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Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health

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ABSTRACT

The objective of this study was to describe relationships among compost bedded pack barn **(CBP)** measurements (moisture, internal temperature, nutrient content, and bedding bacterial counts), ambient weather conditions, and udder health. Data was collected every 2-weeks (n=25 visits) from 8 Kentucky dairy farms with CBP from May 2013 to May 2014. A single observer scored 50 cows per farm for hygiene and collected compost internal temperature, moisture, and compost samples from 9 evenly distributed areas in each barn. Weighted average somatic cell count (**SCC**), high SCC prevalence (**HSP**), and reported clinical mastitis incidence (**RCMI**) were collected from herd records and milking personnel.

Compost internal temperature increased with increasing maximum barn temperature (**BT**). Compost moisture content decreased with increasing BT. Herd hygiene score decreased with increasing BT and increased with increasing compost moisture content. Herd SCC and HSP both increased with increasing BT but were unaffected by compost measurements. As compost internal temperature increased, staphylococci, streptococci, and bacilli species growth in the pack area decreased and coliform species growth increased. Low CBP moisture and high CBP temperature reduced bacteria levels. Cow hygiene and udder health indicators had a stronger relationship with BT than with CBP internal temperature and moisture.

1. Introduction

Attracting global interest, the compost bedded pack barn (**CBP**) is a loose housing system without the stalls and partitions found in freestall and tie-stall housing. Without stalls, the cows' resting and exercise areas are combined potentially reducing ammonia emissions and building costs, and improving cow locomotion (Janni et al., 2006; Barberg et al., 2007a; Shane et al., 2010; Galama, 2011). The large, open resting areas (6.8 ± 1.1–9.0 ± 2.2 m^2 per cow) are generally separated from a concrete feed alley by a 1.2-m retaining wall (Janni et al., 2006; Barberg et al., 2007a; Black et al., 2014). The composting process allows feces and urine to be stored as a solid semi-compost as the bedding base for 6–24 months (Janni et al., 2006; Black et al., 2014).

Compost bedded pack barns require periodic bedding addition and a recommended twice daily tilling with a roto-tiller or deep-tillage tool (Janni et al., 2006; Barberg et al., 2007a; Black et al., 2013). Tilling incorporates manure and air into the pack while exposing greater pack surface area for drying (Janni et al., 2006; Shane et al., 2010). This process promotes microbiological activity, heating and drying the pack and providing a fresh, dry surface for cattle to lie on (Shane et al., 2010).

For effective composting, adequate temperature and moisture content must be maintained. The recommended internal temperature for CBP at depths of 15–31 cm ranges from 43.3 to 65.0 °C and optimum moisture content from 40 to 60% (NRAES-54, 1992, Janni et al., 2006; Bewley et al., 2013). Maintaining a temperature of 54–65 °C for 3–4 days may even inactivate some pathogens and viruses, destroy weed seeds and fly larvae, and decrease odor emanating from the pack.

Inactivating mastitis pathogens was of particular interest to producers and researchers (Janni et al., 2006). In stall-based facilities, increases in bedding bacterial count have been linked to increased SCC and clinical mastitis incidence (Hogan et al., 1989b; Hogan and Smith, 1997; Zdanowicz et al., 2004). A linear relationship has been reported between total rates of clinical mastitis and Gram-negative bacteria and *Klebsiella* spp. counts in bedding (Hogan et al., 1989a). Similarly, Zdanowicz et al. (2004) reported positive correlations between bedding bacterial counts and bacterial counts present on teat ends. Black et al. (2014); Barberg et al. (2007a) reported a total CBP bedding bacteria count in of 5.2 ± 5.1 to 8.2 ± 0.4 $\log_{10}$ cfu/g of dry matter. Lobeck et al.

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(2012); Petzen et al. (2009) reported no differences between CBP bedding bacteria counts and other housing systems, indicating that CBP maintained similar pathogen exposure levels to other housing environments. Similarly, clinical mastitis, SCC, high SCC prevalence (**HSP**), and bulk tank SCC (**BTSCC**) of lactating cows housed in CBP facilities was comparable to that of lactating cows housed in other housing conditions (Lobeck et al., 2011; Black et al., 2014; Eckelkamp et al., 2016).

To the authors' knowledge, no continuous long-term study of CBP effect on observed clinical mastitis cases (**RCMI**), weighted average SCC, HSP, or BTSCC has been published. The objective of this study was to describe the relationships among compost bedded pack moisture and temperature, bedding bacterial counts, ambient weather conditions, and udder health throughout the year.

2. Materials and methods

The study was conducted using data from 8 Kentucky dairy farms with CBP housing 90 ± 14 to 455 ± 23 cows from May 2013 to May 2014. Each farm was visited once every 2-weeks over a 3-day period for a total of 26 visits per farm across the study period. The same group of farms were visited on the same day throughout the study. All farms were assigned an identification number 1–8) that will be used from this point forward. Included in the study were CBP moisture and temperature effects on cow hygiene, mastitis indicators, compost nutrient profile, and bedding bacteria counts. Ambient temperature, humidity, and rain level effects on moisture content, compost internal temperature (**CIT**) at 20 cm depth, and surface temperature were also examined. All barns were used as the lactating cows' housing facility. All herds on this study were enrolled in **DHIA** (Dairy Herd Information Association; Raleigh, NC). To be enrolled in the study, herds had to maintain a yearly mean SCC $<300{,}000$ for the year before enrollment. Over the study, the same observer scored 48 ± 5 cows at every farm visit. If fewer than 50 cows were housed in the CBP, all cows were scored. If herd size was 100–150, cows with even identification numbers were scored. If herd size was 150 to ≥ 200, cows with identification numbers ending in 3, 6, or 9 were scored. All cows in this study were cooled by fans in the barn and fed a farm-specific TMR. Compost bedded pack barns were tilled 2 ± 1 times per day and bedding was added when compost moisture content reached 55–60%.

