Comparison of the effect of different management strategies on the quality of recycled sand used for bedding in free stalls in southeastern Pennsylvania

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Abstract

The effect of different management strategies on dry matter (DM), organic matter (OM) and bacterial populations in newly recycled sand (RS) were compared on three commercial dairy farms in southeastern Pennsylvania during late spring and summer. DM increased by 0.98%, OM decreased by 0.05%, and bacterial numbers were not different comparing arranging RS into one windrow and turning 3 times to forming a static pile. RS stored outside had fewer coliform bacteria compared to when stored inside under cover. RS should be stored for a minimum of 22 days before placement back into the cow stalls to minimize the numbers of bacteria associated with mastitis.

Key words: recycled sand, bedding, processing, mastitis

Introduction

Improving the control of environmental mastitis is a high priority on dairy farms from an animal welfare, milk quality, and economic perspective. Bacterial counts in bedding are associated with bacterial counts on teats[1,2,3,4,5] and rising evidence suggests increased bacterial counts in bedding increase the risk for intra-mammary infections from environmental bacteria, specifically coliforms, *Streptococcus* spp. and *Staphylococcus* spp.[6,7] Sand, either recycled or new, is a popular bedding material because it is inorganic and bacterial counts are generally reported to be lower in sand compared to organic bedding, such as straw, sawdust, and manure solids.[6,8] In addition, hock lesions and the prevalence of lameness are less in animals housed in sand bedded free stalls compared to those housed in stalls with solid rubber mats, rubber crumb- filled mattresses, or bedded with sawdust or straw.[9,10] Sand can be safely recycled, either through passive or mechanical systems, and reused as bedding which saves money for the producer and is environmentally friendly.[11]

In general, greater bacterial counts in ready to use newly RS and new sand (NS) are associated with higher bacterial counts in bedding following use in free-stalls.[7] Goals suggested for DM and OM for ready to use RS to minimize bacterial counts are >95% and <1.5%, respectively.[7] Limited evidence suggests a benefit to using RS bedding with high DM and low OM but the association between DM, OM and bacterial counts in RS requires further research.[6] Both lower DM and higher OM have been associated with increased bedding counts of coliforms and *Streptococcus* spp. in ready -to -use RS and NS and higher OM in RS was associated with at least one negative udder health outcome measure.[a] Because RS generally has higher OM and lower DM compared to NS,[7,11] dairy farmers have resorted to using various labor, capital, and equipment intensive methods to process RS before putting it back into the cow stalls. These methods involve using a McLanahan[b] de-watering screen after separation, mixing and moving RS to different piles, letting sand sit for various periods of time to dry the RS, and storing RS either outside or in a shelter under a roof. The objective of this study is to compare the effects of: 1). The processing technique of churning the RS (forming one windrow and turning it over 3 times) or leaving it dormant in static piles and 2.) The storage technique of storing the RS either outside or in a shelter under a roof on DM, OM, and bacterial counts in the RS.

Materials and methods

Three commercial Holstein dairy farms in southeastern Pennsylvania using RS were selected for this project in the late spring and summer of 2021 based on the producer's flexibility with different treatment options for their RS during the study and their proximity to the University of Pennsylvania School of Veterinary Medicine's New Bolton Center – 20 miles, 3.6 miles, and 54 miles, respectively. Basic farm descriptions are displayed in Table 1. Farm 1 recycled sand from manure using a McLanahan mechanical separation system. Farm 2 and 3 recycled sand from manure using gravity flow sand lanes. Farm 1 and 2 used a McLanahan de-watering sand screen or "shaker" at the time of separation to remove moisture from the RS, producing a drier sand.

Study organization

Objective 1: Compare organizing the RS into one windrow, turned 3 times, to forming a static pile of RS.

The three farms were instructed to continue initially their traditional sand processing techniques and time frames during the late spring and summer of 2021. The traditional sand processing techniques of each farm involved organizing the RS into one windrow which was moved and turned over 3 times. At the time when windrow formation would normally begin on each farm, the producers were instructed to divide the RS into two treatment groups: a churned pile (C) and a static pile (SP). The recycling process in the C group was defined as arranging the sand into one windrow that would be moved and turned 3 times in the traditional time frame used by each farm. RS in the SP group would remain dormant in the pile through the same time frame.

Table 1: Characteristics of the 3 dairy farms in southeastern Pennsylvania included in this study.

Farm	Milking cows (n)	Average milk (kg/cow/day)	Average SCC (cells/mL)	Re-bedding frequency	Sand separation technique	Use of sand shaker
1	500	47	90,000	Twice a week	Mechanical	Yes
2	1100	45	127,000	Twice a week	Gravity	Yes
3	244	39	221,000	Twice a week	Gravity	No

Objective 2: Storing the RS either outside or inside under shelter under roof.

