DYNAMICS OF BACTERIAL POPULATIONS
IN BEDDING MATERIALS

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for the Degree Master of Science

by

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INTRODUCTION

There is increasing concern for the incidence of environmental mastitis which is caused by coliform organisms and species of streptococci other than Streptococcus agalactiae. This concern is based on the trend towards more confinement housing of dairy cows and the increased control of Staphylococcus aureus and S. agalactiae infections (12,17,24). Confinement housing of dairy cows results in increased cow density per unit area and the usage of bedding materials which may support bacterial growth. Thus, cows have an increased exposure to environmental pathogens. Level of exposure of the teat end to mastitis pathogens is a major factor relating to the incidence of new infection (16,24). The more efficient control of S. aureus and S. agalactiae infections may result in an increased number of uninfected quarters to be infected with environmental pathogens (55).

A relationship has been shown to exist between the level of exposure of teat ends to bacteria and the incidence of new IMI (33,44). Bedding materials are in close and prolonged contact with the teat apex and are capable of supporting bacterial populations. Rendos et
al. (51) have indicated that the number of bacteria in bedding materials may influence the number of new IMI. Therefore, a study of bedding materials and the dynamics of bacterial populations in the beddings would be useful in understanding environmental mastitis.
LITERATURE REVIEW

The estimated economic loss to the dairy industry in the United States due to mastitis is $182 per cow or greater than $2 billion annually to the dairy industry (30). The estimated impact of mastitis in Ohio is $69 million. Major economic loss is decreased milk production which accounts for 65 to 70% of the total monetary loss (8,30). Natzke et al. (40) showed a decrease of 1600 lbs. milk per lactation or $224 for one infected quarter in a study over a 3 year period involving 24 herds. Natzke et al. (42) reported that losses of milk production in quarters designated as 1, 2 and 3 by the California Mastitis Test were .19, .29 and .67 kg/qtr/milking. Additional losses resulting from mastitis are related to milk quality, such as antibiotic residue and nutritional quality of milk, premature culling of dairy cows, veterinary costs and additional labor costs (8,30).

Mastitis is an inflammation of the mammary gland usually caused by bacterial infection which alters the composition of mammary secretions. Mastitis results from an interaction of the cow, its environment and
microorganisms (33). Major mastitic pathogens consist of *Staphylococcus aureus*, *Streptococcus agalactiae*, coliform organisms and other streptococci. The pathogenic properties of bacteria which cause mastitis vary with individual organisms (15).

*Staphylococcus aureus* which can be found on the skin of teats, udder and human hands, is invasive and adheres readily to the ductal and acinar epithelial cells, thus colonizing the parenchyma of the mammary gland (19,24,50). *Staphylococcus aureus* produces several toxins and the alpha toxin is most damaging causing vasoconstriction which leads to necrosis of tissues (24). *Streptococcus agalactiae* is considered to be an obligate parasite of the lactating mammary gland, however, the organism can survive for a short period of time outside the mammary gland (24). *Streptococcus agalactiae* also adheres readily to the ductal and acinar epithelial cells (19). The primary source of infection of *Staphylococcus aureus* and *Streptococcus agalactiae* is infected quarters, therefore, they are considered to be contagious and can be transmitted during the milking process from an infected quarter to an uninfected quarter. Teat dipping and dry cow therapy can markedly reduce the incidence of *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections (IMI) in a dairy herd (19,23).

Environmental pathogens such as coliform bacteria and streptococci species other than *Streptococcus agalactiae* are
present throughout the cows environment. Coliform bacteria are a part of the normal flora in the bovine gut and can also be isolated from manure, dirt, bedding, polluted water and alleyways of the barn (19,50). Coliform bacteria are gram negative fermentative bacilli in the family Enterobacteriaceae and include the lactose positive fermenters Escherichiae, Klebsiella and Enterobacter which are indicated most frequently as the cause of mastitis (19). Endotoxin is an integral part of the cell wall of coliform organisms and is released into the mammary gland following death of the bacterium. The release of endotoxin results in an inflammatory process, thus causing an influx of neutrophils. Endotoxin is thought to be involved in acute coliform mastitis (24). Environmental streptococci, such as S. uberis and S. dysgalactiae, are ubiquitous in the cows environment and can be found on the cows lips, vagina, skin of belly and udder (50). Primary source of coliform and environmental streptococcal organisms is the environment. An increased level of exposure of cows to bacterial populations can influence the rate of IMI.

The bovine mammary gland has several bacterial defense mechanisms. Prevention of bacterial invasion is a function of the teat canal which is the primary line of defense (23,24). Increased numbers of bacteria near the teat orifice increases the probability of organisms
gaining access to the gland and subsequently causing infection (11,16,19,24). Infection results from the passage of pathogens through the teat duct and into the teat cistern (19,24). Patency of the teat sphincter muscle affects the ease with which bacteria may enter the gland (17,49). Physical changes which occur in the teat throughout lactation such as lengthening and dilating with increasing lactation age may influence the rate of IMI (34,35). The cellular defense mechanism, polymorphonuclear neutrophils, and non-cellular defense mechanisms such as immunoglobulins, lactoferrin and lactoperoxidase/thiocyanate/hydrogen peroxide system, within the gland provide further protection against infection (33).

Predominant pathogens which cause mastitis may have changed over the past 20-30 years. Environmental streptococcal and coliform bacterial infections are of increasing concern in herds which have a low incidence of S. agalactiae and S. aureus infection. Teat dipping and dry cow therapy are effective control measures against S. aureus and S. agalactiae therefore elimination of quarters infected with these pathogens may allow quarters to be infected with environmental organisms (55). Teat dipping and dry cow therapy as control measures are less effective against environmental streptococci and virtually ineffective against coliforms (10,29,54).
Teat dipping and dry cow therapy in a mastitis control program serve two purposes. They decrease the rate of new intramammary infection (IMI) and also decrease the duration of existing infections (55). Post milking teat dipping decreases the number of organisms present at the teat orifice and thus effectively reduces the new infection rate. Dry cow therapy decreases the duration of existing infections and also reduces the new infection rate during non-lactating period by inhibiting further bacterial growth in the gland (55). Teat dipping and dry cow therapy have been shown to be more effective when used together in a control program as opposed to using either separately (23,37). Teat dipping and dry cow therapy can eliminate infections caused by S. agalactiae and can markedly reduce those infections caused by S. aureus, however contamination with coliforms and environmental streptococci can occur other than at milking and therefore teat dipping has very little effect on these organisms (19). The antibiotics available for use to the dairyman in a dry cow therapy program have reduced efficacy for the environmental streptococci and are ineffective against coliform organisms (17,19,31).

The trend towards more confinement housing of dairy cattle with less time on pasture may also be altering the pattern of exposure to mastitis pathogens (37,55). A change to confinement housing results in an increase in
cow density per unit area and the usage of bedding materials which may support bacterial growth. These factors may result in increased level of exposure to environmental pathogens and relate to the increased concern regarding these organisms (19,23,37,54,55). A comparison of the incidence of mastitis between housing and pasture periods in Sweden showed the new IMI rate was higher during the housing period (20). Jasper (27) and Morris (37), have stated that New Zealand and Australia, which have predominantly pastured herds, do not have a major environmental mastitis problem. However, in the United States, there is an increasing concern for environmental mastitis problems in the dairy industry. The major factor resulting from confinement housing is increased level of exposure to environmental organisms. The use of bedding materials, weather conditions and herd characteristics may also affect the rate of IMI (12,51,58).