2.1. Data collection

Performance records from DHIA were collected with the permission of participating producers including: test day milk production (kg/cow), weighted average herd SCC (cells/mL), HSP (percentage of herd), and number of lactating animals in the herd. Bulk tank SCC (cells/mL) from every milk pick-up was obtained from fluid-milk buyers with producer permission. Producers were provided a Tycon ProWeatherStation (model # TP1080WC; Tycon Systems, Buffdale, Utah) to record barn temperature (**BT**) and humidity (**BH**) and ambient temperature and humidity. Data collected from the weather stations were downloaded biweekly. Ambient temperature, humidity, and precipitation data were retroactively collected from University of Kentucky's Weather Center stations nearest the farms to account for any data gaps from the on-farm weather stations. Stocking density was determined by dividing the available resting area in the barn by the number of cows in the herd (m^2 per cow).

2.2. Cow hygiene and barn analysis

The same observer scored cow hygiene and collected CBP information at all visits on all farms. Hygiene evaluation of the udder, lower leg, and upper leg and flank was conducted using the 4-point system of Cook and Reinemann (2007) where 1=clean, 2=moderate dirt, 3=plaques of dirt with hair visible, and 4=confluent plaques of dirt with no hair visible.

Each CBP was divided into 9 evenly distributed sections (adapted from Black et al., 2013). At the center of each section, surface temperature was measured using an infrared thermometer (accuracy of ± 1 °C; Fluke, model 62, Everett, WA, USA). Internal temperature at 20 cm within the pack was collected using a thermocouple attachment to the infrared thermometer (0.22 m length, accuracy of ± 2.2 °C; Fluke Inc., model 87, Everett, WA, USA). This depth corresponds to the edge of the tilled layer of compost, where the greatest temperature occurs (personal communication with Taraba, 2013). The mean of all sections was used as the compost surface or internal temperature. Samples of compost material were collected at the same site using a 59 cm^3 measuring cup for a 118 cm^3 composite sample in a 4 L plastic bag. Each bag was thoroughly mixed and stored on ice until transfer to a 2 °C refrigerator at the University of Kentucky. University of Kentucky Regulatory Services laboratory personnel evaluated moisture, P, K, Ca, Mg, Zn, Cu, Mn, and Fe concentrations by procedures defined by Peters et al. (2003). Nitrate and ammonium concentrations were analyzed by University of Kentucky Soil and Plant Analysis laboratory by procedures defined by Crutchfield and Grove (2011).

2.3. Bacterial analysis

Four of the 8 producers agreed to participate in an additional analysis of bedding bacterial counts. Samples were collected from the surface of each of the 9 sampling locations using a 59 cm^3 measuring cup for a 118 cm^3 composite sample in a 4 L plastic bag. Samples were stored on ice until transfer to a -40 °C freezer and stored (42 ± 14 d) until bacterial analysis could be performed at the University of Kentucky Animal and Food Sciences microbiology laboratory. Bedding was thawed at room temperature then diluted (1:10) using 25 g of bedding and 225 mL of 0.1% peptone solution. The dilution was hand mixed until bedding was thoroughly suspended in peptone solution. Countable plates were acquired through further serial dilution (10^2–10^5). Total coliforms were determined by addition of 1 mL of the appropriate dilution to 3 M Petrifilm Coliform Count Plates in duplicate (3 M Microbiology Products, St. Paul, MN), and incubation at 37 °C for 24 h. *Klebsiella* species were determined by addition of 1 mL of the appropriate dilution to each half of a MacConkey-inositol-carbenicillin (MCIC) agar (Becton, Dickinson and Company, Franklin Lakes, NJ) prepared according to manufacturer directions using an agar spreader. Colony forming units (cfu) were counted manually and averaged for each duplicate, obtaining a coliform and *Klebsiella* species count.

Table 1.
Mean, standard deviation, minimum, and maximum values for compost bedded pack moisture and nutrient content of compost bedded pack barn material collected from 8 Kentucky compost bedded pack barns from May 2013 to May 2014 (n=26 visits per barn over the year).

Variable	N	Mean	Standard deviation	Minimum	Maximum
Moisture (%)	207	60	6	44	79
Carbon (%)	207	44	2	34	54
Nitrogen (%)	207	2.3	0.6	0.9	3.8
Phosphorous	207	0.5	0.1	0.2	0.8
Potassium (%)	207	1.7	0.5	0.7	3
Calcium (%)	207	1.7	0.5	0.7	3
Magnesium (%)	207	0.6	0.2	0.3	1
Zinc (ppm)	207	156	52	49	310
Copper (ppm)	207	38	21	8	245
Manganese (ppm)	207	224	154	103	2256
Iron (ppm)	207	1289	866	360	7445
Nitrate (ppm)	207	38	24	11	251
Ammonium (ppm)	207	37	79	1	702

Researchers determined streptococci, staphylococci, and bacilli counts using TKT agar, Difco Staph 110, and Difco MYP Agar Mannitol-Egg Yolk Polymyxin B (Becton, Dickinson and Company, Franklin Lakes, NJ), respectively, prepared in the lab according to manufacturer instructions. The appropriate dilution (10^2–10^3) was spiral plated onto two plates of each media type (Eddy Jet, IUL Instruments, I.L.S., Leerdam, The Netherlands). Plates were incubated for 48 h at 35 °C. Colony forming units were counted automatically using a colony counter (Flash & Go, IUL Instruments, I.K.S., Leerdam, The Netherlands).

