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Longitudinal Trends and Associations between Compost Bedding Characteristics and Bedding Bacterial Concentrations

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Abstract

The objectives were to quantify bedding concentrations of total bacteria and selected groups of mastitis pathogens (coliforms and streptococci), to identify bedding factors associated with these bacterial populations, and to describe longitudinal variation of bedding characteristics. Bedding samples from the superficial and deep (20 cm) layers were collected biweekly during 1 year from 3 compost bedded pack (CBP) dairies. Bedding bacterial concentrations and physical-chemical characteristics (moisture, organic matter, carbon-nitrogen ratio (C/N), pH, and density) were determined. Mixed models were used to identify predictors for bacterial concentrations. Shewhart control charts were produced to describe longitudinal variation of bedding characteristics and define alerts of out-of-control variation. Bedding temperature was greater in the deep layer than on the surface (difference = 27.0, 12.1, and 14.4 °C for farms A, B and C, respectively). Except for farm B, bacterial concentrations were greater on the surface than in the deep layer. Organic matter and dry density were associated with concentration of total bacteria and coliforms. For all farms, C/N (positive association) and dry density (negative association) were associated with bedding concentration of streptococci. Deep temperature and moisture remained within the control limits defined in farm A and B during most of the period, whereas 7 and 9 alerts for temperature and moisture were observed on farm C, respectively. For all farms, organic matter, C/N, and pH exhibited great variation, resulting in several out-of-control alerts. Results of this study can be used to manage the CBP towards reducing cows' exposure to mastitis pathogens.

Keywords: compost bedding; bacterial count; mastitis; process control analysis

1. Introduction

The compost bedded pack system (CBP) has been increasingly used worldwide to house dairy cows. The CBP is based on principles of traditional composting, in which organic materials are degraded by means of aerobic microbiological decomposition (Janni, Endres, Reneau, & Schoper, 2007). The CBP barns are bedded with an organic substrate (such as sawdust), to which feces and urine are constantly added by cows. Bedding is aerated twice a day between milking time to incorporate animal waste and ensure that the composting process continues aerobic and results in a comfortable, dry, and clean bedding surface to the animals. New bedding material is added systematically and after periods as long as 1 year, the bedding can be entirely replaced and used as fertilizer (Barberg, Endres, & Janni, 2007; Janni et al., 2007).

Results of North American cross-sectional surveys indicate that the main reasons why farmers shifted from other housing systems to the CBP were cow comfort and its consequences such as increase in milk production, longevity, and decrease in the occurrence of hock and foot lesions, easier management, less investment costs, decrease in environmental contamination by disposal of animal waste, and use of bedding as fertilizer (Barberg et al., 2007; Black et al., 2014). Reduction of animal waste disposal into the environment is of special interest to farmers and government agencies because waste management has been an increasing problem that has limited the expansion of the dairy industry.

Due to the organic nature of the bedding, one of the main concerns for housing cows in the CBP is the potential increase in exposure to environmental mastitis pathogens. It has been consistently demonstrated that bedding bacterial concentration is positively associated with bacterial contamination of the teat skin and risk of intramammary infection in lactating cows (Hogan et al., 1989; Hogan & Smith, 1997; Zdanowicz, Shelford, Tucker, Weary, & von Keyserlingk, 2004).

As a result of the traditional composting process, which is aimed to degrade organic material and produce humus, the concentration of pathogenic bacteria can be greatly minimized due to the high temperature (> 60 °C) reached during the process, and biological unavailability of the material (NRAS-54, 1992). Nonetheless, results of recent studies indicated that temperatures observed in the deep layers of the CBP (< 48.1 °C) are not high enough to substantially decrease bacterial concentrations in the material (Barberg et al., 2007; Shane, Endres, & Janni, 2010; Black et al., 2014). Despite using different laboratory methodologies, results of recent studies demonstrated that compost bedding contains high bacterial concentrations. Barberg et al. (2007) and Black et al. (2014) reported that concentration of total bacteria on the CBP bedding surface was 9.1×10^6 cfu/cc and 158×10^6 cfu/g, respectively. Lobeck, Endres, Janni, Godden, and Fetrow (2012) reported that mean concentration of coliforms, *Klebsiella* spp, and streptococci were 14000, 280, and 3×10^6 cfu/mL of bedding solution, respectively.

In this context, little research has been conducted to identify factors that could be managed by farmers to minimize bedding contamination, and consequently reduce cow's exposure to mastitis pathogens. Black et al. (2014) conducted a cross-sectional study and reported that ambient temperature was the only variable associated with bedding concentration of *Escherichia coli*, streptococci, and staphylococci in multivariable models.

Most studies published to date were performed in the United States and were cross-sectional. Longitudinal studies designed to follow the behavior of bedding variables over a period of time that encompasses all seasons of the year could be valuable not only to identify factors associated with bedding bacterial concentrations, but also to describe and identify bedding characteristics that are more difficult to control. Such studies are necessary to assess the biosecurity of the CBP and could be used to suggest management practices towards minimizing bacterial exposure to cows. Therefore, the objectives of this study were to quantify bedding concentrations of total bacteria and selected groups of mastitis pathogens (coliforms and streptococci), to identify bedding characteristics associated with these bacterial populations, and to describe longitudinal variation of bedding manageable characteristics.

2. Method

2.1 Farm Selection and Sampling Strategy

At the beginning of the study, there were supposedly 4 farms in Brazil that had adopted the CBP as a sole system to confine lactating cows. Three of these farms located in Sao Paulo state were conveniently selected to participate in the study based on the distance to the university (up to 300 km), participation in a dairy herd improvement (DHI) testing program, and willingness to comply with the study protocol.

Farms A, B and C had 33, 53 and 145 lactating cows (milked twice a day) and used peanut shell, sawdust and wood shavings as bedding, respectively. The CBP area on farm A was 290 m^2 (11 m^2/cow) and 0.24 m^3 of new bedding were added monthly per m^2 of bedding area. There was a concrete feeding alley from which cows had free access to the bedding area. Bedding was revolved twice a day between milking processes by use of a deep cultivator. There were 4 fans installed over the bedding area.

The bedding area on farm B was 1000 m^2 (19 m^2/cow) and 0.03 m^3 of new bedding were added monthly per m^2 of bedding area. Bedding was revolved twice a day between milking processes by use of a deep cultivator and there was no fans installed over the bedding area. There was no paved alley and cows were fed on a feed bunk located on the longer side of the barn.

