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BACTERIAL CONCENTRATIONS AND SAND USAGE IN FREE STALLS BEDDED WITH FRESH OR RECYCLED SAND

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ABSTRACT

Many dairy operations using a flush system to clean the free stall barn collect sand in a settling basin and recycle it for bedding. This practice reduces bedding cost, but it is not clear if recycled sand increases exposure to mastitis causing bacteria. A 12-month experiment was conducted to determine the effect of using fresh or recycled sand on bacterial exposure and sand usage in free stalls fitted with four commercial sand retaining devices. Free stalls were bedded each week and sand samples collected for analyses of dry matter (DM), organic matter (OM), and bacterial concentrations. The DM and OM content of fresh and recycled sand used for bedding averaged 96.6 and 96.3% DM and 0.6 and 1.1%, respectively, for fresh and recycled sand. Samples of sand collected from each of the bedding systems contained similar concentrations of DM and OM which averaged 98.0 and 98.2% DM and 1.1 and 1.1% OM, respectively for fresh and recycled sand. Concentrations of *Bacillus cereus, Bacillus subtilis, Bacillus* gram negative and gram positive species, and *Klebsiella sp.* were higher ($P < 0.05$) in recycled sand used for bedding. Concentrations of most other organisms were numerically higher in recycled sand. Concentrations of *Bacillus cereus*, *Bacillus subtilis, Bacillus* gram negative and *Staphylococcus sp.* were higher ($P < 0.05$) and *Streptococcus dysgalactiae* lower ($P < 0.01$) in free stalls bedded with recycled sand compared with fresh sand. Concentrations of *Bacillus cereus, Bacillus subtilis,* and *Streptococcus dysgalactiae* were higher ($P < 0.01$) for free stalls fitted with either Pack Mat or Sand Mizer than control or Sand Trap sand retaining devices. Although concentrations of coliform tended to be higher ($P < 0.10$) for recycled sand, no differences were observed in concentrations for sand retaining devices. Even though differences were observed between fresh and recycled sand, concentrations of all bacteria were below the threshold normally thought to cause mastitis. The amount of sand required to maintain free stalls in our study ranged from 28 lb/d for stalls fitted with the Sand Trap ($P < 0.01$) to 40.8 to 41.5 lb/d for the control stalls or those fitted with the Pack Mat or Sand Mizer. These results indicate that recycled sand can be used for bedding free stalls without risk of increasing the incidence of mastitis or somatic cell count when free stalls are properly maintained.

KEYWORDS. Dairy housing, Free stall, Bedding, Sand, Bacteria

INTRODUCTION

Sand is commonly used to bed free stalls because of it desirable characteristics (Stowell and Inglis, 2000). As an inorganic material, sand does not contain nutrients needed to support significant bacterial growth. Sand has a low water holding capacity and has a loose texture which shifts with the cow and provides good footing. Although one field study reported greater preference for free stalls fitted with mattresses (Heisner et al., 1998), other studies indicate similar preference for free stalls fitted with mattresses or bedded with sand (Thoreson et al., 2000) or

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greater preference for free stalls fitted with sand (Bernard, unpublished data). Differences in these observations may be related to the potential for sand to conduct heat away from the body of the cow, providing a cooler surface, which would be more advantageous in hotter climates.

In a recent survey of Wisconsin dairy producers (Bewley et al., 2001), sand was rated highest for udder health, cow cleanliness and cow comfort by respondents. However, respondents were less satisfied with bedding usage and manure management when sand was used for bedding compared with mattresses. To reduce the amount of sand in manure, some producers have incorporated equipment or facilities into their waste handling system to collect sand. Recycling sand collected from dairy waste would reduce bedding cost and increase return on investment in sand collecting equipment or facilities. However, data are limited for organic matter (OM) content and concentration of bacteria in recycled sand. Rates of clinical mastitis caused by environmental organisms are related to bacterial concentrations in free stall bedding (Hogan et al., 1989). No differences were detected in somatic cell counts of cows housed in free stalls bedded with recycled sand collected from a settling basin compared with that of cows housed in free stalls bedded with fresh sand (Meriwether et al., 2000). It has been suggested that pathogen densities greater than one million per gram of bedding are required before an organism would potentially cause mastitis (Bramley, 1985). Bacteria concentrations in free stalls bedded with recycled sand were not reported by Meriwether et al. (2000), so it is not known what impact the use of recycled sand had on exposure to pathogenic organisms.

Several sand retaining devices are marketed to dairy producers to reduce the amount of sand required to maintain free stalls and to maintain the desired slope in the free stall. Producers question the effectiveness of these retaining devices; if sand retaining devices promote greater retention of OM, greater bacterial concentrations in the free stall bedding could result. This trial was conducted to determine the effect of using recycled sand in free stalls fitted with sand retaining devices on bacterial concentrations in the free stall. A secondary objective was to measure the effectiveness of sand retaining devices on sand usage.