2.4. Clinical mastitis identification

All participating producers recorded clinical cases of mastitis based on the guidelines of Hogan et al. (1989a). A clinical case of mastitis was recorded if abnormal milk (flakes, clots, or watery appearance) without swelling of the affected quarter, normal or abnormal milk and swelling of the affected quarter, or abnormal milk with systemic signs (fever, reduced rumen function, dehydration, weakness, depression, loss of appetite, or rapid pulse) were present. Each dairy producer was trained on recording mastitis cases and pertinent information at the initial visit.

2.5. Statistical analysis

2.5.1. Inclusion criteria

To limit bias from lack of data, inclusion criteria were decided before analyses. All analyses including DHIA data required a minimum of 6 DHIA tests on file for the study period. All RCMI analyses required 13 weeks of data for the study period. The CORR procedure of SAS (Version 9.3, SAS Inst. Inc., Cary, NC) determined inclusion criteria for all MIXED models (r $0.1<0.9$, $P<0.05$; Eckelkamp, 2014). Variables with an r-value >0.9 were eliminated to avoid confounding effects in analyses.

2.5.2. Herd and pack information

The MEANS procedure of SAS was used to determine mean (± SD) number of cows per herd, DHIA test day milk yield, stocking density, hygiene score, BTSCC, SCC, HSP, RCMI, moisture content, CIT, and compost nutrient values for the study period as described in Eckelkamp et al. (2016). The MIXED procedure of SAS was used to develop all models for analyses among farms. Stepwise backward elimination was used to remove non-significant interactions ($P\geq 0.05$). All main effects remained in the model regardless of significance. Explanatory variables for pack moisture and CIT were test day milk yield, BT, BH, stocking density, mean weekly rain level (cm), and all 2-way interactions. The C:N ratio for all herds across the study period was adjusted using the UNIVARIATE procedure of SAS for 5th and 95th percentile to remove extreme outliers and normalize data. Variables were repeated by visit with herd as subject.

Explanatory variables for hygiene score included pack moisture, CIT, BT, BH, and all 2-way interactions. Bulk tank SCC, HSP, and SCC explanatory variables were pack moisture content, CIT, BT, and BH. High SCC prevalence was calculated as a herd percentage for each visit (Barberg et al., 2007b; Lobeck et al., 2011). Hygiene score was not included in these models to avoid confounding effects with pack moisture content. Variables were repeated by visit with herd as subject.

Reported clinical mastitis incidence was calculated as a herd percentage for each week as follows: RCMI=(number of isolates over 2-wk period/number of animals in herd)/2. Explanatory variables for RCMI were pack moisture, CIT, BT, and BH. Variables were repeated by visit with herd as subject.

2.5.3. Bacterial analysis

Staphylococci, streptococci, *Klebsiella* species, bacilli, and total coliform counts ($\log_{10}$ cfu/g dry matter) analyses included explanatory variables of pack moisture, CIT, C:N ratio, stocking density, and all 2-way interactions. Staphylococci bedding bacterial counts were adjusted for 5th and 95th percentiles to remove outliers. Variables were repeated by visit with herd as subject.

2.5.4. Changes over the year

The GLM procedure of SAS was used to determine differences among CBP across time. Hygiene score, compost moisture content, CIT, C:N ratio, SCC, and BTSCC were included in the analysis, with the LSMeans (± SE) for each barn compared by each visit.

3. Results and discussion

3.1. Compost results

3.1.1. Temperature

The CBP moisture and nutrient data are presented in Table 1. Across the year, 75% of the mean ± SD CIT for all barns were within the recommended range (43.2 ± 8.9–47.2 ± 6.1 °C; recommended 43.3–65.0 °C; Bewley et al., 2013; Janni et al., 2006). Fluctuations throughout the year followed the changes in ambient temperature (Fig. 1). Barn ambient temperature was a significant predictor of CIT

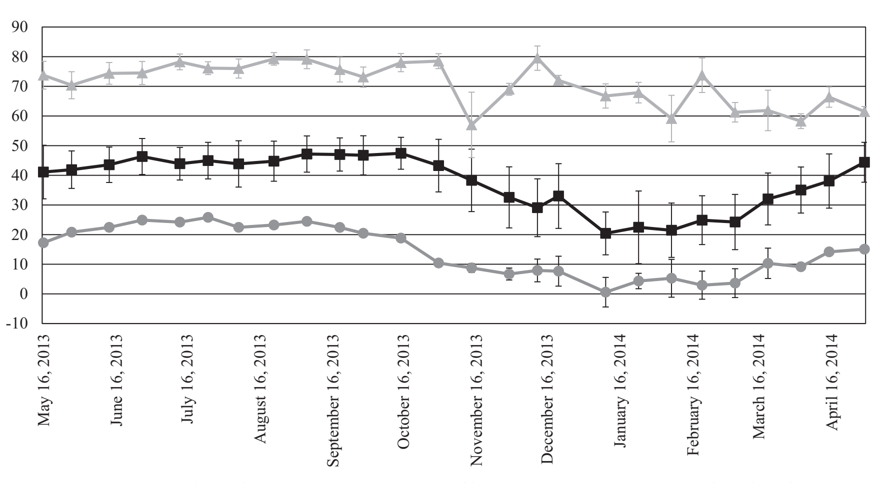

Fig. 1. Mean (± SD) compost bedded pack barn 20 cm internal temperature (°C), ambient temperature (°C), and relative humidity (%) for 8 Kentucky compost bedded pack barns at each visit (n=26 visits per barn) from May 2013 to May 2014.

Table 2.
Estimated coefficients for models and interactions for compost internal temperature and moisture, herd hygiene, bulk tank somatic cell count, somatic cell count, high somatic cell count prevalence, and bedding bacterial counts for 8 Kentucky compost bedded pack barns from May 2013 to May 2014 (n=26 visits per barn over the year).