The CBP area on farm C was 1580 m^2 (12 m^2/cow) and 0.04 m^3 of new bedding were added monthly per m^2 of bedding area. Bedding was revolved twice a day between milking processes by use of a deep cultivator and a rototiller was used approximately twice a week to further break bedding clusters. Twenty-four fans were installed over the bedding area (equally distributed) at the 7th month of the study. Cows were fed in a separate concrete-paved shed, outside the CBP.

Farms were visited monthly between May 2013 and June 2014 for data and sample collection, and verification of compliance with the study protocol. Bedding samples were collected biweekly by a trained farm personnel, as described by Barberg et al. (2007). All samples were collected immediately before milking time (approximately 10 hours after bedding was last revolved) and kept frozen until monthly pick up. The bedding area was divided into 12 equal squares, from which 1 sample was collected from the superficial and deep (20 cm) layers. All samples from each layer were then mixed to create composite superficial and deep samples, which were used for microbiological analysis. Subsequently, the superficial and deep bedding samples were mixed to create a composite sample that represented the bedding as a whole. This sample was used to determine bedding physical-chemical characteristics. Bedding temperature was measured at both depths of each square using a spear tipped digital thermometer. Bedding characteristics, based on visual observations (such as moisture and presence of compacted areas) and management events (such as bedding replacement or addition) were recorded at each visit.

2.2 Microbiological Analysis of Bedding

Bedding samples were processed in the Sao Paulo State University's Mastitis Research Laboratory within 2 weeks from each farm visit. Microbiological analyses of bedding was performed by adding 90 mL of 0.1% peptone water to 10 g of bedding (Zdanowicz et al., 2004). Samples were mixed thoroughly for 1 minute and let settle for 2 minutes. One hundred µL of diluted samples (10^{-1} to 10^{-6}) were plated on blood agar, MacConkey, and Edward's media and incubated for 24 hours to determine concentrations (expressed as log10 cfu/g of bedding) of total bacteria, coliforms and streptococci, respectively.

2.3 Analysis of Bedding Physical-Chemical Characteristics

Bedding samples were sent to the Sao Paulo State University's Fertilizers and Correctives Laboratory for determination of moisture (%), organic matter (%), carbon (%), nitrogen (%), carbon-nitrogen ratio (**C/N**), pH, and wet and dry densities (kg/m^3). All analysis were performed according to Brazilian official methods (Brasil, 2007).

2.4 Statistical Analysis

Shewhart control charts for individual measurements (Montgomery, 2007) were produced to describe longitudinal variation of bedding physical-chemical characteristics and identify difficulties in maintaining variation under control. The process variability of each farm was estimated based on the moving range of two successive observations. The central line of the charts represents the average of the measurements and the upper and lower control limits were defined as follows (Montgomery, 2007):

$$\text{Control limits} = \bar{X} \pm 3\sigma \tag{1}$$

Where $\bar{X}$ is the average of the individuals measurements, $\sigma = (\overline{MR}/\mathrm{d}_2)$, $\overline{MR}$ is the average moving range for 2 successive observations, and d_2 is an anti-biasing constant (1.128) for n = 2 successive observations.

Two tests of interest were used to identify out-of-control variation, according to the following definitions: Test 1 (T1) = 1 point outside the control limits ($\bar{X} \pm 3\sigma$), and Test 2 (T2) = 2 out of 3 consecutive points beyond the limits determined by $\bar{X} \pm 2\sigma$. Control charts were produced with Prism 6 (GraphPad Software Inc, La Jolla, CA, USA) using limits and tests derived from the PROC SHEWHART of SAS (SAS Institute, Cary, NY, USA).

The distribution of study variables was examined to identify departures from a normal distribution. All bacterial counts were not normally distributed and were transformed to a log10 scale for analysis. For each farm, a paired T-test was used to compare mean bedding temperature (°C) and bacterial concentrations (log10 cfu/g) between the superficial and deep layers (Table 1). Subsequently, linear mixed models (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006) were used to compare the means of the bedding variables listed in Table 2 among seasons of the year (summer = December, January, and February; fall = March, Abril, and May; winter = June, July, and August; and spring = September, October, and November). Farm was included in the models as the random term to account for the correlation between repeated observations within the same farm. Bedding age was forced in these models as a covariate because it was associated with most physical-chemical bedding characteristics, and could, therefore, confound seasonal effects. Bedding age was a categorical variable based on the time interval between a given visit day and the last bedding total replacement. Levels were defined as 1 (≤ 4), 2 (5-8), and 3 (≥ 9 months) from the last total bedding replacement).

As an exploratory analysis, linear mixed models (Littell et al., 2006) were used to identify unconditional associations between each explanatory variable and study outcomes Explanatory variables were bedding moisture (%), organic matter (%), C/N, pH, wet density (kg/m^3), dry density (kg/m^3), surface temperature (°C), and deep temperature (°C). Outcome variables were bedding concentrations of total bacteria, coliforms or streptococci (log10 cfu/g). Variables associated with each outcome at a significance level of 0.15, as well as interactions with farm, were considered for multivariable analyses.

Multivariable linear mixed models were then constructed to identify conditional associations between explanatory variables and bedding concentration of total bacteria, coliform and streptococci. A backward model selection procedure was used to select a final multivariable model for each study outcome. Interaction terms between farm and each explanatory variable were included in the models and remained if significant. Farm was included in the models as the random term to account for the correlation between repeated observations within the same farm. Bedding age was not included in the multivariable models described above because it was greatly associated with most bedding physical and chemical characteristics (collinearity).

To assess changes in bedding characteristics resulting from the maturation of the composting process, separate linear mixed models were constructed to compare mean bedding organic matter, density, moisture, and C/N among bedding age categories. Statistical analyses were performed using SAS (SAS Institute, Cary, NY, USA) and significance was set at $P < 0.05$.

3. Results

Seven, 5 and 1 biweekly bedding samples from farms A, B, and C, respectively, could not be collected by farm personnel (Table 1).