Once the traditional method of processing was complete for each farm, sand in the C and SP was further divided into two treatment groups and producers were asked to keep the sand for an additional 21 days. The RS from both the C and SP was either stored outside (O) or inside (I) a shelter under a roof.

Sand processing and sampling schedules by farm

Farm 1: Samples of RS were taken by the herd veterinarian in the summer from 7-2-21 through 7-31-21. The RS was reclaimed using a McLanahan sand separator and put through a McLanahan de-watering screen. Samples of RS were taken at the start of the study (day 0) both prior to and after the McLanahan screen and the RS was divided into two treatment groups, - C and SP. Additional RS was added over a 3-day period to the C group according to the farm's traditional processing. The RS in treatment group C was arranged into one windrow that was moved and turned 3 times over 8 days and sampled. The RS in SP was left unaltered as a static pile. At day 8, RS in C and SP was further divided into treatment groups O and I, creating four treatment piles (CO, CI, SPO, and SPI), which were sampled on day 8 and then at approximately weekly intervals for 21 days with the final sample taken on day 29.

Farm 2: Samples of RS were taken by the herd veterinarian in the late spring and summer from 5-19-21 through 7-16-21. The RS was re-claimed from gravity sand lanes and put through a McLanahan de-watering screen. Additional water was added at the screen to clean the RS. Samples of RS were taken at the start of the study (day 0) prior to and after the McLanahan screen. Additional RS was added to the pile over a 3-day period according to the farm's traditional processing and samples of RS were taken on day 1 and 8. On day 14, the RS was divided into the two treatment groups, - C and SP, – and sampled. The RS in C was arranged into a windrow that was moved and turned on a weekly basis over 3 weeks. The RS in SP was left as a static pile. The RS was sampled in C and SP on a weekly basis over 3 weeks. At day 39, RS in C and SP was further divided into treatment groups O and I, creating four treatment piles (CO, CI, SPO, and SPI), which were sampled on day 39 and then approximately weekly over 19 days with final samples taken on day 58.

Farm 3: Samples of RS were taken by the trained producer over the summer from 6-22-21 through 8-4-21. The RS was reclaimed from gravity sand lanes. At the start of the study (day 0) the RS was sampled. On day 1 the RS was divided into the two treatment groups, – C and SP, - and the RS was sampled in SP. Additional RS was added to the RS in C over a 3-day period according to the farm's traditional processing. The RS in C was sampled and organized into one windrow on day 3, moved and turned three times over a period of 11 days, and sampled on day 7 and 14. On day 22, the RS in C was further divided into treatment groups O and I, creating three treatment piles (CO, CI, and SPO), which were sampled on day 22 and then at approximately weekly intervals for 21 days with the final RS samples taken on day 43. All the RS in SP was stored outside. The RS in SP was sampled on day 1, day 14, and then at approximately weekly intervals with the last sample taken on day 34. The producer did not have space to store the RS under cover for SP and, therefore, did not complete the SPI pile.

Sample collection

A standard operating procedure was established to minimize variability among samples due to individual variation in sampling technique. A 33" soil probe sampler[c] was used on each farm to collect the RS samples. The soil sampler was inserted to its full depth at an estimated center point in the height of the pile and windrow, 1-3' from the ground dependent on total pile or windrow height, and emptied into new one-quart storage freezer bags. This technique was repeated moving around the circumference of the pile 10 times until a full quart of RS was collected from the pile. All of the RS samples were frozen at -18° C (0°F).

Laboratory analysis and bacteria quantification

Frozen RS samples were submitted for bacterial identification and quantification for *Streptococcus* spp., *Staphylococcus* spp., coliforms, and non-coliforms at Cornell University's Quality Milk Production Services (QMPS[d]) Laboratory as well as detection of other pathogens associated with mastitis.

RS samples

Frozen RS samples were allowed to thaw at refrigeration temperature (2°C–8°C) for 4 hours. The RS sample was placed into a large, clean, zip-type bag that allowed thorough mixing and breaking up of any clumps. Using a weight-verified scale, 10 ± 1% (9.90–10.10) grams (g) of the RS sample was weighed into a sterile vial by taking small subsamples from at least three random locations within the mixed RS sample. Ninety ml of sterile PBS was added to the 10-g test sample and vortexed for 40 seconds at setting 7 (1,800 rpm). Approximately 10 ml of this suspension was decanted into an empty sterile dilution tube. This was the 10^{-1} dilution. The 10^{-2} dilution was made by vortexing the 10^{-1} dilution for a minimum of 4 seconds and removing 1 ml using a micropipette and adding it to 9 ml of PBS. This dilution process continued until the 10^{-5} dilution.

Plate inoculation and incubation parameters

For each RS sample, 50 µl of each dilution was inoculated on different selective media. Edwards media was inoculated to test for *Streptococcus* spp. and "streptococci-like" organisms.

MacConkey media was inoculated to test for coliforms and other Gram-negative organisms. Trypticase soy agar with 5% sheep blood and 0.1% esculin was inoculated to test for other Gram-positive organisms.

