12-2015

Effects of bedding with recycled sand on lying behaviors, udder hygiene, and preferences of lactating Holstein dairy cows

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To the Graduate Council:

I am submitting herewith a thesis written by Heather DeAnna Ingle entitled "Effects of bedding with recycled sand on lying behaviors, udder hygiene, and preferences of lactating Holstein dairy cows." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Effects of bedding with recycled sand on lying behaviors, udder hygiene, and preferences of lactating Holstein dairy cows

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Heather DeAnna Ingle

December 2015

DEDICATION

To my family. Much love.

ACKNOWLEDGEMENTS

I would like to thank the Department of Animal Science and Dr. Peter Krawczel for taking me on as a graduate student. Thank you to Dr. Michael Smith and Dr. Hank Kattesh for being on my committee and contributing to my program as a whole. Thank you to everyone that helped on my project.

Never could I have made it this far without the help from fellow graduate students (Randi, Sierra, Nicole).

Thank you Bee for pulling me through and supporting me every step of the way. I appreciate you and your family. The prayers I received from you guys was unbelievable and so truly appreciated. Sisters for life Bee.

Thank you Jess for calming me down during one of the most stressful times in my life. You helped me have fun and relax even when things didn't seem bright at all. You made me realize the world wouldn't end if I failed. So, thank you for all of your support.

And most importantly thank you to my family. The love and support I've received through these years has been such a blessing. Thank you and love you guys with all of my heart.

Mom, Dad, I can't thank you guys enough and truly appreciate everything you've done for me and can't wait to see where life takes me. Love you guys with all my heart.

And thank you God for guiding me through this difficult time in my life and giving me the strength to carry on.

ABSTRACT

Effects of bedding with recycled sand and season on lying behaviors, stress, hygiene, and preferences of late-lactation Holstein cows were studied. Cows (n=64) were divided into 4 groups (n=8 per group) per season. In summer (Aug-Sept), cows were balanced by days in milk (268.1±11.9 d) and parity (2.0±0.2). In winter (Jan-Feb), mean DIM was 265.5±34.1 d. Cows were assigned to one of two treatments (trt) using a crossover design with each trt lasting 7-d (no-choice phase): bedding with recycled sand (RS; n=32) or control (CO; clean sand; n=32). Stocking density was maintained at 100%. Choice phase allowed a cow to have each treatment. Accelerometers recorded daily lying time/d, number of lying bouts/d, lying bout duration (min/bout), and total steps/d. Blood, teat swabs, milk, sand samples, and udder hygiene scores were collected on d 0, 3, and 7 of each experimental week. Blood was used to assess levels of cortisol. Samples were cultured for Streptococci, Staphylococci, and gram-negative bacteria. Video data was used to assess bedding preferences. All data were analyzed using the MIXED and GLIMMIX procedures of SAS 9.3. Lying time was not affected by treatment, but there was an increase in steps during winter. Cortisol was higher for control cows in summer and recycled sand in winter. Bacterial counts were higher for cows on recycled sand. Hygiene scores were higher for cows on recycled sand during the summer. There was not a preference for control or recycled sand.

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CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Animal welfare issues have been increasingly apparent in the industry today for agriculture animals. Producers are now doing their best to identify these issues and make corrective actions. The meaning of animal welfare includes mental and physical needs of an animal and ensuring that those are met. These needs have changed throughout the years dependent on each animal. Specifically for this research, dairy cow welfare is of importance. Many aspects have been considered for the welfare of dairy cows. Research has indicated many different areas that must be met to provide an adequate environment for dairy cows. Among these were adequate diet and appropriate housing. Within the housing environment many variables affect cows. Housing needs to provide comfortable lying surface for dairy cows. The focus of this research was bedding materials for lactating Holstein dairy cows.

LYING BEHAVIORS

Factors that Affect Lying Behaviors

Lying time has been proven to be important to dairy cows as they willingly give up other aspects such as feeding and socialization (Munksgaard et al., 2005) to spend more time lying down. A high producing dairy cow spend on average, 4.3 ± 1.1 h/d feeding, 2.7 ± 1.1 h/d milking, and 11.9 ± 2.4 h/d lying (Gomez and Cook, 2010) ; it important to note that almost half of the day was spent lying down. This need for high amounts of lying time has been proven to help production as when dairy cows laid down blood flow to the udder increased by 28%

(Metcalf et al., 1992).This increased amount of flow to the udder was only one factor that positively contributed to production.

Among other factors that contributed to milk production, diet was identified as one of the major contributing factors. It was hard to assess differences among farms concerning production as most use different total mixed rations (TMR). However, a study conducted by Bach et al. (2008) took a novel approach by providing 47 dairy herds with the same feed ration to assess other factors that contributed to milk production. A few of the other major factors that contributed to milk production were how heifers were reared, whether feed refusals (provided enough feed to keep at ad libitum) were available, and stall availability (Bach et al., 2008). Within that study, as stall availability increased there was an increase in milk yield. This indicated that stall availability had an impact on lying time as there was probably decreased lying time when stall availability was low which contributed to the lower milk yield.

Stall availability has also been known to affect lying behaviors. When 12 cows were given 8 freestalls to lie in, lying time decreased by an hour when stocking density reached 150% and tendency to lie down after milking decreased by 13 minutes (Fregonesi et al., 2007b). Results were similar for a study that had a total of 34 cows per pen, where lying time decreased by an hour when stocking density reached 142% (Hill et al., 2009). These studies presented details that despite the number of cows, when stocking density reached a certain level, lying time decreased. This is consistent with a study by Krawczel et al. (2012) which reported that when stocking densities reached 131% and 142% lying time decreased; however, there was only a 30 minute decrease in lying time for the higher stocking densities. Lying behaviors were altered by stocking density, but the relatively low decrease in lying time may have not been biologically significant with respect to production. The short duration of the studies need to be considered in the interpretation of their data.

Seasonal Weather Effect on Lying Behaviors

Dairy cows changed lying behaviors between seasons. It was found that lying time was highest in winter months (December – March) and lowest in summer months (June – September); there were no differences in lying time for spring (April – May) or in the autumn months of October – November (Brzozowska et al., 2014). These results are similar to those reported by De Palo et al. (2006) where lying time decreased when temperatures increased. The decreased lying times for these studies were likely due to heat-stress in the warmer months.

The upper critical air temperature for dairy cows was stated to be between 25 - 26°C by Berman et al. (1985), but later it was reported that heat-stress for dairy cows can begin at as low as 21°C. Once cows started experiencing heat stress, lying behaviors were affected. In a study comparing heat stress in three different states, Arizona, California, and Minnesota, it was noted that cows located in Arizona were potentially heat-stressed for the duration of the study because cows in Arizona stood longer than cows in California and Minnesota (Allen et al., 2015). These cows may have stood longer as it has been stated that the larger the surface area a cow exposes allowed more heat to dissipate (Berman, 2003). Another study found that when temperatures ranged from 18 - 24°C there was a three hour decrease in lying time (Cook et al., 2007), it was suggested that strategies such as fans and sprinklers be used once temperatures reach 21°C.

