CSIRO PUBLISHING

Animal Production Science
http://dx.doi.org/10.1071/AN16308

Comparison of bacterial populations in bedding material, on teat ends, and in milk of cows housed in compost bedded pack barns

R. L. Albino[A], *J. L. Taraba*[B], *M. I. Marcondes*[A,D], *E. A. Eckelkamp*[C] *and J. M. Bewley*[C]

[A]Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil.
[B]Biosystems and Agricultural Engineering Department, University of Kentucky, Lexington, KY 40546, USA.
[C]Department of Animal and Food Science, University of Kentucky, Lexington, KY 40546, USA.
[D]Corresponding author. Email: marcosinaciomarcondes@gmail.com

Abstract. The objective of this study was to observe relationships among somatic cell count (SCC) and bacteria counts in milk, on teat ends of lactating cows, and in compost samples from the aerated layer of the compost bedded pack. Twenty-nine lactating cows were used in this study. Clinically mastitis cows were not selected for the trial. The correlation between total bacteria count (TBC) and *Streptococcus* spp. of teat end and hygiene score was (r = 0.49) and (0.44, P = 0.01), respectively. In addition, there was a positive correlation (0.40, P = 0.03) between TBC on teat ends and somatic cell score. When analysing bacterial populations on teat ends and in milk, there was a positive correlation (0.39, P = 0.03) between *Escherichia coli* at the teat end and coliform counts in milk and also a positive correlation (0.38, P = 0.04) between coliform counts at the teat end and milk. Furthermore, *Streptococcus* spp. counts on teat end were positively correlated (0.38, P = 0.04) with TBC in milk. Although correlations were observed between hygiene score and SCC with bacterial population, all correlations were moderate. Therefore, hygiene score was not an efficient tool to estimate bacterial populations on teat end and milk.

Additional keywords: bacteria, dairy cows, milk quality.

Received 11 May 2016, accepted 21 January 2017, published online 15 March 2017

Introduction

The compost bedded pack (CBP) barn is a housing system for lactating dairy cows consisting of a large, open resting area, usually bedded with sawdust or dry, fine wood shavings that are composted in place, along with manure (Janni *et al.* 2007). In comparison with free-stall barns, reported benefits of the CBP system included improved cow comfort, cleanliness, and reduced feet and leg problems (Somers *et al.* 2003; Black *et al.* 2013). However, additional research is needed to assess the impact of the CBP on milk quality.

Feed alley cleaning method and frequency, bedding type and size, floor humidity, and neck rail height are challenges in free-stall systems that affect the cow's comfort and hygiene score. Dirty and uncomfortable cows can show higher values for somatic cell count (SCC) compared with clean and comfortable cows (Reneau *et al.* 2003; Justice-Allen *et al.* 2010).

Moisture, mud, and manure present in the environment of the cow are the primary sources of exposure for environmental mastitis pathogens, and cow hygiene score provides visible evidence of exposure to these potential sources. Schreiner and Ruegg (2003) observed in a free-stall system a 1.5 times increased risk for intramammary infection when cows were scored with high values compared with low values of hygiene score. Hygiene scores of cows housed in a CBP system ranged between 2.2 and 2.6 (Shane *et al.* 2010; Black *et al.* 2013). However, a deeper understanding of the bacterial population dynamics within the CBP barn resting area and their relationship with SCC and hygiene score is needed.

Therefore, we have conducted a study to understand the relationship between hygiene score, milk quality, and bacterial populations in milk and teat end. Our first hypothesis was that cows with high hygiene score and SCC contained elevated bacterial population on teat ends and milk, and that specific bacteria on teat end and milk was highly correlated with SCC. Our second hypothesis was that bacterial population on teat end was strongly correlated ($r \geq 0.68$) with bacterial population in milk.

The objectives of this study were (1) to investigate the relationship between SCC and hygiene score with bacteria counts in milk and on teat ends of lactating cows housed in CBP, and (2) to describe the distribution of bacterial population within the compost area.

Materials and methods

The experiment was conducted on a commercial dairy farm in Kentucky housing 128 Holstein cows in a CBP, from 12 March to 19 March 2013. The mean milk production per cow was 35 kg/day. Twenty-nine multiparous cows that were free of clinical

mastitis and not undergoing any antibiotic treatment were randomly chosen for the study. The selected cows had mean ± s.d. lactation number of 3 ± 1, 189 ± 127 days in milk (DIM) and a somatic cell score (SCS) of 3.28 ± 0.66.