Table 1. Descriptive statistics for bedding variables by farm and bedding layer

Variable	FARM A				FARM B				FARM C			
	N	MEAN[1]	SD	CV	N	MEAN	SD	CV	N	MEAN	SD	CV
Temperature (°C)												
Surface[2]	17	26.9^{Aa}	3.0	11.1	25	32.7^{Ba}	2.2	6.8	29	28.0^{Aa}	2.2	8.0
Deep layer[2]	17	53.9^{Ab}	2.7	5.0	25	44.8^{Bb}	3.2	7.2	29	42.4^{Bb}	5.9	13.8
Concentration of total bacteria (log10 cfu/g)												
Surface	18	8.8^{Aa}	0.5	6.0	24	8.4^{Ba}	0.4	4.6	28	8.9^{Aa}	0.5	8.4
Deep layer	18	8.0^{Ab}	0.7	8.4	24	8.3^{Aa}	0.4	5.2	28	8.3^{Ab}	0.7	5.4
Concentration of coliforms (log10 cfu/g)												
Surface	18	6.7^{Aa}	0.7	10.7	24	6.0^{Ba}	0.7	10.7	28	6.7^{Aa}	0.8	13.9
Deep layer	18	5.4^{Ab}	0.8	15.2	24	6.0^{Ba}	0.5	7.9	28	5.9^{ABb}	0.8	12.5
Concentraton of streptococci (log10 cfu/g)												
Surface	18	6.7^{Aa}	1.1	16.9	24	5.6^{Ba}	0.9	15.7	28	6.8^{Aa}	0.6	12.1
Deep layer	18	5.6^{ABb}	1.6	27.9	24	5.3^{Aa}	1.3	25.1	28	6.2^{Bb}	0.7	8.4
Organic matter[3] (%)	18	41.8^{A}	7.5	18.0	24	31.4^{B}	3.4	10.8	28	35.1^{B}	8.4	24.0
Carbon (%)	18	23.2^{A}	4.1	17.8	24	17.5^{B}	1.9	11.0	28	19.1^{B}	3.9	20.2
Nitrogen (%)	18	1.8^{A}	2.6	148.0	24	0.7^{B}	0.2	30.0	28	0.9^{B}	0.3	28.5
Carbon-nitrogen ratio	18	26.6^{A}	4.0	15.1	24	26.6^{A}	9.4	35.4	28	22.9^{A}	8.8	38.4
Moisture (%)	18	37.4^{AB}	4.0	10.6	24	40.4^{A}	6.4	15.8	28	35.5^{B}	8.4	23.7
pH	18	9.0^{A}	0.5	5.7	24	8.8^{A}	0.5	6.0	28	8.9^{A}	0.4	4.6
Wet density (kg/m^3)	18	389.4^{A}	158.6	40.7	24	539.3^{B}	52.1	10.0	28	458.9^{A}	103.9	22.6
Dry density (kg/m^3)	18	240.7^{A}	93.8	39.0	24	324.4^{B}	56.5	17.4	28	298.0^{B}	90.3	30.3

Note. [1]Different upper case letters within the same row indicates a significant difference ($P < 0.05$) between means. Different lower case letters within the same column indicates a significant different between means ($P < 0.05$); [2]Bedding samples were collected from 12 equally divided areas of the bedding and mixed to create composite superficial and deep (20 cm) samples; [3]Bedding physical-chemical characteristics were estimated on composite samples created by mixing the superficial and deep bedding samples.

3.1 Bedding Changes during the Study Period

During the study period, the most frequent management issues reported by farmers were improper aeration of the bedding deep layer (revolving too shallow), bedding compaction, and difficulties to maintain moisture under control. Compacted bedding was observed on 25, 20, and 18% of the visits to farms A, B and C, respectively. Moist bedding was recorded on 38, 30, and 9% of the visits to farms A, B and C, respectively.

The bedding of farm B was entirely replaced during the last month of the study, when the farmer reported the material as compacted, humid, and more difficult to manage. The bedding of farm C was replaced once in the 1st month of the study due to lack of cultivation. Before replacement, bedding was reported as compacted, humid, rotten (blackened with foul odor), and cold at the deep layer. For farms B and C, bedding replacements were characterized by a drop followed by a gradual increase in deep temperature, a decrease in pH, and a sharp increase in moisture, C/N, organic matter (only for farm C), and the concentrations of total bacteria, streptococci, and coliforms (Figures 1 and 2).