In addition to the organisms that were quantified, the following bacteria of mastitis significance were identified and counted as detected or not detected: *Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Klebsiella* spp., and a category of other bacteria which included *Truperella pyogenes, Corynebacterium* spp., *Pseudomonas* spp., and Gram-negative and -positive bacillus. Technicians experienced in microbiology used visual cues and biochemical tests[e] along with colony morphology to identify these pathogens. The presence of even one colony would be considered as detected.

Plates were incubated at 35°C–38°C. After 18–24 hours of incubation, plates were observed using standard microbiology procedures. At 18–24 hours, the lactose-positive, Gram-negative colonies were counted and *E. coli* and *Klebsiella* spp. were observed and recorded. Plates were placed back in the incubator at 35°C–38°C for an additional 18–24 hours.

Bacteria counts calculation

Plates were removed from the incubator and the number of colony-forming units (CFU; CFU/g for RS samples) were counted by an experienced laboratory technician from the dilution plate (up to 10^{-5} for bedding samples) that presented 25–250 colonies whenever possible. All counts and the dilution plate were recorded in an internal form. Bacterial counts were calculated on a dry bedding weight basis based on the dilution factor.

Moisture content (DM content) estimation

The drying dish was weighed. The scale was tared and 10 ± 1% (9.90–10.10) g of RS material was added and evenly spread. The dish containing the 10 g of bedding was placed into the oven and dried for at least 4 h at 100 ± 10°C. After drying, the sample was weighed, and the total weight to two decimals was recorded.

Organic matter (OM content) estimation

A drying beaker was weighed and tared and 5-10 g of a subsample of bedding material was added to the beaker and total weight was recorded. The beaker with sample was placed into a drying oven set at 105 +/- 5 C° for at least 4 hours to remove moisture and then cooled to room temperature in a desiccator and weighed. The beaker and sample were then put in a 500 C° muffle furnace for at least 4 hours reducing the sample to ash, an inorganic material. The ash in the beaker was cooled to room temperature in a desiccator and the final weight was recorded. Ash content and organic content were calculated on a DM basis.

Particle size analysis

A sand sieve analysis was performed at QMPS on the initial sand samples from each farm to quantify the amount of particles falling within the recommended range of 0.1-1 mm in diameter.

Statistical analysis

All analyses were conducted with Stata 17MP[f] with two-sided tests of hypotheses and a *P*-value < 0.05 as the criterion for statistical significance. Descriptive analyses included computation of means (with 95% confidence intervals [95%CI]), standard deviations, medians, interquartile ranges (IQR) of continuous variables and tabulation of categorical variables. The Shapiro-Wilk test was performed to determine the extent of skewness. Frequency counts and percentages were used for categorical variables. Inference statistical analysis was conducted using a multilevel mixed-effects linear regression model. The outcomes of interest included DM, OM and bacterial counts (total bacteria, total coliforms, *Klebsiella* spp., *E. coli, Streptococcus* spp., *Staphylococcus* spp. and other bacteria). Each of the outcomes was analyzed with a mixed effects linear regression model with the following fixed effects: "Churned" as a categorical variable (churned (C), static pile (SP), or other (OT) to identify the pre-and-post-dewatering screen changes), "Storage cover" (inside under cover (I) or outside (O)), and "Time" (categorical variable where each time point is considered a separate category). The "Churned" category OT was used to classify sand that was neither churned nor static at that point in the processing and to absorb some of the differences between farms such as usage of a de-watering screen and either stage 1 or stage 2 lagoon water to reclaim the sand prior to grouping RS into C and SP. Random effects were set on the level of individual farm. To correct for possible departures from normality of the outcome, robust estimation of the variance was used. Post-hoc pairwise analysis was performed on the model adjusted marginal means, abbreviated as "marginal means". The least significant difference method was used to correct for multiple comparisons. All marginal means were reported with their respective 95% CI.

Results

Samples of RS were obtained from 3 farms during the late spring and summer from 5-19-21 through 8-4-21. Weather data, from a central airport to the study farms, during the study time is detailed in Table 2. The final data set included a total of 68 RS samples, with 22 samples from Farm 1, 28 samples from Farm 2, and 18 samples from Farm 3. Particle size analysis of initial sand samples from each farm revealed 92.7%, 98.6%, and 98.70% of particles falling within the recommended 0.1-1 mm particle size on each farm respectively. Selective data by treatment group for the beginning of the study (day 0), days from the start of the study when sand was grouped into I and O, and the end of the study and overall ranges of the data are presented in Table 3 and 4 respectively for each farm. Total days of RS processing ranged from 29 to 58 days (Table 3). DM on day 0, when the sand was first reclaimed, ranged from 80-83% but increased to 89% on Farm 1 and 85% on Farm 2 on day 0 after processing the sand through the de-watering screen (Table 3). Farm 2 added water at the de-watering screen step as a way to further clean the RS. Initial day 0 *Streptococcus* spp. counts were at least 6 times higher on Farm 1 than on Farm 2 and 3 (Table 3). Over the course of the study DM ranged from 80 to 98% and OM ranged from 0.4 to 1.8% (Table 4).