The barn had a bedding area 18.23 m wide and 59.15 m long equivalent to 1081 m^2 (Fig. 1). The space per cow of the commercial farm was 8.40 m^2/cow, which is higher than the minimum of 7.4 m^2 per cow recommended by Janni *et al.* (2007).

The CBP was stirred with a rototiller with a 20-cm penetration capacity twice daily during the morning and the afternoon milking and 5 cm of a kiln-dried mixture of shavings and sawdust was added once per week.

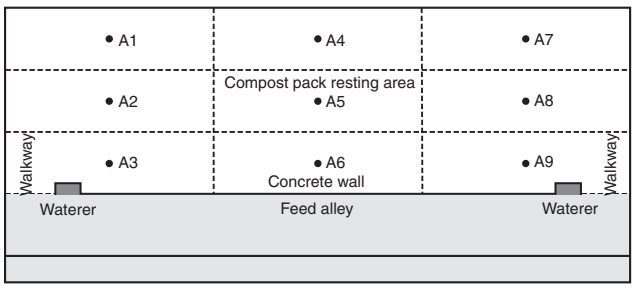

Fig. 1. Layout of pre-defined divisions in the compost bedded pack barn. Bedding samples were taken individually on each one of nine spots to represent the variation of bacterial population.

The barn was divided into nine pre-defined quadrants (Fig. 1). Inside each quadrant, three bedding samples were collected on the surface of the pack to form a combined sample, which were placed in a sterile plastic bag and mixed thoroughly, to analyse bacterial population. At the same location, the temperature was recorded at three different depths: 0 cm, 10.2 cm and 20.3 cm and a bedding sample was collected to determine moisture content. To analyse moisture and change of bacteria three samples were taken in a profile of 20 cm deep inside each spot (Fig. 1), pooled together in a plastic bag and mixed for 5 min. Then, a unique sample was taken to represent the bedding material from each spot.

The bedding samples were collected on Day 0 and Day 7, immediately following afternoon tilling. Samples were kept on ice until the following day, when they were analysed at the University of Kentucky Animal and Food Science Microbiology Laboratory.

The udder and leg hygiene score was performed on Day 0 and Day 7 using procedures described by Schreiner and Ruegg (2002). Udders and legs were assigned a subjective score based upon following criteria: (1) completely free of dirt or has very little dirt; (2) slightly dirty; (3) mostly covered in dirt; or (4) completely covered, caked-on dirt.

Duplicate milk samples were collected from each cow on both Day 0 and Day 7. The first sample was a combined product of five streams of milk taken from each quarter before milking unit attachment during the afternoon milking (Andrew 2001). The second sample was collected from weigh jars after agitation of

Table 1. Descriptive results (n = 29) of bacteria analysis on teat end and milk, somatic cell count, and hygiene score of lactating dairy cows managed on compost bedded pack system

Ec, *Escherichia coli*; Kb, *Klebsiella* spp.; Cc, coliform counts; Ssp, *Streptococcus* spp.; TBC, total bacteria count

			Bacteria on milk raw					Bacteria on teat end				
Unidity	SCS[A] log SCC/mL	Hygiene[B] –	Ec cfu/mL	Kb cfu/mL	Cc cfu/mL	Ssp cfu/mL	TBC cfu/mL	Ec cfu/mL	Kb cfu/mL	Cc cfu/mL	Ssp cfu/mL	TBC cfu/mL
Mean	5.3	2.2	2	0	3	1.831	5.554	29	9	34	6.410	27.685
s.e.m.	1.0	0.4	0	0	1	340	1.031	5	2	6	1.190	5.141
Maximum	6.6	4.0	51	5	60	48.850	95.575	238	75	285	55.175	62.625
Minimum	4.2	1.0	0	0	0	1	1	1	0	1	376	7.128
Number of zero[C]	0	0	26	28	19	0	0	0	22	0	0	0

[A]Somatic cell score.
[B]Hygiene score from leg and udders.
[C]Number of plates with no growth colonies.