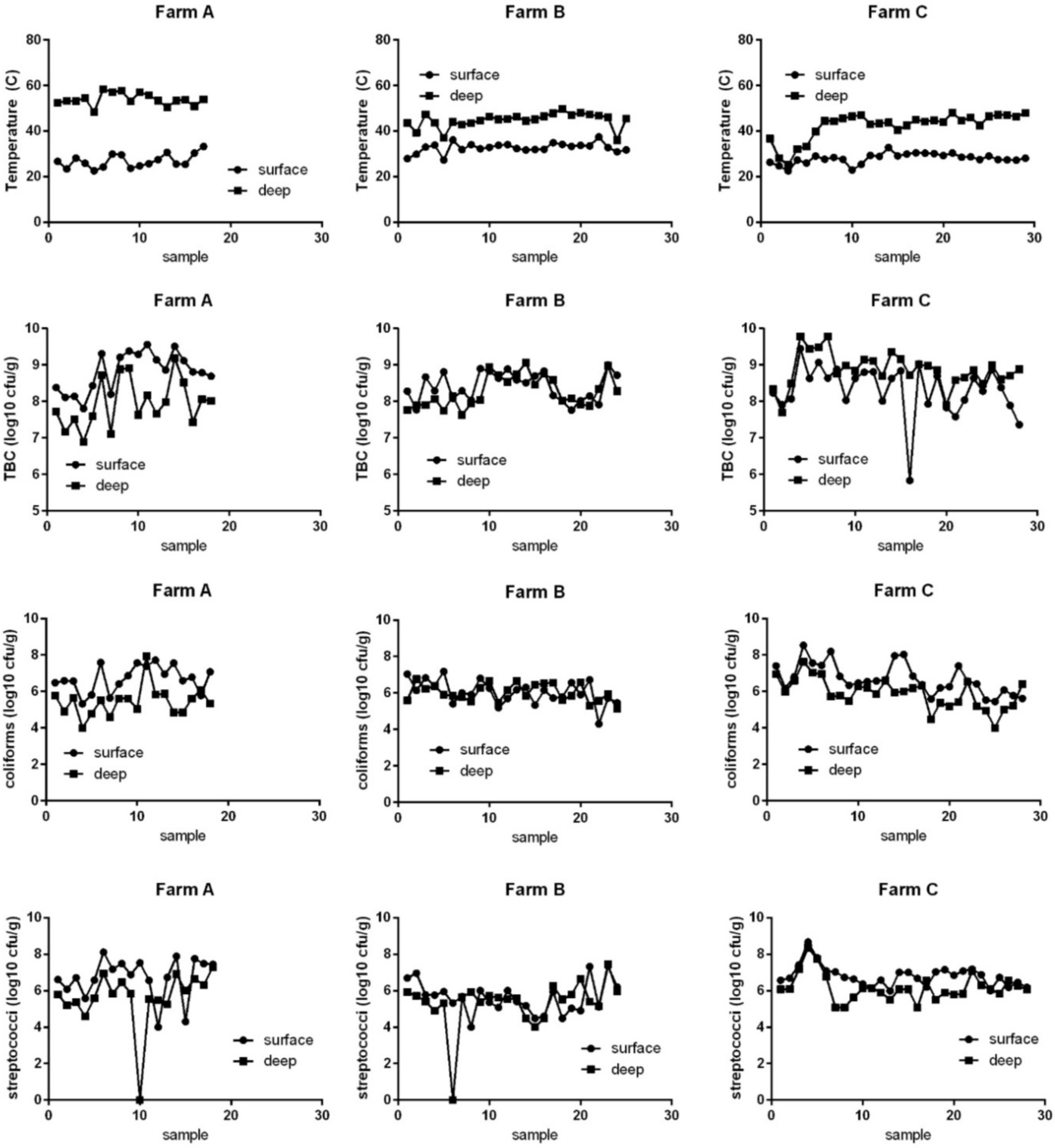

Figure 1. Longitudinal variation of bedding concentrations of total bacteria, coliforms, and streptococci, and bedding temperature, by bedding layer. Samples were collected biweekly from the superficial and deep (20 cm) layers of the bedding

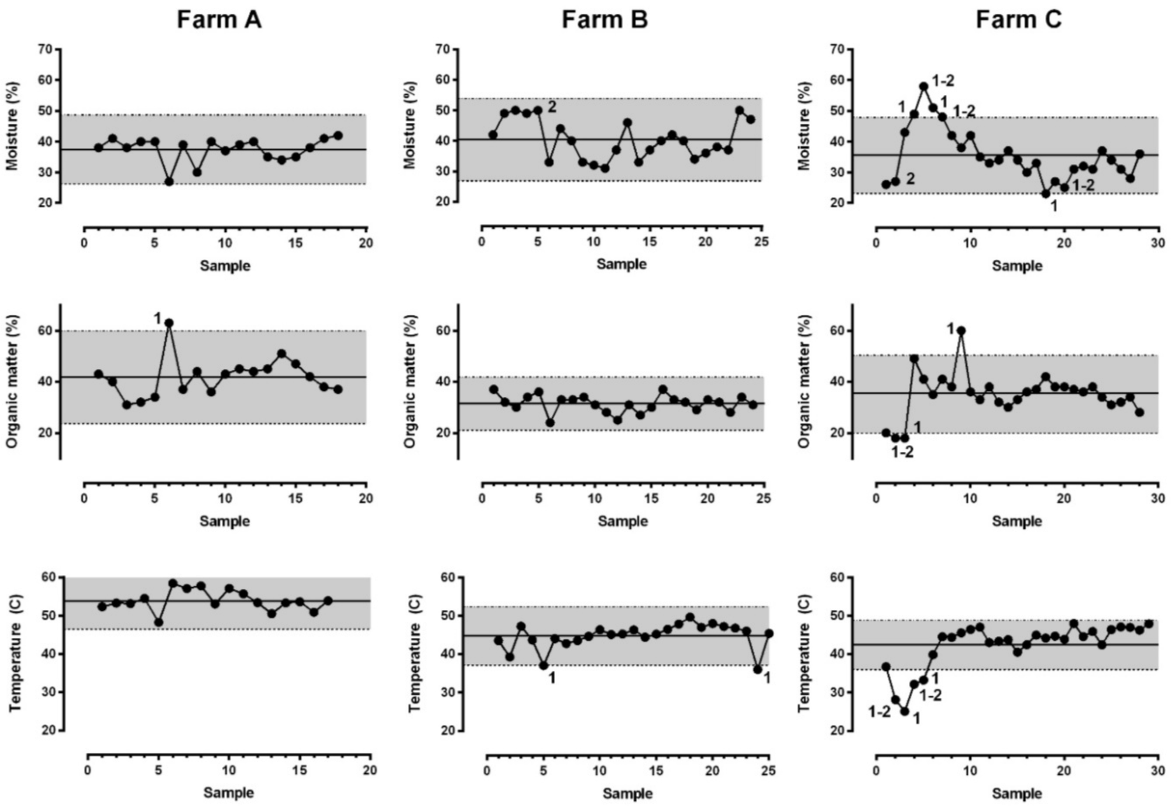

Figure 2. Shewhart control charts for individual measurements of bedding manageable characteristics. Bedding samples were collected biweekly. The central line indicates the mean of the observations. The upper and lower horizontal lines indicate the upper and lower control limits ($\bar{X} \pm 3\sigma$). Test 1 = 1 point beyond the mean ± 3σ (control limits); Test 2 = 2 of 3 consecutive points beyond the mean ± 2σ. Temperature was measured at the deep layer (20 cm) of the bedding

3.2 Bedding Temperature

For all farms studied, temperature measured in the deep layer (20 cm) of the bedding was greater than that measured on the surface (Table 1). Nonetheless, the difference between the superficial and deep layer was greater for farm A (27.0 °C), as compared with farms B (12.1 °C) or C (14.4 °C), due to the greater deep temperature found on farm A (Table 1 and Figure 1). For all farms, bedding temperature at both layers varied little during the study period (coefficient of variation (CV) by farm ranged from 5.0 to 13.8%) and the temperature on the surface was not different among seasons of the year. Bedding superficial temperature of farm B was 5.8 and 4.7 °C greater than those observed on farms A and C, respectively (Table 1). Deep layer temperature was greater in spring than in winter for all farms (Table 2). No alerts of out-of-control variation occurred for bedding deep temperature of farm A. Two T1 alerts (temperature less than the lower limit) were signaled on farm B, coinciding with major bedding replacements, and 7 alerts (4 T1 and 2 T2) occurred on farm C during the study period (Figure 2).