Type of Housing Effect on Lying Behaviors and Preferences

Housing structures have been shown to affect lying behaviors of dairy cows. These housing structures included types of confined housing as well as pasture based housing. For instance, when 25 cows were placed on pasture and freestalls bedded with washed river sand for 2 day periods and then had a 3 day choice period, cows spent almost 2 hours longer lying down when placed in freestalls (Legrand et al., 2009). However, when given the choice between pasture and freestalls, cows chose to spend most of their time on pasture, especially during the night, but use of pasture was affected by weather. When pastures were wet from heavy rainfall cows chose to spend nights inside the freestalls. Cows likely spent time indoors during the day due to temperature or the fact that feed was available indoors. Another study compared the use of pasture versus indoor cubicles, but found there were no differences in average daily lying times which were roughly 9.5 h/d (O'Driscoll et al., 2009). The inconsistent results of these studies were likely due to the time of year each study was conducted, summer and winter respectively. The type of indoor housing system may also have contributed to the differences in uses as different types of confinement housing such as tie-stall barn or composted bedded pack have affected lying behaviors.

Different types of confinement housing influence lying behaviors. When cows were placed in tie-stalls with concrete covered with chopped straw and box stalls with mattress flooring covered with straw, cows spent four more hours lying in the box stall than in tie-stall (Haley et al., 2000). The difference in lying time was likely due to the comfort level of the box stalls with the addition of mattress flooring compared to concrete. In a study that compared freestalls and an open pack that were bedded with a geotextile base covered with washed river sand, cows spent less time lying down when restricted to stalls, but lying time was adequate at 12.5 h/d (Fregonesi et al., 2009b). When cows were given the choice between the open pack and freestall, cows chose to spend more time in the open pack area (Fregonesi et al., 2009b). These differences in preferences may in part have been due to the type of bedding provided with cows choosing which was most the most comfortable.

Freestall Design Change Lying Behaviors

Particular stall designs have affected lying behaviors of dairy cows. For instance, when two freestall designs were compared, one with Dutch-style partitions 1.2 m wide with a neck rail positioned 1.1 m above stall surface and one without partitions and neck-rails, there were no differences in lying time, but cows spent more time standing in the stalls without partitions and neck-rails (Abade et al., 2015). Although there were no differences in lying time, cows preferred to spend a majority of the lying time in conventional freestalls with partitions and neck-rails (Abade et al., 2015). The position of partitions within the stall has affected lying behaviors of dairy cows as well. When partitions were wide (132 cm) lying time and lying bouts were longer than when partitions were 112 cm wide (Tucker et al., 2004). Although lunge space to allow for cows natural movements when rising availability did not have an effect on lying time when partitions were 132 cm wide, there has been a recommended space allowance for cows to lunge when rising. The recommended lunge space for a Holstein cow was 300 cm of longitudinal space and 109 cm of lateral space (Ceballos, 2003). The lunge space provided.

Another aspect of the stall that has affected lying behavior was neck rail placement. Less time was spent standing with front two hooves in the stall when the neck rail was placed further from the curb, but there were no differences in lying time (Fregonesi et al., 2009a). Similar results were found when neck rails were placed 140, 175, and 233 cm from the curb, where cows spent more time standing in the stall when the neck rail was furthest from the curb (Tucker et al., 2005). The more space a cow was allotted allowed for more moveable space, but this space has been affected by the addition of the brisket board. When a wooden brisket board was placed 20 cm high and 227 cm from the rear curb, cows spent an hour less time lying in stalls with a brisket board than a stall without one (Tucker et al., 2006). It is possible that cows may have spent less time lying in stalls with a brisket board as it made the stall more restrictive.

Bedding Materials Altered Lying Behaviors

Producers have used many different types of bedding materials, such as straw, mattresses, and sand. During an on farm survey, dairies with sand had 50% of cows lying down compared to only 40% of cows lying down on bedding types such as sawdust, straw, or composted manure (Lombard et al., 2010).This greater lying time with sand may have been due to when samples were taken, but there was also an indication that lying time was increased when stalls had recently been bedded (Lombard et al., 2010). It has also been reported by van Gastelen et al (2011) that lying bouts were longer on sand (92.0 ± 12.9 min) when compared to mattresses (47.9 ± 7.4 min) and box compost (46.1 ± 18.5 min). These increased lying bouts on sand may have been indicative of a greater comfort level with this type of bedding.

In other studies cows have expressed a preference for straw bedding when compared to sand (Manninen et al., 2002); but, cows that had previous exposure to sand bedding were more likely to accept sand as a bedding material than those just housed on straw (Norring et al., 2008).

In contrast, sand or sawdust were the preferred bedding materials when compared to mattresses (Tucker et al., 2003). In that study, lying time was lowest on sand bedding for a choice phase experiment compared to sawdust and mattresses, but lying time was very low for two of the cows while placed on the sand treatment signifying that these two cows specifically preferred sawdust or mattresses. When stall bases of rubber mats, sand, and concrete were covered with a small amount of straw bedding, lying time was highest on rubber mats compared to sand and concrete based stalls (Norring et al., 2008). Preferences may have been affected by maintenance of stalls as it has been known to alter lying behaviors.

During wet weather, cows were placed in a “stand-off” area to lower damage to grazing area, and cows preferred a wood chip pad over a concrete pad as lying time was higher (Fisher et al., 2002, Schütz and Cox, 2014). When cows were given a geotextile mattress and concrete flooring in tie-stalls, cows spent more time lying on the geotextile mattress (Haley et al., 2001) as this provided a more comfortable lying surface. When comparing straw bedding and soft lying mats, there were no differences in lying time, lying bout length, or number of lying bouts (Wechsler et al., 2000).

Effects of Bedding Maintenance on Lying Behaviors

Stall maintenance has a direct effect on lying behaviors of dairy cows. Drissler et al. (2005) found that lying time decreased when sand bedding became concave over a 10 d period. Regular maintenance of bedding level was important in the depth of bedding available to cows, as bedding was pulled or kicked into the alleyways often after bedding was added. Depth of straw and shavings affected lying time, as for each additional kilogram of bedding, lying time increased (Tucker et al., 2009). Moisture also affected lying behaviors of cows. Fregonesi et al. (2007) found that when cows were placed on wet sawdust bedding, lying time decreased by 5 h/d, and when given the choice between dry or wet sawdust bedding, cows spent 12.5 ± 0.3 h/d on dry bedding while only spending 0.9 ± 0.3 h/d on wet bedding. Dairy calves decreased lying time when wet sawdust bedding was provided, and calves preferred the driest sawdust bedding available (Camiloti et al., 2012). When different levels of dry matter were used for bedding, cows increased lying time as dry matter increased; however, lying time in the summer was lower than in the winter despite the dry matter percent (Reich et al., 2010). This indicated that seasons had an effect on lying time.