Table 2. Correlation analysis between concentration of bacteria on teat end and milk with hygiene score and somatic cell score of lactating dairy cows managed on compost bedded pack system

Ec, *Escherichia coli*; Kb, *Klebsiella* spp.; Cc, coliform counts; Ssp, *Streptococcus* spp.; TBC, total bacteria count

Variables	Milk					Teat					
	Ec	Kb	Cc	Ssp	TBC	Ec	Kb	Cc	Ssp	TBC	
Hygiene[A]	–0.06	–0.03	–0.18	–0.02	0.16	0.25	0.08	0.24	0.44	0.49	r
	0.74	0.85	0.34	0.88	0.39	0.18	0.66	0.20	0.01	0.02	P-value
SCS[B]	0.25	0.05	0.25	0.18	–0.03	> –0.01	0.18	0.13	–0.27	0.40	r
	0.19	0.78	0.19	0.36	0.88	0.97	0.34	0.50	0.15	0.03	P-value

[A]Hygiene score.
[B]Somatic cell score.

milk to analyse SCC. The SCC was obtained by flow cytometry, using a CombiSystem 2300 device (Bentley Instruments Inc., MN, USA) (IDF Standard 148A: 1995). The milk samples were collected using aseptic techniques following guidelines published by the National Mastitis Council (2004).

Teat bacterial samples were collected individually from all quarters by rotating a swab around the exterior orifice immediately before afternoon milking. Each swab was immersed in maintenance broth before swabbing. Maintenance broth was prepared by adding 0.85% sodium chloride and 0.10% protease-peptone to distilled water. The maintenance broth also contained 0.20% sodium thiosulfate as a neutraliser for the iodine remaining on the teats from the post dip of the previous milking (Rendos *et al.* 1975). The teat was not washed or pre-dipped before swabbing but was cleaned with dry paper towel when visible amounts of bedding material were present (Rendos *et al.* 1975).

The four swabs of each cow were put inside a cooler, transported to the laboratory, and then cultured within 24 h of collection (Quirk *et al.* 2012). At the laboratory, four swabs per cow were pooled together in a 20 × 150-mm tube (Corning Inc., New York, NY, USA) containing 5.2 mL of sterile 0.85% NaCl, 0.10% peptone and 0.20% sodium thiosulfate broth. An additional 1 : 10 dilution was made with a phosphate buffer. The two samples (no dilution and 1 : 10) were plated on the media described below.

Bedding, milk, and teat bacteria analyses followed the methods described by Hogan *et al.* (1989). Briefly, 25 g of each bedding sample were added to 225 mL (1 : 10 dilution) of a 0.10% peptone solution in a 500-mL plastic bag and mixed manually for 1 min. The contents of the bag sat for 3 min and the supernatant was collected. Serial dilutions (1 : 100, 1 : 1000, and 1 : 10 000) of the liquid phase were conducted in a phosphate buffer, and then plated on the surface of each media: Petri film, MacConkey-inositol-carbenicillin agar, thallium sulfate-crystal violet B toxin blood agar and standard aerobic plate count agar.

The 3M™ Petri film™ *E. coli*/Coliform Count Plate was used to enumerate *Escherichia coli* (*E. coli*) and coliform counts species (Cc). The MacConkey-inositol-carbenicillin agar was used to enumerate *Klebsiella* spp. The standard aerobic plate count agar was used to obtain to total bacterial count (TBC). Colony forming units (cfu) were counted manually, obtaining Cc, *E. coli*, and *Klebsiella* spp. count per gram of bedding after incubating for 24 h at 35°C. *Streptococcus* spp. was enumerated using thallium sulfate-crystal violet B toxin blood agar prepared in the laboratory and spiral plated (Eddy Jet, IUL Instruments, I.L.S., Leerdam, The Netherlands) with the diluted material. The standard aerobic plate count agar was prepared using the same methods described for thallium sulfate-crystal violet B toxin blood agar. Both plates were analysed after being incubated for 48 h at 35°C.