Marginal means

Marginal means and 95% CI for DM, OM, and bacterial populations for specific RS population variables are listed Tables 5, 6, 7 and 8. The random effect variance was higher in comparison to the residual variance, indicating high variability between the farms.

In spite of the systemic noise, statistically significant results were found. DM increased by 0.98% in C compared to SP (Table 5) and by 0.5% when stored O compared to I (Table 6). OM content decreased by 0.05% in C compared to SP (Table 5).

There were no significant differences in bacterial populations (cfu/gram) for *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella* spp., *E. coli* or total bacterial populations detected based on processing (C vs SP) or storage (O vs I) (Table 7, 8). Coliform bacteria decreased by 8,684 cfu/gram in RS stored O compared to I (Table 8).

Effect of duration of processing

Using pairwise comparisons of marginal means for adjacent time points, and controlling for the effects of treatment groups, a specific time could be identified when further changes in OM, DM and bacterial populations ceased to be significant. Significant changes in DM and OM ceased to occur between 15-20 days of processing (Figures 1, 2). Optimal reference values suggested by Patel et al. 2019,[7], are superimposed on the graphs (Figures 1, 2). Significant decreases in total bacteria, *Staphylococcal* spp., *and Klebsiella* spp. (cfu/gram of bedding) ceased after 13 days of processing. Significant decreases in *Streptococcal* spp. ceased after 15 days and in total coliform bacteria after 22 days of processing. Thus, most bacterial populations stopped changing significantly after 15 days and complete cessation of significant changes in bacterial populations occurred by 22 days of processing (Figure 3).

Discussion

This study focused on the effect of commonly used post-reclamation processing techniques of RS on DM, OM, and bacterial populations. Management strategies evaluated consisted of forming one windrow and moving and turning RS three times compared to static piles, storing RS outside compared to inside under cover and evaluating the effect of time while controlling for these processing techniques. Many dairy producers expend an enormous amount of time, energy and expense in processing their RS, with limited information to validate and guide their efforts. Thus, this study aimed to provide a better understanding of the effect of these techniques for producers to consider when assessing the labor and equipment required to perform them. However, several caveats apply when extrapolating the data from this study to others. Such caveats include the small number of farms included, the tightly defined geographical location of these farms, and the specificity of the time of year that the study encompassed. Individual farms should be advised to perform their own repeated analysis of their RS throughout each stage of their individual processing protocols to better understand the unique characteristics of their farm's RS.

DM content

The DM content increased by 0.98% when RS was organized by C compared to SP and DM increased by 0.5% when stored O compared to I, although the clinical impact of such a small change is unknown. Significant increases in DM ceased to occur at approximately 15-20 days in the study. The DM content of bedding has significant implications on the bedding system's ability to support bacterial growth as well as the comfort of cattle utilizing the bedding.[12,13] The suggested goal for DM for newly RS to minimize bacterial counts is >95%.[7] Putting RS through a McLanahan de-watering screen increased DM on Farm 1 from 80 to 89% and on Farm 2 from 83% to 85% (Table 3), although notably the bacteria numbers remained high in RS samples taken from Farm 1 and 2 immediately after the McLanahan de-watering screen (Table 3). There was variation from 3 to 51 days as to when each farm first reached the DM suggested goal of 95%. Although not specifically studied, Farm 2 probably did not benefit from adding water at the de-watering screen. Godden et al. 2019, reported RS samples were drier in the summer compared to winter, showing that achieving a higher DM content in RS is easier in the summer than winter.

OM Content

The OM content decreased when RS was organized as C compared to SP. The decrease in OM content by 0.04% in C was numerically small and likely not clinically significant. Significant decreases in OM ceased to occur by approximately 15-20 days in the study. The OM values in this study were low at the beginning of the study and decreased over time; ranging at day 0 between 0.9 and 1.3% and at the end of the study between 0.4 and 1% (Table 4) – well below the goal of <1.5% to minimize bacteria counts suggested by Patel et al. 2019.7. Nonetheless, these results suggest that total days processing RS before placing back into the cow stalls had a greater effect on lowering OM than other methods used to process the RS.