MATERIALS AND METHODS

The study was conducted as a randomized block design with a 2 x 4 factorial arrangement of treatments. Treatments included two sources of sand for bedding (fresh or recycled) and three commercial sand retaining devices plus a control. Two sections of 16 free stalls adjacent to the feed alley in a four-row free stall barn were blocked into groups of four. One section was bedded with fresh sand and the second section was bedded with recycled sand. Within each section, each block of four free stalls was randomly fitted with one of four free stall treatments. A description of the sand retaining devices is provided below.

> Pack Mat®: Multi-celled mattress filled with uniformly sized rubber crumbs with a needle-punch polypropylene cover treated to repel liquid. The mattress lies on a packed clay base with approximately 2% slope. Cover is anchored to the brisket board and bedded with approximately 3 in of sand. (Promat LTD.)
>
> Sand Trap™: Strips of tire tread cut to avoid exposing steel belting with plastic inserts held together with stainless steel cable connected to a treated 2 x 4 board. Sand Trap™ is filled with sand plus an additional 2 in of sand. (Topper, Inc.)
>
> Sand Mizer: A 32 oz. fabric placed on a packed clay bed and anchored to a treated 4 x 4. Fabric is covered with approximately 4 in. of sand. (Don Themm Enterprises, Inc.)

Free stalls were stocked at 90 to 100% of capacity throughout the year with a combination of lactating Holstein (approximately 75%) and Jersey (approximately 25%) cows. The fresh sand used for bedding was dug from a pit and delivered to the dairy by a local contractor. The recycled sand was collected from a sand settling basin after each flush. This basin is located at the end of a cross alley on the west side of the free stall barn and is approximately 2 ½ inches deep. All flush water runs through this basin before going into a gravity separator designed to collect manure solids and any sand washed out of the sand settling basin. The recycled sand was pushed out of the basin twice daily, allowed to drain, and piled outside until needed for bedding. Free stalls were bedded each week to maintain a constant amount of sand in each stall. The amount of sand used on each block of stalls was recorded weekly from November 16, 2000 through May 15, 2001. Free stalls were raked twice daily to remove any manure or wet sand. All stalls were raked twice daily to maintain the desired shape of the free stall surface. The free stall barn is equipped with fans and a high pressure mister system that operates when the ambient temperature is above 75E F and the relative humidity is below 80%.

Samples of sand used for bedding were collected each week during bedding. A sample of sand was collected each week from each block of free stalls from the back 25% of the free stall, prior to rebedding. All samples were frozen until analyses of dry matter (DM) and ash content (AOAC, 1990). One set of samples each month was analyzed for bacterial concentrations at the All Florida Veterinary Laboratory, Inc. (Archer, FL). Data were analyzed for significance using PROC MIXED procedure of SAS (1989). The model included effects of sand source, sand retaining device, sample data and the appropriate interactions. Significance was declared when P # 0.05.

RESULTS AND DISCUSSION

The DM concentration of the fresh and recycled sand used for bedding was similar (Table 1). Recycled sand contained more ($P < 0.001$) OM and had greater concentrations of *Bacillus cereus* ($P < 0.01$), *Bacillus subtilis* ($P < 0.01$), *Bacillus* gram negative ($P < 0.001$) and gram positive ($P < 0.01$), and *Klebsiella sp.* ($P < 0.01$) than fresh sand. Concentrations of coliform tended to be higher ($P < 0.10$) and concentrations of most other bacteria were numerically higher ($P > 0.10$) in recycled sand than fresh sand. Concentrations of *Bacillus* gram positive were higher ($P < 0.05$) during May, June, and July than other months of the year for both fresh and recycled sand, but the increase was greater for the recycled sand. A similar increase ($P < 0.05$) was observed for *Coryne* sp. from May through September.

Sand is an inorganic material generally considered to be lacking in substrates needed to support bacterial growth, but bacteria were isolated from the fresh sand. The fresh sand used in this study was dug from a pit and had not been washed prior to use. Concentrations of bacteria and OM observed in the fresh sand were minute and would not be considered to be a problem. The settling basin used to collect the recycled sand effectively removed most of the manure based on OM concentrations. As noted previously, all flush water is channeled through this settling basin and the recycled sand is removed from the settling basin twice daily. This differs greatly from some systems used on farms that are not as effective in removing manure solids from the recycled sand. Although differences in bacteria concentrations were detected, bacteria were present in concentrations of less than 100,000 cfu/g of sand. The reason for increased concentrations of *Bacillus* gram positive and *Coryne* sp. during the summer months is not clear, but may be related to ambient temperatures. Both fresh and recycled sand were piled outside in the sun and exposed to normal temperatures which range from a high of 85 to 95E F to lows in the upper 60s to low 70s.