Parameter	Estimate	SE	T	Significance
CIT[1]				
Intercept	21	14	1.53	0.17
BT[k]	0.61	0.11	5.38	< 0.01
BH[l]	0.07	0.06	1.14	0.26
Milk yield[m]	−0.07	0.32	−0.21	0.83
Stocking density[n]	6.09	0.06	0.69	0.49
Rain level[o]	−0.09	0.10	−0.92	0.36
Compost moisture[b]				
Intercept	0.82	0.08	9.92	< 0.01
BT[k]	−0.003	0.001	−4.02	< 0.01
BH[l]	0.001	0.0004	−1.17	0.24
Milk yield[m]	0.002	0.002	1.08	0.28
Stocking density[n]	0.06	0.05	1.20	0.23
Rain level[o]	0.001	0.001	1.26	0.21
Herd hygiene score[c]				
Intercept	1.91	0.22	8.82	< 0.01
BT[k]	−0.007	0.003	−2.54	0.01
BH[a]	−0.001	0.001	−0.70	0.48
CIT[a]	−0.002	0.002	−1.04	0.30
Compost moisture[b]	0.88	0.26	3.40	< 0.01
Herd BTSCC[d]				
Intercept	239,227	66,343	3.60	0.01
BT[k]	−3,334	1,986	−1.68	0.09
BH[a]	−97.45	365	−0.27	0.79
CIT[a]	−965.89	798.39	−1.21	0.23
Compost moisture[b]	3,4280.28	80,395	0.04	0.97
CIT[a]*BT[k]	129.98	53.05	2.45	0.01
Herd SCC[e]				
Intercept	315,826	96,982	3.26	0.02
BT[k]	2,691	1,166	2.31	0.02
BH[a]	−326	474	−0.69	0.49
CIT[a]	58	831	0.07	0.94
Compost moisture[b]	−141,410	118,947	−1.19	0.24
Herd HSP[f]				
Intercept	0.36	0.12	2.91	0.03
BT[k]	0.02	0.01	2.75	< 0.01
BH[a]	< 0.01	< 0.01	0.07	0.94
CIT[a]	−0.0002	0.001	−0.22	0.82
Compost moisture[b]	0.14	0.17	0.86	0.39
Compost moisture[b]*BT[k]	−0.02	0.01	−2.39	0.02
Compost coliform counts[g]				
Intercept	11.63	3.26	3.56	0.04
CIT[a]	−0.15	0.07	−2.25	0.03
Compost moisture[b]	−8.50	5.11	−1.67	0.10
C:N[p]	0.01	0.02	0.31	0.76
Stocking density[n]	−0.36	0.80	−0.45	0.65
Compost moisture[b]*CIT[a]	0.26	0.11	2.41	0.02
Compost streptococci counts[h]				
Intercept	6.62	1.42	4.66	0.02
CIT[a]	−0.02	0.01	−2.64	0.01
Compost moisture[b]	2.90	1.87	1.55	0.12
C:N[p]	0.01	0.02	0.56	0.58
Stocking density[n]	−0.32	0.78	−0.42	0.68
Compost staphylococci counts[i]				
Intercept	8.08	1.29	6.29	0.01
CIT[a]	−0.02	0.01	−2.56	0.01
Compost moisture[b]	−0.81	1.48	−0.55	0.59
C:N[p]	0.01	0.02	0.30	0.77
Stocking density[n]	−0.58	0.78	−0.75	0.46
Compost bacilli counts[j]				
Intercept	7.67	1.33	5.77	0.01
CIT[a]	−0.02	0.01	−2.17	0.03
Compost moisture[b]	0.13	1.41	0.09	0.93
C:N[p]	0.03	0.01	2.32	0.02
Stocking density[n]	−0.20	0.80	−0.25	0.81

[a] CIT = mean compost bedded pack barn internal 20 cm temperature (°C) over the 2 week visit period.
[b] Mean compost bedded pack barn moisture content (%) over the 2 week visit period.
[c] Mean herd hygiene score collected using the 4-point system of Cook and Reinemann (2007) where 1=clean, 2=moderate dirt, 3=plaques of dirt with hair visible, and 4=confluent plaques of dirt with no hair visible. Fifty cows in each herd were scored at each visit.
[d] Mean herd bulk tank somatic cell count (cells/mL) over the 2 week visit period.
[e] Mean herd somatic cell count (cells/mL) over the 2 week visit period.
[f] Mean herd high somatic cell count prevalence (% of herd with SCC > 200,000 cells/mL) over the 2 week visit period.
[g] Mean compost bedded pack coliform counts ($\log_{10}$ cfu/g) over the 2 week visit period.
[h] Mean compost bedded pack streptococci counts ($\log_{10}$ cfu/g) over the 2 week visit period.
[i] Mean compost bedded pack staphylococci counts ($\log_{10}$ cfu/g) over the 2 week visit period.
[j] Mean compost bedded pack bacilli counts ($\log_{10}$ cfu/g) over the 2 week visit period.
[k] BT = mean barn temperature (°C) over the 2 week visit period.
[l] BH = mean barn humidity (%) over the 2 week visit period.
[m] Mean milk yield (kg/cow/d) over the 2 week visit period.
[n] Mean stocking density (m^2/number of cows in the herd) over the 2 week visit period.
[o] Mean precipitation amount (cm) over the 2 week visit period.
[p] C:N = mean compost bedded pack carbon to nitrogen ratio over the 2 week visit period.