Mastitis Incidence Altered by Lying Behaviors

Dairy cows that were infected with mastitis changed lying habits. Cows that had clinical mastitis increased total number of steps and decreased amount of time spent lying, but lying time was only decreased during the acute phase of mastitis which was during the first 20 h (Siivonen et al., 2011). In another study with clinical mastitis, cows with clinical mastitis had decreased lying time on d 2 (Medrano-Galarza et al., 2012). When cows were challenged with *Escherichia coli*, cows increased time spent standing on d 0, but there were no differences in standing time between mastitic cows and control on d 1 and 2 (Fogsgaard et al., 2012). Lying time decreased on challenge day compared to lying time prior to inoculation, but cows had no preference on which side they laid on when mastitis occurred (Cyples et al., 2012). It was an interesting find that cows had no preference to which side they laid on as it was expected they would avoid the mastitis side due to pain.

MICROBIOLOGY

Mastitis is and continues to be a leading cause of money loss in the dairy industry. Over 90% of cases of clinical mastitis were recommended to be treated with cost of each treatment priced around $50 (Bar et al., 2008). However, total costs lost due to clinical mastitis were roughly $180 per case, with most loss due to milk (Bar et al., 2008). Mastitis occurres when bacteria enteres through the teat canal into the mammary gland and multiplies, resulting in inflammation (Harmon, 1994). Many bacteria have been identified as mastitis causing agents. Bacteria which generally cause mastitis have been split into two major categories, contagious and environmental. The major bacteria considered contagious were *Staphylococcus aureus* and *Streptococcus* spp, which are spread mainly through the milking parlor (Wilson et al., 1997, Barclay and Ji, 2014). Preventative measures suggested improved hygiene in the parlor, including teats cleaned properly, one towel per cow, and changed milking unit liners. The bacteria for environmental mastitis were coliforms and environmental streptococci (Hogan and Smith, 2012). The key was to reduce exposure of these pathogens from dairy cows, but this was difficult to accomplish as the major reservoir for these bacteria was the area in which they were housed (Hogan and Smith, 2012).

Type of Housing and Relation to Mastitis Incidence

Different types of housing affect rate of mastitis. For example, cows housed in confinement developed clinical mastitis 1.8 times more often compared to cows on pasture; the incidence rate of mastitis on pasture was 24% while 43% was in confinement housing (Washburn et al., 2002). This study was representative of three years on pasture and confinement, which allowed weather to be considered. Weather was important to include as during wet weather pasture based cows were likely to lower in hygiene due to mud and standing water.

Clinical mastitis occurred at different rates when various confinement housing systems were compared. When 533 freestalls and 59 tie stall barns were compared, tie stall barns had a higher rate for clinical mastitis (Valde et al., 1996).The occurrence of intramammary infections detected by milk sample culture were different between housing. Occurrence of intramammary infections in tie stalls (22.2%) was higher than those in freestalls (12.8%), bedded packs (12.8%), and paddocks (7.1%). The pathogen that was most isolated from milk samples that caused mastitis was *Staphylococcus aureus* (Ferguson et al., 2007).

Hygiene and Factors in Cow's Habitat

Hygiene of the cow's udder was one of the most significant factors to be considered when mammary infections were a problem, especially the teat end (Neave et al., 1966). Methods were developed to assess the level of contamination to which cows were subjected. One of the most used tools was to score udder contamination from the rear-view of the cows. The scoring system by Cook and Reinemann (2007) was 1 (free of dirt), 2 (slightly dirty), 3 (moderately covered with dirt), and 4 (covered with caked on dirt). When hygiene scores increased, potential problems arose as there was an association of isolating major mastitic pathogens between a score of 2 and 4 (Schreiner and Ruegg, 2003). Another scoring system also assessed leg and flank cleanliness (Reneau et al., 2005). This score sheet had 5 areas (tail head, thigh, abdomen, udder and hind limbs) and scaled 1 (clean) to 5 (dirty). This system was more detailed and provides a better understanding of the different areas of concern.

Facilities Affected Hygiene. Cows were exposed to contaminated areas within housing, such as the alleyways and the freestalls themselves. When cows were housed in freestalls, scores for lower legs increased (Cook, 2002). When housed in tie stalls upper leg and flank areas became a concern as hygiene decreased due to the location of defecation and lying area (Cook, 2002). These different housing systems presented different areas of concern, but alleyways were the most problematic.

Cleanliness of alleyways was an important factor as manure built up and provided an area for bacteria to grow. Scraping of the alleyways improved hygiene. When alleyways were scraped with an automatic hydraulic scraper, udder scores improved by dropping 27% and teat end scores dropped 37% when housed in freestalls (Magnusson et al., 2008). This specific study used a different method of scoring where a 100 mm ruler was used to measure how much of an area was covered in dirt (Magnusson et al., 2008). The results from this study showed improvement, however, the alleyways were scraped 12 times a day. This is not likely to occur at other facilities with different types of alley scrapers.

Stall Design and Cleanliness. Design of freestalls affected cleanliness of stalls. When stall measurements were compared, dirtiness of stalls increased as width of stalls increased (Tucker et al., 2004). There was a positive factor with the increased width of stall as lying time increased; however, standing with all four hooves in the stall increased as well, which contributed to the uncleanliness of the stalls. A part of the stall that helped to alleviate this unclean condition was restrictive neck-rail placements. When freestalls had less restrictive neck-rails positioned at 190 cm from the rear curb, there was an increase in udder scores and stall cleanliness decreased (Fregonesi et al., 2009a). The most restrictive neck-rail at 130 cm from the rear curb provided the cleanest freestalls and improved udder hygiene. Another study had similar results when freestalls had neck-rails restricted to 130 cm from the rear curb stall cleanliness improved (Bernardi et al., 2009). There was also a decrease in the time it took to prep teat ends for milking, which was likely due to the improvement of stall cleanliness. When cows were allotted more space to stand in freestalls, they were more likely to urinate and defecate within the stall which likely allowed adherence of bedding to the udder to increase.

Bedding Material and Cleanliness. When cows were housed on comfort mats, concrete, and rubber mats, the mats provided cleaner areas compared to concrete (Herlin, 1997); however, mats were probably cleaner than concrete due to the comfort level provided by the mats. When cows were not provided with a comfortable lying surface, standing within the stall increased which likely increased defecation and urination within the stall. In another study differences in stall and cow hygiene between compost packs, rubber filled mattresses, sand, and waterbeds across multiple states were observed (Fulwider et al., 2007). They found that hygiene scores were lower on mattresses and waterbeds when compared to sand, and compost packs were similar to hygiene with cows on waterbeds (Fulwider et al., 2007). Hygiene was likely affected by the frequency of bedding added and number of times areas were cleaned. When stall cleanliness was compared between sand and straw bedding there were no differences, but cow cleanliness for midleg, upper legs, and abdomen were cleaner on sand (Norring et al., 2008).