There were no interactions of sand source and sand retaining device, so main effect means are reported and discussed. The fresh sand collected from the free stalls each week had slightly lower concentrations of DM ($P < 0.05$) and OM ($P < 0.001$) than the recycled sand (Table 2). It is doubtful that these differences have any biological significance given the minute difference in actual values. Concentrations of *Bacillus cereus* ($P < 0.001$), *Bacillus subtilis* ($P < 0.05$), *Bacillus* gram negative ($P < 0.05$), and *Staph. sp*. ($P < 0.05$) were higher and *Streptococcus dysgalactiae* ($P < 0.01$) and yeast ($P < 0.05$) were lower in recycled sand than fresh sand collected from free stalls.

There was an interaction of sampling time and sand source for concentrations of DM ($P < 0.001$), OM ($P < 0.001$), *Bacillus cereus* ($P < 0.001$), and *Staph. sp*. ($P < 0.01$). The DM content of the recycled sand was 2% lower at the beginning of the trial in November 2000 and was 5% lower on one sampling date in July, 2001 than fresh sand, but the DM concentration was similar for both sands at all other sampling dates. Recycled sand had higher concentrations of OM than fresh sand except during early February and August through early September when OM concentrations were similar. Concentrations of *Bacillus cereus* in recycled sand increased significantly in June, peaked in July and then declined through November 2001 whereas concentrations in the fresh sand only increased slightly during July 2001. *Staph. sp*. concentrations were higher in recycled sand during March, but were similar to that of fresh sand the rest of the year.

Hogan et al. (1989) monitored bacterial concentrations in free stall bedding materials for a year in commercial dairy herds in Ohio. Mean concentrations of gram-negative bacteria, coliform, *Klebsiella* species, and streptococci bacteria isolated from free stalls bedded with washed sand were less than 10^7 cfu/g of sand. These researchers reported that concentrations of bacteria were similar throughout the year. Concentrations of bacteria in free stalls bedded with either fresh or recycled sand were lower than those reported by Hogan et al. (1989). Although differences were observed between fresh and recycled sand and interactions of sand source and sampling time occurred, concentrations of bacteria were well below the concentration of one million bacteria per gram of bedding thought to potentially cause mastitis (Bramley, 1985).

Concentrations of DM and OM were not affected by sand retaining devices in the free stalls and averaged 98.1% DM and 1.4% OM (Table 3). Minor differences in bacterial concentrations were observed for *Bacillus cereus* ($P < 0.01$), *Bacillus subtilis* ($P < 0.01$), *Proteus* sp. ($P < 0.001$) and *Step. dysgalactiae* ($P < 0.01$). In general, concentrations of these bacteria were highest in free stalls fitted with Pack Mat and lowest for the control free stalls or those fitted with Sand Trap, but differences in concentrations were relatively small. Interactions ($P < 0.01$) of sand retaining device and sampling date were observed for *Bacillus cereus*, *Bacillus subtilis*, *Coryne* sp*., Proteus* sp., and *Pseudomonas aeruginosa*. Concentrations of these bacteria were similar during the initial months of the study and then increased during the warmer months before declining in cool weather. The increase tended to be greater in free stalls fitted with Pack Mat compared with Sand Trap. In the case of *Coryne* sp., concentrations were higher for Sand Trap during March and then declined for the duration of the study whereas concentrations isolated in the control free stalls and those fitted with Sand Mizer were much higher from June through September than the remainder of the study.

The amount of sand required to maintain free stalls was lower ($P < 0.01$) for those stalls bedded with fresh sand (35.7 lb/stall/d) compared with those bedded with recycled sand (39.5 lb/stall/d). The recycled sand had a more crystalline texture which was probably a result of the flush washing out any clay residues that were in the fresh sand. When hand raking the free stalls, those bedded with recycled sand were easier to level than those bedded with the fresh sand.

Control free stalls required 41.5 lb/stall/d of sand to maintain the desired fill and slope. The amount of sand required to maintain the free stalls was lowest ($P < 0.01$) for stalls fitted with the Sand Trap (28.0 lb/stall/d) compared to all treatments. Free stalls fitted with the Pack Mat and Sand Mizer required 40.1 and 40.8 lb/stall/d, respectively.