Herd characteristics which may affect the incidence of environmental organism mastitis consist of stage of lactation, parity and age of the animals. The new infection rate increases around calving and during the early dry period (15,31,36,49,52). At these times the mammary gland is undergoing transition and is highly susceptible to infection, whereas the fully involuted mammary gland is resistant to coliform infections due to the antibacterial properties of the secretions (56). Neave
et al. (43) indicated that the new infection rate in the early dry period was seven times higher than during lactation. The cessation of milking may reduce the ability of the mammary gland to eliminate existing organisms. *S. aureus* and *S. agalactiae* infections can be reduced and eliminated during the early dry period by dry cow therapy. Smith and Todhunter (55) reported that in dry treated cows, coliform infected quarters increased three fold from drying off to calving, while environmental streptococcal infected quarters decreased 50%. Sharma and Packer (53), McDonald and Anderson (35,36) and King (31) found similar results, although no dry treatment was reported in these studies. The rate of infection is higher during the first few weeks post drying off and decreases as the dry period increases (15,48,52). The number of dry period infections increases with age of the animal (15,47,48,52). Pearson and Mackie (49) in a study of three dairy herds over three years showed that of the total number of cows infected with mastitis, 51% were in lactation 4 or greater and 19% were first lactation cows. As parity increases, the number of infections increase (23). Oliver and Mitchell (48) reported that a positive relationship exists between IMI and dry period number. They found 2.6% of cows completing the first or second dry period had intramammary infections while 23.8%
of cows completing a third or greater dry period were infected (48).

An increase in the number of organisms at the teat apex increases the probability of infection, thus level of exposure to organisms may influence rate of IMI (24,28). DeHart et al. (16) studied rates of infection in udders of cows exposed to an E. coli broth culture ($10^9$ CFU/ml) at milking time for 3 weeks by either dipping teat ends pre or post milking in broth, or spraying the udder during milking. They found no differences in rate of IMI among treated groups although all treatments were significantly higher than the control untreated group. Neave and Oliver (44) also showed that the new infection rate in dry cows may be related to the amount of contamination of the teats by pathogens at drying off and the persistence of these pathogens at the orifice of the teat canal.

An increase in mastitis during the summer months when cows were housed suggests that season of year and housing may play an important role in the incidence of IMI (6,45,60). Pearson and Mackie (49) reported that clinical mastitis consisting of many cases of acute environmental mastitis occurred three times greater in winter months when animals were housed, than in summer when cows were on pasture. Sharma and Packer (53) reported that infections from S. uberis were high during
winter months and least during summer months. Smith and Todhunter (55) reported an increase in incidence of clinical mastitis that was related to season of year regardless of stage of lactation in housed dairy cattle. However, King (31) indicated that udder infection was not directly influenced by season of year. Oliver (47) also stated that he saw no evidence of a seasonal trend in infection which was independent of stage of lactation or age.

Housing conditions such as temperature, humidity and ventilation exert an influence on the animal. The thermal comfort zone for adult cattle is 10-26.7°C with 10-20 ventilation changes per hour and a relative humidity of 40-80% (2,22). A heat stress formula for livestock is used to calculate the amount of additional stress on animals under certain temperature and humidity conditions (39). Heat stress may predispose the animal to a diseased state such as mastitis. Temperature and humidity exert their influence on the bacterial organisms in addition to the host. Optimum temperature for growth of coliform and streptococcal organisms is 37°C (22). Consequently, higher ambient temperatures would be expected to influence the growth of these organisms in the cows environment.

Bedding materials have a close and prolonged relationship with the teat apex and are capable of
supporting bacterial growth. Thus bedding materials influence rate of IMI (12). Characteristics and properties of bedding materials vary. Factors influencing the growth of bacteria in any media are temperature, moisture and nutrient availability. In addition, pH, method of processing the material and bacterial populations of unused materials influence the growth in bedding materials (12).

Various bedding materials have been studied. Dairy waste solids (DWS) have been shown to be an acceptable bedding material after proper preparation measures are undergone. The process of composting DWS results in a lower coliform bacterial count than a washed recycled manure product (5,14,25). Eberhart (18) reported a Klebsiella count in washed recycled manure of $2 \times 10^5$ CFU/g and a total coliform count of $1.3 \times 10^6$ CFU/g. Carroll and Jasper (14) in a study of coliform populations in bedding materials, showed that prior to composting, DWS contained a coliform count of approximately $10^6$ CFU/g with Klebsiella and Enterobacter predominating. Following composting, the total coliform count was $10^5$ CFU/g to $10^7$ CFU/g on the compost pile surface. However, internal temperature of the compost pile was 75°C and total coliform count at this temperature was 0 to $10^4$ CFU/g. Janzen (25) showed that a decrease in total bacterial count from $10^8$ CFU/g to less than $10^4$ CFU/g occurred
following composting. Major factors involved in composting DWS to decrease bacterial contamination are temperature, moisture and degree of aeration (14). Temperature in composting piles generally peak at approximately 71.5°C and level off at about 57°C (1,5,25). Thermal death of coliforms and environmental streptococci occur at approximately 55-60°C (22). Initially total bacterial count decrease in the compost pile over 4-6 days with a relatively constant bacterial count following 6 days (5,25). Bacterial populations in DWS have an inverse relationship with temperature during composting (5). Freshly voided feces rarely contain higher than $10^5$ CFU/g wet weight bacterial populations (1,13). Janzen (25) and Bishop et al. (5) composted DWS for 2 weeks and determined a moisture content of 70-80%, a decrease of 5-10% from uncomposted DWS. Janzen (25) suggests that temperature in bedding increases if moisture is present.

The use of sawdust as a bedding material has also been studied. Sawdust has been implicated in harboring Klebsiella organisms (4,13,46,51). Newman and Kowalski (46) compared bacterial populations of milk samples from cows bedded on sawdust with the bacterial population in the sawdust. They isolated Klebsiella from 54% of the milking cows and also 75% of the bedding samples. These same stalls were then changed to sand and straw bedding, and three months later samples were again obtained.
Results on second sampling showed only 9% of cows harboring Klebsiella organisms in the mammary gland and 40% of bedding samples to contain Klebsiella (46). Fairchild et al. (21) reported that sawdust had a much higher Klebsiella count \((3.4 \times 10^6 \text{ CFU/g})\) than paper \((2.5 \times 10^4 \text{ CFU/g})\), sand \((<1.0 \times 10^3 \text{ CFU/g})\) or lime \((<1.0 \times 10^3 \text{ CFU/g})\). They also indicated that total coliform counts were higher in sawdust \((4.1 \times 10^6 \text{ CFU/g})\) and paper \((8.7 \times 10^4 \text{ CFU/g})\) than in sand or lime \((<1.0 \times 10^3 \text{ CFU/g})\). The dry matter of sand and lime throughout the trial was approximately 99% whereas sawdust was about 60%. A pH of 9.5 for unused lime was found, whereas unused sawdust plus lime and sawdust alone had much lower pHs at 7.2 and 4.5 respectively. They demonstrated a direct relationship between bacterial teat end populations and bacterial populations in beddings (21). Rendos et al. (51) showed similar results, indicating that sawdust bedded cows have higher coliform and Klebsiella populations on teat ends than cows bedded on wood shavings or straw. They also showed that streptococcal and staphylococcal organisms were most numerous on straw bedded cows.