($P<0.01$; Table 2). Mean milk yield (kg/cow/day), stocking density (%), BH (%), and rain level (cm), had no significant effects on CIT ($P=0.83$, 0.49, 0.26, and 0.36 respectively). Compost bedded pack barns require a narrow range of moisture and temperature for optional functionality (Bewley et al., 2013). With increasing BT, an increase in CIT occurred (Fig. 2(a)). This was probably because greater BT allowed the pack to maintain a greater heat level. Minnesota researchers reported seasonality in CIT, with warmer months (summer) having greater CIT and cooler months (winter) have lower CIT (Barberg et al., 2007a; Shane et al., 2010).

3.1.2. Moisture

Mean moisture content over the year for all CBP was 59.9 ± 6.6%, within the recommended range of 40–65% (NRAES-54, 1992; Table 3). Moisture content never fell below 44%, and remained within the recommended range 77% of all observations for all barns. Moisture content in the current study was unaffected by milk yield ($P=0.28$), stocking density ($P=0.23$), BH ($P=0.24$), or rain level ($P=0.20$). As BT increased, pack moisture content decreased ($P<0.01$; Fig. 2(b); Table 2). Comparing the BT graphs, CIT increases while moisture decreases with increasing BT (Fig. 2a and b). The increased CIT and increased BT will drive drying of the pack through evaporative water loss. As mentioned previously, the increase in moisture deposited on the pack is also evaporated at a greater rate when CIT and BT are high. For these reasons, the moisture content of the pack should be expected to decrease over the summer, increasing the length of time a single load of bedding will last (Janni et al., 2006; Eckelkamp et al., 2014). These results corroborate previous research that colder ambient temperatures were inhospitable to CBP establishment (Bewley et al., 2013). Dairy producers with CBP also observed poorer pack perfor-

