimize exposure or risk because CBP management through monitoring of moisture, temperature, C:N ratio, and space per cow may also reduce composting efficiency.

Escherichia coli

Tests of significance and estimated coefficients for the *E. coli* model are given in Table 4. *Escherichia coli* showed strong positive correlations with CT ($r = 0.54$; $P < 0.05$) and ambient temperature ($r = 0.46$; $P < 0.05$) but a strong negative correlation with moisture ($r = -0.45$; $P < 0.05$). However, only the relationship with ambient temperature was maintained when accounting for all factors. In low ambient temperature conditions (−3°C), *E. coli* counts increased above 9 $\log_{10}$ cfu/g when the C:N ratio decreased to below 14:1 and moisture decreased to below 29%. However, *E. coli* counts decreased to below 4 $\log_{10}$ cfu/g when the C:N ratio increased above 35:1 and moisture decreased below 34%. In high ambient temperature conditions (27°C), *E. coli* counts increased above 3 $\log_{10}$ cfu/g when the C:N ratio increased above 38:1 and moisture decreased below 32%.

Escherichia coli are gram-negative coliform bacteria with a rod shape (Dufour, 1977). This facultative anaerobic species resides in normal gut flora and is

Table 4. Estimated coefficients for the model of *Escherichia coli* counts ($R^2 = 0.49$)

Variable	Estimate	SE	*t*-value	*P*-value
Intercept	15.5447	6.14	2.53	0.02
Ambient temperature, °C	−0.6299	0.30	−2.11	0.04
Moisture, %	−0.1551	0.09	−1.65	0.11
Space per cow,[1] m²/cow	−0.0158	0.04	−0.44	0.66
C:N	−0.3610	0.25	−1.46	0.16
Composite temperature,[2] °C	0.0171	0.01	1.60	0.12
Moisture × C:N	0.0055	0.00	1.48	0.15
Ambient temperature × C:N	0.0261	0.01	2.18	0.04
Ambient temperature × moisture	0.0103	0.00	2.22	0.03
Ambient temperature × moisture × C:N	−0.0004	0.00	−2.29	0.03

[1]Total compost bedded pack area divided by number of cows housed on the pack.

[2]Mean of surface and 10.2-cm depth pack temperatures.

Table 5. Estimated coefficients for the model of staphylococci counts ($R^2 = 0.37$)

Variable	Estimate	SE	*t*-value	*P*-value
Intercept	8.6151	0.76	12.82	<0.01
Ambient temperature, °C	0.0232	0.01	2.05	<0.05
Moisture, %	−0.0000	0.01	−0.00	1.00
Space per cow,[1] m^2/cow	−0.0248	0.03	−0.87	0.39
C:N	−0.0209	0.01	−1.94	0.06
Composite temperature,[2] °C	−0.0073	0.01	−0.96	0.34

[1]Total compost bedded pack area divided by number of cows housed on the pack.

[2]Mean of surface and 10.2-cm depth pack temperatures.

continually excreted in the feces (Lehtolainen, 2004). Many of the strains living in the normal flora are nonpathogenic; however, some mastitic strains can be found in the intestinal flora (Linton and Robinson, 1984). Because of this, the CBP will contain *E. coli* because manure is a substrate in the system, and some of those bacteria will be mastitic pathogens. Ward et al. (2002) explained that *E. coli* are affected by 3 temperature ranges: the bacteria will survive with minimal multiplication in temperatures below 15°C, survive and multiply optimally between 15 and 45°C, and begin to die in temperatures above 45°C. In the current study, CBP temperature did not play a role in *E. coli* bacterial counts; however, 45°C was not within the CT range modeled, meaning that the CBP surface never reached temperatures high enough to destroy *E. coli* bacteria. Had CBP CT reached this level, composting would have reached the temperature necessary for optimal biodegradation (Stentiford, 1996); however, the CBP surface may have been too hot for cows to lie on. When the lying surface is hotter than that of the cow, heat is conducted toward the cow, raising the body temperature. When ambient conditions are warm, this additional heat conductance may prompt cows to stand instead of lying down. Managing the lower CBP layers for optimal composting may be a better management strategy than trying to achieve the high temperatures needed to destroy *E. coli* bacteria on the surface because of the effects on the cow.