The effects of various chemical applications to sawdust in an effort to reduce bacterial populations have been studied. Bashandy and Heider (4) applied paraformaldehyde spray, slaked lime and orthophenylphenol to sawdust twice at seven day intervals and monitored the
bacterial population, pH and moisture content of each treatment. Slaked lime and paraformaldehyde spray treatments resulted in a significant reduction in total bacterial and coliform counts as compared to the control. A significant decrease in bacterial teat end populations for cows bedded on both slaked lime treated sawdust and paraformaldehyde treated sawdust was observed. The 5% paraformaldehyde spray and slaked lime treatments decreased the total bacterial count in beddings and on teat ends for 5-7 days, and reduced coliform counts for approximately 12 days. Moisture content was significantly lower and pH was higher with slaked lime and paraformaldehyde treated sawdust than the untreated sawdust.

Several experiments with chemical application to DWS have been done in an attempt to control bacterial populations. Janzen et al. (26) reported that although bacterial counts in composted DWS treated with 50% limestone did not differ significantly from untreated composted DWS, bacterial populations of the teat ends were significantly lower for cows bedded with treated DWS than with untreated DWS. Streptococcus spp., Staphylococcus aureus, E. coli and Enterobacter counts were all significantly lower in crushed limestone bedding, teat swabs and milk than untreated DWS or 50:50 mixture. The pH of limestone bedding was higher than untreated DWS or 50:50 mixture.
Janzen et al. (26) suggested that crushed limestone treatment decreased bacterial populations by producing an alkaline condition and thus creating an antimicrobial environment.

The present study was done to determine the dynamics of bacterial growth in three different bedding materials. Factors directly associated with each bedding material such as bedding pH and dry matter content were examined for their relationship to bedding bacteria populations. Temperature, humidity, contamination by animal and usage over time were also evaluated for possible effects on bacterial growth in beddings. Simultaneously, a control study was done using routine bedding materials.
MATERIALS AND METHODS

Experimental Design

Housing. Two separate housing facilities, tie and free stalls were used throughout the experimental period from May to September 1984. Two trials per month were conducted. The tie stall facility (comfort barn) had a capacity for 60 cows. Each stall measured 2.8 m², was equipped with a rubber mat over a cement floor and had no retaining wall at the rear of the stalls. The same nine adjacent stalls were used throughout all trials. The free stall barn was a separate facility consisting of 8 pens with 20 stalls per pen, and a total capacity of 160 cows. Free stalls had concrete floors and measured 2.3 m². One half of a free stall pen, consisting of 10 stalls, was used throughout the experimental period. Lactating Holstein cows were maintained in experimental stalls in each facility during all trials.

Bedding Materials. Three different bedding materials were used in both tie and free stalls: 1) recycled manure (RM); 2) pelleted corn cobs (PCC); and 3) lime treated recycled manure (TRM). Recycled manure was obtained from the TRU-hydrasieve separator system (Babson
Bros. Co., Oakbrook, Ill.) present at the site. Manure was flushed to a pit below the TRU machine, and then elevated by a pump and passed through rollers which separated the solids from the liquid. Dairy waste solids were collected outside in an open holding shed adjacent to the comfort stall barn. Pelleted corn cobs were purchased from Andersons' Inc. (Maumee, Ohio) and stored in a grain bin at the barn. Lime treated recycled manure was prepared by adding hydrated agricultural lime, Ca(OH)$_2$ (United States Gypsum, Chicago, Ill.) to TRU-recycled manure solids at ratio of 1 kg lime/20 kg TRU-recycled manure solids.

All experimental stalls were scraped and swept clean at the beginning of each trial period. Each bedding material was placed in three adjacent stalls in each housing facility. Initially 20 kg of RM and TRM were added to each of the tie stalls and free stalls. Stalls bedded with PCC were bedded to a depth of 2.5-5.0 cm in tie stall housing and 5.0-7.5 cm in free stall housing. Accumulated manure and heavily soiled bedding were removed twice daily from all stalls and unused bedding material added daily as required to maintain the initial volume of bedding. Bedding materials were rotated amongst stalls within a housing facility every trial to insure bedding every stall with each material.
Sample Collection. Bedding samples were collected at 0 (unused), 12, 24, 48 and 96 hrs throughout each trial period. Collection at each sampling period was made prior to adding additional bedding to stalls. Approximately 1 kg sample of each bedding type was placed in a steripak bag using plastic gloves. Bedding samples were collected from the rear 1/3 of the center stall in each treatment group. Adjacent similar treatment stalls were used as buffer stalls to ensure a homogeneous bedding sample in the center sampling stalls. Analysis of samples was performed immediately following collection.

Control Study

Housing and Management. Two bedding materials were used routinely at this facility. Both tie and free stalls not used in the experimental study were bedded with recycled manure solids. The back end of tie stall were scraped clean twice daily of manure and heavily soiled bedding. Unused RM was added once daily as needed to tie stalls. Free stalls were bedded twice weekly with unused RM. Stalls were raked daily to clean and level bedding. Maternity box stalls were bedded with PCC. Manure and heavily soiled bedding were removed once per day and unused PCC added.
Collection of Control Samples. Approximately 1 kg sample of each bedding was placed in a steripak bag using sterile plastic gloves. Random samples were obtained weekly in each housing facility throughout the experimental period. Analysis of samples was performed immediately following collection.

Microbiological Analysis. A 10 g quantity of each bedding sample was added to 90 ml sterile Phosphate Buffered Saline (PBS) adjusted to pH 7.1. The mixture was agitated vigorously for 30-45 seconds and solids were allowed to settle for 2-3 minutes. Appropriate dilutions (10 fold) for plating were prepared in sterile microtiter plates. Wells of microtiter plates contained 250 ul PBS and dilutions from $10^{-2}$ to $10^{-5}$ were prepared. Appropriate dilutions were then plated on the surface of various specialized agar plates for microbial determination. Samples were plated using 100 ul of the original $10^{-1}$ dilution on 1/2 of a solid agar plate, and 40 ul of dilutions $10^{-1}$ to $10^{-5}$ dispensed in four-10 ul quantities on 1/2 of a solid agar plate. After appropriate dilutions were spotted on solid agar, the plates were incubated at room temperature for 15 mins to permit some drying, then incubated at 37°C for 18-24 hrs. Colony forming units/ml for each sample were determined by counting the lowest dilution countable.
Total growth on MacConkey agar (Difco Laboratories, Detroit, MI) was designated as total gram-negative bacteria. Coliform bacteria were determined by counting only the lactose positive colonies (red) on MacConkey agar. The number of *Klebsiella* spp. was determined using MacConkey-Inositol-Carbenicillin agar (3). Concentration of carbenicillin was 75 µg/ml, and was based on results of preliminary studies showing less background growth when compared to agar containing 50 µg/ml as used by Bagley and Seidler (3). The number of *Streptococci* spp. were determined using TKT/FC agar (Gibco Laboratories, Madison, WI) containing 5% blood plasma.

**Dry Matter Determination.** A minimum quantity of 10 g per sample was measured into a pre-weighed drying pan. Samples were then placed in a drying oven at 100°C for 18-24 hrs. Samples were removed, placed in a dessicator, cooled to room temperature, and reweighed.