The plates that showed a range of bacteria colonies between 20 and 250 were considered countable plates. Serial dilutions were done until a countable number of colonies was possible. The bacteria counted on teat end milk, SCC and compost area were log-transformed. Pearson correlation coefficients among teat end bacteria, milk bacteria, and SCS were calculated using the CORR procedure (SAS 9.3; SAS Institute Inc., Cary, NC, USA). The correlation *P*-value was estimated using Fisher's transformation (Fisher 1973). The hygiene score, a nonparametric variable, was compared with milk and teat end bacteria through Spearman Correlation Coefficients. A correlation coefficient (r) of $r \geq 0.9$ was rated as very high, $r = 0.68$–1.0 as strong or high, $r = 0.36$–0.67 as moderate, and $r \leq 0.35$ as weak correlation (Taylor 1990). Bacteria distribution throughout the bed was analysed descriptively using mean and standard deviation using PROC MEANS (SAS 9.3; SAS Institute Inc.).

Results and discussion

The SCS was equivalent to the SCC of 516 569 cells/mL (data not shown) indicating that although we have observed a low concentration of TBC in milk, the mammary gland was affected by bacteria which had little influence on cfu (Table 1). The TBC mean on raw milk was extremely low and *Streptococcus* spp. was the main bacteria responsible for contributing to this value (Table 1). The same standard was observed for bacteria concentration on teat end, which was for the most part affected by *Streptococcus* ssp. (Table 1).

Bedding bacterial composition may affect bacterial composition in milk and on teat ends. The major pathogens associated with bedding materials are the environmental streptococci (including *Streptococcus uberis*) and coliforms such as *E. coli*, and *Klebsiella* spp. (Hogan *et al.* 1989; Smith and Hogan 2000). However, only *Streptococcus* ssp. on teat ends was correlated ($r = 0.44$, $P = 0.01$) with hygiene score (Table 2). The correlation observed between TBC on teat end ($r = 0.49$, $P = 0.02$) and hygiene score was likely influenced by bacteria from the streptococci genus. Nevertheless, the correlations observed were only moderate.

In addition, no significant correlations were observed between *E. coli*, *Klebsiella* spp., *Streptococcus* ssp., and Cc with SCS. However, there was a positive correlation ($r = 0.40$, $P = 0.03$) between SCS and TBC on teat end (Table 2). Therefore, this moderate correlation between SCS and TBC on teat end may be due to an accumulated effect of all bacteria present on the teat end, thus not all bacteria will impact SCS.

When evaluating the presence of specific bacteria on teat ends and in milk, *Streptococcus* spp. on teat ends was correlated (0.38,

Table 3. Correlation analysis between bacteria on teat end and milk of lactating dairy cows managed on compost bedded pack system

Ec, *Escherichia coli*; Kb, *Klebsiella* spp.; Cc, coliform counts; Ssp, *Streptococcus* spp.; TBC, total bacteria count

		Teat					
		Ec	Kb	Ssp	Cc	TBC	
Milk	Ec	0.32	0.41	0.32	0.30	0.21	*r*
		0.09	0.02	0.09	0.11	0.27	*P*-value
	Kb	–0.01	–0.10	–0.20	> –0.01	–0.37	*r*
		0.94	0.58	0.30	0.99	0.04	*P*-value
	Ssp	–0.32	–0.19	>0.01	–0.28	0.15	*r*
		0.09	0.31	0.97	0.14	0.42	*P*-value
	CC	0.39	0.31	0.32	0.38	0.03	*r*
		0.03	0.10	0.08	0.04	0.88	*P*-value
	TBC	0.09	0.07	0.38	0.17	0.35	*r*
		0.65	0.71	0.04	0.36	0.06	*P*-value

$P = 0.04$) with TBC in milk, and *E. coli* on teat end was correlated (0.39, $P = 0.03$) with Cc in milk (Table 3). However, no association was observed between hygiene score or SCS and *E. coli* or Cc in milk or on the teat end (Table 3). This result indicates that indirect measures such as hygiene score do not provide reliable information for all type of bacteria concerning contamination on teat ends and milk.

Figure 2 shows the bacteria distribution on the compost barn using a bubble graph. The concentration of *E. coli* and coliform counts between spots inside the compost area did not show great numeric difference. A greater number of *Streptococcus* spp. was observed on spots 3 and 9, which are high traffic areas where cows enter the CBP from the feed alley. It is important to notice that spot 9 had a greater bacterial count for almost all bacteria analysed. These areas usually contained the highest moisture and a less deep aeration layer, which might be a reflection of the difficulty of tilling these areas.

