Methods of processing recycled manure solids bedding on Midwest dairy farms I: Associations with bedding bacteria counts, milk quality, udder health and milk production

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Abstract

This observational study investigated the relationships between method of processing recycled manure solids (RMS) bedding and 1) bacteria counts in RMS bedding, 2) bacteria counts in bulk tank milk, and 3) udder health and milk production. A convenience sample of 29 farms in Minnesota and Wisconsin were recruited to represent 4 RMS processing systems; green (GRN, n = 7), anaerobic digestion (DIG, n = 6), composting (COM, n = 4), and hot air drying (DRY, n = 12). Premises were visited twice (summer/winter) to collect fresh bedding and bulk tank milk (BTM) samples for culture, test-day records, and a herd management questionnaire.

Coliform and *Klebsiella* spp. counts were lower, or tended to be lower in DRY, COM or DIG versus GRN RMS samples. Counts of *Streptococci* and Strep-like organisms (SSLO) were statistically or numerically lower in COM or DRY as compared to GRN or DIG RMS samples. Coliform and SSLO counts in BTM were lower, or tended to be lower, in DIG, COM or DRY as compared to GRN RMS samples. Test-day average linear score, proportion of cows with infection, and proportion of cows with chronic infection was lower, or tended to be lower, in herds using DRY or COM versus GRN RMS bedding. Average 305ME was higher, or tended to be higher, for herds using DRY versus GRN or DIG RMS. Adoption of COM or DRY processing could allow producers to mitigate the negative impacts of high bacteria levels in GRN RMS bedding, though more research is needed.

Key words: recycled manure solids bedding, bacteria, milk quality, udder health

Introduction

Bedding material selection and management can impact cow health and well-being through a variety of mechanisms including comfort and lying time, foot and leg health, and udder health.[1] Because cows spend 12 to 14 hours per day laying down, bedding is an important source of teat end exposure to environmental mastitis pathogens.[2] Multiple studies have reported that bedding bacteria counts (BBC) are associated with bacterial load on the teat end and with risk for intramammary infection (IMI), whether measured as clinical mastitis or subclinical mastitis (i.e. elevated SCC).[3-12] In a recent observational study of 168 herds from 17 U.S. states, Patel et al., (2019) identified 4 major groups of mastitis pathogens – total coliforms, *Klebsiella* spp., environmental *Streptococci* and Strep-like organisms (SSLO), and non-aureus *Staphylococci* (*Staph*. spp.) – as being associated with impaired udder health.[9]

While traditional bedding materials such as wood shavings, straw and sand are still prevalent, the dairy industry has observed a rapid increase in adoption of recycled manure solids (RMS) over the past couple of decades. The national 2014 NAHMS survey reported that RMS was used as bedding on 5% of U.S. dairy farms, but accounting for 26% of cows, given that RMS are more likely to be adopted by larger herds.[13] Reported reasons for producer interest in RMS include availability, economics, and improved cow comfort.[14] Green (GRN) or raw RMS is most commonly produced by putting raw (undigested) slurry through a screw press to separate solids from liquid. The resulting GRN solids do not receive any processing prior to being used as bedding under cows. Despite its perceived advantages, one major risk in using GRN RMS bedding is exposure of the teat end to higher levels of environmental mastitis pathogens, resulting in increased risk for impaired udder health and milk quality, as compared to the use of inorganic or organic non-manure bedding materials.[9]

While many Midwest dairies use either GRN pressed solids, some others first process slurry through an anaerobic digester (DIG) prior to solid-liquid separation. Anaerobic digestion is a microbial process that degrades organic matter to produce biogas and digestate. While designs vary, plug flow systems are commonly used on Midwest dairy farms. Contents from each successive addition, move through the long, narrow digester vessel as a plug with a retention time typically between 15-30 days. While temperatures vary, they frequently are mesophilic 86°F (30°C) to 108°F (42°C). If destined to be used as bedding, effluent is often put through a screw press after exiting the digester.

On many farms, GRN pressed solids will be used as bedding. However, some herds have adopted other secondary processing techniques in an effort to lower BBC and minimize detrimental impacts on udder health (relative to GRN). One such option is composting (COM). In the Midwest, composting is typically done mechanically, with pre-pressed solids passing through a slowly rotating drum that mixes solids with hot air, heating contents to over 150°F for approximately 24 hr. A different secondary processing technique adopted on some farms is the use of hot air rotary drum dryers (DRY). Dryers take about 12-15 minutes to process pre-pressed solids. Solids are exposed to approximately 700°F (371°C) at entry and 130°F (54°C) at exit, and may be between 45-50% DM when exiting the dryer.

Testimonials from individual producers suggest that the adoption of DIG, DRY or COM processing can reduce BBC in RMS, resulting in better udder health characteristics as compared to use of GRN RMS bedding. However, studies to investigate this assertion are extremely limited. One observational study that included 33 U.S. herds, reported that herds using hot air dryers had a lower clinical mastitis incidence than herds using GRN bedding.[9] However, this study included only 8 DRY systems and only 2 of these were in the Midwest. As such, Midwest-based studies are needed to compare the efficacy and impact of different RMS processing systems on BBC and udder health. The objectives of this Midwest-based study were to identify if associations exist between method of processing RMS and 1) BBC in fresh RMS bedding, 2) microbial quality of bulk tank milk, and 3) udder health parameters and milk production.

Materials and methods

Herd enrollment, data and sample collection

This cross-sectional observational study was conducted using a convenience sample of 29 free stall premises in Minnesota and Wisconsin. Sample size was limited by budget and availability of appropriate farms within the region. Farms were recruited to achieve representation from 4 different processing systems including GRN, DIG, COM and DRY. Premises were visited twice, once in Aug-Sept, 2019, and again in January 2020, to collect bedding samples, electronic herd records, and to complete a management questionnaire describing facilities and management, including but not limited to manure and bedding management, milking procedures and mastitis control practices. Milk production metrics captured from the most recent DHIA test-day preceding each herd visit included average 305ME (lb/cow). Udder health metrics captured included test-day average linear score (AVLS), the proportion of cows with an intramammary infection (IMI), the proportion of cows with new IMI (NIMI) and the proportion of cows with a chronic IMI (CRON). Intramammary infection was defined as LS ≥ 4.0, new IMI was defined as LS changing from < 4.0 to ≥ 4.0 in the last 2 tests (adjusted for a 30-day interval between tests), and CRON was defined as a LS ≥4.0 on the last 2 tests. The monthly cumulative incidence of clinical mastitis (CLXM) was also recorded for the 30-day period preceding sample day. Producers were also invited to collect duplicate bulk tank milk (BTM) samples daily for 3 consecutive days within ±7 d of when technicians visited the farm. Milk samples were collected either from the on-farm bulk tank or, for farms that directly loaded into tankers from a single dairy, from the tanker truck as the milk was unloaded at the milk processing facility. Samples were frozen immediately after collection and stored at -20°C until transported on ice to the laboratory for analysis.