**Determination of pH.** Each sample was prepared by adding 10 g of bedding material to 90 ml deionized distilled water. Samples were stirred on a magnetic stir plate and the pH determined using a 2-point calibration curve pH meter (Corning pH Meter 125, Medfield, MA).

**Temperature and Humidity Data Collection.** Barn temperature (T°C) and relative humidity (H%) for each
housing facility were obtained at each of the five sample collection time periods for all trials. Temperature and H% were monitored using a 0°C Psychrometer (Model No. 3312-40, Cole Parmer Instrument Co. Chicago, Ill.) placed on the alleyway to the rear of experimental tie stalls and on the floor of treated free stalls. Wet and dry bulb readings were obtained and relative humidity determined.

Ambient T°C and H% were also recorded throughout all trials at each time point. Ambient T°C data was obtained from the Ohio Monthly Weather Report of the Ohio Agricultural Research and Development Center (OARDC) Weather Station located within 1 km of the facility. Relative humidity data was obtained from the Monthly Summary Local Climatological Data published by the National Weather Service office located at Lahm Municipal Airport in Mansfield, Ohio. Humidity data was unavailable from the OARDC Weather Station, however, data was obtained from Lahm Municipal Airport which is the closest weather station available.

Statistical Analysis. Number of bacteria was expressed as colony forming units/g wet weight, \( \log_{10} \) (CFU/g) and analyzed by Least Squares Analysis and Analysis of Variance tests (59). Tukey's procedure was then applied at a .05 significance level (59). A quadratic regression
model comparing bacterial growth with pH and dry matter content was used to determine existing relationships. A linear regression model was utilized in examining correlations between bacterial growth and temperature and humidity.
RESULTS

Analysis of data indicated that housing type had no effect (P>.05) on the number of bacteria in RM or TRM during the experimental period. However, the number of *Klebsiella* spp. and gram-negative bacteria in free stall PCC was approximately 0.5 CFU/g higher (P<.05) as compared to PCC in tie stalls. As a result of minimal effect of housing type on bedding bacteria populations, data from both tie and free stalls for all trials were combined to determine bacteria changes in bedding with time.

**Time Effect.** Numbers of bacteria in all bedding materials were lowest at 0 hr, and 0 hr CFU/g were consistently lower in TRM and PCC when compared to RM (Tables 1-4). All four bacteria populations measured, increased (P<.05) with time. Maximal increase in CFU/g occurred from 0 to 24 hrs (P<.05). This trend was consistent among all three bedding materials. Any change in CFU/g in all beddings from 24 to 96 hrs was generally less than 1.0 CFU/g, log_{10}.

Gram-negative bacteria in PCC were lower (P<.05) at all times when compared to RM or TRM (Table 1). Maximum numbers in PCC were at 96 hr but CFU/g did not change
Table 1. Colony forming units (CFU) of Gram-negative bacteria in bedding materials from 0 to 96 hrs. Means represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
<td>X</td>
<td>SE</td>
<td>X</td>
</tr>
<tr>
<td>RM</td>
<td>7.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.04</td>
<td>7.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.10</td>
<td>8.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TRM</td>
<td>4.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.28</td>
<td>6.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.21</td>
<td>8.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCC</td>
<td>3.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.18</td>
<td>4.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.12</td>
<td>5.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Determined using MacConkey Agar (all colonies counted).
<sup>2</sup>Means are CFU/g wet wt., log<sub>10</sub>.
<sup>3</sup>RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.
<sup>4</sup>All means among beddings were different (P<.05) at this time.
<sup>5</sup>Mean for PCC was lower (P<.05) at this time than RM or TRM; however, no difference existed between RM and TRM (P>.05).
<sup>a,b,c,d</sup>Means within a row with different superscripts differed (P<.05).
Table 2. Colony forming units (CFU) of coliform bacteria\(^1\) in bedding materials from 0 to 96 hrs. Means\(^2\) represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding(^3)</th>
<th>-----0(^4)-----</th>
<th>-----12(^4)-----</th>
<th>-----24(^4)-----</th>
<th>-----48(^4)-----</th>
<th>-----96(^4)-----</th>
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<tr>
<td></td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>RM</td>
<td>5.41(^d)</td>
<td>.07</td>
<td>6.27(^c)</td>
<td>.06</td>
<td>6.59(^b)</td>
</tr>
<tr>
<td>TRM</td>
<td>2.78(^d)</td>
<td>.22</td>
<td>4.62(^c)</td>
<td>.18</td>
<td>5.32(^b)</td>
</tr>
<tr>
<td>PCC</td>
<td>2.68(^d)</td>
<td>.15</td>
<td>3.71(^c)</td>
<td>.17</td>
<td>4.57(^b)</td>
</tr>
</tbody>
</table>

\(^1\) Determined using MacConkey Agar (only lactose positive colonies).
\(^2\) Means are CFU/g wet wt., log\(_{10}\).
\(^3\) RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.
\(^4\) All means among beddings were different (\(P<.05\)) at this time.
\(^{a,b,c,d}\) Means within a row with different superscripts differed (\(P<.05\)).
Table 3. Colony forming units (CFU) of *Klebsiella* spp.\(^1\) in bedding materials from 0 to 96 hrs. Means\(^2\) represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding(^3)</th>
<th>Time (h)</th>
<th>(\bar{x})</th>
<th>SE</th>
<th>(\bar{x})</th>
<th>SE</th>
<th>(\bar{x})</th>
<th>SE</th>
<th>(\bar{x})</th>
<th>SE</th>
<th>(\bar{x})</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM</td>
<td>0(^4)</td>
<td>5.11(^{d})</td>
<td>.09</td>
<td>5.78(^{c})</td>
<td>.11</td>
<td>6.08(^{b,c})</td>
<td>.08</td>
<td>6.28(^{a,b})</td>
<td>.08</td>
<td>6.48(^{a})</td>
<td>.08</td>
</tr>
<tr>
<td>TRM</td>
<td>12(^5)</td>
<td>2.22(^{d})</td>
<td>.16</td>
<td>3.71(^{c})</td>
<td>.18</td>
<td>4.64(^{b,c})</td>
<td>.12</td>
<td>4.91(^{a,b})</td>
<td>.11</td>
<td>5.40(^{a})</td>
<td>.12</td>
</tr>
<tr>
<td>PCC</td>
<td>24(^5)</td>
<td>2.14(^{d})</td>
<td>.11</td>
<td>2.67(^{c})</td>
<td>.13</td>
<td>3.25(^{b})</td>
<td>.16</td>
<td>3.75(^{a,b})</td>
<td>.16</td>
<td>4.37(^{a})</td>
<td>.22</td>
</tr>
<tr>
<td></td>
<td>48(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Determined using MacConkey-Inositol-Carbenicillin Agar (selective for *Klebsiella* spp.).

\(^2\) Means are CFU/g wet wt., log\(_{10}\).

\(^3\) RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.

\(^4\) Mean for RM was higher (\(P<.05\)) at this time than PCC or TRM; however, no difference (\(P>.05\)) existed between PCC and TRM.

\(^5\) All means among beddings were different (\(P<.05\)) at this time.