Staphylococci

Tests of significance and estimated coefficients for the staphylococci model are given in Table 5. Staphylococci showed a strong positive correlation with ambient temperature ($r = 0.53$; $P < 0.05$) and strong negative correlations with moisture ($r = -0.44$; $P < 0.05$) and C:N ratio ($r = -0.52$; $P < 0.05$). However, only the relationship with ambient temperature remained a significant variable influencing staphylococci when other variables were accounted for. Ambient temperature significantly affected staphylococci ($P < 0.05$; Table 5), indicating that staphylococci exhibit some heat intolerance. Staphylococci counts increased as ambient temperature increased ($P < 0.05$). *Staphylococcus aureus* survives in temperatures between 6 and 48°C, with an optimum temperature of 37°C (Vandenbosch et al., 1973). The wide temperature survival range combined with the additional CBP heat generation indicated that staphylococci might survive well in many climatic conditions. However, CBP temperature, moisture, C:N ratio, and space per cow had no significant effect on staphylococci counts. Consistent CBP management for optimal moisture, temperature, C:N ratio, and space per cow to achieve successful composting conditions may not influence the total staphylococci count in the bedding material. Staphylococci counts may increase in colder weather because of the increased survival in lower ambient temperatures. Producers should concentrate on preventative mastitis methods, such as proper milking procedures and dry-off treatment.

Staphylococci are gram-positive bacteria with a cocci shape, forming clusters (Chauhan et al., 2012). As with other bacteria, some species are harmless, whereas others can cause disease. *Staphylococcus aureus* is a cause of contagious mastitis in dairy herds (Barkema et al., 2006). Bedding can be a *Staph. aureus* source (Roberson et al., 1994), but replacement heifers (Roberson et al., 1994) and milking equipment (Zadoks et al., 2002) likely contribute more to the spread. Coagulase-negative staphylococci are usually considered a minor mastitis pathogen because mastitis cases are typically mild and subclinical (Taponen et al., 2006); however, CNS mastitis has become the most common mastitis type in many countries (Pitkälä et al., 2004; Tenhagen et al., 2006). Some CNS species may be environmental opportunists, but most CNS species causing IMI reside on the udder (Pyörälä and Taponen, 2009). When dealing with a *Staphylococcus aureus* or CNS mastitis outbreak, improved management within the parlor, at dry off, and during calving should be considered.

Streptococci

Tests of significance and estimated coefficients for the streptococci model are given in Table 6. Streptococci

Table 6. Estimated coefficients for the model of streptococci counts ($R^2 = 0.68$)

Variable	Estimate	SE	*t*-value	*P*-value
Intercept	10.0377	5.76	1.74	0.09
Ambient temperature, °C	−1.0474	0.30	−3.54	<0.01
Moisture, %	−0.0158	0.10	−0.16	0.88
Space per cow,[1] m²/cow	−1.9800	0.52	−3.83	<0.01
C:N	2.0993	0.43	4.88	<0.01
Composite temperature,[2] °C	−0.1191	0.04	−2.88	<0.01
C:N × C:N	−0.0533	0.01	−4.82	<0.01
Moisture × C:N	−0.0320	0.01	−4.36	<0.01
Moisture × space per cow	0.0299	0.01	3.68	<0.01
Space per cow × ambient temperature	0.1029	0.03	3.66	<0.01
Moisture × ambient temperature	0.0157	0.00	3.44	<0.01
Composite temperature × C:N	0.0038	0.00	2.81	<0.01
Moisture × C:N × C:N	0.0008	0.00	4.54	<0.01
Moisture × space per cow × ambient temperature	−0.0015	0.00	−3.37	<0.01

[1]Total compost bedded pack area divided by number of cows housed on the pack.

[2]Mean of surface and 10.2-cm depth pack temperatures.

showed a moderate negative correlation with space per cow ($r = -0.38$; $P < 0.05$), although this relationship was not maintained when accounting for other variables. Streptococci grow in temperatures between 25 and 42°C (Hardie and Whiley, 1995). Achieving CBP temperatures greater than 42°C may reduce streptococci counts. This management practice can also help reduce pack moisture by increasing moisture evaporation from the pack through increased temperature.