On the day of the herd visit, study technicians collected post-processed ready-to-use (RTU) bedding samples. Wearing clean disposable neoprene gloves, the sampler collected RTU bedding from the bedding storage area by collecting grab samples from the top 5 cm of bedding from 15 random locations in the pile. After mixing in a clean bucket, composite samples were transferred to two 1-quart (946 mL) Ziploc®[a] bags. The age of the RTU bedding (number days that it had been in the pile) was recorded. Though not the focus of this manuscript, used bedding was also collected from a representative number of stalls. Briefly, used bedding was collected as a grab sample from the top 5 cm of bedding in the back one-third of 15 randomly selected stalls or locations in the yard, representing up to 5 lactating pens, and then mixed well in a clean bucket before transferring into 2 1-quart Ziploc bags. Samplers avoided manure pats. If more than 5 lactating pens existed, then samples were collected from 5 pens housing early or peak lactation cows and heifers. The used bedding sample age was recorded as the days since fresh bedding was most recently added to the stall or resting area. Following collection, all bedding samples were immediately placed on ice, then frozen at -20°C within 8 hr. of collection.

Laboratory analysis

Bedding cultures

Frozen bedding samples were transported on ice to the Laboratory for Udder Health (Saint Paul, MN) for analysis. After thawing at room temperature, 50 cm^3 of bedding material was firmly packed into a 50 cm cup, transferred into a new Whirl-Pak®[b] bag, mixed with 250 mL of sterile water[c], and left to stand for 10 min. Serial 10-fold dilutions of the samples were made using sterile water[c] and dilutions plated onto MacConkey agar (gram-negative bacteria selection) and colistin naladixic acid agar[d] (gram-positive bacteria selection) plates and incubated overnight at 37°C. For the MacConkey plates, lactose fermenting (pink) colonies were counted as coliform bacteria and all other colonies were counted as non-coliform gram-negative bacteria. Colonies with a confluent appearance on MacConkey agar were identified to the genus level using a MALDI Biotyper[e], and colonies identified as *Klebsiella* spp. were counted and reported as a percentage of total coliform count. For colistin naladixic acid plates, colony morphology in conjunction with catalase reaction and Gram stain were used to differentiate colonies of *Staphylococcus* spp., SSLO, and *Bacillus* spp. Total bacteria count and counts of *Bacillus* spp., coliforms, *Klebsiella* spp., non-coliform gram-negatives, *Staphylococcus* spp. and SSLO were recorded as colony-forming units per cubic centimeter of wet bedding with a minimum limit of detection being 25 cfu/cm^3 (reported as zero). Duplicate bedding samples were also submitted to other laboratories for determination of various bedding characteristics (e.g. dry matter percentage, organic matter, nutrients) and results reported in a companion manuscript.

Bulk tank milk culture

After thawing to room temperature, BTM and a 10-fold dilution of the BTM sample were plated onto MacConkey, Factor[f] (selective for gram-positive bacteria), and Focus[g] (selective for SSLO bacteria) media plates and incubated for 2 d at 37°C. Lactose fermenting (pink) colonies on MacConkey medium were counted and reported as coliform bacteria. All beta-haemolytic colonies on Focus® media were counted and identified to the species level using a MALDI Biotyper, as these colonies were suspect for *Streptococcus agalactiae*. All colonies on Focus® media that were not identified as *S. agalactiae* were counted and recorded as *Streptococc*i spp. or Strep-like organisms (SSLO). Beta-haemolytic colonies on Factor® media were counted and identified to the species level using a MALDI Biotyper, and those with a confidence score ≥2.0 for *S. aureus* were counted and reported as such. Non-hemolytic colonies of *Staphylococcus* spp. (based on colony morphology, catalase reaction, or Gram stain) were counted and reported as non-aureus *Staphylococci* (NAS). For *Mycoplasma* spp., 0.1 mL of BTM was swabbed across the entire surface of a *Mycoplasma* agar plate and incubated for 7 d in a 7% CO_2 incubator at 37°C.

Plates were examined for *Mycoplasma* spp., and colonies were counted by a trained microbiology technician. For each BTM sample, total counts of coliforms, SSLO, and NAS were recorded as colony-forming units per milliliter of milk. Culture results were recorded as positive or negative for *Staph. aureus*, *Strep. agalactiae* and *Mycoplasma* spp. The minimum limit of detection for the BTM culture protocol was 5 cfu/mL.

Data analysis

Descriptive analysis

Data were entered into an Excel[h] database. Statistical analysis was performed using SAS[i] software, with the farm being the unit of analysis and 2 observations (summer/winter) available for all but 1 farm. Information recorded for each observation included herd ID, season, sample collection date, information captured in the herd management questionnaire, DHI test results, CLXM, and culture results for RTU RMS and BTM samples. Descriptive statistics were calculated to evaluate the distribution of data and to identify missing data. Because BBC (cfu/cm^3) and BTM culture results (cfu/mL) are not normally distributed, results were transformed ($\log^{10}$) before further analysis. In addition to describing measures for BBC, BTM culture results, and udder health and production, descriptive statistics were also generated to describe dry matter (DM) percent of RTU bedding, herd characteristics, lactating cow housing, bedding and manure management, milking procedures, and routine mastitis control practices.

Objective 1: Association between RMS processing method and bedding bacteria counts

Univariable linear regression (PROC MIXED) was initially used to describe unconditional relationships between bedding processing system (explanatory variable of interest: GRN, DIG, COM, DRY) and each of the following BBC count variables in RTU bedding ($\log^{10}$, cfu/cc): coliform bacteria, *Klebsiella* spp., SSLO and *Staph.* spp. Additional potential confounders or explanatory variables investigated included season, herd size, housing, bedding and manure management practices, parlor procedures, and routine mastitis control practices. Any explanatory variable that was unconditionally associated with one or more of the BBC outcomes of interest at $P < 0.20$ was offered into the final multivariable models investigating the relationship between RMS Processing method and BBC. The only variable forced into these multivariable models was RMS processing method. Herd was included as a random effect in all models. A backward stepwise variable selection process was used, with the least significant variables being removed one by one until all remaining predictors had $P \leq 0.10$. At each removal step, the potential for confounding was investigated by examining the effect of each explanatory variable on the estimate of the association between BBC and udder health (UH) parameters. A variable was identified as a confounder and retained in the model if its inclusion resulted in >15% change in the estimate of the effect of BBC on the UH outcome. Final models were selected based on lowest Akaike's Information Criteria, and final model fit was assessed by plotting the deviance residuals. Overall significance was declared for Type 3 *P* value at $P \leq 0.05$ and a tendency (trend) at $0.05 < P \leq 0.10$. However, the Bonferonni correction factor was used to adjust the final critical *P* value for multiple contrasts between the 4 RMS processing systems (critical $P \leq 0.0083$; trend $P \leq 0.017$).

Objective 2: Association between RMS processing method and bacteria counts in bulk tank milk

Mixed linear regression analysis, using the same process as what was previously described for objective 1, was used to describe the relationship between bedding processing system (explanatory variable of interest) and the following 3 dependent continuous variables in BTM ($\log_{10}$, cfu/mL): coliform counts, SSLO counts and NAS counts. Mixed logistic regression (PROC GLIMMIX) was used to describe the relationship between bedding processing system and odds of a bulk tank milk sample being positive for either *Mycoplasma spp.* or *Staph. aureus*.