\(^a,b,c,d\) Means within a row with different superscripts differed (\(P<.05\)).
Table 4. Colony forming units (CFU) of Streptococcus spp.\textsuperscript{1} in bedding materials from 0 to 96 hrs. Means\textsuperscript{2} represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding\textsuperscript{3}</th>
<th>Time (h)</th>
<th>0\textsuperscript{4}</th>
<th>12</th>
<th>24\textsuperscript{5}</th>
<th>48\textsuperscript{5}</th>
<th>96\textsuperscript{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>RM</td>
<td>6.56\textsuperscript{c}</td>
<td>.06</td>
<td>7.34\textsuperscript{b}</td>
<td>.12</td>
<td>7.50\textsuperscript{a}</td>
<td>.08</td>
</tr>
<tr>
<td>TRM</td>
<td>4.57\textsuperscript{c}</td>
<td>.20</td>
<td>6.37\textsuperscript{b}</td>
<td>.15</td>
<td>7.67\textsuperscript{a}</td>
<td>.04</td>
</tr>
<tr>
<td>PCC</td>
<td>3.01\textsuperscript{c}</td>
<td>.13</td>
<td>4.66\textsuperscript{b}</td>
<td>.15</td>
<td>5.44\textsuperscript{a}</td>
<td>.13</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Determined using TKT Agar containing 5% blood plasma (selective for Streptococcus spp.).
\textsuperscript{2} Means are CFU/g wet wt., log\textsubscript{10}.
\textsuperscript{3} RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.
\textsuperscript{4} All means among beddings were significantly different (\(P<.05\)) at this time.
\textsuperscript{5} Mean for PCC was lower (\(P<.05\)) at this time than RM or TRM; however, no difference existed between RM and TRM (\(P>.05\)).
\textsuperscript{a,b,c} Means within a row with different superscripts differed (\(P<.05\)).
significantly (P>.05) beyond 24 hrs. The number of gram-negative bacteria in TRM was lower (P<.05) as compared to RM at 0, 12 and 24 hrs, but no difference (P>.05) existed between gram-negative bacteria in TRM or RM at 48 and 96 hrs. Maximum number of gram-negative bacteria was at 48 hrs for both RM and TRM.

The number of coliform bacteria (Table 2) differed (P<.05) among the three bedding materials at all times. Coliform populations were consistently highest in RM and lowest in PCC. Maximum coliform numbers were at 96 hrs for all three bedding materials and coliform numbers increased (P<.05) from 24 to 96 hrs in contrast to number of gram-negative bacteria in bedding materials. Coliform CFU/g was 5.41 in RM at 0 hr and exceeded 6.0 CFU/g by 12 hrs. Number of coliform bacteria in TRM at 0 hr (2.78 CFU/g) was low and did not exceed 6.0 CFU/g until 96 hrs. While coliform numbers did differ (P<.05) between RM and TRM at 96 hrs, the difference was only .62 log_{10} units. Coliforms in PCC never exceeded 6.0 CFU/g during these trials.

The relative number of Klebsiella spp. (Table 3) among bedding materials and the increase in number with time was generally comparable to that observed for the coliform bacteria. Klebsiella spp. were consistently lower (P<.05) in TRM when compared to RM and number was lowest in PCC. Number of Klebsiella spp. in RM exceeded
6.0 CFU/g by 24 hrs while the number in TRM or PCC never exceeded 6.0 CFU/g.

Number of *Streptococcus* spp. (Table 4) in the three bedding materials was lowest at 0 hr and increased (P < 0.05) through 24 hrs. Bedding populations of streptococci did not change (P > 0.05) between 24 hrs and 96 hrs. *Streptococcus* spp. were consistently lower (P < 0.05) in PCC when compared to either RM or TRM. Treated recycled manure contained fewer (P < 0.05) *Streptococcus* spp. than RM at 0 and 12 hrs but CFU/g did not differ at 24, 48 or 96 hrs. Maximum number of *Streptococcus* spp. was at 48 hrs for both RM and TRM and at 96 hrs for PCC.

**Dry Matter Content of Bedding Materials.** Analysis of dry matter content (DM%) in bedding materials (Table 5) did not differ (P > 0.05) between housing types. Thus, DM% for all trials and both housing types was combined to determine change in DM% with time. Mean DM% differed (P < 0.05) among bedding materials at all times. Dry matter content of PCC (87%) was consistently higher than TRM (38%) or RM (34%) throughout the 96 hr experimental period. The dry matter content of PCC decreased with time while DM% of RM and TRM increased with time. However, the DM% of each bedding material varied by less than 10% from 0 to 96 hrs.

A quadratic regression model was used to analyze the relationship of DM% and bacterial numbers in the beddings
Table 5. Dry matter content\(^1\) of bedding materials from 0 to 96 hrs. Means represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding(^2)</th>
<th>Time (h)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0(^3)</td>
<td>12(^3)</td>
<td>24(^3)</td>
<td>48(^3)</td>
<td>96(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
<td>SE</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>RM</td>
<td>30.1(^c)</td>
<td>.4</td>
<td>32.8(^b)</td>
<td>.4</td>
<td>35.3(^a)</td>
<td>.6</td>
<td>36.2(^a)</td>
</tr>
<tr>
<td>TRM</td>
<td>32.5(^d)</td>
<td>.5</td>
<td>36.5(^c)</td>
<td>.5</td>
<td>37.9(^b, c)</td>
<td>.7</td>
<td>40.1(^a, b)</td>
</tr>
<tr>
<td>PCC</td>
<td>90.5(^a)</td>
<td>.5</td>
<td>88.5(^a, b)</td>
<td>.8</td>
<td>86.2(^b, c)</td>
<td>.6</td>
<td>86.1(^b, c)</td>
</tr>
</tbody>
</table>

\(^1\)Means are percent dry matter.
\(^2\)RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.
\(^3\)All means among beddings were different (\(P<.05\)) at this time.
\(^a, b, c, d\)Means within a row with different superscripts differed (\(P<.05\)).
(Figure 1). The relationship was derived using only the CPU/g, \( \log_{10} \) values determined at 24, 48 and 96 hrs. Changes in bacterial numbers with time were minimal during this period.

The relationship of DM% and bacterial numbers in bedding was also analyzed using a linear regression model. Data indicated a relationship existed (\( P < .05 \)), however, \( r^2 \) values were low (<.35) suggesting minimum linear correlation. The \( r^2 \) values for the quadratic regression model (Figure 1) indicated a high correlation existed (\( P < .05 \)) between bedding DM% and bacteria numbers. Thus, a non-linear relationship existed.

Maximum number of *Streptococcus* spp., coliforms and *Klebsiella* spp. was associated with a dry matter content of 40% while maximum numbers of gram-negative bacteria was at 50% dry matter.

The number of all bacteria in RM and TRM increased with time (Tables 1-4) as did the DM% (Table 5). Whereas, the DM% of PCC decreased with time (Table 5), the number of all bacteria increased (Tables 1-4). The number of bacteria in RM and TRM increased as the DM% increased to approximately 40% and the number of bacteria in PCC increased as DM% decreased.