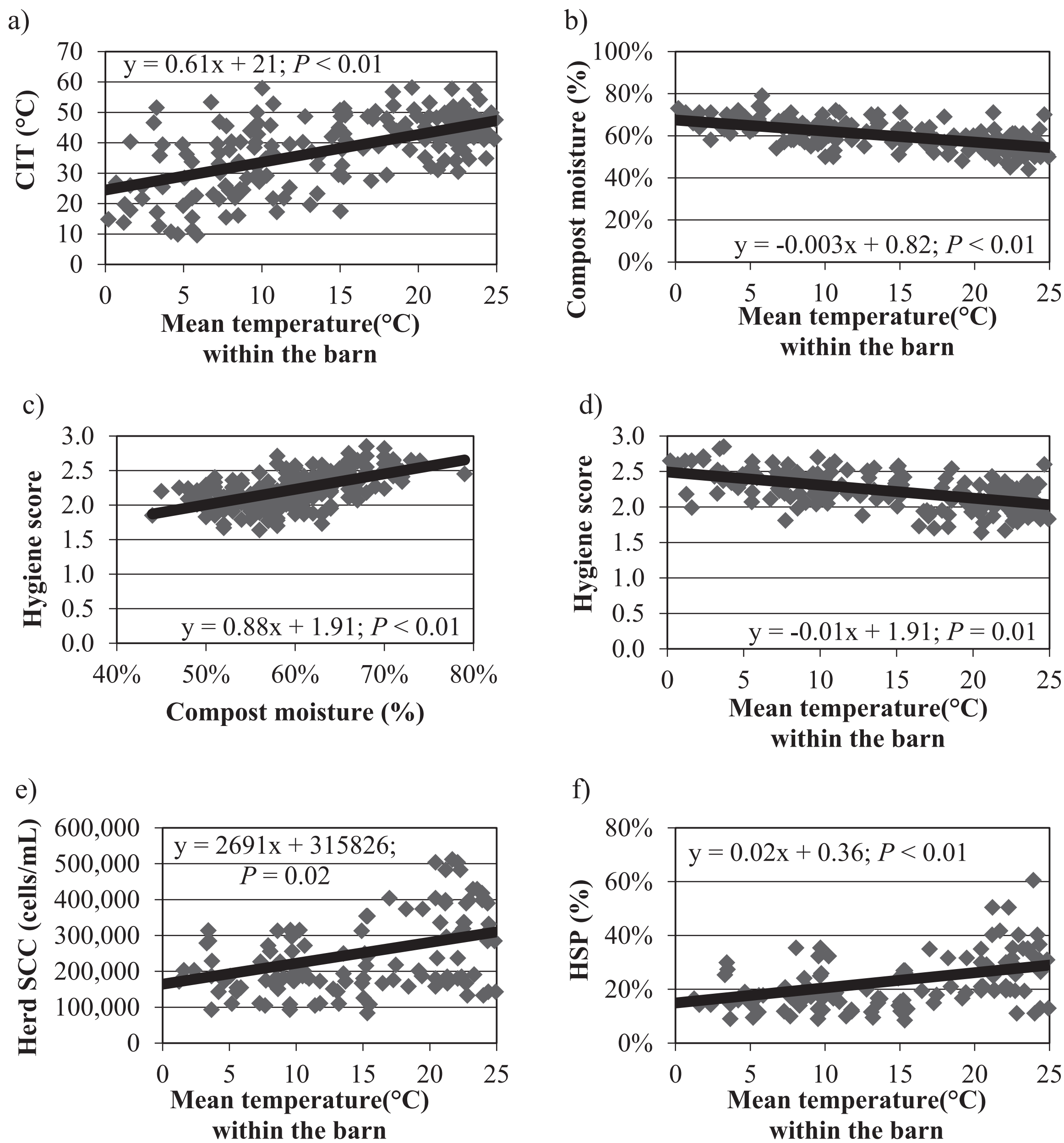

Fig. 2. Statistically significant relationships (a–f) among compost bedding features, environmental factors, and udder health issues for 8 compost bedded pack barns from May 2013 to May 2014 (n=26 visits per barn over the year). (a) Maximum barn temperature (BT; °C) for the 2-week visit period as a predictor of compost bedded pack 20 cm internal temperature (CIT; °C); (b) BT as a predictor of compost bedded pack barn moisture content (%); (c) compost bedded pack barn moisture content (%) as a predictor of mean herd hygiene score collected using the 4-point system of Cook and Reinemann, (2007) where 1=clean, 2=moderate dirt, 3=plaques of dirt with hair visible, and 4=confluent plaques of dirt with no hair visible. 48 ± 5 cows in each herd were scored at each visit; (d) BT as a predictor of mean herd hygiene score collected using the 4-point system of Cook and Reinemann, (2007) where 1=clean, 2=moderate dirt, 3=plaques of dirt with hair visible, and 4=confluent plaques of dirt with no hair visible. 48 ± 5 cows in each herd were scored at each visit; (e) BT as a predictor of mean herd somatic cell count (SCC; cells/mL); (f) BT as a predictor of mean herd high SCC prevalence (% of herd with a SCC > 200,000 cells/mL per month).

mance through lower CIT and higher moisture at colder ambient temperatures (Barberg et al., 2007b). Maintaining a CIT ≥ 38 °C and a moisture count ≤ 55% in the warmer months (high BT) and keeping fans on even below heat stress levels in cattle (temperature humidity index of 68) over the year will encourage moisture evaporation from the pack and increase bedding life in the colder months of the year (Eckelkamp et al., 2014).

3.2. Cow results

3.2.1. Cleanliness

Herd hygiene remained below a score of 3 (solid plaques of dirt with hair visible; mean ± SD 2.2 ± 0.2, 1.6–2.8). Herd hygiene score was unaffected by CIT (P=0.17) and BH (P=0.48). However, pack moisture content and BT were predictors of herd hygiene score (P < 0.01 and 0.01, respectively; Table 2). Herd hygiene score increased with increasing pack moisture content and decreased as BT increased (Fig. 2c and d). As reported previously, pack moisture also decreased with increasing BT (Fig. 2(b)). The decrease in pack moisture decreased the ability of CBP material to adhere to animals, resulting in a lower hygiene score at greater BT. Similar to these results, Minnesota researchers noted a difficulty in maintaining a clean and dry barn environment in the winter season, when moisture content and hygiene scores were greater (Barberg

Table 3.
Mean (±SD) and range of compost bedded pack moisture content (%), 20 cm internal pack temperature (°C), and carbon and nitrogen ratio (C:N ratio) for 8 Kentucky compost bedded pack barns from May 2013 to May 2014 (n=26 visits per barn over the year.

Farm	Moisture content (%)		20 cm internal pack temperature (°C)		C:N ratio	
	Mean (± SD)	Range	Mean (± SD)	Range	Mean (± SD)	Range
8	60 (±6)	51–70	30 (±12)	10–46	22:1 (±4)	17:1–33:1
5	61 (±5)	53–69	32 (±6)	20–45	21:1 (±4)	14:1–31:1
2	64 (±8)	50–79	33 (±13)	10–48	15:1 (±2)	12:1–19:1
6	63 (±5)	54–71	35 (±11)	16–50	18:1 (±3)	14:1–24:1
3	60 (±6)	51–72	37 (±12)	10–52	24:1 (±8)	16:1–51:1
1	54 (±5)	45–64	43 (±7)	22–54	16:1 (±2)	12:1–20:1
4	59 (±7)	44–71	44 (±12)	15–58	27:1 (±6)	20:1–41:1
7	59 (±6)	48–70	46 (±8)	28–55	22:1 (±3)	17:1–27:1

et al., 2007b; Lobeck et al., 2011). This may indicate difficulty in maintaining low hygiene scores over the winter in CBP without more frequent bedding additions. This may also be useful for producers to aid in decision making before colder temperatures arrive.

3.2.2. Mastitis indicators

Pack moisture content, CIT, BH, and BT had no effect on reported clinical mastitis incidence ($P=0.67$, 0.51, 0.78, and 0.52, respectively) or BTSCC ($P=0.97$, 0.23, 0.79, and 0.09, respectively). However, the interaction of CIT and BT was a significant predictor of BTSCC ($P=0.01$; Table 2). Barn ambient temperature was also a significant predictor of herd SCC ($P=0.02$; Table 2) and HSP ($P<0.01$; Table 2), while compost moisture content, CIT, and BH were not significant predictors (SCC, $P=0.23$, 0.94, and 0.49, respectively; HSP, $P=0.39$, 0.82, and 0.94, respectively). Overall, mean BTSCC remained below 300,000 cells/mL for all CBP. With increasing BT and CIT, BTSCC increased, unless CIT was <25 °C. When CIT was <25 °C, BT was generally low. In this case, the BTSCC decreased. There may be a confounding effect of BT in this relationship, as CIT increased with increasing BT (Fig. 2(a)). Herd SCC and HSP both increased with increasing BT (Fig. 2e and f). Increased SCC, which HSP and BTSCC are related to, has been proven to increase as temperature humidity index increased (Dohoo and Meek, 1982; Morse et al., 1988). Similar results were identified by Bouraoui et al. (2002) with SCC doubling during periods of heat stress (410,000–860,000 cells/mL from no heat stress to heat stress; $P<0.05$). Based on the current study and the literature, the effects on BTSCC, HSP, and SCC may be because of the environmental effect on the animal more than the effect of CIT on the animal.

The interaction of compost moisture content and BT was a significant predictor of HSP ($P=0.02$; Table 2). With increasing moisture content and increasing BT, the HSP increased. High SCC prevalence was derived from the percentage of animals within the lactating herd with a SCC >200,000 cells/mL on DHIA test day. Factors known to affect SCC should be expected to affect HSP. Reneau et al. (2005) reported that SCC increased with increasing herd hygiene score. Increasing moisture content of the pack increased the hygiene scores of the animals, resulting in an increased HSP.