CONCLUSION

Results of this study indicate that recycled sand can be used to bed free stalls without greatly increasing exposure to potential pathogens. Although differences in bacteria concentrations were observed, concentrations did not approach the one million per gram concentration that has been suggested to increase the incidence of mastitis. The data do suggest that concentrations of some bacteria, *Bacillus* organisms and *Coryne* sp., will increase during the summer when temperatures are higher. Although concentrations did not exceed one million, additional attention to free stalls may be needed to minimize potential bacterial growth. Use of sand retaining devices did not greatly alter bacteria concentrations and only the Sand Trap reduced the amount of sand required to maintain free stalls.

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Table 1. Analysis of fresh and recycled sand used to maintain free stalls.

Item	Fresh	Recycled	SE	P <
DM, %	96.6	96.3	0.3	0.48
Ash, % of DM	99.4	98.9	0.09	0.001
OM, % of DM	0.6	1.1	0.09	0.001
	---------------- cfu/g of sand ----------------			
Bacillus cereus	3,818	22,898	4,010	0.01
Bacillus subtilis	1,455	9,855	1,806	0.01
Bacillus gram negative	5,271	79,471	10,480	0.001
Bacillus gram positive	32,363	60,663	5,615	0.01
Coliform	0	400	156	0.10
Coryne sp.	23,091	31,191	4,317	0.22
Nocardia sp.	182	0	141	0.34
Proteus sp.	3,817	0	2,976	0.36
Strep. uberis	3,273	4,873	1,329	0.42
Staph. sp.	1,364	7,164	3,815	0.31
Mold	182	982	418	0.21
Yeast	0	800	432	0.22
Klebsiella sp.	1,273	9,673	2,648	0.05

Table 2. Analysis of fresh and recycled sand collected from free stalls.

Item	Fresh	Recycled	SE	P <
DM, %	97.97	98.21	0.08	0.05
Ash, % of DM	98.86	98.36	0.03	0.001
OM, % of DM	1.14	1.64	0.03	0.001
	----------------- cfu/g of sand -----------------			
Bacillus cereus	14,519	22,734	1,692	0.001
Bacillus subtilis	9,931	12,126	1,009	0.05
Bacillus gram negative	79,888	121,749	9,598	0.05
Bacillus gram positive	272,217	331,123	177,042	0.21
Coliform	40,668	21,062	10,373	0.29
Coryne sp.	76,496	85,731	6,896	0.34
Nocardia sp.	2,607	2,785	339	0.82
Proteus sp.	58	96	67	0.31
Pseudomonas	94	53	51	0.31
Strep. dysgalactiae	5,406	1,498	749	0.01
Strep. faccalis	5,854	1,942	1,827	0.16
Strep. uberis	26,999	23,437	2,385	0.49
Staph. sp.	18,630	30,573	3,367	0.05
Mold	3,028	3,700	598	0.48
Yeast	2,525	1,304	409	0.04
Klebsiella sp.	2,649	4,171	576	0.20

Table 3. Analysis of sand in free stalls equipped with sand retaining devices.

	Control	Pack Mat	Sand Mizer	Sand Trap	SE	P <
DM, %	98.10	98.15	97.95	98.14	0.11	0.52
Ash, % of DM	98.63	98.58	98.56	98.66	0.04	0.25
OM, % of DM	1.37	1.42	1.44	1.34	0.04	0.25
	------------------------ cfu/g of sand --------------------------					
Bacillus cereus	14,674[b]	22,875[a]	21,531[a]	15,427[b]	2,404	0.01
Bacillus subtilis	9,489[b]	14,292	10,512[b]	9,822[b]	1,434	0.01
Bacillus gram negative	128,902	87,875	106,960	79,538	13,640	0.25
Bacillus gram positive	332,973	368,708	254,729	250,272	33,422	0.10
Coliform	43,530	18,625	47,861	13,445	14,742	0.27
Coryne sp.	87,191	86,833	86,611	63,820	9,800	0.15
Nocardia sp.	3,524	2,792	2,444	2,023	483	0.27
Proteus sp.	83[ab]	250[a]	0[b]	0[b]	92	0.00
Pseudomonas	26	0	85	185	73	0.13
Strep. dysgalactiae	2,794[b]	5,458[a]	5,506[a]	50[c]	1,064	0.01
Strep. faccalis	3,774	667	8,372	2,790	2,596	0.14
Strep. uberis	22,212	29,500	24,770	24,391	3,390	0.49
Staph. sp.	26,812	30,833	23,394	17,367	4,785	0.13
Mold	4,256	3,917	3,330	1,950	850	0.18
Yeast	1,521	2,292	2,073	1,770	581	0.80
Klebsiella sp.	4,021	2,666	2,293	4,659	819	0.35

[a,b]Means within a row with unlike superscripts differ (P < 0.10)