Objective 3: Association between RMS processing method, udder health and milk production

Mixed linear regression, using the same process as what was previously described for objective 1, was used to describe the relationship between bedding processing system (explanatory variable of interest) and the following dependent variables from the test-day preceding each herd visit: AVLS, IMI, NIMI, CRON, CLXM and 305ME.

Results

Herd characteristics

Of the 29 participating dairies, 8 were in Minnesota and 21 in Wisconsin. Processing systems included GRN (n = 7), COM (n = 4), DIG (n = 6) and DRY (n = 12). One of the GRN dairies was visited only once (summer), while all other premises were visited twice (summer/winter). As such, 29 and 28 herd visits (57 total) were conducted in summer and winter months, respectively. Because one of the DRY premises transported RTU bedding to a second premise within the same farm system, only 1 (not 2) bedding sample observations per season were included in the analysis for this farm system. It should be noted that of the 12 premises using DRY RMS, 4 used a dryer as the sole processing step, while 8 used some combination of 2 processing steps: 7 farms first processed slurry through an anaerobic digester prior to drying the pressed solids, and 1 farm first processed pressed solids through a mechanical composter prior to drying. However, because analysis showed no important difference in outcomes when comparing single-step drying vs combination (2-step) systems, the observations from all 12 premises were combined into the DRY category for the final analysis.

The median (range) number of milking cows in herds using GRN, COM, DIG or DRY RMS bedding was: GRN 969 (235 to 2,558), 469 (390 to 875), 2,260 (1,537 to 4,455) and 2,420 (511-5,467), respectively. The predominant breed in study herds included Holstein (93%), Jersey (3.5%) and Crossbred (3.5%). The average (SD; range) days in milk, parity, and 305ME on test-day was 183 (11; 160 to 201) days, 2.1 (0.2; 1.8 to 2.5) lactation, and 28,010 (3,024; 19,697 to 32,846) lbs, respectively. Mean days in milk and parity did not differ among herds for the 4 processing methods (results not shown). Herds reported having used their current RMS processing system an average of 6.3 (± 4.1; 1 to 15.0) years. Ventilation systems for lactating cow barns included cross-ventilated (3.8%), natural (66.7%), tunnel (26.4%), or mixed (3.8%), with air quality subjectively described by the operator to be either poor or fair (14.8%) or good (85.2%). Stall surface was described as deep bedded (70.4%), shallow bedded (concrete = 7.4%; mattress = 14.8%), or mixed (7.4%). For the purposes of later analysis, stall surface

was categorized as deep bedded or shallow (included mixed systems). Twelve premises (41%) reported using hydrated lime as a bedding conditioner top dressed on RMS. Alleys were reportedly scraped an average of 4.8 (± 4.35; 2 to 20) times per day, with manure and contaminated bedding scraped from the back of stalls an average of 2.7 (± 0.73; 0 to 3) times per day. Fresh bedding was added to stalls an average of 3.7 (± 2.0; 0.7 to 7.0) times per week. Milking frequency was 3X (88.9%) or 2X (7.4%), with one herd using an automatic milking system (3.7%). In describing parlor routines, manual forestripping was conducted in 92.3% of herds, with an automated teat scrubber used in 26% of parlors. Predipping, postdipping and use of individual towels to dry teats prior to unit attachment was described in 100%, 100% and 81% of parlors, respectively. Milk cultures were reported to be routinely done for clinical mastitis cases, high SCC cows, or at freshening in 55.6%, 11.2% and 22.2% of herds, respectively. Blanket or selective dry cow therapy was reported in 84.6% and 15.4% of herds, respectively, with 92.2% of herds routinely using a teat sealant at dry-off. Mean (±SE) DM% was higher for DRY (44.6 ± 2.16) as compared to GRN (30.8 ± 2.5), DIG (32.5 ± 2.7) or COM (30.3 ± 3.63) RTU bedding samples ($P = 0.009$).

Objective 1: Relationship between RMS processing method and bedding bacteria counts (Figure 1).

Fifty-six RTU bedding samples were cultured (DRY = 23, COM = 8, DIG = 12, GRN = 13). Although results varied by bacteria group, RMS processing system was associated with counts of coliform bacteria, *Klebsiella* spp. and SLLO in RTU samples. Specifically, the adjusted mean coliform count (± SE) was lower in herds using DRY solids, and tended to be lower in COM or DIG, as compared to herds using GRN solids, but with no difference between DRY, COM and DIG (DRY = 1.97 [0.36], COM = 2.32 [0.61], DIG = 2.77 [0.50], GRN = 4.20 [0.48], Type 3 $P = 0.009$). *Klebsiella* spp. counts were lower in herds using DRY, COM or DIG as compared to GRN solids, but with no difference between DRY, COM and DIG (DRY = 0 [0.17], COM = 0 [0.19], DIG = 0.12 [0.24], GRN = 1.89 [0.23], Type 3 $P < 0.0001$). Counts of SSLO were lower in herds using COM as compared to GRN or DIG, and were lower or tended to be lower in herds using DRY as compared to GRN or DIG solids, respectively, but with no difference between DRY and COM, or between DIG and GRN (DRY = 3.42 [0.38], COM = 2.37 [0.64], DIG = 5.06 [0.53], GRN = 5.78 [0.50], Type 3 $P = 0.0005$). There was no statistically significant association between processing method and *Staph* spp. bacteria counts in RTU bedding (DRY = 0.76 [0.45], COM = 1.55 [0.77], DIG = 1.58 [0.63], GRN = 2.47 [0.59], Type 3 $P = 0.17$). After controlling for herd as a random effect, no main effect covariates other than processing method, were retained in any of the final models for BBC.

Objective 2: Relationship between RMS processing method and bacteria counts in bulk tank milk (Figure 2).