**Bedding pH and Effect on Bacteria Populations.** Analysis of pH of bedding materials revealed no difference (\( P > .05 \)) between housing types (Table 6). Maximum variation of
FIGURE 1. Correlation between the change in dry matter content (DM%) of all bedding materials and the number or bacteria in beddings. Points plotted represent mean colony forming units (CFU/g) from 24 through 96 hrs during the experimental period from May to September 1984.
Table 6. Change in pH of bedding materials from 0 to 96 hrs. Means represent a total of 10 trials from May to September 1984, duplicate samples, in both tie and free stall facilities (n=40).

<table>
<thead>
<tr>
<th>Bedding¹</th>
<th>-----0²-----</th>
<th>-----12²-----</th>
<th>-----24²-----</th>
<th>-----48²-----</th>
<th>-----96²-----</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x   SE</td>
<td>x   SE</td>
<td>x   SE</td>
<td>x   SE</td>
<td>x   SE</td>
</tr>
<tr>
<td>RM</td>
<td>8.34ᵇ  .05</td>
<td>8.73ᵃ  .03</td>
<td>8.00ᵃ  .02</td>
<td>8.89ᵃ  .05</td>
<td>8.83ᵃ  .04</td>
</tr>
<tr>
<td>TRM</td>
<td>11.88ᵃ  .06</td>
<td>10.45ᵇ  .13</td>
<td>9.77ᶜ  .07</td>
<td>9.86ᶜ  .01</td>
<td>9.83ᶜ  .01</td>
</tr>
<tr>
<td>PCC</td>
<td>5.45ᵈ  .04</td>
<td>5.59ᶜ,ᵈ  .04</td>
<td>5.78ᵇ,c  .05</td>
<td>6.00ᵃ,b  .08</td>
<td>6.09ᵃ  .10</td>
</tr>
</tbody>
</table>

¹RM = recycled manure; PCC = pelleted corn cobs; TRM = lime treated recycled manure.
²All means among beddings were different (P<.05) at this time.
ᵃ,b,c,dMeans within a row with different superscripts differed (P<.05).
bedding pH between housing types, occurred in RM. The pH of RM in free stalls was higher by 0.5 pH units as compared to RM in tie stalls. Thus, data was combined for all trials and both housing types to determine changes in bedding pH with time. Mean pH differed (P<.05) among bedding materials at all times. The pH of TRM was consistently higher (10.36) than RM (8.72), and PCC was lowest (5.78) throughout the experimental period. The pH of TRM decreased with time while the pH of RM and PCC increased. Unused TRM had a high pH (11.88) and decreased to 9.8 by 96 hrs. The pH of PCC and RM varied by approximately 0.6 pH from 0 to 96 hrs. Maximum change in pH of RM occurred by 12 hrs, as compared to maximum change in pH of TRM by 24 or PCC by 48 hrs.

A quadratic regression model was used to analyze the relationship of pH and bacteria numbers in the bedding (Figure 2). Analysis was made using only CFU/g, log_{10} values for 24, 48 and 96 hrs. Changes in bacteria number were minimal with time during this period.

A linear regression model was also used to examine the relationship of bedding pH and bacteria populations. Data indicated a correlation existed (P<.05), however, r^2 values were low suggesting a non-linear relationship may exist. The correlation coefficient, r^2 values, for the quadratic regression model indicate a non-linear
FIGURE 2. Correlation between the change in pH of all bedding materials and the number of bacteria in beddings. Points plotted represent mean colony forming units (CFU/g) from 24 through 96 hrs during the experimental period from May to September 1984.
relationship exists (P<.05) between pH and CFU/g in bedding materials.

Gram-negative bacteria and Streptococcus spp. attained maximum numbers at a bedding pH of 9-10. Maximum number of coliforms and Klebsiella spp was reached at pH of 8. The number of coliform and Klebsiella spp. was greater than 10^6 CFU/g at bedding pH of 6.5-9.5. However, the number of gram-negative and Streptococcus spp. was greater than 10^6 CFU/g at pH 6.0 and remained above 10^6 as pH rose to 11. Numbers of Streptococcus spp. varied least with change in pH.

**Correlation Between Temperature and Humidity vs. Growth.** Analysis of data indicated differences (P<.05) existed among trials with barn and ambient T°C and H% (Table 7). Barn T°C varied with a low of 12.8°C at the end of May to a high of 23.8°C in the early part of August. Humidity was also lowest in May and highest in August. Barn and ambient T°C were similar throughout all trials. Maximum T°C and H% were indicated during June, July and August, and both May and September were significantly lower (P<.05).

A linear regression model was used to analyze bedding CFU/g and the variation due to T°C and H%. The relationship was derived using a mean of values for ambient T°C and H% from 0 to 96 hrs in each trial. The CFU/g in beddings was calculated using data for both
Table 7. Mean values of barn and ambient temperature (T°C) and relative humidity (H%), including temperature humidity index (THI) during each trial period from May to September 1984, a total of 10 trials. Each value represents a mean of five sampling times from 0 to 96 hrs, duplicate samples, in both tie and free stall facilities (n=20).

<table>
<thead>
<tr>
<th>Month</th>
<th>Trial</th>
<th>---T°C-----</th>
<th>---H%-----</th>
<th>---THI-----</th>
<th>---T°C-----</th>
<th>---H%-----</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X SE</td>
<td>X SE</td>
<td>X SE</td>
<td>X SE</td>
<td>X SE</td>
</tr>
<tr>
<td>May</td>
<td>1</td>
<td>14.5c,d .6</td>
<td>54.8d,e 1.7</td>
<td>58.2e,f .8</td>
<td>11.2f,g .7</td>
<td>62.0c,d 3.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.8d .4</td>
<td>51.8e 1.2</td>
<td>55.7f .4</td>
<td>9.8g .9</td>
<td>62.8c,d 3.5</td>
</tr>
<tr>
<td>June</td>
<td>3</td>
<td>21.1a,b .4</td>
<td>63.8c,d 1.7</td>
<td>67.7b,c .5</td>
<td>20.9b,c .7</td>
<td>52.8d 2.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>22.5a .2</td>
<td>74.8a,b 1.2</td>
<td>70.6a,b .4</td>
<td>23.0a,b .5</td>
<td>66.6b,c,d 2.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>21.1a,b .2</td>
<td>72.1a,b,c 1.1</td>
<td>68.1b .2</td>
<td>20.3c .2</td>
<td>67.6b,c 3.6</td>
</tr>
<tr>
<td>July</td>
<td>6</td>
<td>21.2a,b .3</td>
<td>68.6b,c 1.0</td>
<td>68.1b .4</td>
<td>20.3c .6</td>
<td>68.2b,c 1.2</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>23.8a .2</td>
<td>80.6a .6</td>
<td>73.1a .3</td>
<td>23.6d .2</td>
<td>91.0a 2.8</td>
</tr>
<tr>
<td>August</td>
<td>8</td>
<td>19.5b .3</td>
<td>63.2c,d 1.5</td>
<td>64.4c,d .4</td>
<td>17.2d .1</td>
<td>76.6b 3.8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>16.3c .4</td>
<td>64.8b,c 1.1</td>
<td>61.3d,e .4</td>
<td>14.4e .6</td>
<td>78.0a,b 3.4</td>
</tr>
<tr>
<td>September</td>
<td>10</td>
<td>14.9c,d .4</td>
<td>62.1d 1.2</td>
<td>59.1e,f .5</td>
<td>13.1e,f .7</td>
<td>72.0b,c 3.4</td>
</tr>
</tbody>
</table>

1Week which sampling occurred, beginning 1st week of May through 2nd week of September, 2 trials per month.
2Calculated using barn T°C and H% values (39).
a,b,c,d,e,f,g,Means followed by similar superscripts within a column were not significantly different (P>0.05).
housing types at 48 hrs. The number of bacteria in beddings was maximum at this time. Data indicated that no linear relationship (P>.05) existed with bedding CFU/g and T°C or H%. However, number of *Klebsiella* spp. had a positive relationship (P<.05) with humidity. The r² value suggested 66% of variation in number of *Klebsiella* organisms in bedding is attributed to humidity.