Table 2. Least square means for bedding characteristics by season of the year, adjusted by bedding age.

Variable	Fall			Winter			Spring			Summer		
	N	Mean[1]	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Concentration of total bacteria[2] (log10 cfu/g)	16	8.68^{ab}	0.21	21	8.38^{a}	0.21	16	8.97^{b}	0.21	17	8.92^{b}	0.21
Concentration of coliforms[2] (log10 cfu/g)	16	6.33^{a}	0.22	21	6.37^{a}	0.31	16	6.54^{a}	0.32	17	6.77^{a}	0.33
Concentration of streptococci[2] (log10 cfu/g)	16	6.74^{a}	0.46	21	6.30^{a}	0.45	16	6.35^{a}	0.46	17	6.17^{a}	0.46
pH[3]	16	8.68^{a}	0.09	21	8.71^{a}	0.08	14	8.87^{ab}	0.09	19	9.18^{b}	0.09
Carbon-nitrogen ratio	16	27.55^{a}	1.68	21	25.39^{a}	1.42	14	22.90^{a}	1.72	19	26.98^{a}	1.63
Organic matter (%)	16	35.83^{a}	3.62	21	31.66^{a}	3.50	14	37.10^{ab}	3.63	19	40.75^{b}	3.60
Wet density (kg/m^3)	16	436.11^{ab}	55.03	21	471.03^{ab}	52.58	14	528.86^{a}	55.33	19	402.38^{b}	54.70
Dry density (kg/m^3)	16	267.73^{a}	33.52	21	281.78^{a}	31.46	14	338.30^{a}	33.78	19	253.30^{a}	33.24
Moisture (%)	16	38.83^{a}	1.80	21	40.44^{a}	1.56	14	34.89^{a}	1.83	19	37.46^{a}	1.76
Surface temperature (°C)	15	29.04^{a}	1.90	21	28.31^{a}	1.86	16	29.19^{a}	1.89	19	30.13^{a}	1.89
Deep temperature (°C)	15	47.63^{ab}	3.74	21	44.09^{a}	3.68	16	48.75^{b}	3.72	19	48.28^{ab}	3.73

Note. [1]Different upper case letters within the same row indicates a significant difference ($P < 0.05$) between means; [2]Bacterial concentrations on the bedding surface. Bedding samples were collected from 12 equally divided areas of the bedding and mixed to create a composite sample; [3]Bedding physical-chemical characteristics were estimated on composite samples created by mixing the superficial and deep bedding samples.

3.3 Bedding Moisture

Although mean bedding moisture only varied between 35.5% (farm C) and 40.4% (farm B) among farms, the within farm variation (as estimated by the CV) was 2.3 and 1.5 greater for farm C, as compared with farms A and B, respectively (Table 1 and Figure 2). Bedding moisture was not different among seasons of the year (Table 2). No alerts of out-of-control variation occurred for bedding moisture of farm A, and 1 T2 alert was observed at the beginning of the study for farm B. Farm C had more difficulty maintaining moisture within the control limits and experienced 9 alerts during the study period (5 T1 and 4 T2 alerts, Figure 2).

For all farms, bedding moisture was negatively associated with bedding deep temperature ($P < 0.01$). A one-unit increase in deep temperature was associated with a decrease of 0.5 units (percentage points) in bedding moisture (coefficient = -0.54, standard error = 0.16). For farms B and C, moisture decreased as bedding became older ($P < 0.01$ for the interaction between farm and bedding age, Figure 4).

3.4 Bedding Bacterial Concentrations

Except for farm B, concentrations of total bacteria, coliforms and streptococci were greater on the surface than in the deep layer of the bedding (Table 1 and Figure 1). Concentrations of total bacteria, coliforms and streptococci were less on the bedding surface of farm B than those observed on farms A e C, between which the concentrations were not different (Table 1). For all farms, concentration of total bacteria was less in winter than in spring or summer (Table 2), whereas no differences were found for the concentrations of streptococci and coliforms among seasons of the year (Table 2).

Of all explanatory variables, bedding organic matter and dry density were associated with concentration of total bacteria on the bedding surface in the final multivariate model (Table 3). Nonetheless, both associations were farm dependent (there was a significant interaction between each variable and farm). There was a positive association between bedding organic matter and the concentration of total bacteria of farm A. A one-unit increase in organic matter was associated with a 4.8 % increase in the concentration of total bacteria. This association was not evident on farms B and C (Table 3). A negative association between dry density and the concentration of total bacteria was identified for farms B and C (wood-based bedding), whereas a positive association was found on farm A (peanut shell-based bedding) (Table 3).

Organic matter and dry density were associated with bedding concentration of coliforms in the final model. A one-unit increase in organic matter was associated with a 6.2 and 6.8 % increase in the concentration of coliforms on farms A and B, respectively. In contrast, a negative association was found on farm C (Table 3). A negative association between dry density and the concentration of coliforms was identified for farms B and C, whereas a positive association was found on farm A (Table 3).

Bedding C/N and dry density were both associated with the concentration of streptococci in the final model. For all farms, C/N was positively associated with the concentration of streptococci. A one-unit increase in C/N was associated with a 3.5 % increase in the concentration of streptococci. A negative association between dry density and the concentration of streptococci was identified for all farms (Table 3).