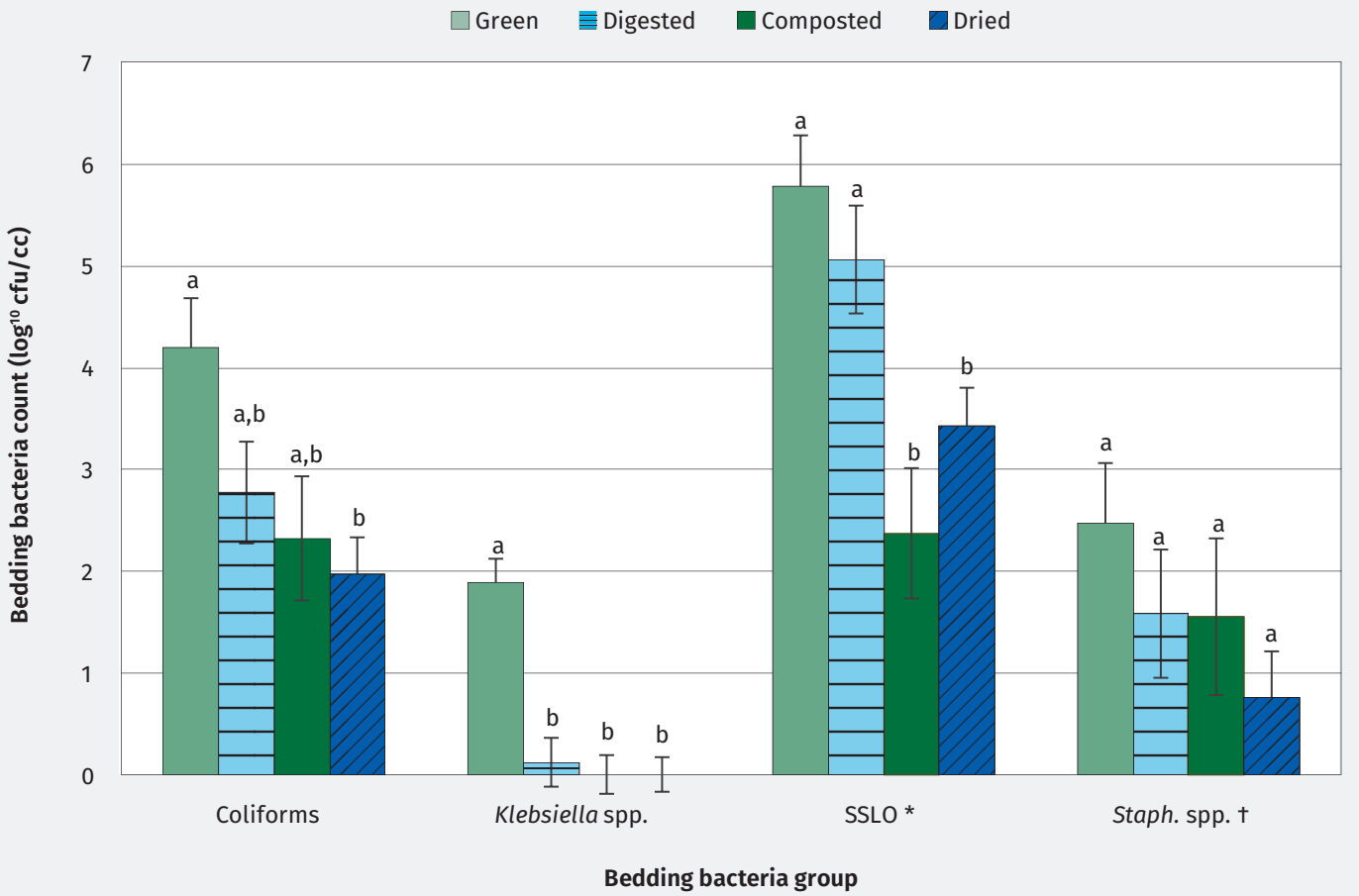
A total of 44 BTM samples were submitted for culture (DRY = 16, COM = 5, DIG = 10, GRN = 13). Of these, 40.9%, 6.8%, and 0% were positive for *Staph. aureus*, *Mycoplasma* spp., or *Strep. agalactiae*, respectively. When using mixed linear regression, the adjusted mean (SE) coliform count in BTM was highest in herds using GRN (2.38 [0.25]) as compared to DIG (1.54 [0.28]), COM (0.97 [0.42]) or DRY (1.72 [0.23]) RMS (Type 3 $P = 0.04$). However, after adjusting the critical P value for multiple contrasts, only COM tended to have a statistically lower coliform count than GRN. Counts of SSLO in BTM were higher in herds using GRN (3.31 [0.15]) as compared to herds using either DIG (2.61 [0.18]) or COM (2.31 [0.25]) RMS. There was no difference in SSLO in BTM between DIG, COM and DRY (2.94 [0.14]) RMS (Type 3 $P = 0.01$). There was no difference in NAS counts in BTM among the 4 processing systems (GRN = 1.66 (0.15); DIG = 1.96 (0.17); COM = 1.82 (0.24); DRY = 1.71 (0.13) (Type 3 $P = 0.59$). Mixed logistic regression showed no relationship between RMS processing system and odds for presence of *Staph aureus* in BTM (GRN = 53.9% (7 of 13); DIG = 20.0% (2 of 10); COM = 20.0% (1 of 5); DRY = 50.0% (8 of 16); Type 3 $P = 0.29$) or *Mycoplasma* spp. in BTM (GRN = 0% (0 of 13); DIG = 0% (0 of 10); COM = 0% (0 of 5); DRY = 18.8% (3 of 16); Type 3 $P = 1.0$). After controlling for herd as a random effect, no other covariates, other than processing method, were retained in any of the final models for bacteria in raw BTM.

Objective 3: Relationship between RMS processing method, udder health and milk production (Table 1; Figure 3).

Not all study herds were on a routine DHIA testing program or recorded clinical mastitis data. Furthermore, recording systems changed within some herds between the summer and winter testing periods. As a consequence, of the 29 herds visited in summer months, DHIA test day data and clinical mastitis event records were only available for 23 and 20 herds, respectively. Similarly, of the 28 herds visited in winter months, DHIA test day data and clinical mastitis event records were only available for 20 and 18 herds, respectively. Although results varied by method, RMS processing system was associated with AVLS, IMI, CRON and 305ME, but not NIMI or CLXM. Specifically, the adjusted mean AVLS (± SE) was lower in herds using DRY, and tended to be lower in COM, as compared to herds using GRN solids, but with no difference between DRY and COM (DRY = 2.12 [0.17], COM = 2.15 [0.26], DIG = 2.63 [0.22], GRN = 2.89 [0.16], Type 3 $P = 0.006$). The proportion of cows with IMI was lower in herds using DRY, and tended to be lower in COM, as compared to GRN solids, but with no difference between COM and DRY, or between DIG and GRN (DRY = 17.31 [2.32], COM = 14.46 [4.64], DIG = 23.82 [2.97], GRN = 25.92 [2.15], Type 3 $P = 0.015$). The proportion of cows with CRON was lower in herds using DRY, and tended to be lower in COM, as compared to DIG or GRN solids, but with no difference between COM and DRY, or between DIG and GRN (DRY = 8.66 [1.61], COM = 8.59 [3.28], DIG = 14.95 [2.06], GRN = 16.10 [1.50], $P = 0.003$). The incidence of NIMI was not different between the 4 types of processing systems (DRY = 9.02 [1.55], COM = 9.78 [2.88], DIG = 13.10 [2.02], GRN = 12.84 [1.47], Type 3 $P = 0.15$). The monthly cumulative incidence of CLXM was not different among herds using the four processing methods (DRY = 4.13% [0.98], COM = 1.26% [2.58], DIG = 3.93% [1.14], GRN = 3.60% [1.07], Type 3 $P = 0.76$). Finally, Avg305ME (lbs/cow) was higher or tended to be higher for herds using DRY as compared to GRN or DIG solids, respectively, but with no difference between DRY versus COM, or between DIG versus GRN (DRY = 30,381 [886], COM = 28,113 [1,773], DIG = 27,595 [1,123], GRN = 25,770 [949], Type 3 $P = 0.02$).