For maximum aerobic composting, the optimum moisture range wet basis is between 40% and 60% (Haug 1993). Nevertheless, no differences in bedding moisture were found by position or sampling date ($P > 0.05$, data not shown) and the average bedding moisture content was 69 ± 1.4% (wet basis; $n = 18$, range = 67–72%). The bedding moisture was slightly above maximum threshold (60%) and this fact may be linked with barn temperature, once the barn temperature increases the bedding moisture decreases (Eckelkamp *et al*. 2016). As the trial was conducted during the winter, the low temperatures made it difficult for bedding to dry.

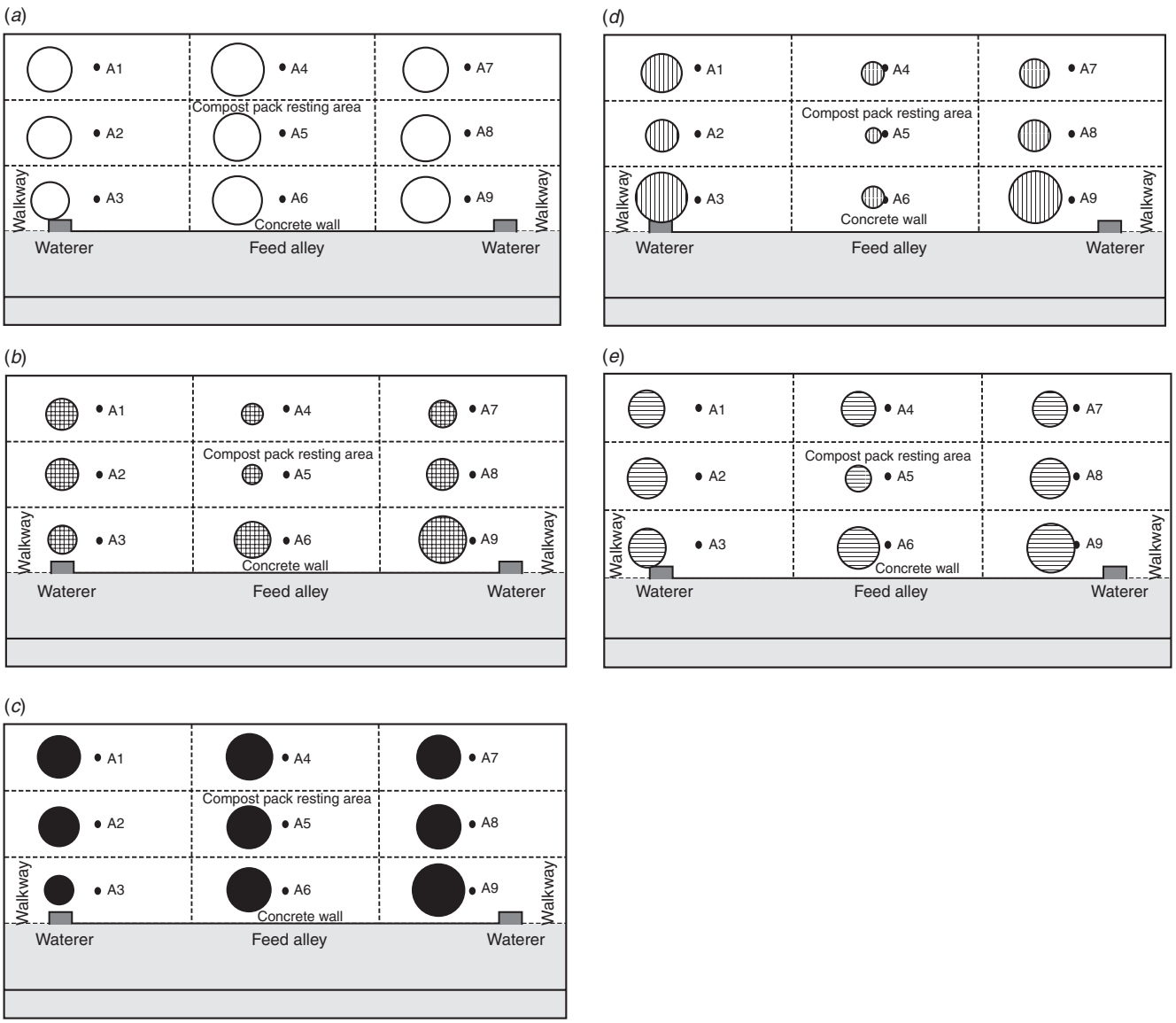

Fig. 2. Distribution of concentration of bacteria (*a*) *Escherichia coli*, (*b*) *Klebsiella*, (*c*) total coliform, (*d*) *Streptococcus* sp. and (*e*) total bacteria count throughout the compost barn using the bubbles graph. The bigger bubbles indicate the higher concentrations; conversely, smaller bubbles indicate the lower concentration.