Table 3. Multivariable mixed models used to identify bedding factors associated with bedding bacterial concentrations (log10 cfu/g)

Explanatory variables	Coefficient	SE	P-value
Total bacteria			
Intercept	8.458	1.325	
Organic matter (%)	-0.002	0.024	0.15
Organic matter*farm			< 0.01
Organic matter*farm A	0.050	0.026	
Organic matter*farm C	-0.003	0.026	
Organic matter*farm B	0	Reference	
Dry density (kg/m^3)	-0.001		0.11
Dry density*farm			< 0.01
Dry density*farm A	0.002	0.002	
Dry density*farm C	-0.004	0.002	
Dry density*farm B	0	Reference	
Coliforms			
Intercept	6.092	2.196	
Dry density (kg/m^3)	-0.002	0.003	0.05
Dry density*farm			0.01
Dry density*farm A	0.003	0.003	
Dry density*farm C	-0.005	0.003	
Dry density*farm B	0	Reference	
Organic matter (%)	0.068	0.042	0.07
Organic matter*farm			< 0.01
Organic matter*farm A	-0.006	0.046	
Organic matter*farm C	-0.101	0.046	
Organic matter*farm B	0	Reference	
Streptococci			
Intercept	6.229	0.625	
C/N ratio	0.035	0.014	< 0.01
Dry density (kg/m^3)	-0.005	0.001	0.06
Dry density*farm			< 0.01
Dry density*farm A	0.003	0.001	
Dry density*farm C	0.004	0.001	
Dry density*farm B	0	Reference	

3.5 Bedding Physical-Chemical Characteristics

Mean bedding organic matter, C and N were greater for farm A (peanut shell-based bedding), as compared with farms B and C (wood-based bedding, Table 1). Bedding organic matter was greater in summer than in fall or winter (Table 2). Bedding C/N and pH were not different among farms. For all farms, mean pH was greater in summer than in fall or winter and C/N was not different among seasons (Table 2). Bedding wet density was less in summer than in spring and dry density was not different among seasons of the year. Both wet and dry densities were greater for farm B (sawdust bedding), as compared with farms A (peanut shell) or C (wood shavings, Table 1). For all farms, several alerts of out-of-control variation for bedding organic matter, C/N, pH, were detected (Figures 2 and 3). Opposite longitudinal trends were observed for pH (increasing) and C/N (decreasing) during the study period (Figure 3).

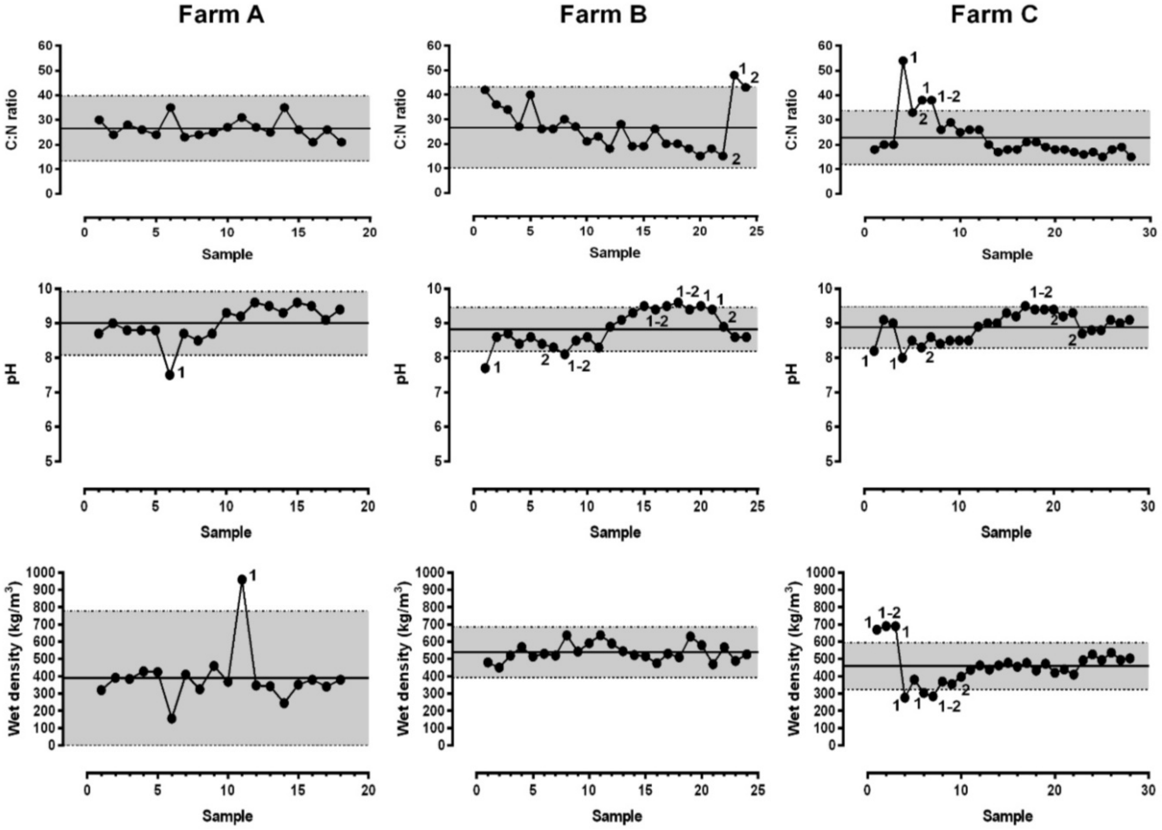

Figure 3. Shewhart control charts for individual measurements of bedding manageable characteristics. Bedding samples were collected biweekly. The central line indicates the mean of the observations. The upper and lower horizontal lines indicate the upper and lower control limits ($\bar{X} \pm 3\sigma$). Test 1 = 1 point beyond the mean ± 3σ (control limits); Test 2 = 2 of 3 consecutive points beyond the mean ± 2σ. Temperature was measured at the deep layer (20 cm) of the bedding

Bedding organic matter, dry density and C/N were associated with bedding age, but these associations depended on the farm studied ($P < 0.01$ for the interaction between farm and organic matter, density, or C/N). For farms, B and C, C/N decreased with bedding age and there was a decrease in organic matter over time (which was significant only on farm C, Figure 4). For farm C, dry density of old bedding was greater than that of new bedding (Figure 4).