Streptococci counts peaked to a level above 8 $\log_{10}$ cfu/g when the C:N ratio ranged from 20:1 to 22:1 ($P < 0.05$), a range slightly less than that which is ideal for composting (Gray et al., 1971; NRAES, 1992), at all concentrations of moisture modeled. This result indicates that streptococci may thrive in a carbon concentration environment similar to that of composting microbes. Similar to the interaction between C:N ratio and moisture, a peak in streptococci counts occurred when the C:N ratio was between 16 and 18 at all concentrations of moisture modeled ($P < 0.05$). In low ambient temperature conditions (−3°C), streptococci counts increased above 9 $\log_{10}$ cfu/g when space per cow decreased below 8.5 m^2/cow and moisture decreased below 35%. Streptococci counts decreased below 5 $\log_{10}$ cfu/g when space per cow increased above 12 m^2/cow and moisture decreased below 29%. In high ambient temperature conditions (27°C), streptococci counts increased above 9 $\log_{10}$ cfu/g when space per cow increased above 11 m^2/cow and moisture decreased below 31% or when space per cow increased above 11 m^2/cow and moisture increased above 66%. Streptococci counts decreased below 5 $\log_{10}$ cfu/g when space per cow decreased below 5 m^2/cow and moisture decreased below 28%.

Streptococcus species are gram-positive, spherical shaped bacteria, which grow in chains. *Streptococcus uberis* resides on many cow body sites (Cullen, 1966; Cullen and Little, 1969; Kruze and Bramley, 1982) and in the environment, including the bedding (Bramley, 1982). *Streptococcus agalactiae* are contagious mastitis pathogens, but are susceptible to penicillin therapy and can be eradicated from a herd (McDonald, 1977). These results imply that streptococci thrive in the environment ideal for composting bacteria and microbes. Considering this, an ideal management strategy for streptococci count reduction may be to provide adequate space per cow in winter weather, while being careful to maintain recommended moisture levels (50 to 60%; Gray et al., 1971; NRAES, 1992).

Bacillus Species

Tests of significance and estimated coefficients for the *Bacillus* spp. model are given in Table 7. In low ambient temperature conditions (−3°C), *Bacillus* spp. counts increased above 9 $\log_{10}$ cfu/g when space per cow decreased below 8 m^2/cow and moisture increased above 34%, and when the C:N ratio decreased below 23:1 and moisture decreased below 38%. *Bacillus* spp. counts decreased below 5 $\log_{10}$ cfu/g when the C:N ratio increased above 35:1 and moisture decreased below 39%, and when space per cow increased above 11.5 m^2/cow and moisture decreased below 30%. Conversely, in high ambient temperature conditions (27°C), *Bacillus* spp. counts increased above 9 $\log_{10}$ cfu/g when the C:N ratio decreased below 16:1 and moisture increased above 66%, the C:N ratio increased above 37:1 and moisture decreased below 33%, and space per cow increased above 11 m^2/cow and moisture decreased below 31%. *Bacillus* spp. counts decreased below 5 $\log_{10}$ cfu/g when the C:N ratio decreased below 12:1 and moisture decreased below 27%, and when space per cow decreased below 5 m^2/cow and moisture decreased below 28%.

Table 7. Estimated coefficients for the model of *Bacillus* spp. counts ($R^2 = 0.40$)

Variable	Estimate	SE	*t*-value	*P*-value
Intercept	41.1956	11.58	3.56	<0.01
Ambient temperature, °C	−1.7969	0.57	−3.15	<0.01
Moisture, %	−0.5046	0.18	−2.82	<0.01
Space per cow,[1] m²/cow	−1.8681	0.58	−3.21	<0.01
C:N	−0.5968	0.31	−1.95	0.06
Composite temperature,[2] °C	−0.0019	0.01	−0.15	0.88
Ambient temperature × space per cow	0.1005	0.03	3.40	<0.01
Ambient temperature × moisture	0.0282	0.01	3.20	<0.01
Ambient temperature × C:N	0.0316	0.01	2.18	0.04
Moisture × space per cow	0.0291	0.01	3.17	<0.01
Moisture × C:N	0.0087	0.00	1.87	0.07
Ambient temperature × moisture × space per cow	−0.0016	0.00	−3.26	<0.01
Ambient temperature × moisture × C:N	−0.0005	0.00	−2.32	0.03

[1]Total compost bedded pack area divided by number of cows housed on the pack.