Cow cleanliness on different beddings was important as these were the areas that housed environmental pathogens linked with mastitis.

Bedding Material and Bacterial Populations

Bacterial growth is different between bedding materials and it is important to understand these are udders are exposed to this environment, and specifically teat ends. Bacteria growth excel when housed in organic bedding materials under high temperatures and humidity conditions. For example, when sand, foam mattresses with sawdust bedding, horse manure, and box compost were compared, sand had the lowest bacterial colonies of gram-positive bacteria followed by box compost then did horse manure (van Gastelen et al., 2011). There was an interesting finding in that gram-negative bacteria in sand and box compost were similar (van Gastelen et al., 2011), which is an unusual occurence. Four different types of bedding material representing clean sand, shavings, recycled sand, and separated post digested manure solids, were used to evaluate growth of *Enterococcus faecium* and *Klebsiella pneumoniae* (Godden et al., 2008). *Klebsiella pneumoniae* grew the most in the manure solids and least in clean sand. Although *Enterococcus faeciumi* growth was low in manure solids and recycled sand, growth was even lower in clean sand and shavings (Godden et al., 2008). Similarly, Sorter et al. (2014) found that bacterial growth was high in deep-packed recycled manure bedding, but when cleaned and the back third of the stall was replaced, bacteria like *Klebsiella* spp. were lowered but not streptococcal counts. Bacterial growth was still high throughout the study with daily replacement of bedding (Sorter et al., 2014). To obtain lower bacterial counts, daily replacement of bedding was required, but it was determined that was not a favorable option for dairy producers as costs and labor would have increased.

Several studies have examined the effects of conditioners and disinfectants on bedding in an effort to lower bacterial loads in different types of bedding exhibiting high bacterial growth. Hogan et al. (1999) tested three different commercially available conditioners, granulated alkaline conditioner, granulated acidic conditioner, and hydrated lime, on bacteria growth in recycled manure and sawdust. Hydrated lime and alkaline conditioner were best for recycled manure, while an acidic conditioner was best for sawdust; however, the effect of the conditioner started to wear off between d 2 and 6, meaning conditioners needed to be added often in these bedding to obtain lower bacterial growth. A similar study tested hydrated lime, coal fly ash, kiln-dried wood shavings, acidic bedding conditioner, and no bedding on mattresses which were added on d 1, 3, and 5 (Kristula et al., 2008). Hydrated lime was the only treatment that lowered gram-negative bacteria such as *Klebsiella* spp., but skin irritation occurred when hydrated lime was used. Teat swabs were collected from cows housed on pasture and fresh straw and bacterial growth was tested with the use of iodine. Although, low concentrations of iodine were able to inhibit growth of bacteria such as *Staphylococcus aureus*, it did not inhibit growth of *Klebsiella pneumoniae* (Waltemyer et al., 2014). *Mycoplasma* species, which can cause mastitis, metritis, pneumonia, and death, was able to replicate significantly in recycled sand; however, numbers were reduced when bedding was treated with 0.5% sodium hypochlorite or 2% chlorhexidine (Justice-Allen et al., 2010). The sand that was collected for the use in this study, tested positive for *Mycoplasma* as the dairy it came from was experiencing clinical mastitis. The use of sodium hypochlorite and chlorhexidine were able to diminish the bacteria; however, because of its caustic properties, it would need to be tested for effects on skin before it should be used on dairy operations.

The depth of bedding was also important as bacterial growth was different throughout the layers of bedding. Bacterial populations were higher in depths of 50 – 75 mm when compared to the top portion at 25 mm in clean sand that had been propane flamed for control of bacterial populations (Hogan et al., 2012). Propane flaming also helped reduce bacterial loads in recycled sand, with the greatest decrease in bacterial populations occurring 25 mm below the top portion of the bedding. The decreased bacterial loads at different levels was important because cows kicked or raked bedding out before lying which exposed the lower levels of bedding.

Bedding Adhered to Teat Ends

The anatomy of the mammary gland helps to protect itself from bacteria invasion. The streak canal was identified as the primary defense against pathogen entry (Sordillo et al., 1997). The streak canal, covered with stratified squamous epithelium, protects the mammary gland in two ways: closed entryway for bacteria and formed a keratin plug that block bacteria entry. These are very important mechanisms that helped reduce intramammary infections. The teat canal remains open up to an hour after milking, which increases the ability of bacteria to enter. Producers are able to add another step of preventative measures with post-dipping after milking (Dahl et al., 2007). The streak canal and post-dip helped provide protection against bacteria that are potentially housed in bedding.

Bedding material used for dairy cows housed a multitude of bacteria. Dependent upon the type of bedding, adhesion of bacteria to the teat ends were higher. For instance, teat swabs collected from cows housed on sawdust had higher coliforms and *Klebsiella* than shavings and straw, but *Streptococci* and *Staphylococci* were highest in straw bedding (Rendos et al., 1975).

Gram-negative bacteria, coliforms, *Klebsiella* species and staphylococcal counts were higher on pelleted corn cobs than chopped newspaper when compared between chopped newspaper and wood shavings, but all counts were higher on chopped newspaper than wood shavings (Hogan et al., 1990). Teat swabs collected from cows had higher counts of *Klebsiella* and coliforms when housed on sawdust bedding compared to sand; however, *Streptococcus* spp. were 10 times higher in sand (Zdanowicz et al., 2004). Different bedding materials provided different growths of bacteria.

PHYSIOLOGICAL MEASURES

There are many definitions of stress, but Moberg (2000) defined stress as "the biological response elicited when an individual perceives a threat to its homeostasis." Stress happens when there is a stressor which is the actual event or threat against the animal. Stress is been difficult to measure in animals. It is especially difficult to separate acute and chronic stress. Acute stress has been defined as relatively short and a single stressor; whereas chronic stress was multiple acute stressors that acted upon the animal for a period of time (Moberg, 2000). Acute stress affect animals in one of two ways. There is either 1) a disruption of critical biological events, for example isolation disrupted ovulation, or 2) diversion of resources away from biological functions, for example growth was slowed down when youths were restrained for multiple hours (Moberg, 2000).

A popular indicator for stress has been the measure of cortisol concentrations within the blood. Cortisol has played multiple roles in the body and has been identified as the "stress hormone." When stressed, cortisol is released in excess. The reaction behind cortisol release start with the activation of the hypothalamus (Matteri et al., 2000). The hypothalamus releases corticotropin-releasing factor that activates the anterior pituitary which in turn, releases adrenocorticotropin. This hormone then activates the adrenal cortex which then released more cortisol into circulation.