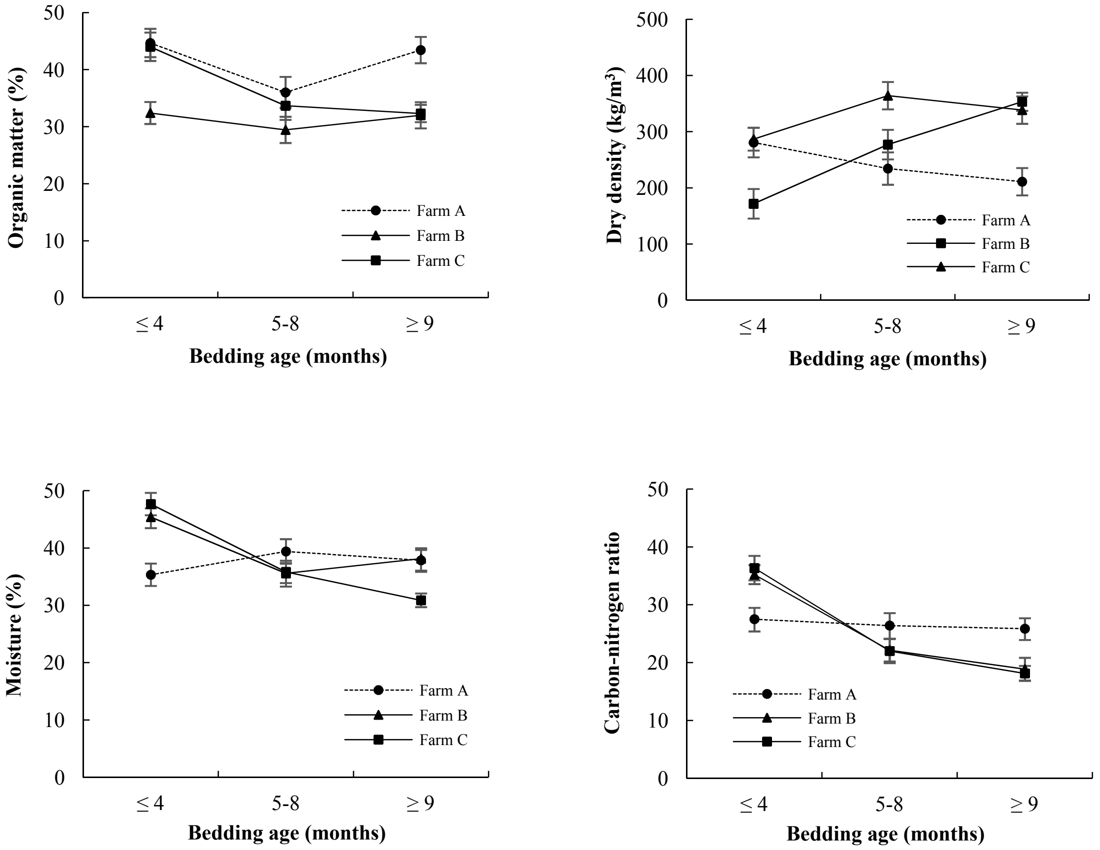

Figure 4. Mean bedding organic matter, dry density, moisture, and carbon-nitrogen ratio, by bedding age. Significant differences ($P < 0.05$) between means were found for the following contrasts: Organic matter and dry density: ≤ 4 × ≥ 9 on farm C. Moisture: ≤ 4 × 5-8 on farms B and C, and ≤ 4 x ≥ 9 on farm C. Carbon-nitrogen ratio: ≤ 4 × 5-8 on farms B and C, and ≤ 4 × ≥ 9 months on farm C

4. Discussion

Little research has been conducted towards validating management practices that could be used to minimize CBP bacterial concentrations. Most studies were cross-sectional, in which bedding characteristics and bacterial concentrations were determined at a fixed point in time from a population of farms. While cross-sectional studies are useful for capturing variation among farms, longitudinal designs also allow capturing of within farm variation, which can be used to identify seasonal trends and bedding characteristics that are easier or more difficult to control. In addition, longitudinal studies are more valid in determining causal associations because a temporal relationship between explanatory and response variables can be determined. Because the CBP system was recent on all farms at the beginning of the study, we could study bedding characteristics at various stages of bedding maturity.

Monitoring of key indicators such as organic matter, moisture, deep temperature, C/N, density, and pH is important to assess the quality of the composting process. Results of the present study indicate that variation in the bedding characteristics studied greatly depended on the bedding management of each farm. A greater number of out-of-control alerts were observed on farm C (as compared with farms A and B) for all indicators studied. Most alerts occurred at the beginning of the study when bedding was neglected and had to be entirely replaced. Once the managers learned more about CBP management, the bedding of farm C was consistently maintained within the farm goals (little moisture, loose, deep layer temperature > 40 °C, and little adhesion to cows) until the end of the study. Except for such extreme situations, bedding characteristics that can be measured and managed on the farms (moisture, temperature, and density) varied little during the course of the study. For all farms, the several alerts of out-of-control variation observed for bedding organic matter and C/N ratio could be explained by the periodic addition of new bedding to the CBP.

4.1 Factors Associated with Bedding Bacterial Concentrations

One hypothesis frequently raised by CBP users is that the increase in bedding temperature resulting from the composting process would substantially decrease bedding bacterial concentrations. However, mean concentration of total bacteria on the bedding surface was mostly > 8.0 log10 cfu/g for all farms during the course of the study. Likewise, Barberg et al. (2007) and Black et al. (2014) reported that mean concentration of total bacteria on the CBP bedding surface was 7.0 log10 cfu/cc and 8.2 log10 cfu/g, respectively.

Results of this study indicate that cows housed in the CBP are exposed to a contaminated surface, which might increase the pressure of intramammary infection if there is a transfer of pathogens to the teat skin (Rendos, Eberhart, & Kesler, 1975; Hogan et al., 1989). Bedding characteristics such as moisture and particle size, which could influence bedding adhesion to cows, need to be studied to assess the risk of mastitis in the CBP.

Bedding organic matter was positively associated with bedding concentration of total bacteria (Farm A) and coliforms (Farms A and B), which indicates that an increase in available nutrients favors the growth of bacterial populations in the CBP. Main sources of organic matter to the CBP are new bedding and animal waste, which are frequently added to the CBP. Two farmers reported during the study that addition of large amounts of new bedding resulted in a temporary increase in clinical mastitis incidence. These reports should be further investigated because we observed that new wood-based bedding was not only a source of organic matter, but also was more humid than old bedding. Results of previous studies demonstrate that concentration of coliforms (especially *Klebsiella* spp) is greater in sawdust bedding than other bedding materials (Hogan et al., 1989; Hogan & Smith, 1997).