[2]Mean of surface and 10.2-cm depth pack temperatures.

Bacillus bacteria are rod-shaped, gram-positive, spore-forming bacteria, which may be aerobic or anaerobic (Parrott-Sheffer and Rogers, 2012). *Bacillus* bacteria are rarely the cause of mastitis (Brown and Scherer, 1957; Howell, 1972; Jones and Turnbull, 1981); however, *Bacillus* spores can survive pasteurization, reducing milk shelf life (Jones and Turnbull, 1981; Griffiths, 1992). *Bacillus* spp. survive at a wide temperature range, with maximum growth temperatures ranging from 31 to 76°C. The optimal growth temperature is typically 6°C below the maximum growth temperature. This characteristic makes *Bacillus* a difficult pathogen to destroy. *Bacillus* plays an active role in composting (Beffa et al., 1996), increasing the likelihood of the teats contacting the bacteria. *Bacillus* spp. thrive in environments similar to composting bacteria, making *Bacillus* spp. reduction while maintaining active composting difficult. Two management strategies are to provide more space per cow or add additional carbon sources during colder weather to avoid excessive moisture addition to the CBP.

Management

Mastitis-causing bacteria thrive in similar conditions to that of composting bacteria and microbes, making elimination of these bacteria difficult in an active composting environment. In commercial composting, material is sanitized because the process is fully completed, killing bacteria in the different heating stages (Stentiford, 1996). However, in the CBP system, producers attempt to manage the CBP at a consistent stage to promote material degradation, making the system a semi-composting process. Additionally, bedding, manure, and urine are added to the CBP regularly, supplying carbon and nitrogen to mastitis-causing and composting bacteria alike.

Bacteria levels are not likely to be reduced by managing CBP moisture, temperature, C:N ratio, and space per cow. Therefore, the producer's aim should be to provide a dry lying surface to prevent dirty cows and increased SCC. This should be achieved by managing the composting process and through adequate bedding addition to reduce moisture on the surface layer. Increased moisture and nutrient availability in sawdust bedding increased bacterial counts (Fairchild et al., 1982; Zdanowicz et al., 2004). Further, a correlation existed between bedding bacterial counts and stall cleanliness in freestalls (Zdanowicz et al., 2004). However, contrary to previous belief, managing cows to remain standing after milking did not reduce the odds of IMI (DeVries et al., 2010) making a dry lying surface even more crucial. In periods of inadequate composting activity and high CBP moisture, cow cleanliness should take precedence. Additional bedding should be added to reduce the risk of IMI from increased exposure to pathogens (Neave et al., 1969) when housed on bedding with high bacterial counts (Hogan et al., 1989; Hogan and Smith, 1997; Zdanowicz et al., 2004).

The lying environment of cows housed on the CBP contained high bacteria levels compared with fresh bedding (Fairchild et al., 1982; Hogan and Smith, 1997) or pasture (*Strep. uberis*; Lopez-Benavides et al., 2007). Therefore, attention must be paid to other management areas where preventative measures can be taken, such as during the dry period, at calving, and with replacement heifers. Additionally, meticulous parlor procedures (USDA-APHIS, 2003) are necessary to prevent contagious pathogen spread during milking.

CONCLUSIONS

Mastitis-causing bacteria thrive in the CBP environment, which meets the moisture and nutrient demands of the bacteria. Managing the CBP system for moisture, temperature, C:N ratio, and space per cow may help to reduce some bacterial species counts, but the bacterial load in the bedding will likely remain high. Producers should manage the CBP for moisture to maintain a dry resting surface for cows to help prevent increased SCC and IMI. The CBP provides a comfortable environment for cows but must be carefully managed to ensure that udder health is not compromised.

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