However, the normal cycles of cortisol need to be taken into consideration when studies are being conducted. The normal ultradian rhythm for cortisol has a period of 120 min with a very weak circadian rhythm (Lefcourt et al., 1993). There have been contradictions concerning diurnal patterns of cortisol. Hudson et al. (1975) found no diurnal patterns, while Wagner and Oxenreider (1972) found circulation cortisol concentrations to be significantly lower between 1800 – 0200 h. In addition, Wagner and Oxenreider (1972) and Macadam and Eberhart (1972) found no early morning peaks in the pattern, but Hudson et al. (1975) found a peak in cortisol concentration when cows were awakening. These differences were likely due to the difference in environments or the cow itself as concentrations varied among cows (Moberg, 1985).

Handling Caused Stress

There is an association between animal fear and stockperson attitudes. When the stockperson showed moderate negative interactions there was a significant increase in milk cortisol (Hemsworth et al., 2000). Handling cows for blood sampling can cause an increase in cortisol concentrations (Hopster et al., 1999). When blood was collected once a day, cows that had previous exposure to handling remained steady but, primiparious dairy cows that had less handling had increased cortisol concentrations during blood collection. Therefore it was determined that routine handling of cows affectes cortisol concentrations. When cortisol concentrations were measured after different types of handling, cows experienced no increase in cortisol concentrations after restraint for 30 minutes but, there was a slight increase after milking and cleaning of stalls (Bertoni et al., 2010). This indicates that the handling or presence of the personnel affected cortisol concentrations. There has also been an occurrence of increased cortisol concentrations when cows were in isolation. Cows placed in isolation with and without human contact had higher cortisol concentrations compared to control cows but, no differences in cortisol concentrations occurred between isolation cows (Rushen et al., 2001). This indicates that isolation from other cows may cause more stress than handling from personnel.

Lying Deprivation Causes Changes in Cortisol Concentrations

When the lying behaviors of cattle were altered, a stress response can occur. Young dairy bulls that were deprived of roughly 14 hours of lying time by having a girth strap wrapped around the middle portion of their body tended to have a higher cortisol release when challenged with ACTH (Munksgaard et al., 1999). However, there were no differences in cortisol concentrations between isolated, lying deprived, and control cows (Munksgaard and Simonsen, 1996). In another study, when cows were deprived of lying with an electrical girth strap, cortisol concentrations were higher for cows deprived of lying (Fisher et al., 2002). It was reported that once cows had been shocked they would rarely try to lie down again while the strap was on. It was stated that the increased cortisol was from deprivation of lying and not from the electrical girth strap.

There are contradictory studies on overstocking and cortisol concentrations. Cows that were exposed to 0.37 and 0.50 freestalls per cow for 7 d, had higher cortisol concentrations compared with cows exposed to 0.75 and 0.63 freestalls per cow (Friend et al., 1979). However, when fecal cortisol metabolites were measured, there were no differences between stocking densities of 100, 113, 131, and 142% (Krawczel et al., 2012). Similarly, when cortisol concentrations were compared between control (1 stall per cow) and overstocked (1 stall per 2 cows), cortisol concentrations did not differ (Huzzey et al., 2012). These results may or may not have been affected by the duration of the studies as they may have been too short to cause a response by no decrease in lying time.

Housing Areas Affected Cortisol Concentrations

Different types of bedding materials affect cortisol concentrations. For instance, when cows were moved from pasture to a “stand-off” area during the wet season to help lower soil damage, cortisol concentrations decreased with bedding materials used (Schütz and Cox, 2014). Cortisol concentrations decreased when removal area had 12-mm rubber mat, 24-mm rubber mat, concrete, or wood chips cortisol concentrations decreased on all types, but 24-mm rubber mat cortisol concentrations decreased by 45% compared to 34% on wood chips, 27% on concrete, and 22% on 12-mm rubber mat. When indoor and outdoor housing with a wood chip surfaces were compared, cows had higher concentrations of cortisol when outdoors than when indoors, but the change in concentration could have been attributed to the decreased lying time while outdoors as cows decreased lying time to 4 h/d(Tucker et al., 2007) . These factors are important to take into consideration when providing the most adequate and comfortable bedding for dairy cattle.

CHAPTER II

EFFECTS OF BEDDING WITH RECYCLED SAND ON LYING BEHAVIORS, UDDER HYGIENE, AND PREFERENCES OF LACTATING HOLSTEIN DAIRY COWS

INTRODUCTION

Providing adequate lying surface for dairy cows is of high importance as dairy cows spend 12 – 13 h/d lying (Jensen et al., 2005). Lying time is one of many factors that contribute to their health and production, but is important in milk production as blood flow increases while cows lie down which aids in production (Metcalf et al., 1992). However, many factors, including bedding material, affect lying time.

Different bedding materials that are used for comfortable lying surfaces affect length of time, as well as quality of lying time. For instance, lying time was higher on straw when given the choice between straw and sand (Norring et al., 2008). However, lying time differences were likely due to previous exposure or preference for one bedding material over the other. Cows that had previous exposure to sand spent equal amounts of time on sand and sawdust while cows that spent most of their time on sawdust as they had previous exposure to sawdust (Tucker et al., 2003). Not only did bedding type affect choice of bedding, but quality of bedding has played a role.

Quality of bedding has affected time spent lying, as when bedding was wet cows reduced lying time by five hours (Fregonesi et al., 2007c). Reich et al. (2010) had similar results for wet sawdust bedding, but lying time decreased for all bedding during the summer likely due to heat stress, there is the possibility to induce stress as cows that were deprived of lying time had an increase in cortisol concentrations (Fisher et al., 2002). As these studies had a decrease in lying time, potential physiological stressors may have appeared as they did with deprivation of lying time (Fisher et al., 2002). There was also the potential for reduced hygiene levels due to the increased wetness of bedding material in contact with the udder.

Hygiene is a concern as udders placed in areas with high bacterial load could lead to potential mastitis risks. There have been different reports for cows housed on sand. Zdanowicz et al. (2004) reported that rear udders were dirtier on sand bedding when compared to sawdust bedding. Conversely, Norring et al. (2008) found that when cows were housed on sand, udder cleanliness and hoof health was better compared to cows housed on sawdust. Dependent upon cleanliness of the bedding, this could be a potential problem as there is an association with isolating pathogens and higher udder scores (Schreiner and Ruegg, 2003).

Some bedding materials had higher bacterial loads than others, which is important as a cows' teat ends are in close proximity with bedding. Bacterial counts on teat ends of cows was relatively weak with cows housed on sand compared to cows housed on sawdust bedding; however, streptococci was higher on teat ends when cows were housed on sand bedding (Zdanowicz et al., 2004). Furthermore, sawdust bedding that had been treated with alkaline conditioner versus non-treated had no association with teat skin and teat canal bacterial counts of *Stapylococcus aureus* (Paduch et al., 2013). Clean sand had the lowest growth of *Klebsiella pneumonia*, while post digested manure solids had the highest growth (Godden et al., 2008). These differences in bacterial loads were important as they gave an indication of growth in sand, but little research has been completed on recycled sand.