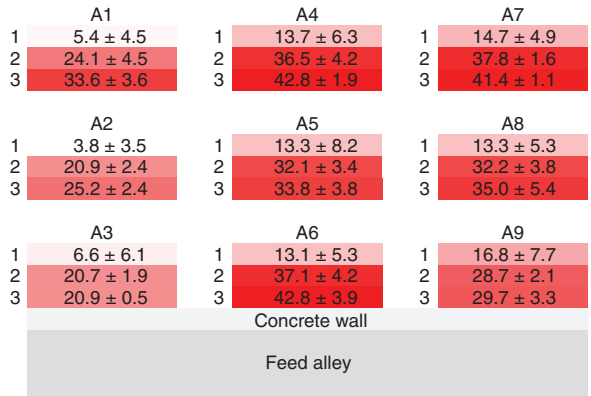

Fig. 3. Temperature distribution (mean ± s.d.) in degrees Celsius of the bedded pack at three depths inside each spot (A1 to A9), obtained from two collection days, in the compost bedded pack system. The shading indicates the temperature variation in shaded scales, where white indicates cooler, and warmer temperature are closer to darker shading. The numbers positioned on the left of each spot represents the different layers, where 1 is the superficial layer, 2 is equal to a 10.2-cm depth and 3 is equal to a 20.3-cm depth.

From the information presented in Fig. 3 we observed that the mean temperature of sampling locations had not reached a minimum recommended temperature of 55°C (Black *et al.* 2014). Spots 1, 2 and 3 had lowest mean temperatures (Fig. 3). The reduced internal temperatures may be the result of high moisture content (>60%) and further may be justified by the environment temperature, as the experiment was conducted in the winter and the mean temperature outside the barn was 5.2°C (range from 1°C to 9.4°C), which might have impaired the bed heat losses to the environment.

The high concentration of these bacteria could be controlled through increased tillage of these spots. Increasing tillage frequency can reduce overall farm efficiencies due to increased time spent tilling. However, this management should be seen as a treatment to prevent cows developing clinical mastitis. Greater amounts of dry bedding in these areas could reduce moisture level and improve the composting process (Black *et al.* 2013). Also directing fans on these spots will effectively reduce moisture content. Usually, maintaining recommended moisture levels (50–60%) will control the *Klebsiella* spp. and *Streptococcus* spp. population (NRAES 1992).

Conclusion

The hygiene score was not an efficient tool to determine the bacterial population on teat end and in milk; however, we should not state that this tool would not be useful in other situations, once that cause and effect had not been established in this trial. Further work should be conducted to understand when hygiene score will become a powerful tool to evaluate milk quality in CPB systems. Moreover, bacterial population on teat ends do not necessarily impact SCC. However, bacteria analysis on teat ends may indicate the presence of *Streptococcus* spp. and Cc in milk.

In addition, *Streptococcus* spp. was the only genus that showed correlation between hygiene score and milk quality, therefore attention should address bacteria of this species when high hygiene scores are observed in cows at CPB.

Acknowledgements

The authors gratefully acknowledge technical assistance from Kabby Akers of the University of Kentucky and Robert and Angie Klingenfus for the opportunity to conduct this work. We also acknowledge CAPES for providing the scholarship for Ronan Lopes Albino.