Effect of time and processing techniques on bacterial populations

The results of this study suggest that total days before newly RS is placed back into the cow stalls has a large effect on decreasing bacterial populations. There was a lack of significant differences in bacterial populations (cfu/gram) between C and SP and only a small decrease in total coliform bacteria was detected in RS stored O compared with I. The decrease in coliform bacteria in sand stored outside could be due to the negative effect of the sun's ultraviolet radiation on coliform bacterial populations. Significant decreases in bacterial populations associated with known mastitis pathogens, such as

Table 2: Monthly weather data through the study time frame from a central airport within the region of the study farms.[g]

	May 2021		June 2021		July 2021	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Minimum daily temperature (°F)	39	71	49	75	58	74
Average daily temperature (°F)	48	81	60	85	68	85
Maximum daily temperature (°F)	52	93	67	97	75	94
Precipitation (in)	0	1.15	0	0.78	0	1.99
Wind speed (mph)	0	28	0	31	0	32

Table 3: Descriptive data of recycled sand (RS) in each treatment group on day 0, day moved outside (O) or inside (I) and final day of sampling for each of the 3 study farms. Bacterial counts are reported on a dry weight basis.

Farm	Days	Group	DM %	OM %	Total CFU (cfu/g)	*Streptococcus* (cfu/g)	*Staphylococcus* (cfu/g)	*Coliform* (cfu/g)	*Klebsiella* (cfu/g)	*E. Coli* (cfu/g)	Other bacteria (cfu/g)
1	0	Pre-screen	80	1.1	5,469,250	3,750,000	1,625,000	500	0	0	93,750
1	0	Post-screen	89	0.9	6,688,764	3,932,584	1,685,393	3,371	0	0	1,067,416
1	8	C O	95	0.7	7,693,475	284,211	1,894,737	28,211	7,158	0	5,486,316
1	8	C I	95	0.6	4,425,262	242,105	2,315,789	105,263	52,632	52,632	1,762,105
1	8	SP O	92	0.8	8,277,174	3,152,174	3,260,870	70,652	0	16,304	1,793,478
1	8	SP I	90	0.7	8,574,443	4,444,444	3,111,111	74,444	0	18,889	944,444
1	29	C O	96	0.4	144,374	1,458	64,583	0	0	0	78,333
1	29	C I	95	0.4	811,789	10,947	694,737	0	0	0	106,105
1	29	SP O	97	0.4	813,196	6,598	701,031	5,979	0	2,680	99,588
1	29	SP I	96	0.5	122,917	3,333	47,917	0	0	0	71,667
2	0	Pre-screen	83	1.3	4,294,458	530,120	3,180,723	1,446	0	1,446	582,169
2	0	Post-screen	85	1.8	10,570,353	658,824	5,647,059	4,235	0	4,235	4,260,235
2	39	C O	91	1.1	5,488,792	8,352	4,835,165	1,319	0	0	634,956
2	39	C I	93	1	4,347,957	17,634	3,870,968	3,441	0	645	455,914
2	39	SP O	91	1	1,190,769	17,143	483,516	0	0	0	690,110
2	39	SP I	91	1.1	4,504,176	13,407	2,901,099	2,857	0	0	1,586,813
2	58	C O	95	0.9	564,001	1,053	442,105	632	0	0	120,211
2	58	C I	94	1	911,063	5,319	829,787	1,702	0	0	74,255
2	58	SP O	97	1	845,361	1,237	288,660	825	0	0	554,639
2	58	SP I	95	1	621,685	632	204,211	0	0	0	416,842
3	0	no screen	82	0.9	1,593,171	609,756	463,415	1,220	0	1,220	518,780
3	22	C O	90	0.8	588,889	5,556	33,333	2,444	0	0	547,556
3	22	C I	91	0.7	406,813	20,440	32,967	1,758	0	0	351,648
3	22	SP O	93	0.9	1,290,107	73,118	645,161	6,237	0	0	565,591
3	22	SP I									
3	43	C O	96	0.7	161,042	3,542	45,833	0	0	0	111,667
3	43	C I	94	0.7	415,106	6,596	36,170	55,319	0	0	317,021
3	35	SP O	95	0.7	889,475	46,316	357,895	1,053	0	0	484,211
3	35	SP I									

Definitions:
Pre-screen – Prior to RS running through shaker
CO – Churned RS stored outside
SP O – Static pile RS stored outside
Post-screen – After RS has run through shaker
CI – Churned RS stored under cover
SP I – Static pile RS stored under cover

Table 4: Ranges for descriptive data of recycled sand (RS) for the 3 study farms including dry matter (DM), organic matter (OM), and bacterial information throughout the study time frame. Bacterial counts are reported on a dry weight basis.

Farm	Range	DM %	OM %	Total CFU (cfu/g)	*Streptococcus* (cfu/g)	*Staphylococcus* (cfu/g)	*Coliform* (cfu/g)	*Klebsiella* (cfu/g)	*E. Coli* (cfu/g)	Other bacteria (cfu/g)
1	Minimum	80	0.4	122,917	1,458	47,917	0	0	0	66,250
1	Maximum	98	1.1	5,469,250	4,444,444	4,946,237	105,263	52,632	9,684	5,486,426
2	Minimum	83	0.9	564,001	632	204,211	0	0	0	74,255
2	Maximum	97	1.8	33,146,068	658,824	28,539,326	7,356	0	7,356	4,453,933
3	Minimum	81	0.7	161,043	220	28,571	0	0	0	111,667
3	Maximum	96	1.2	2,622,221	716,049	790,123	55,319	449	2,444	1,116,049

Streptococcus spp., *Klebsiella* spp., *E. coli, Staphylococcus* spp. and total coliforms, ceased to occur by approximately 22 days after the sand was reclaimed.