Kristula et al. (2005) determined that bacterial counts for clean sand and recycled sand were similar a week after bedding was added and Harner et al. (2009) reported that bacteria in recycled sand peaked at 72 hours. It was argued that recycled sand had potential as a bedding material for dairy cows. Justice-Allen et al. (2010) found that recycled sand bedding is a source for *Mycoplasma* spp., bacteria that causes mastitis; however, when recycled sand was cleaned with a common disinfectant, *Mycoplasma* spp. could no longer be isolated. These studies are limited in the fact that there was not a cow component, and how the use of recycled sand may affect lying behaviors, hygiene, or preferences.

There have been no reports on how recycled sand affects lying behaviors or cleanliness of dairy cows. The first objective of this study was to determine the effects of using recycled sand as bedding on lying behaviors, stress hormones, and hygiene of late-lactation Holstein dairy cows. The second objective was to determine the preference between recycled sand and clean sand among late-lactation Holstein dairy cows.

MATERIALS AND METHODS

This study was conducted at the University of Tennessee's Little River Animal and Environmental Unit (Walland, TN) during August-September 2014 and January-February 2015, and was approved by the Institutional Animal Care and Use Committee. Late-lactation Holstein dairy cows (n = 64) were split evenly between seasonal experiments. For each season, cows were assigned to 1 of 4 groups with each group consisting of 8 cows and balanced by days in milk (Summer: 268.1 ± 11.9 d; Winter: 265.5 ± 6.0 d) and parity (Summer: 2.0 ± 0.2). All cows were pregnant during the summer; however, in the winter, 16 cows were non-pregnant. Cows were milked twice daily between 0700 and 0900 h and 1730 and 1900 h. Cows were housed in a 4-row free stall barn. Two experimental pens were split into two smaller pens, where stalls were blocked off with gates to obtain one stall per cow totaling in 8 useable stalls per pen. An equal number of freestalls were available on the back and feedbunk alley. The bed length was 2.4 m with a width of 1.2 m. Neck rail height was 1.2 m with the brisket board 1.7 m from the rear curb. Alleyways were flushed with water twice daily to rid alleys of manure and other debris.

Feed bunk headlocks were blocked off to provide 8 useable headlocks per experimental pen. Cows were fed fresh total mixed ration (TMR) two times/d (0700 h and 1530 h), and feed was pushed up two time/d. The TMR was comprised of 60% corn silage, 25% pelleted premix grain concentrate, 12% small grain silage, and 3% dry hay. Water was available for ad libitum consumption from a ball trough.

Treatments

Freestall bedding was either control (clean, unused sand) or recycled sand (reclaimed from the dairy's flushing system). Recycled sand was collected from the gravity slope of the flushing system, and stored in an uncovered area. When sand was to be added, treatments were turned with a skit steer to ensure that recycled sand treatment was properly mixed. Control sand was stored under a covering. Bedding was added daily to maintain level with the curb. Stalls were raked twice daily during each milking.

Two phases were completed for this study: a no-choice phase and a choice phase. For the no-choice phase cows (n = 8 per experimental pen) were provided with either recycled sand or control sand for week one. A cross-over design was used which allowed for each cow to be their own control. Cows switched treatment for week two. The choice phase allowed cows (n = 8 per 16 stalls) to choose between control or recycled sand for one week.

No-Choice Phase

The no-choice phase consisted of a weeklong treatment (control or recycled sand) where stocking density was kept at 100%, where one stall was available per cow. At the end of the first week, cows switched treatments Cows were placed on clean or recycled sand for one week, then a crossover occurred and treatments switched within groups.

Lying Behaviors. IceTag data loggers (IceRobotics Ltd., Edinburgh, Scotland) were attached to the rear fetlock while in the milking parlor two days prior to start of the experiment to allow for habituation (MacKay et al., 2012). Data loggers remained on the cows for the duration of the study. Data loggers collected daily lying times (h/d), lying bout frequency (number/d), lying bout length (min/bout), and total steps (number/d). Each measurement was used to calculate a daily average for each variable.

Lying behaviors were monitored by video for later analysis. Behavior was recorded for a continuous 24 h for each week using an EZ Bullet VF Platinum Weatherproof IR camera (EZWatch, Louisville, Kentucky) positioned above the each experimental pen that included two of the smaller pens. The camera was mounted above each pen and Turbo View 16 Channel Platinum HD DVR (EZWatch, Louisville, Kentucky) was used to record data. During the summer, due to technical difficulties, the three best days of video for each week were chosen, and lights were left on each night. In the winter, the last three days of each week were collected and infrared lights were used for nighttime analysis. Video data were analyzed using 10-min scan samples to assess how many cows were lying in a stall, lying in the alleyway, standing in a stall, standing in the alleyway, perching in the stall (front two hooves located in the stall), or at the feed bunk or waterer (Overton et al., 2002).

HOBO 4-channel analog data loggers (UX120-006M, Onset Computer Corporation, Bourne, MA) were placed in each experimental pen to collect sand temperatures of clean and recycled sand at 15 min intervals. Temperature probes were placed under the railings of stalls to ensure that the temperature collected was that of the sand and not the cow.

Dry Matter. Dry matter samples were collected from each pen on days 0, 3, and 7 of each experimental week. A metal scoop was assigned to each treatment and was used to obtain a small amount of bedding from the back third of each stall, in each pen per treatment. Samples were put in a plastic bag (Ziploc ®, Ziploc Storage Gallon Bags, Racine, WI) in a cold room (4°C) until ready for analysis. Samples were thoroughly mixed by agitating bag for at least 1 min, when a representative sample of 25 g was removed from bag. Samples were oven-dried at 55°C for 48 h and weighed.

Physiological Measures. Blood samples were collected on days 0, 3, and 7 before or after morning milking of each experimental week. Blood was collected via coccygeal vein while cows were restrained in the headlocks or a freestall. Each blood sample was centrifuged at a speed of 3800 rpm for 10 minutes; plasma was harvested, and stored in -80 °F freezer for later analysis. Serum total cortisol concentrations (ng/mL) were analyzed by using a radioimmunoassay (RIA) kit following procedures of Cortisol (MP Biomedicals, LLC, Orangeburg, NY). Samples were analyzed in duplicate and counted for 1 minute in a gamma counter (Cobra II Auto-gamma counter, Model D5005, Packard Instrument Co., Meriden, CT;

(Doherty et al., 2007). Intra- and interassay coefficients of variation were 104.2 and 12.4% for low and 63.7 and 14.5% for high cortisol samples.