Additional explanatory variables describing season, the frequency of scraping manure/wet bedding from the back of stalls (times per day), the producer's perception of ventilation quality (good versus poor or fair), and use of a bedding conditioner, were retained in 1 or more of the models for various udder health or milk production outcomes. When considering season, the proportion of cows with IMI (%) was estimated (SE) to be 2.1% (0.65) ($P = 0.005$) higher in summer months than in winter months. Season was also associated

Figure 1: Bacteria counts in ready-to-use recycled manure solids bedding from Midwest herds using different recycled manure solids bedding processing systems. Green solids = Light green bar; Digested = Horizontal light blue lines; Composted = Dark green bar; Dried = Diagonal blue lines.



[a,b] Superscripts within bacteria group: Statistical difference $P \leq 0.0083$; (Bonferonni adjustment of critical P values to account for multiple contrasts)

* SSLO: *Streptococci* or streptococci-like organisms

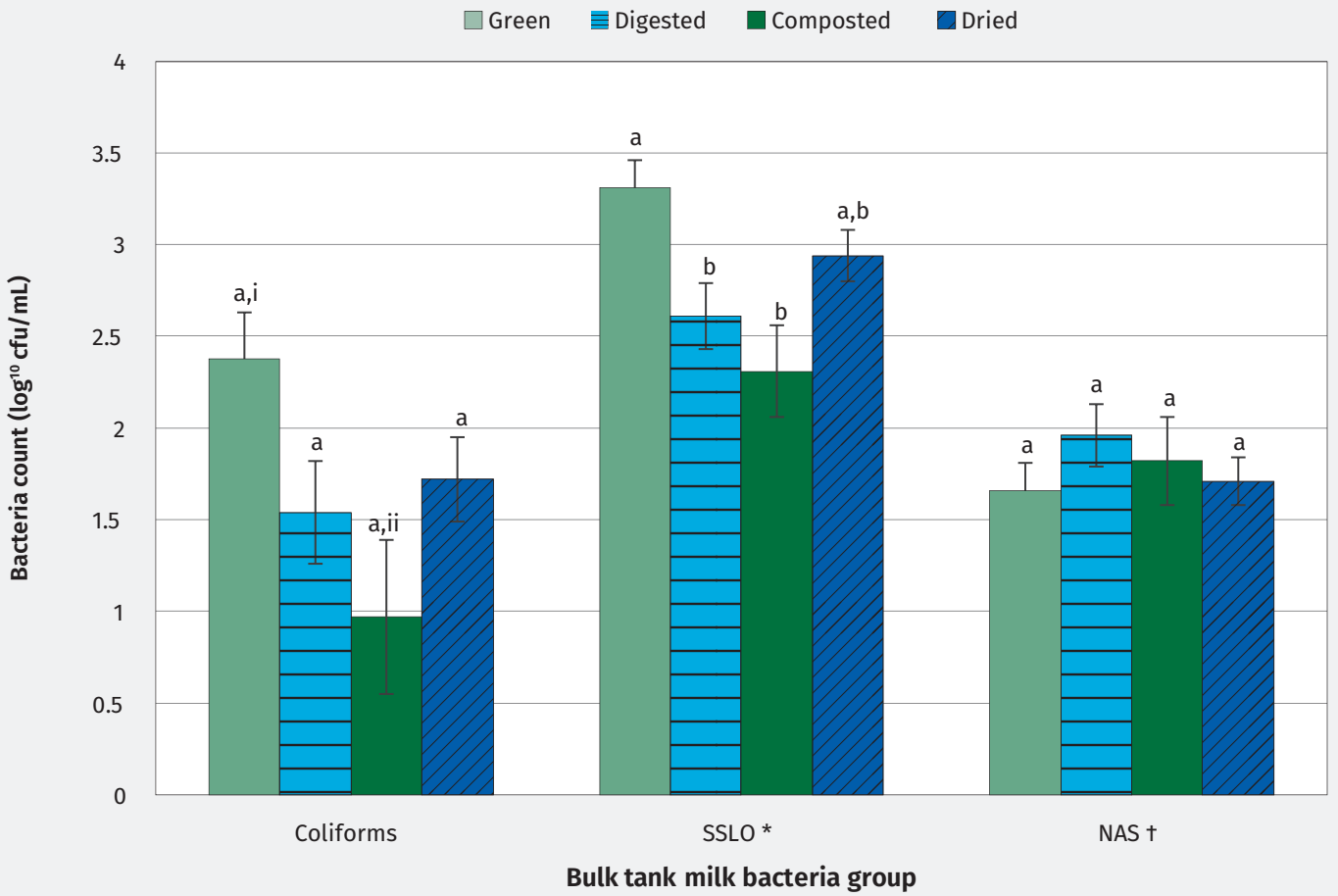
† *Staph* spp.: *Staphylococci* spp.

with 305ME, with an estimate (SE) of 586 (154) lb more milk produced in winter versus summer months ($P = 0.001$). When considering the frequency of scraping manure/wet bedding from the back of stalls, each additional scraping event per day was associated with a 3.25% (1.69) reduction in proportion of cows with IMI ($P = 0.07$), and with a 2.71% (1.18) reduction in proportion of cows with CRON ($P = 0.03$). The producer's assessment of ventilation quality in the lactating barn tended to be important in several models, with poor or fair (versus good) ventilation being associated with a 0.45 (0.26) ($P = 0.096$) increase in AVLS, a 6.81% (3.50) ($P = 0.07$) increase in proportion of cows with IMI, a 4.38% (2.40) ($P = 0.08$) increase in NIMI, and a 4.91% (2.44) ($P = 0.06$) increase in proportion of CRON cows. The use of lime as a bedding conditioner in stalls tended to be associated with a 2.56% (1.34) ($P = 0.07$) reduction in CLXM, but was not associated with any other udder health or milk production outcome.

Discussion

Certain mastitis pathogens may be ubiquitous in some bedding materials, while other pathogens, such as *Escherichia coli* or *Klebsiella* spp., may arrive after contamination of bedding by fecal material, water or feed.[15] Because raw RMS is derived from manure, and because it provides high levels of organic matter and moisture, it can support high levels of bacterial growth, and therefore pose a greater risk to udder health as compared to other organic and inorganic bedding materials.[9,16,17] This is the first study, to our knowledge, designed to compare BBC, milk quality, udder health, and milk production in Midwest herds using 4 different processing systems to produce RMS bedding: GRN, DIG, COM or DRY. Study strengths include the sampling of 29 commercial herds in MN and WI, and sampling in both summer and winter months. However, several limitations must be acknowledged. Budget constraints and availability of local dairies using RMS limited sample size, particularly for COM (n = 4). Although only large Midwest dairies were sampled, study herds were typical of Midwest herds that have adopted RMS bedding. We believe our findings can be extrapolated to other similar humid regions (e.g. the northeast U.S. and most of Canada), but may not apply to regions with very different climates. Finally, because this was an observational study, causal inferences for detected associations must be viewed with caution. While we did attempt to capture and control for possible confounding variables in our models (e.g. herd characteristics, facilities,

Figure 2: Bacteria counts in raw bulk tank milk from Midwest herds using different recycled manure solids bedding processing systems. Green solids = Light green bar; Digested = Horizontal light blue lines; Composted = Dark green bar; Dried = Diagonal blue lines.