3.3. Bedding bacteria results

Bedding bacteria counts were 7.22 ± 0.72 $\log_{10}$ cfu/g, 6.34 ± 0.50 $\log_{10}$ cfu/g, 7.74 ± 0.62 $\log_{10}$ cfu/g, 4.52 ± 1.15 $\log_{10}$ cfu/g, 6.20 ± 0.62 $\log_{10}$ cfu/g on a dry matter basis for streptococci, staphylococci, bacilli, *Klebsiella* species, and coliforms, respectively (Table 4).

3.3.1. Coliforms

Coliforms were relatively stable over the year, fluctuating between mean counts of 6–7 $\log_{10}$ cfu/g. Total coliforms ($\log_{10}$ cfu/g) experienced an increase as CIT increased ($P=0.02$; Fig. 3; Table 2). Stocking density, pack moisture content, and C:N ratio had no significant effect on coliforms ($P=0.65$, 0.10, and 0.76, respectively). However, *Klebsiella* species had no significant relationships with pack moisture ($P=0.83$), CIT ($P=0.48$), C:N ratio ($P=0.30$), or stocking density ($P=0.84$). When CIT and pack moisture content were considered together, total coliforms changed depending on CIT ($P=0.02$; Table 2). Below 60% moisture, total coliform counts decreased with increasing CIT. At or above 60% moisture, total coliform counts increased with increasing CIT. The lowest and highest coliform counts occurred at 10 °C when pack moisture was 70% and 40%, respectively (5.8 and 7.4 $\log_{10}$ cfu/g). At all CIT, 60% pack moisture content maintained a relatively stable coliform count, similar to the counts over the year (Fig. 3; 6.3–6.4 $\log_{10}$ cfu/g from 10 to 58 °C). This result is understandable as mean pack moisture was between 50% and 60% over the study (Table 3). At low temperatures, high moisture content may be inhospitable to coliform growth, whereas high moisture content at high temperatures promoted coliform growth.

Black et al. (2014) reported similar findings, with internal temperature positively correlated with coliform count ($r=0.42$, $P<0.05$) and pack moisture negatively correlated with coliform count ($r=-0.34$, $P<0.05$). The current study showed that increasing CIT would moderately increase the coliform bacteria in the pack (Fig. 3). The differences between studies may be attributed to multiple visits per farm tracking the whole year in this study, unlike Black et al. (2014). The same may be true for the response of coliform bacteria to an interaction of CIT and pack moisture content. Barberg et al. (2007b) noted similar behavior with coliforms increasing in the summer (5.29 $\log_{10}$ cfu/g) compared to the winter (4.61 $\log_{10}$ cfu/g), corresponding to warmer internal pack temperatures and lower moisture content in the summer. Despite coliform species growth increasing when CIT was high, udder health parameters were not different ($P\geq0.05$) between CPB and sand-bedded freestall barns in a companion study by Eckelkamp et al. (2016). This may be linked to BT influencing changes in udder health parameters, which would occur at similar levels in both housing types (Eckelkamp et al., 2016).

Table 4.
Mean (±SD) and range for total coliform species, Klebsiella species, Streptococcus species, Staphylococcus species, and Bacillus species bedding bacterial counts (log10 cfu/g) for 4 Kentucky compost bedded pack barns from May 2013 to May 2014 (n=26 visits per barn over the year).

Farm	Total coliform species ($\log_{10}$ cfu/g)		Klebsiella species ($\log_{10}$ cfu/g)		Streptococcus species ($\log_{10}$ cfu/g)		Staphylococcus species ($\log_{10}$ cfu/g)		Bacillus species ($\log_{10}$ cfu/g)	
	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range	Mean (±SD)	Range
1	6.0 (±0.5)	5.2–6.8	4.5 (±1.2)	2.3–6.4	6.7 (±0.8)	4.9–8.1	6.4 (±0.8)	3.4–7.4	7.6 (±0.7)	6.1–8.5
3	6.6 (±0.4)	5.7–7.4	5.4 (±0.9)	3.4–6.5	7.5 (±0.6)	6.2–8.2	6.4 (±0.6)	5.0–7.8	8.1 (±0.5)	6.6–8.6
4	6.1 (±0.7)	4.8–7.1	4.1 (±0.9)	2.3–6.2	7.2 (±0.9)	5.1–8.3	6.1 (±0.6)	5.4–7.5	7.5 (±0.7)	6.5–8.5
8	6.1 (±0.7)	4.5–7.1	4.1 (±1.0)	4.5–7.1	7.5 (±0.6)	5.9–8.4	6.6 (±0.7)	5.2–7.8	7.9 (±0.5)	6.2–8.6