Rectal temperatures were collected to obtain core body temperature on days 0, 3, and 7 of each experimental week while cows were in headlocks or freestalls. Temperatures were collected with a calibrated thermometer (GLA M500HPDT Thermometer and GLA M207R Probe, GLA Agricultural Electronics, San Luis Obispo, CA).

Udder Hygiene. Teat end samples were collected on days 0, 3, and 7 of each experimental week. To assess bacteria loads on teat ends, a sterile cotton swab in 4.5 mL Phosphate Buffered Saline (PBS) was run across the back left teat before teats were prepped for milking on days 0, 3, and 7 for week one and two. Samples were placed on ice until later analysis, and teat swabs were plated within 12 h of collection of sample. Teat swab samples were prepped by placing 100 µ of original sample into 4.5 mL of PBS for serial dilutions until countable plates were obtained (Hogan, 1999). For each further dilution, 100 µ was placed in 4.5 mL of PBS until countable plates were achieved. All media was prepared by following standard operating procedures (Forbes et al., 2002), and media was prepared and plated in a similar manner for milk samples and sand samples. For isolation of gram-negative bacteria, 1 mL of the appropriate dilution was plated on MacConkey agar (Oxoid Ltd., Basingstoke, Hants, England) that was prepared according to manufacturer's directions. Baird-Parker agar (Oxoid Ltd., Basingstoke, Hants, England) was used to isolate *staphylococcus spp.* by adding 1 mL of the appropriate dilution to each corresponding plate. For *streptococcus spp.*, Modified Edwards Medium (Oxoid Ltd., Basingstoke, Hants, England) was used to isolate bacteria by adding 1 mL of the appropriate dilution to each plate. Modified Edwards Medium was prepared to manufacturer's directions with the addition of 0.5 g of ferric citrate to each liter of agar. All samples were then incubated at 37 °C for 48 h (Hogan, 1999). Colony forming units (cfu) were manually counted for each agar plate.

After teats were prepped for milking, an aseptic milk composite sample was collected from each quarter on days 0, 3, and 7 of each experimental week. An alcohol swab was run across each teat end to ensure it was clean before collection of the milk sample, and then each teat was stripped to obtain a composite milk sample. Samples were frozen at -20°C until later analysis (Schukken et al., 1989). Once thawed to room temperature, composite milk samples were prepped by diluting 100 µ of original sample into 4.5 mL of PBS for serial dilutions until countable plates were obtained (Hogan, 1999). Samples were plated as stated prior in teat-swab analysis, and colony forming units (cfu) were manually counted for each agar.

A representative sample of sand was collected from each stall on days 0, 3, and 7 of each experimental week and thoroughly mixed before a representative sample was placed in a 120 mL wide mouth bio-tite specimen container (Fisher Scientific, Pittsburgh, PA). Samples were placed on ice until later analysis. Sand samples were plated within 12 h of collection of sample. For preparation of plating the bedding samples, 10 g of sand was placed in 90 mL of PBS and stirred on a hot plate with a stir bar for constant agitation to obtain 5 mL of the solution that was placed in 45 mL of PBS; this was the 10^{-2} dilution (Gooch et al., 2006). Samples were further plated as stated prior for teat-swab analysis, and colony forming units (cfu) were manually counted for each agar plate.

Udders were scored for hygiene on days 0, 3, and 7 of each week in the experimental pen. At the beginning of the study and between treatments, udders were cleaned with a brush. Each cow was visually scored from the rear on a scale of 0 to 3. A score of 0 was splashes of manure covered < 50% of the area, score of 1 was fresh splashes of manure covered > 50% of the area, score of 2 was dried and fresh manure covered > 50% of the area and a score of 3 was the entire area was covered with dried caked manure (adapted by Cook, 2011).

Choice Phase

In order to test preference for sand type, cows were given the option between clean and recycled sand for one week. Each pen had a total of 16 stalls available to allow each cow to have a stall with each treatment (Figure 1). Bedding treatments were placed in a checkerboard design to ensure that each treatment was spread evenly throughout the pen. Two groups were moved to another pen where they maintained grouping away from the herd for a week while the other two groups were participating in the experiment. A week later the earlier non-participating cows were moved back to the experimental pen. IceTag data loggers were left on during this experiment to collect similar data as previously mentioned. Data loggers were removed on the last day of the study. Video analysis was used to assess activity of cows within stall and treatment with which was being used to determine preferences of bedding. Location of stall was recorded to assess whether there was a stall preference for cows located in the front or back alley. Activity within the stall included lying, perching (front two hooves in stall), and standing (all hooves in stall).

Statistical Analysis

Data were analyzed as a cross-over design. Fixed effects were treatment and sample day for lying behaviors, cortisol concentrations, bacterial counts, and hygiene. The random effects were cow within treatment and sequence of events. The MIXED procedure of SAS was used for analysis. A log-transformation was used to normalize skewed data. Mean separation was configured by the GLIMMIX procedure to assess mans. Least square means are reported for continuous data, and the Frequency procedure was used for presence/absence percentages for variables with excessive zeros. A mixed model was used for video analysis with cow (n = 8) as the observational unit during the choice phase. Fixed effects for video analysis were location within pen, treatment, and activity. Random effects included pen within location, activity, and treatment. A Poisson distribution was used to assess frequency of activities within a given time.

RESULTS

An injury during the summer session resulted in a cow being removed during the second week of the study and data from that week was excluded from analysis. Data presented for teat end and bedding bacteria are only representative of winter due to the loss of summer samples. Sand temperatures were collected, however, due to cows digging up probes and cords being chewed data was not able to be analyzed.

No-Choice Phase

Lying behavior. There were no effects of treatment on lying behaviors during the summer session (Table 1). There was a sequence of events effect for frequency of lying bouts where cows that started on control sand had an average of 10.0 ± 0.6 number of lying bouts/d, while cows that started on recycled sand had 11.7 ± 0.6 number of lying bouts/d ($P = 0.04$). Cows on clean sand had greater number of steps during the winter session ($P = 0.03$; Table 2). There were no other sequences of events effects for the summer or winter session ($P > 0.05$).

Dry Matter. The mean dry matter values for control and recycled sand was 94.9 ± 0.9% and 92.3 ± 0.9% , respectively; and samples tended to differ between treatments ($P = 0.07$; Table 3). The mean dry matter values for control sand for the winter session was 93.9 ± 1.3% and 90.5 ± 1.3% for recycled sand. There was a treatment effect where dry matter was lower for recycled sand samples ($P = 0.02$; Table 4).

Physiological Measures. Cows had higher serum cortisol concentrations while on control sand than recycled sand during the summer session (0.75 ± 0.2 vs 0.34 ± 0.1 ng/mL, $P = 0.02$). Cortisol concentrations changed over time for summer ($P < 0.001$; Figure 2). Cows that started on recycled sand had higher cortisol concentrations ($P = 0.03$; Figure 3). Cortisol concentrations were higher for cows on recycled sand compared to control during the winter session (0.53 ± 0.1 vs. 1.3 ± 0.3 ng/mL; $P = 0.02$). There were changes over time in cortisol concentrations for winter session ($P < 0.0001$; Figure 4). Many of the samples were below detectable limits of the assay.