Significance for RMS processing methods (Bonferonni adjustment of critical *P* values to account for multiple contrasts)

[a,b] Superscripts within bacteria group: Statistical difference $P \leq 0.0083$

i,ii Superscripts within bacteria group: Tendency for difference $P \leq 0.017$

* SSLO: *Streptococci* or streptococci-like organisms

† NAS: Non-aureus *Staphylococci* spp.

manure and bedding management, parlor procedures, routine mastitis control practices), it is always possible that unmeasured factors could confound the presence or magnitude of associations observed in these types of studies. Despite these limitations, we documented important differences in BBC, milk bacteria counts and udder health outcomes among the 4 RMS processing methods evaluated.

Objective 1: Relationship between RMS processing method and bedding bacteria counts

As expected, BBC, including coliforms, *Klebsiella* spp., and SSLO, were highest in GRN samples while processing reduced or tended to reduce BBC. The BBC of DIG samples were intermediate relative to GRN and the COM and DRY samples. Digested samples had lower coliform and *Klebsiella* spp. counts than GRN, while SSLO counts were similar. The latter observation regarding DIG samples is consistent with results from 7 farm-scale digesters that were sampled on a biweekly basis for 9 months and demonstrated that pathogen inactivation was highly variable.[18] In the latter study, pathogen reduction (log-removal values), was up to 2 times less than pathogen reduction values previously reported primarily from bench top studies.[18] In addition, population exponential decay coefficients at mesophilic temperatures varied dramatically among pathogens ranging from 3.8 ± 4.8/day for *Escherichia coli* to 0.65 ± 0.38/day for *Streptococcus* spp.[18] The results indicated less than optimal removal of pathogens from RMS and the authors concluded that full-scale anaerobic digestion of cattle manure requires optimization for pathogen inactivation, and that future research should focus on identifying potential causes (e.g., overloading, poor mixing and poor temperature control) of this suboptimal performance.

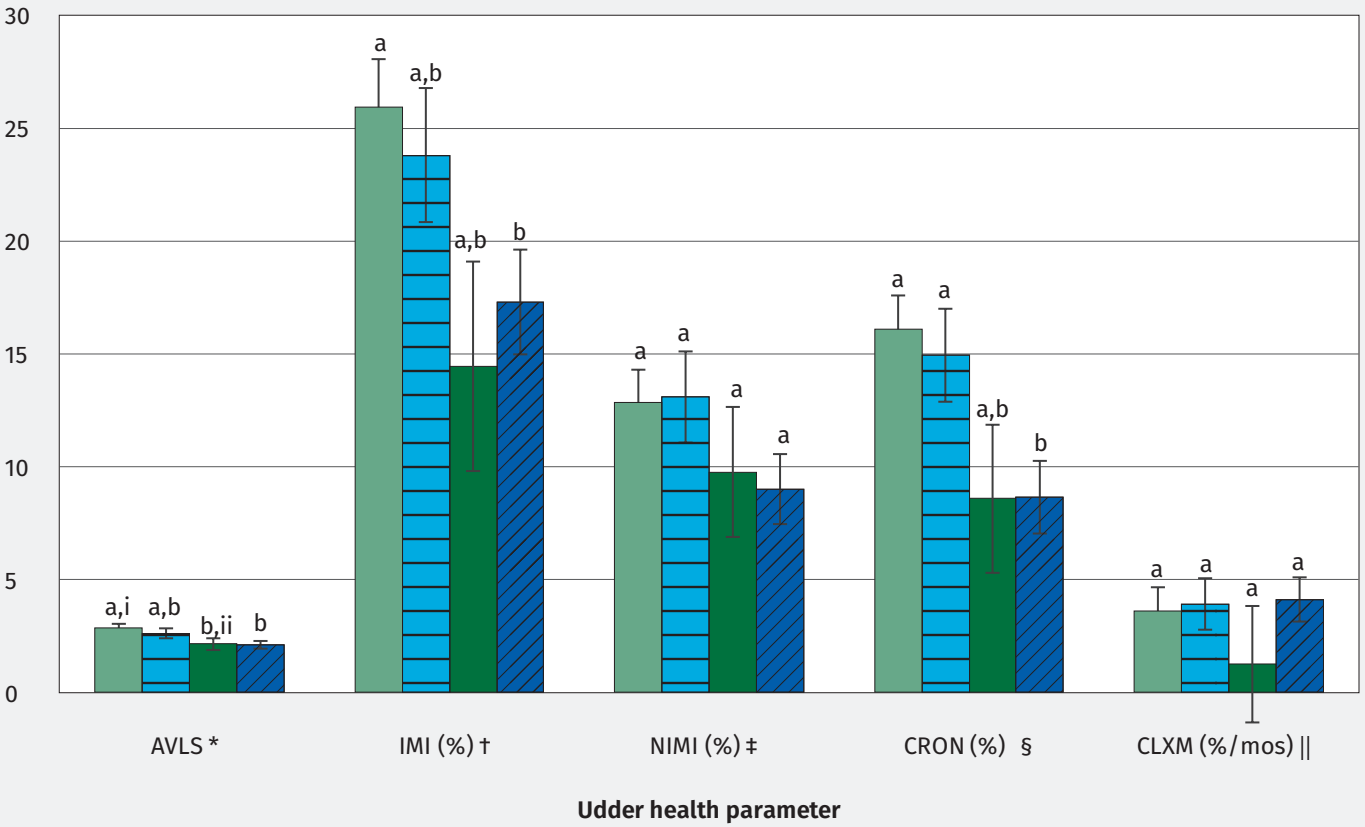
In general, the COM and DRY samples had lower BBC than the GRN samples. Composting can be accomplished by a variety of methods including windrow composting, static pile composting or aerated pile composting, with temperatures ranging between 40-65°C.[19] A more common approach use at dairies in the Midwest and Northeast is mechanical (drum) composting due to frequent precipitation and high ambient humidity. A lab-based experiment reported fairly similar performance of 4 composting methods (static windrow, turned

Table 1: Final multivariable models describing the relationship between RMS processing method and test-day udder health outcomes.