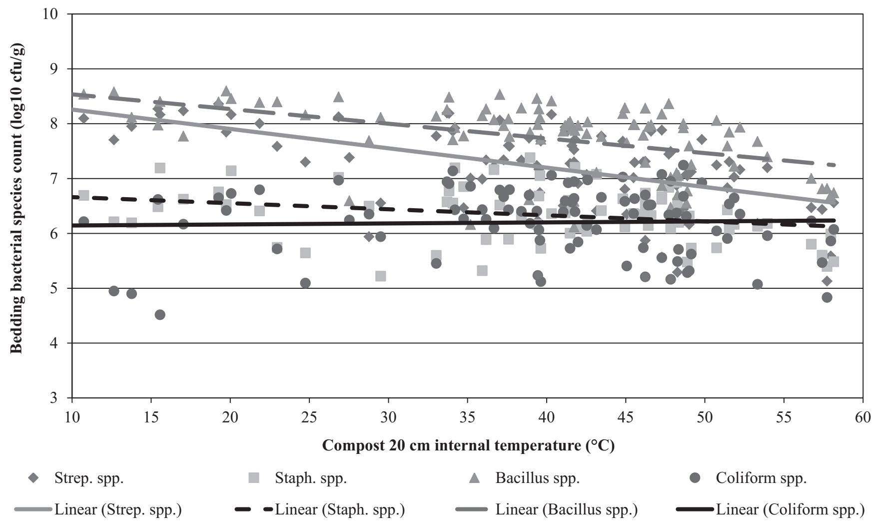

Fig. 3. Compost bedded pack barn 20 cm internal temperature (°C) for the 2-week visit as a predictor of mean bedding bacterial counts for 8 Kentucky compost bedded pack barns at each visit (n=26 visits per barn) for May 2013 to May 2014.

3.3.2. Streptococci

Streptococcus dysgalactiae and *Strep. uberis*, two well-known environmental mastitis pathogens, are present in the environment and bedding of the cow (Bramley et al., 1996). Similar to total coliforms, streptococci responded to CIT ($P=0.01$; Table 2), but not pack moisture content ($P=0.12$), C:N ratio ($P=0.58$), or stocking density ($P=0.68$). As CIT increased, streptococci decreased (Fig. 3). Over the year, streptococci counts were greatest from November 2013 to February 2014, when the CITs were lowest. Conversely, Black et al. (2014) reported no effect of CIT on *Strep.* spp. counts. However, Black et al. (2014) reported *Strep.* spp. in the compost bedding exhibited a moderate negative correlation with stocking density (m^2 per cow; $r=-0.38$; $P<0.05$) and C:N ratio ($b^2=2.1$; $P<0.05$). Reduction in *Strep.* spp. concentrations occurred in a low moisture and high C:N ratio environment, but increased in a low moisture and a C:N ratio between 20:1 and 22:1 ($P<0.05$, respectively; Black et al., 2014). The larger number of farms with greater variation on Black et al. (2014) study may have allowed for the significance of these variables. Black et al. (2014) recommended managing for low pack moisture conditions to decrease streptococci growth. Managing for decreased moisture and increased internal pack temperature may limit streptococci growth based on the current study and the published literature (Shane et al., 2010; Black et al., 2014).

3.3.3. Staphylococci

Staphylococci cause contagious and environmental mastitis infections. *Staphylococcus aureus* is a highly contagious mastitis pathogen (Barkema et al., 2006) while coagulase-negative staphylococci are generally opportunistic invaders of the udder present on the teat skin and in the teat canal (Cook, 2002; Verbist et al., 2011). Staphylococci experienced a slight decrease with increasing CIT ($P=0.01$; Fig. 3; Table 2). The lowest staphylococci counts occurred in the summer months when CIT was greatest. Staphylococci counts were not affected by pack moisture content, C:N ratio, or stocking density ($P=0.38$, 0.60, and 0.19, respectively). Unlike the current study, Black et al. (2014) noted *Staph.* spp. counts held a positive correlation with ambient temperature and negative correlations with moisture and C:N ratio ($r=0.52, -0.44$, and -0.52; $P<0.05$, respectively). In a MIXED model, *Staph.* spp. increased with increasing ambient temperature ($r=0.52$; $P<0.05$; Black et al., 2014). Although BT was not included in the streptococci model to avoid confounding affects, it can be inferred that streptococci would decrease at higher BT as well as CIT (Table 2; Fig. 2(a)).

3.3.4. Bacilli

. Bacilli responded to changes in CIT (Fig. 3) and C:N ratio ($P=0.03$ and 0.02, respectively; Table 2), but not to pack moisture content or stocking density ($P=0.93$ and 0.81, respectively). With increasing CIT, bacilli within the pack decreased. Conversely with increasing C:N ratio, bacilli within the pack increased. Over the year, bacilli counts were greatest from November 30, 2013 to March 6, 2014 (8.07–8.43 $\log_{10}$ cfu/g), corresponding to the lower CIT present during that period (Fig. 1). All farms monitored by Shane et al. (2010) produced greater *Bacillus* ssp. counts in summer than in winter (6.12 vs. 4.17 $\log_{10}$ cfu/g, respectively). Unlike the current study, *Bacillus* spp. counts increased at warmer internal temperatures and lower C:N ratios that occurred in summer (Shane et al., 2010). In a previous Kentucky study, when ambient temperature was low (−3 °C), *Bacillus* spp. increased when space per cow decreased below 8 m^2 per cow and moisture increased over 34%, and when moisture and C:N ratio decreased below 38% and 23:1 (Black et al., 2014). At high ambient temperatures (27 °C), *Bacillus* spp. increased under several different conditions: C:N ratio below 16:1 and moisture above 66%, moisture below 33% and C:N ratio over 37:1, and moisture below 31% and space per cow above 11 m^2 per cow (Black et al., 2014).

4. Conclusions

Similar to previous research, high CIT did not ensure reduction in bacteria levels. Increasing CIT corresponded to a decrease in *Staphylococcus*, *Streptococcus*, and *Bacillus* spp. counts, whereas coliform spp. increased. Although all bacterial counts but *Klebsiella* species were above 6.0 $\log_{10}$ cfu/g, udder health was not compromised. Hygiene in the study herds was indicative of the CBP ability to maintain adequate cleanliness, corresponding to the overall low levels of subclinical and clinical mastitis infection in the CBP herds included in this study. Herd and bulk tank SCC were below 300,000 cells/mL, and unaffected by the pack moisture content and CIT. Pack moisture and internal temperature was affected by barn ambient temperature. This may support the need to build the compost bedded pack barns according to industry recommendations.

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