References

Andrew SM (2001) Effect of composition of colostrum and transition milk from Holstein heifers on specificity rates of antibiotic residue tests. *Journal of Dairy Science* **84**, 100–106. doi:10.3168/jds.S0022-0302(01)74457-8

Black RA, Taraba JL, Day GB, Damasceno FA, Bewley JM (2013) Compost bedded pack dairy barn management, performance, and producer satisfaction. *Journal of Dairy Science* **96**, 8060–8074. doi:10.3168/jds.2013-6778

Black RA, Taraba JL, Day GB, Damasceno FA, Newman MC, Akers KA, Wood CL, McQuerry KJ, Bewley JM (2014) The relationship between compost bedded pack performance, management, and bacterial counts. *Journal of Dairy Science* **97**, 2669–2679. doi:10.3168/jds.2013-6779

Eckelkamp EA, Taraba JL, Akers KA, Harmon RJ, Bewley JM (2016) Sand bedded freestall and compost bedded pack effects on cow hygiene, locomotion, and mastitis indicators. *Livestock Science* **190**, 48–57. doi:10.1016/j.livsci.2016.06.004

Fisher RA (1973) 'Statistical methods for research workers.' 14th edn. (Hafner Publishing Company: New York)

Haug RT (1993) 'The practical handbook of compost engineering.' (Lewis Publishers: Boca Raton, FL)

Hogan JS, Smith KL, Hoblet KH, Todhunter DA, Schoenberger PS, Hueston WD, Pritchard DE, Bowman GL, Heider LE, Brockett BL, Conrad HR (1989) Bacterial counts in bedding materials used on nine commercial dairies. *Journal of Dairy Science* **72**, 250–258. doi:10.3168/jds.S0022-0302(89)79103-7

Janni KA, Endres MI, Reneau JK, Schoper WW (2007) Compost dairy barn layout and management recommendations. *Applied Engineering in Agriculture* **23**, 97–102. doi:10.13031/2013.22333

Justice-Allen A, Trujillo J, Corbett R, Harding R, Goodell G, Wilson D (2010) Survival and replication of mycoplasma species in recycled bedding sand and association with mastitis on dairy farms in Utah. *Journal of Dairy Science* **93**, 192–202. doi:10.3168/jds.2009-2474

National Mastitis Council (2004) 'Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality.' (National Mastitis Council: Verona, WI)

NRAES (Northeast Regional Agricultural Engineering Service) (1992) 'On-farm composting handbook, NRAES-54.' (Northeast Regional Agricultural Engineering Service: Ithaca, NY)

Quirk T, Fox LK, Hancock DD, Capper J, Wenz J, Park J (2012) Intramammary infections and teat canal colonization with coagulase-negative staphylococci after postmilking teat disinfection: Species-specific responses. *Journal of Dairy Science* **95**, 1906–1912. doi:10.3168/jds.2011-4898

Rendos JJ, Eberhart RJ, Kesler EM (1975) Microbial populations of teat ends of dairy cows, and bedding materials. *Journal of Dairy Science* **58**, 1492–1500. doi:10.3168/jds.S0022-0302(75)84740-0

Reneau JK, Seykora AJ, Heins BJ, Endres MI, Farnsworth RJ (2003) Association between hygiene scores and somatic cell scores in dairy cattle. *Journal of the American Veterinary Medical Association* **227**, 1297–1301. doi:10.2460/javma.2005.227.1297

Schreiner DA, Ruegg PL (2002) Effects of tail docking on milk quality and cow cleanliness. *Journal of Dairy Science* **85**, 2503–2511. doi:10.3168/jds.S0022-0302(02)74333-6

Schreiner DA, Ruegg PL (2003) Relationship between udder and leg hygiene scores and subclinical mastitis. *Journal of Dairy Science* **86**, 3460–3465. doi:10.3168/jds.S0022-0302(03)73950-2

Shane EM, Endres MI, Janni KA (2010) Alternative bedding materials for compost bedded pack barns in Minnesota: a descriptive study. *Applied Engineering in Agriculture* **26**, 465–473. doi:10.13031/2013.29952

Smith KL, Hogan JS (2000) Bedding's contribution to mastitis in dairy cows. In 'Dairy housing and equipment systems, managing and planning for profitability'. NRAES 129. (Natural Resource Agriculture and Engineering Service)

Somers JGCJ, Frankena K, Noordhuizen-Stassen EN, Metz JHM (2003) Prevalence of claw disorders in Dutch dairy cows exposed to several floor systems. *Journal of Dairy Science* **86**, 2082–2093. doi:10.3168/jds.S0022-0302(03)73797-7

Taylor T (1990) Interpretation of the correlation coefficient: a basic review. *Journal of Diagnostic Medical Sonography: JDMS* **6**, 35–39. doi:10.1177/875647939000600106