A livestock heat stress formula (39) was used to calculate a temperature and humidity index (THI). Barn T°C and H% values were used in the formula (Table 7). Mean THI values were calculated from 0 to 96 hrs for each trial. Analysis of data indicated variation among trials existed (P<.05) with highest index during June, July and August. Mean THI values for each trial were below a livestock alert stage (75-78), however, various individual values indicated livestock alert levels.

A linear regression model was used to determine the relationship between bedding bacterial numbers and THI. Mean THI for both housing facilities, from 0 to 96 hr for each trial was used. Bedding bacterial numbers were calculated using data from both housing types at 48 hr. Data indicated no relationship existed (P>.05) between CFU/g in bedding and THI values during the experimental period.

**Control Study Results.** Analysis of data indicated no differences existed (P>.05) in CFU/g between RM in either
tie or free stalls throughout the experimental period (Table 8). The number of gram-negative bacteria and Klebsiella spp. was lower (P<.05) in PCC than RM in either free or tie stalls. However, no difference existed (P>.05) among bedding materials in the number of coliform organisms or Streptococcus spp. Numbers of gram-negative, coliform, and Streptococcus spp. in each bedding were greater than $10^6$ CFU/g throughout the trial period. Klebsiella spp. in PCC was less than $10^6$ CFU/g as compared to number of Klebsiella in RM.

The pH of bedding materials varied with maximum difference noted in PCC (Table 9). The pH of PCC was 1 pH unit lower (P<.05) as compared to pH of either RM in tie or free stalls. A linear and quadratic regression model was used to analyze CFU/g in bedding and the variation due to bedding pH. Data suggests that no significant (P>.05) relationship exists between CFU/g and pH of bedding.

Dry matter content of all bedding materials were different (P<.05) (Table 9). Pelleted corn cobs had a higher DM% than RM in free stalls, and RM in tie stalls was lowest. However, maximum difference in DM% between tie and free stalls was 3.8%. The relationship between CFU/g and DM% in beddings was analyzed using a linear and quadratic regression model. Data suggested that no
Table 8. Colony forming units (CFU) of bacteria in control bedding material. Mean$^1$ represent a total of 21 weeks from May to September 1984, one sample per week, duplicate samples (n=42).

<table>
<thead>
<tr>
<th>Bedding$^2$</th>
<th>Gram-negative$^3$</th>
<th>Coliform$^4$</th>
<th>Klebsiella spp.$^5$</th>
<th>Streptococcus spp.$^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>SE</td>
<td>$\bar{X}$</td>
<td>SE</td>
</tr>
<tr>
<td>FCC</td>
<td>7.43$^b$</td>
<td>.15</td>
<td>6.85$^a$</td>
<td>.15</td>
</tr>
<tr>
<td>RM-Tie</td>
<td>8.14$^a$</td>
<td>.09</td>
<td>6.68$^a$</td>
<td>.10</td>
</tr>
<tr>
<td>RM-Free</td>
<td>8.15$^d$</td>
<td>.08</td>
<td>6.78$^a$</td>
<td>.12</td>
</tr>
</tbody>
</table>

$^1$Means are CFU/g wet wt., log$_{10}$
$^2$PCC = pelleted corn cobs; RM-Tie = recycled manure in tie stalls; RM-Free = recycled manure in free stalls.
$^3$Determined using MacConkey Agar (all colonies counted).
$^4$Determined using MacConkey Agar (only lactose positive colonies).
$^5$Determined using MacConkey-Inositol-Carbenicillin Agar (selective for Klebsiella spp.).
$^6$Determined using TKT/FC Agar containing 5% blood plasma (selective for Streptococcus spp.).
$^a,b$Means followed by similar superscripts within a column were not significantly different (P>.05).
Table 9. Dry matter content (DM%) and pH of control bedding materials. Means represent a total of 21 weeks from May to September 1984, one sample per week, duplicate samples (n=42).

<table>
<thead>
<tr>
<th>Bedding</th>
<th>pH</th>
<th></th>
<th>DM%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SE</td>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>PCC</td>
<td>7.69b</td>
<td>0.11</td>
<td>66.4a</td>
<td>1.2</td>
</tr>
<tr>
<td>RM-Tie</td>
<td>8.53a</td>
<td>0.05</td>
<td>34.9c</td>
<td>0.5</td>
</tr>
<tr>
<td>RM-Free</td>
<td>8.73a</td>
<td>0.05</td>
<td>38.7b</td>
<td>0.9</td>
</tr>
</tbody>
</table>

1PCC = pelleted corn cobs; RM-Tie = recycled manure in tie stalls; RM-Free = recycled manure in free stalls.

a,bMeans followed by similar superscripts within a column were not significantly different (P > .05).
correlation (P > .05) existed between bedding DM% and
CFU/g.

Temperature and humidity data was analyzed to deter-
mine the relationship with CFU/g in control bedding.
Ambient T°C and H% values were used in this analysis
throughout the experimental period. A linear regression
model was used to analyze data. No correlation (P > .05)
was shown to exist between bedding CFU/g and T°C or H%.
DISCUSSION

A rapid increase in bedding bacterial numbers and maximum change in the physical properties of beddings were observed following contamination by animals. The numbers of bacteria were significantly lower in unused bedding when compared to used bedding following 24 hours use. This trend was consistent among all beddings throughout the experimental period. These results are consistent with those reported by Carrol and Jasper (13), who stated that an increase in bedding coliform counts occurred within 24 hours following bedding contamination with feces. The pH of TRM and PCC also changed significantly during this time and approached a more neutral pH of 7-8. Recycled manure solids maintained a relatively constant pH increasing slightly throughout the experimental period. The dry matter content of TRM and RM increased significantly by 5-10% within 24 hrs, while PCC decreased in dry matter content during this time. All bedding parameters measured changed minimally after 24 hours.