Explanatory variable (level)	Udder health parameter (Dependent variable)							
	Model 1. AVLS *		Model 2. Cows with IMI (%) †		Model 3. Cows with NIMI (%) ‡		Model 4. Cows with CRON (%) §	
	Estimate (SE), Type 3 *P* value	Adjusted mean (SE)	Estimate (SE), type 3 *P* value	Adjusted mean (SE)	Estimate (SE), Type 3 *P* value	Adjusted mean (SE)	Estimate (SE), Type 3 *P* value	Adjusted mean (SE)
Intercept	2.67 (0.17)	.	29.96 (4.89)	.	10.65 (1.52)	.	20.72 (3.40)	.
Processing ‖								
Composted	-0.74 (0.28), 0.006	2.15 (0.26) [b,ii]	-11.46 (4.8), 0.01	14.46 (4.64) [a,b]	-3.06 (3.02), 0.15	9.78 (2.88) [a]	-7.51 (3.38), 0.003	8.59 (3.28) a,b
Digested	-0.26 (0.24)	2.63 (0.22) [a,b]	-2.10 (3.28)	23.82 (2.97) [a,b]	0.27 (2.22)	13.10 (2.02) [a]	-1.15 (2.27)	14.95 (2.06) a
Dried	-0.78 (0.21)	2.12 (0.17) [b]	-8.61 (2.84)	17.31 (2.32) [b]	-3.81 (1.90)	9.02 (1.55) [a]	-7.44 (1.96)	8.66 (1.61) b
Green	Ref	2.90 (0.16) [a,i]	Ref	25.92 (2.15) [a]	Ref	12.84 (1.47) [a]	Ref	16.10 (1.50) a
Season								
Summer			2.09 (0.65), 0.005	21.42 (1.96) [a]				
Winter	.	.	Ref	19.33 (1.99) [b]	.	.	.	.
Ventilation								
Fair or Poor	0.45 (0.26), 0.096	2.68 (0.25) [i]	6.81 (3.50), 0.07	23.78 (3.43) [i]	4.38 (2.40), 0.08	13.38 (2.34) [i]	4.91 (2.44), 0.058	14.53 (2.39) i
Good	Ref	2.22 (0.09) [ii]	Ref	16.97 (1.40) [ii]	Ref	8.99 (0.93) [ii]	Ref	9.62 (0.98) ii
Stall Scrape Freq (x/day)	.	.	-3.25 (1.69), 0.07	.	.	.	-2.71 (1.18), 0.03	.

Significance for RMS processing methods (*P* value adjusted for multiple contrasts):
[a,b] Superscripts within column: Statistical difference $P \leq 0.0083$
[i,ii] Superscripts within column: Tendency for difference $P \leq 0.017$
Significance for other covariates in models:
[a,b] Superscripts within column: Statistical difference $P \leq 0.05$
[i,ii] Superscripts within column: Tendency for difference $0.05 < P \leq 0.10$
* AVLS. Herd average linear score on test-day.
† IMI: Proportion of cows on test-day with Linear Score ≥ 4.0.
‡ NIMI: Proportion of cows with LS < 4.0 on previous test-day and LS ≥ 4.0 on current test-day.
§ CRON: Proportion of cows with LS ≥ 4.0 on both the previous and current test-day.
‖ RMS processing method forced in all models.

Figure 3: Udder health in Midwest herds using different processing systems to produce recycled manure solids bedding. Models adjust for random herd effect and some other covariates (see Table 1). Green solids = Light green bar; Digested = Horizontal light blue lines; Composted = Dark green bar; Dried = Diagonal blue lines.



Significance for RMS processing methods (Bonferonni adjustment of critical P values to account for multiple contrasts)

[a,b] Superscripts within udder health outcome parameter: Statistical difference P≤0.0083

[i,ii] Superscripts within udder health outcome parameter: Tendency for difference P≤0.017

* AVLS: Average linear score on the most recent test-day preceding each herd visit

† IMI: Proportion of cows on test-day with Linear Score ≥ 4.0.

‡ NIMI: Proportion of cows with LS < 4.0 on previous test-day and LS ≥ 4.0 on current test-day.

§ CRON: Proportion of cows with LS ≥ 4.0 on both the previous and current test-day.

‖ CLXM: Cumulative incidence of clinical mastitis for the 30-day period preceding sample day

windrow and drum composting for 24 or 72 hr), but concluded that drum composting for 24 hr provided the best option in terms of product quality, temperature reached, decreased bacterial numbers, and emitted airborne contaminants.[19]

The specific mechanism(s) which to reduce bacteria counts in RMS have not been thoroughly delineated. Although the direct effect of heat on bacterium viability undoubtedly plays a central role, the indirect effects of secondary processing methods, such as increasing DM% of the RMS, could also reduce BBC, or at least delay bacterial proliferation in bedding in stalls. High DM% in bedding has been associated with decreased BBC and improved udder health measures.[9,17] Decades ago, it was reported that dried composted manure was satisfactory material for free stall bedding provided it was dried properly before application.[20] Researchers have since suggest that a realistic goal for fresh RMS is ≥ 35% DM.[21-23] As such, processing strategies that increase DM% may represent one important opportunity to reduce BBC in RMS bedding. In the current study, DM% was significantly higher for DRY samples as compared to GRN, DIG or COM RTU bedding samples.

However, coliform and *Klebsiella* spp. counts were not different between DIG, COM and DRY samples, and SSLO counts were not different between COM and DRY samples. As such, it remains to be determined if any apparent effect of DM% on BBC is either partially or totally confounded by the direct effect of exposure to heat in some of these systems. Put another way, it will be important to sort out whether heating or drying (or both, independent of one another) play a role in affecting BBC in RMS. This question will be explored in a companion manuscript. Finally, it may be possible that other bedding characteristics, such as nutrient/substrate availability for bacteria, could be altered by processing, thereby impacting BBC. We demonstrated that COM and DRY reduced BBC, but our study was not designed to identify the relative contributions of heat and drying. Additional research is needed to evaluate the importance and interdependence of these factors.

Objective 2: Relationship between RMS processing method and bacteria counts in bulk tank milk

Bacteria in BTM arrive from a variety of sources including contaminated milking equipment/system, milk from an infected mammary gland, or contaminated teat skin.[3,8,11] Higher bacteria loads in bedding will be transferred to teat skin. This, when coupled with insufficient cleaning and disinfection of either teat ends or milking equipment prior to milking, could increase BTM bacteria count. However, the relationship between use of RMS bedding (versus other bedding materials) and bacteria levels in BTM remains nebulous, as equivocal results have been reported. For example, 2 recent observational studies, 1 with 325 Wisconsin herds, and 1 with 125 U.K. herds, reported no association between use of RMS (vs. sand or other organic materials) and BTM bacteria counts.[21,24] However, a recent study of 70 herds in Ontario, Canada, reported average raw BTM bacteria counts were higher in herds using RMS than in herds using wood products, straw or sand bedding.[17] Similarly, SSLO counts in BTM have been reported to be higher in herds using RMS compared to herds using new sand or other organic materials.[9]

In the current study, SSLO counts in BTM were statistically lower, or tended to be lower, in herds using DIG, COM or DRY, as compared to herds using GRN RMS bedding. Similarly, coliform counts tended to be lower in COM (vs GRN) solids. Processing method was not associated with NAS counts in BTM. These findings are consistent with the fact that we also observed lower coliform counts in DIG, COM and DRY RMS bedding, and lower SSLO counts were detected in COM and DRY RMS bedding.