This study did not focus on establishing a connection between bedding characteristics and udder health. But the bedding bacterial load was of interest given continuing research attempts to correlate bedding bacterial populations with teat end bacterial populations and their potential for intra-mammary infection.[1,2,4,5]

An interesting option that emerged from this study is to process RS as a SP. While the DM was lower by an average of 0.98% in SP compared to C, bacterial numbers did not differ between the groups. The clinical effect of such a small difference in DM is unknown but the economic and labor savings could be significant by processing RS in piles compared to churning. Potentially RS could be stored in large piles outside in the summer and stockpiled for winter months when processing RS for optimal DM can be challenging. Further research investigating RS quality in static piles focusing on different pile characteristics and in different regions would be beneficial as presumably storing RS in static piles is less labor intensive compared to processing RS in windrows.

Conclusion

Results from this study suggest that, in the late spring and summer in the small geographic region of southeastern Pennsylvania, dairy herds can increase DM in newly RS by 0.98% by organizing RS into one windrow and moving and turning it 3 times and by an additional 0.5% by storing RS outside. The RS stored outside has less total coliform numbers. The RS should be stored for at least 22 days prior to putting the RS back into the cow stalls to decrease the levels of commonly known mastitis bacteria. Further changes in DM and OM ceased by 15-20 days. Results from this study warrant further investigations into the clinical significance of the changes in and relationship between DM, OM, and bacterial populations, the strategy of stockpiling RS into piles, and the economic comparison between these processing techniques.

Footnotes

[a] Personal communication between Michaela Kristula and Sandra Godden. 2020.
[b] McLanahan Corporation. Holidaysburg, PA.
[c] Forestry Suppliers. Jackson, MS.
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[f] StataCorp LLC, College Station, TX.
[g] Weather Underground, weather data for Lancaster, PA

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Table 5 : Effect of processing technique (churned (C) vs static pile (SP)) on dry matter (DM) and organic matter (OM) marginal means (95% CI) in recycled sand (RS).

Variable	SP	C	
	Mean (95% CI)	Mean (95% CI)	*P*
DM (%)	92.13 (89.10 – 95.15)	93.11 (89.72-96.50)	0.004
OM (%)	0.88 (0.48 – 1.28)	0.83 (0.44-1.23)	< 0.001

Table 6: Effect of storage technique (inside (I) vs outside (O)) on dry matter (DM) and organic matter (OM) marginal means (95% CI) in recycled sand (RS).

Variable	I	O	
	Mean (95% CI)	Mean (95% CI)	*P*
DM (%)	91.68 (88.67 – 94.70)	92.18 (89.17 – 95.19)	0.026
OM (%)	0.87 (0.48 – 1.26)	0.87 (0.48 – 1.26)	0.368

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Table 7: Effect of processing technique (static pile (SP) vs churned (C)) on bacterial populations (cfu/g of dry bedding) in recycled sand (RS).

Variable	SP	C	
	Mean (95% CI)	Mean (95% CI)	*P*
Total bacteria	3,499,934 (-435,007 - 7,434,874)	2,929,491 (-1,917,106 - 7,776,087)	0.348
Streptococcus spp.	643,188 (-164,637 - 1,451,012)	241,449 (48,716 - 434,181)	0.305
Staphylococcus spp.	1,949,894 (-1,482,739 - 5,382,527)	1,744,343 (-2,403,501 - 5,892,187)	0.608
Total *coliforms*	12,380 (11,645 - 13,115)	13,822 (7,653 - 19,991)	0.603
Klebsiella spp.	332 (-2,119 - 2,782)	2,546 (559 - 4,533)	0.293
E. coli	2,944 (1,332 - 4,556)	4,404 (2,157 - 6,651)	0.116
Other bacteria	916,971 (-29,963 - 1,863,906)	931,524 (282,477 - 1,580,571)	0.928

Table 8: Effect of storage technique (inside (I) vs outside (O)) on bacterial populations (cfu/g of dry bedding) in recycled sand (RS).