Hydrated lime, Ca(OH)$_2$, significantly reduced the numbers of bacteria when added to unused recycled manure
solids. However, no difference in CFU/g was detected when comparing TRM to RM after 24 hrs. These results agree with those reported by Janzen et al. (26). Janzen et al. (26) used a 50:50 mixture of crushed limestone, CaCO$_3$, and composted dairy waste solids (DWS) as bedding in free stalls. They found no differences in number of *E. coli* and *Streptococcus spp.* in treated DWS when compared to untreated DWS. However, Bashandy and Heider (4) found differing results. They mixed slaked lime, Ca(OH)$_2$, with sawdust bedding at a ratio of 8 lb/100 ft$^2$ twice at 7 day intervals. The total bacterial and coliform counts remained significantly lower for approximately one week in treated sawdust when compared to untreated sawdust bedding. The differences among studies may be a result of the type of bedding material used and type of chemical disinfectant applied. Unused DWS have been reported to have a higher coliform population when compared to unused sawdust (51). Treated recycled manure solids in both studies supported higher numbers of bacteria than treated sawdust bedding. Reduced numbers of bacteria associated with treated sawdust, may be due to the lower bacterial counts of unused sawdust. Therefore, reduced bacterial numbers in treated bedding may be a function of both bedding material and effect of type of chemical disinfectant used.
Lime, CaO, is soluble in water, thus generating heat and forming Ca(OH)$_2$, a strong caustic agent. Hydrated lime lyses the bacterial cell wall, causing a bactericidal effect. Thus, agricultural lime added as a chemical disinfectant to bedding materials may be bactericidal or inhibitory due to the high pH of the bedding. Hydrated lime significantly increased the pH of unused recycled manure solids, however, maximum decrease in pH occurred within 24 hrs. The high alkaline pH of unused TRM resulted in decreased bacterial counts, however, as the pH decreased in used bedding, the numbers of bacteria increased. The major effect of hydrated lime may be an increased bedding pH thus resulting in an antimicrobial environment. Similar results reported by Bashandy and Heider (4) indicated slaked lime treated sawdust had an initial increase in pH followed by a gradual decrease in 5 days. These results differed from those reported by Janzen et al. (26). They detected no significant difference in pH when comparing a 50:50 mixture of crushed limestone and DWS to untreated DWS. The pH of unused sawdust was 6.7 as compared to 6.9 for unused composted DWS (4, 26). Our study showed that unused recycled manure solids had a higher pH of 8.3. Recycled manure solids may be a more efficient buffer, thus resisting change in pH when compared to sawdust.
Optimum pH range for bacterial growth is 6-8 (22). Lower numbers of bacteria were associated with bedding materials having a high basic pH or a pH below 7. Decreased total bacterial and coliform counts were reported in slaked lime treated sawdust which had a pH of 8, as compared to untreated sawdust (4,21). Data from our study indicated PCC maintained a significantly lower pH and CFU/g throughout the experimental period when compared to RM or TRM. Data also suggested that coliform and Klebsiella organisms were more susceptible to the effects of pH than total Gram-negative bacteria and Streptococcus spp. Results suggest that maintaining a bedding pH above 8 or below 6 may significantly reduce bacterial populations in beddings. Good management techniques consisting of prompt removal of heavily soiled bedding, and frequent addition of unused bedding may reduce bacterial numbers in beddings. In addition, further studies to determine the proper ratio of hydrated lime to bedding to reduce the number of bacteria would be beneficial.

Moisture availability is an important factor affecting the number of bacteria in bedding materials (1,11,13,14,21). A positive relationship existed between moisture content and the number of bacteria in beddings. Increased bacterial counts in beddings were associated with a moisture content of more than 40%. Data in our
study indicated recycled manure solids had a moisture content of 65-70% as compared to the 80% reported by Carroll and Jasper (14). The moisture content of PCC (10-15%), was consistently lower than recycled manure solids. The addition of hydrated agricultural lime significantly increased the DM% of recycled manure solids. However, the increase in DM% did not significantly reduce the numbers of bacteria in treated recycled manure solids. The high DM% of PCC in tie and free stalls is probably a contributing factor to the lower CFU/g when compared to recycled manure solids.

Type of housing facility was shown to have minimal effect on the number of bacteria in bedding materials. The number of Gram-negative and Klebsiella organisms in PCC were higher in free stalls when compared to tie stalls. The difference in CFU/g may have been a result of the management and handling of bedding materials within each housing facility. Free stalls bedded with PCC were used less frequently when compared to other stalls. Therefore, unused PCC were added to free stalls approximately every 3 days during each trial period as compared to the daily addition of PCC to tie stalls.

The physical properties of PCC used in the experimental stalls differed when compared to PCC in the maternity box stalls (control studies). Pelleted corn cobs used in the tie and free stalls had a significantly
higher DM content and lower pH when compared to PCC in box stalls. The texture of PCC also varied. Pelleted corn cobs in box stalls appeared to have a consistency similar to sawdust and appeared almost powdery. Whereas, tie and free stall PCC remained in pelleted form. Bramley and Neave (11) suggested that the new IMI rate may be reduced if bedding coliform counts were below 10^6 CFU/g. Pelleted corn cobs in tie and free stalls had a coliform count less than 10^6 CFU/g, however, the number of coliform bacteria in box stall PCC was comparable to number in RM. Therefore, the use of PCC as a bedding material in normal routine management conditions would not indicate reduced CFU/g when compared to RM.

An attempt was made in our study to analyze the relationship between environmental parameters, such as temperature and relative humidity, and the CFU/g in bedding materials. The new IMI rate for environmental organism mastitis has been shown to increase during summer months and decrease during winter months (12,19,20,55). A direct relationship also exists between the level of coliform contamination of bedding and the new infection rate (11,51). Therefore, a relationship would appear to exist between ambient weather conditions and bacterial populations in bedding. All data obtained during the experimental period indicated that variation of CFU/g in bedding materials was independent of ambient T°C and %.

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The only direct correlation shown to exist was between Klebsiella spp. and percent relative humidity. As the percent relative humidity increased, the number of Klebsiella spp. increased. These results support those reported by Thomas et al. (58). An increase in the percent relative humidity would be expected to have a direct effect on the dry matter content of bedding. Increased available moisture in the bedding would potentially result in a more favorable growth medium for bacteria.

A relationship between bedding CFU/g and THI (livestock heat stress index) was also examined. Temperature humidity index can be used to evaluate the amount of heat stress livestock encounter as a result of ambient weather conditions. High THI values are associated with periods of high temperature and relative humidity. Thus, the THI may represent another measure of environmental conditions. Data collected during the experimental period indicated no direct association existed between the THI and bacterial numbers in beddings.

The length of the experimental period may have been a major limiting factor in our inability to show a relationship between environmental conditions and bedding CFU/g. The duration of the experimental period in this study was six months and consisted of late spring and summer seasons. Relatively minor changes occurred during this time in ambient temperature and relative humidity.
An extension of the experimental period to at least one year would result in data representing all seasonal changes and greater variation in environmental conditions.
CONCLUSION

Hydrated agricultural lime significantly reduced all bacterial populations in unused recycled manure solids. The major effect of adding hydrated lime may have been an increased alkaline pH which resulted in an antimicrobial environment. Reduced bacterial numbers in limestone treated recycled manure lasted approximately 24 hours.

Pelleted corn cobs in the tie and free stalls had lower numbers of bacteria than recycled manure. However, the bacterial numbers in PCC used in maternity stalls did not differ when compared to recycled manure. Pelleted corn cobs in the tie and free stalls had a significantly lower pH, and higher dry matter content than PCC in the maternity box stalls (control studies). Data suggested that the management and handling of bedding materials may have an influence on the bacterial populations in beddings.

There was a significant increase in the number of bacteria in all beddings following 24 hours of use. Maximum changes in pH and moisture content of all beddings also occurred within 24 hours. Data suggested that contamination of bedding materials by environment and animal occurs rapidly.
No direct relationship was shown to exist between bedding CFU/g and ambient temperature. A positive correlation was shown to exist between the number of *Klebsiella* spp. in all beddings and the percent relative humidity. The duration of the experimental period was likely insufficient to evaluate a relationship between ambient conditions and the bacterial numbers in beddings.
REFERENCES CITED