One possible implication of our study findings is that adopting DIG, COM or DRY processing could reduce bacterial contamination of BTM for herds using RMS bedding. This could be of specific interest to processors that require low bacteria counts in milk to improve the quality and shelf life of the products they produce. Although not measured in the current study, some (though not all) studies have associated the use of RMS bedding with increased counts of mesophilic and thermophilic spore-forming bacteria in BTM, which can cause spoilage and reduced quality of dairy products.[25-27] It might be possible that 1 or more of the secondary processing systems evaluated in this study could modify the counts of spore-forming bacteria in BTM. However, to our knowledge, this hypothesis has not yet been evaluated. Although additional work is needed to delineate the impacts of BBC and to resolve the specific impacts of RMS processing on BBC and bacteria counts in BTM, clearly producers should pay attention to sufficient cleaning and sanitation of both teat skin and equipment prior to milking.

Objective 3: Relationship between RMS processing method, udder health and milk production

While equivocal findings exist, most studies indicate that herds using RMS generally exhibit impaired udder health compared to herds using other organic or inorganic bedding materials.[1,9,28-31] Furthermore, a considerable herd-to-herd variation is evident within the subset of herds using RMS bedding.[9] For example, an observational study with 168 herds included 33 RMS herds and 25% of these RMS herds achieved reasonably good udder health, with test-day average LS ≤ 2.2, prevalence of IMI ≤ 17%, and incidence new IMI ≤ 7.0%.[9] This supports the concept that management strategies and other mitigating factors exist that allow producers to achieve good results despite using higher risk RMS bedding. Results of the current study indicate that adoption of secondary processing systems such as COM or DRY could contribute to improved udder health in some herds using RMS bedding.

Herds that used DIG RMS did not experience improved udder health or milk production as compared to herds using GRN RMS bedding, despite having lower coliform and *Klebsiella* spp. counts in RMS samples. It is unclear if the similar SSLO and *Staph* spp. counts in GRN and DIG RMS contributed to this lack of improvement in DIG herds. Regardless, the results indicate that using DIG as the sole secondary processing technique for RMS might not reliably reduce bedding pathogen loads sufficiently unless DIG performance is optimized. Additional research is needed to identify constraints, such as overloading, poor mixing and poor temperature control, which influence effectiveness of DIG.

Herd characteristics or management strategies that were statistically associated, or tended to be associated, with one or more measures of improved udder health included: 1) good (vs poor or fair) ventilation, 2) increased frequency of scraping the backs of stalls, and 3) use of an alkalinizing bedding conditioner in stalls. While some of these attributes or practices, such as good ventilation or frequent raking of stalls, have long been recommended by milk quality experts,[23] direct evaluations of their beneficial effects are extremely limited. While the application of some bedding conditioners has been demonstrated to reduce BBC in stalls for less than 1 day, direct evidence has been lacking to demonstrate that this results in improved udder health.[32-34] Our relatively small study may have had insufficient power to identify relationships between udder health metrics, and other commonly recommended practices for manure and bedding management, parlor procedures and mastitis control practices.

Although our results are encouraging, particularly for the adoption of COM or DRY secondary processing systems, they are from a single, relatively small, observational study that included only 4 COM dairies. Furthermore, only 1 form of RMS composting (mechanical drum composting) was evaluated. Although mechanical drum composting is common in the Midwest, other regions with less frequent precipitation and lower relative humidity use other methods to compost RMS. Additional (ideally larger) studies are needed to determine if our results can be replicated and to evaluate the relative efficacy of other RMS composting methods to reduce BBC and enhance udder health. Our results also support the need for investigations to determine how to improve and optimize consistency of the DIG product. Other questions that need to be investigated include the impact of processing on the risk of recycling zoonotic pathogens (e.g. *Salmonella* spp.) in RMS, and relative efficacy of combining different processing methods (e.g. DIG + DRY) on BBC in RMS. Finally, an economic analysis is needed to determine the return on investment and cost-benefit to the producer that choses to adopt specific secondary RMS processing to produce alternative bedding materials.

Conclusions

Midwest herds using secondary processing systems such as COM or DRY had reduced BBC, reduced coliform and SSLO bacteria counts in BTM, improved udder health, and higher milk production (DRY only) as compared to herds using GRN RMS bedding. Herds using DIG RMS had reduced coliform and *Klebsiella* spp. counts in RTU bedding samples and reduced coliform and SSLO counts in BTM, but did not exhibit improvements in udder health or milk production as compared to herds using GRN RMS. Additional study of the impacts of RMS processing on udder health and dairy economics is needed.

Endnotes

[a] Ziploc, SC Johnson, Racine, WI
[b] Whirl-Pak, Nasco, Fort Atkinson, WI
[c] Sterile water, Becton Dickinson and Company, Franklin Lakes, NJ
[d] Colistin naladixic acid agar, Becton Dickinson and Company, Franklin Lakes, NJ (gram-positive selection)
[e] MALDI Biotyper, Bruker Daltonics, Billerica, MA
[f] Factor agar, University of Minnesota, St. Paul, MN (gram-positive selective agar)
[g] Focus agar, University of Minnesota, St. Paul, MN (selective for SSLO bacteria)
[h] Excel, Microsoft Corp., Redmond, WA
[i] SAS version 9.4, SAS® Institute Inc., Cary, NC

Acknowledgements

The authors express their sincere gratitude to the owners and managers of the 29 herds who assisted us with this study, as well as Dr. Brian Stampfl and the staff at Valley Veterinary Clinic (Seymour, WI) who generously allowed us temporary use of their freezers during sampling visits to the Green Bay area. Many thanks also go to our team of student technicians including Nicole O'Sell, Patrice Sorenson, Jenna Frank, Rylee Black, Delaney Cox, Natalie Oestrich, Lindsay Miller and Nick Pitlick.

Funding

This work was funded by a grant from the Upper Midwest Agricultural Safety and Health Center (UMASH) with partial funding from an unrestricted gift from McLanahan Corp. (Hollidaysburg, PA).

Conflicts of Interest

The authors do not have any conflicts of interest to declare.

Author contributions

Dr. Godden contributed to conception and design, acquisition of data, analysis and interpretation, drafting of the manuscript, and approval of the final version to be published.

Dr. Royster, Dr. Crooker and J. Timmerman contributed to conception and design, acquisition of data, manuscript revision, and approval of the final version to be published.

Dr. Peña Mosca contributed to acquisition of data, manuscript revision, and approval of the final version to be published.

Abbreviations

AVLS	Average linear score
BBC	Bedding bacteria counts
BTM	Bulk tank milk
CLXM	Clinical mastitis incidence
COM	Composted
CRON	Chronic intramammary infection
DIG	Digested
DRY	Dried
DM	Dry matter
GRN	Green
IMI	Intramammary infection
NAS	Non-aureus *staphylococci* spp.
NIMI	New intramammary infection
RMS	Recycled manure solids
SSLO	*Streptococci* spp. and Strep-like organisms
Staph	*Staphylococci* spp.
RMS	Recycled manure solids
RTU	Ready-to-use

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