Variable	I	O	
	Mean (95% CI)	**Mean (95% CI)**	***P***
Total bacteria	2,840,503 (-766,160 - 6,447,167)	3,063,864 (-1,768,755 - 7,896,483)	0.726
Streptococcus spp.	455,122 (-106,139 - 1,016,383)	352,629 (-2,797 - 708,055)	0.360
Staphylococcus spp.	1,728,810 (-1,613,067 - 5,070,687)	1,832,470 (-2,386,137 - 6,051,078)	0.823
Total *coliforms*	15,705 (9,289 - 22,121)	7,021 (4,375 - 9,666)	0.040
Klebsiella spp.	3,060 (-849 - 6,969)	40 (-1,556 - 1,636)	0.271
E. coli	4,358 (1,288 - 7,428)	2,002 (804 - 3,200)	0.212
Other bacteria	655,088 (-87,202 - 1,397,378)	860,036 (60,356 - 1,659,716)	0.103

Figure 1: Marginal means (with 95% CI) for dry matter (DM) of recycled sand (RS) by study day following the initiation of sample collection. Threshold line indicated at 95% based on the recommended minimum DM for RS prior to re-introduction[7].

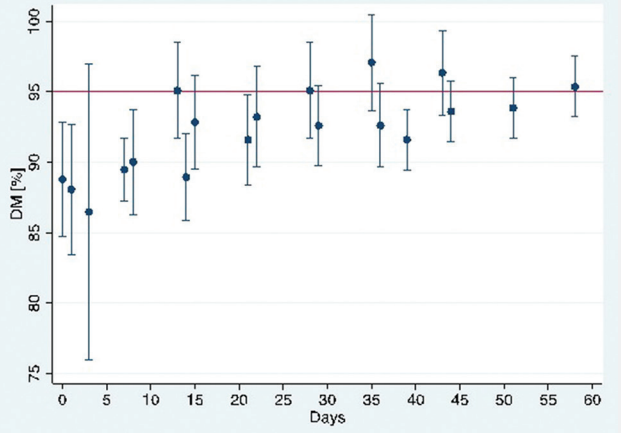


Figure 2: Marginal means (with 95% CI) for organic matter (OM) of recycled sand (RS) by study day following the initiation of sample collection. Threshold lines indicated at 1.5% based on the recommended maximum for RS prior to re-introduction[7] and 1.0%.

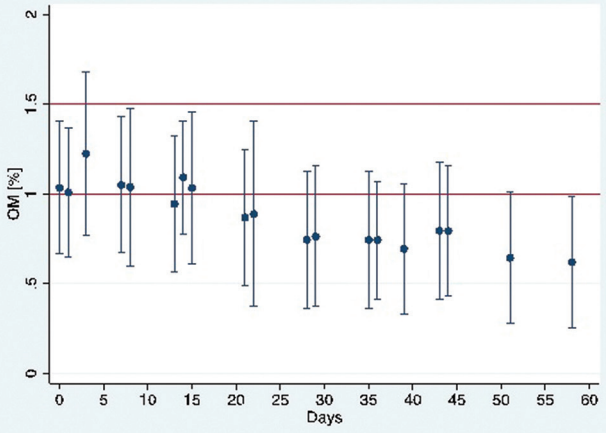
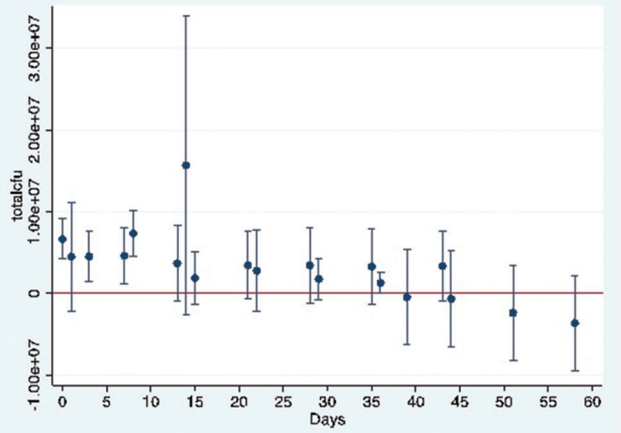
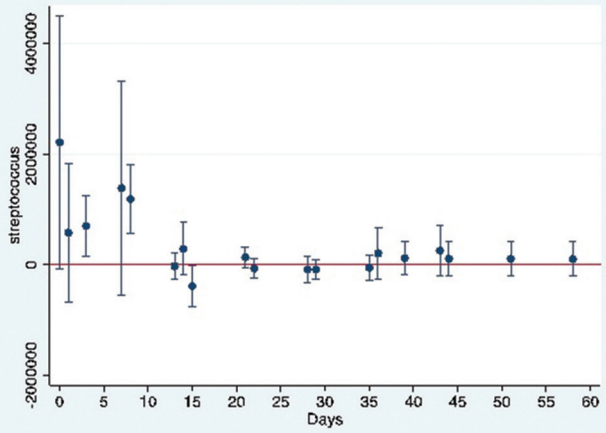


Figure 3: Marginal means (with 95% CI) for bacterial populations (cfu/gram of dry RS) of recycled sand (RS) by study day following initiation of sample collection.