Bedding dry and wet densities are estimated by measuring the amount of bedding that can be placed into a fixed-size container and is mostly influenced by particle size and physical properties of the material. For farms that used wood-based bedding, density was negatively associated with bacterial concentrations. Denser bedding can result in less oxygen penetration and impairment of aerobic conditions. In addition, as bedding becomes mature due to composting, microbiological decomposition results in decreased particle size and bioavailability of nutrients. Thus, older bedding becomes denser and less bioavailable, which may decrease microbial growth. Other characteristics of mature bedding such as increased water retention might result in difficulties to manage the bedding. Studies are necessaries to determine whether management of new and mature bedding should be different and identify the ideal time for total bedding replacement. Composting characteristics of peanut shell seems to differ from wood-based materials and need to be further studied. For instance, bedding density of farm A was lesser than those measured on farms B and C, which might explain the greater composting temperatures observed on farm A. Less dense and more degradable materials allow rapid composting and may favor microbial growth due to greater nutrient availability and bedding aeration.

For all farms, a positive correlation was found between C/N and the concentration of streptococci. Carbon is a limiting factor for bacterial growth and its ratio relative to nitrogen has been used to monitor composting efficiency (Kiehl, 1985). Because nitrogen is constantly added to the CBP by animal waste, and the main source of carbon is new bedding, carbon availability to microbes becomes more limited and C/N decreases as bedding becomes older (Figure 3). During the course of the study, C/N decreased steadily on farms B and C, which used wood-based bedding.

Bedding pH was not associated with bedding bacterial concentrations but an increasing trend was observed for all farms as bedding became older (Figure 3). The pH increases throughout the composting process because acids are neutralized by bases released from organic matter (Kiehl, 1985). Because pH levels greater than 9 were observed on all farms during several consecutive months, possible detrimental effects on teat skin might deserve further investigation.

4.2 Bedding Temperature

In agreement with previous studies conducted in the United States (Barberg et al., 2007; Shane et al., 2010; Black et al., 2014), mean deep layer temperature was greater than that measured on the surface, indicating microbiological activity. Barberg et al. (2007) and Black et al. (2014) reported mean deep temperatures of 42.5 and 36.1 ºC for populations of 12 and 47 CBP in Minnessota and Kentucky, respectively. Shane et al. (2010) studied a group of 6 CBP and found deep layer temperatures ranging from 31.8 to 48.1 ºC in summer, and 13.8 to 40.6 ºC in winter. Nonetheless, deep temperatures reported in the present and previous studies did not reach values (> 55 ºC) capable of substantially reducing bedding bacterial populations.

Although this study was not designed to make comparisons among farms, they can be useful to generate new hypotheses. It was interesting to note that the composting process seemed to depend on the type of organic material. The greater deep temperature observed on farm A (as compared to farms B and C) could be explained by the use of peanut shell, which is less dense than wood and more rapidly degraded by microorganisms. Despite the greater bedding temperature maintained on farm A, bacterial concentrations on the surface were not different among farms. Due to regional availability of materials such as wood, further research is necessary to study the use of alternative bedding types for the CBP.

Bedding surface temperature is a point of concern because it could be detrimental to cow comfort and health. The greater surface temperature observed on farm B (5.8 and 4.7 °C greater than those observed on farms A and C, respectively) can be explained by the lack of fans and suggests that, for the environmental conditions observed during the study, proper ventilation in the CBP area is essential for the welfare of the cows. Both deep and surface temperature had little variation during the study. Deep temperature was greater in spring than in winter, suggesting that weather conditions can influence bedding temperature. One limitation of this study is that associations between weather conditions (air temperature and humidity) and bedding factors could not be studied because these data were not collected.

Bedding deep temperature of farm C decreased to < 25 °C at the beginning of the study, when bedding was neglected. Cooling of the bedding was associated with an increase in bedding density, moisture, and bacterial concentrations, and a decrease in C/N. After bedding was replaced and management was corrected, deep temperature increased rapidly and remained stable until the end of the study. These observations agree with previous reports, suggesting that monitoring temperature is an inexpensive means to assess bedding quality and the efficiency of the composting process (Barberg et al., 2007).

4.3 Bedding Moisture

Moisture is considered one of the critical points for the management of the CBP (Barberg et al., 2007; Lobeck et al., 2012). Although moisture is necessary for the composting process, its excess can result in negative consequences such as bedding compacting, decrease in temperature and aeration, development of an anaerobic environment, and possible particle adherence to cows (NRAS-54, 1992; Barberg et al., 2007).

Except for farm C at the beginning of the study, moisture was maintained within the control limits for most of the study period. Moisture was negatively associated with bedding deep temperature, suggesting that excessive levels can result in a series of events (compaction, less penetration of air, and impairment of aerobic conditions) which resulted in decreased microbial activity in the deep layer and reduction of bedding temperature. The decrease in moisture observed during the process of bedding aging agrees with previous knowledge of traditional composting (Kiehl, 1985), and can be explained by the high moisture levels found in new bedding (Figure 4), and evaporation due to factors such as composting temperatures, bedding aeration, and ventilation.

Results of this study indicate that low moisture levels can be maintained without impairing bedding deep temperature. The low moisture levels (< 35 %) maintained on farm C's bedding (Figure 2) were associated with deep temperatures > 40 ºC. In these conditions, there was little adherence to cows, which remained in excellent hygienic conditions (data shown in the companion paper). Therefore, moisture levels recommended for traditional composting (40-65%; NRAES-54, 1992) may not be ideal for the CBP. The moisture content (> 55 %) observed at the initial phase of the study on farm C was associated with bedding compaction and bacterial counts > 9.5 log10 cfu/g of bedding (Figures 1 and 2). Further studies are necessary to determine minimum moisture levels that could be targeted without compromising the composting process.

5. Conclusions

Results indicated that bedding organic matter, density and C/N were associated with bedding specific bacterial concentrations. Although the temperature observed in the deep layer of the bedding was greater than that measured on the surface, bedding temperatures were not great enough (> 55 ºC) to substantially decrease bedding concentrations of total bacteria, streptococci and coliforms.

In agreement with previous knowledge of traditional composting, bedding aging was characterized by changes in several physical-chemical characteristics. For farms that used wood as bedding, moisture, C/N, and organic matter decreased, whereas density and pH increased over time.

Extreme variation of bedding characteristics occurred during the initial phase of the study. Once farmers learned the principles of composting, bedding manageable characteristics such as temperature, organic matter, moisture, density, and C/N were maintained within control limits during the study period. Results of this research can be used by farmers and consultants to manage the CBP towards reducing cows' exposure to mastitis pathogens.

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