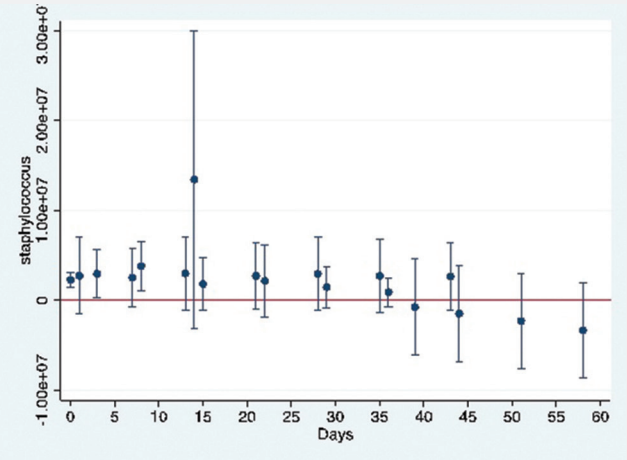


Total bacteria

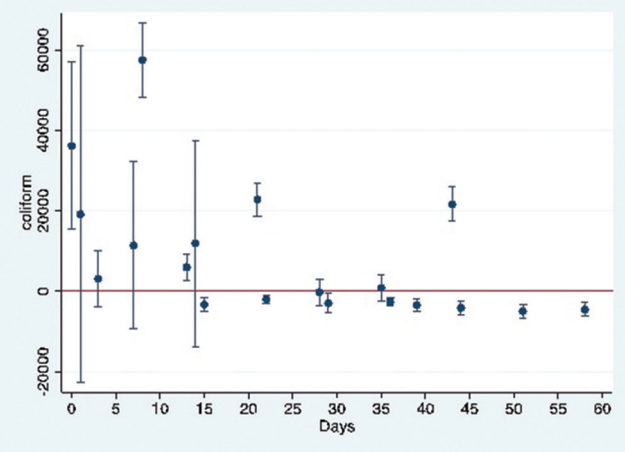


Streptococcus spp.

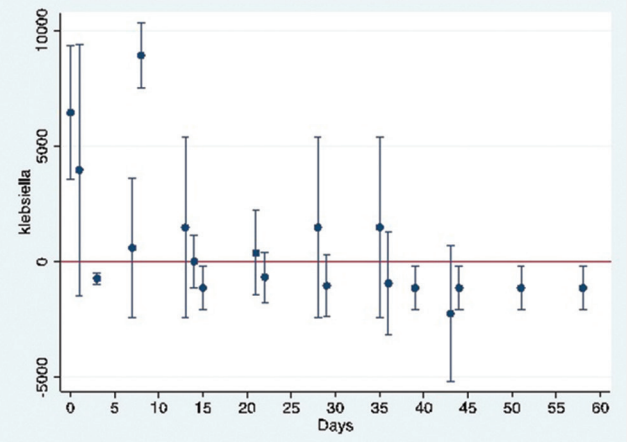
Figure 3: Continued



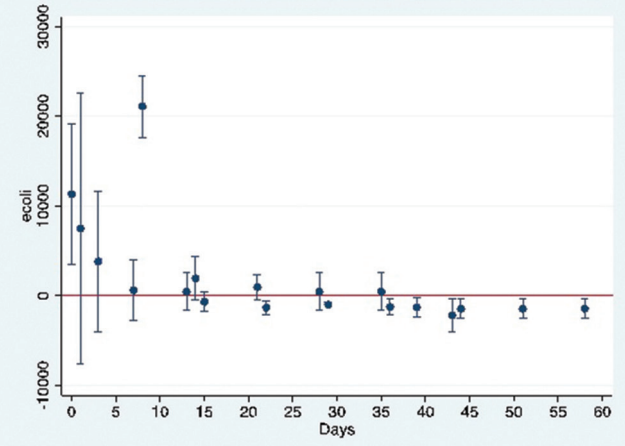
Staphylococcus spp.



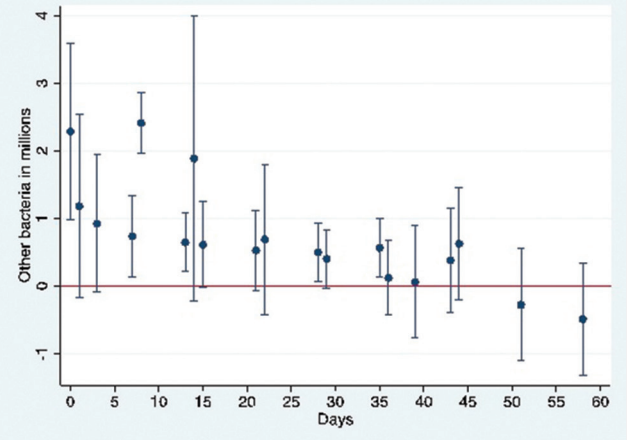
Coliforms



Klebsiella spp.



Escherichia coli



Other bacteria