Body temperatures were the same for cows on control and recycled sand during the summer session (38.5°C; $P = 0.17$).Temperatures differed across sample days for treatments ($P < 0.001$; Table 5). Cows that started on control sand had lower body temperatures than those that started on recycled (38.5 vs 38.7°C; $P = 0.01$). In the summer, cows that started on control sand decreased in body temperature, while recycled sand cows increased (*P* = 0.003; Table 6). Body temperatures did not differ between control and recycled sand during winter session (38.0 vs 38.1°C; *P* = 0.13). Temperatures changed across time for winter (*P* < 0.001, Table 7). Temperatures increased for cows that started on control sand, but did not differ for cows that started on recycled sand (*P* < 0.001; Table 8).

Bacterial Counts. Teat swab samples for the summer session were lost due to technical error. Cows studied during the winter session had higher bacterial counts for MacConkey agar while on recycled sand (248.7 ± 77.5 vs 30.6 ± 78.3 cfu/mL; *P* = 0.05). Bacterial counts were higher for cows on recycled sand compared to cows on control for Baird-Parker agar plates (6,197.7 l counts vs 2,934.5 1 1,509.2 cfu/mL; *P* = 0.05). Bacterial counts increased throughout both experimental weeks, with the highest peaks on d 7 (*P* = 0.05, Figure 5). There were no differences between bacterial counts on control and recycled sand for Modified Edwards medium (1,533.3 ± 654.0 vs 1,525.6 ± 645.1 cfu/mL; *P* > 0.05).

Composite milk samples collected for summer session had no effects between control and recycled sand for bacterial counts on MacConkey agar (0.06 ± 0.03 vs 0.08 ± 0.03 cfu/mL; *P* > 0.05), Baird-Parker (0.46 ± 0.1 vs 0.51 ± 0.1 cfu/mL; *P* > 0.05), or Modified Edwards (0.10 ± 0.04 vs 0.09 ± 0.04 cfu/mL; *P* > 0.05). Bacterial counts were higher on sample d 7 for cows oncontrol sand for Modified Edwards (*P* = 0.03; Figure 6). For winter composite milk samples, no differences were observed for bacterial counts on MacConkey agar, Baird-Parker agar, or Modified Edwards medium (*P* > 0.05; Table 9).

Bacterial counts did not differ between control and recycled sand on MacConkey following culture (316,096.6 ± 181,046.6 vs 1,455,179.3 ± 833,464.6 cfu/mL; $P > 0.05$) or Baird-Parker (1,412,765.1 ± 863,455.4 vs 6,339,798.9 ± 3,874,765.7 cfu/mL, $P > 0.05$). Bacterial counts steadily increased through the experiment, but peaked on d 7 ($P = 0.04$; Figure 7). Bacterial counts did not differ between control and recycled sand on Modified Edwards (5,172,000 ± 3,460,527 vs 5,950,000 ± 3,460,527 cfu/mL; $P > 0.05$).

Hygiene. Hygiene scores were higher for cows on recycled sand compared to cows on control sand in the summer session (clean: 0.05 ± 0.03; recycled: 0.14 ± 0.03; $P = 0.02$). Hygiene scores did not differ between control and recycled sand for the winter session (0.15 ± 0.04 vs 0.10 ± 0.04; $P > 0.05$). Few cows were scored higher than a zero for both summer and winter.

Choice Phase

There was no preference between control and recycled sand during the summer session ($P = 0.80$). During any 10-min interval, 34.8% ± 0.1 of cows could be found in control while 32.0% ± 0.1 could be found in recycled sand. Cows spent more time lying in stalls than standing or perching during any 10-min interval ($P < 0.0001$; Figure 8). There was not a preference between control and recycled sand for the winter session ($P = 0.89$). During any 10-min interval, 25.9% ± 0.04 of cows could be found on control sand 25.5% ± 0.04 of cows could be found on recycled sand. Similarly in the winter session, cows spent more time lying in stalls than perching or standing for any 10-min interval ($P < 0.0001$; Figure 9).

DISCUSSION

In the current study, cows, regardless of treatment spent 11.7 ± 0.3 h/d lying in the summer and 11.9 ± 0.4 h/d lying in the winter. This behavior was consistent with the time budget of a lactating dairy cow averaging between 10 and 14 h/d lying previously reported (Cook et al., 2004, Ito et al., 2009). Obtaining sufficient rest was important as it was suggested that lying time facilities milk production. Blood flow increased by 28% to the udder when cows were lying (Metcalf et al., 1992). An evaluation of the primary nondietary factors affecting milk production reported stall maintenance and stall availability explained 38% of the variation in milk yield across the 45 enrolled farms (Bach et al., 2008). Additionally, when stall availability was below 0.8 stalls/cow, there were no farms within the high production range (Bach et al., 2008). Previous studies reported that when stocking density reached 131% and 142% cows reduced lying time by 30 to 84 min/d (Fregonesi et al., 2007; Krawczel et al., 2012). This indicates that stall availability may affect milk yield. Beyond milk yield, the ability to meet her time budgets needs was likely important to a cow's overall well-being. Cows indicated that lying time was a priority over other activities by sacrificing feeding and social interactions for lying time (Munksgaard et al., 2005). These factors are important for lying behaviors, and many factors may have contributed to the lying time within this study.

The lying time for this study suggests that stall bedding and stall design were comfortable as both have affected lying behaviors. When stalls were wider at 126 cm, cows spent more time lying down (Tucker et al., 2004); and when neck rails were placed further from the curb, cows spent more time standing in the stall (Tucker et al., 2005). The measurements for freestalls in the current study were consistent with the recommended measures for Holstein dairy cows of 680.4 kg. As reported by Cook (2014) bed lengths were 2.4 m long, 1.2 m wide, with neck rail height at 1.2 m, and brisket board located 1.7 m from the curb. Prior experience with different bedding materials drives preferences of dairy cows. It was suggested that cows with previous exposure to sand are more acceptable of this bedding type (Norring et al., 2008). Cows have also reduced lying time in sand bedding as bedding became concave (Drissler et al., 2005), to ensure this did not occur in the current study, bedding was added each day. Bedding quality was a major component for lying time. A study comparing dry matter at 86% and 26% reported lying time decreased by 5 h/d (Fregonesi et al., 2007a). Another study observed lying times were decreased an hour when dry matter dropped below 60% (Reich et al., 2010). However, dry matter for the current study was 88% at the lowest, which indicates that dry matter may not have been low enough to negatively affect lying behaviors. However, Fregonesi et al (2007) and Reich et al. (2010) used sawdust for bedding. It is possible that the threshold at which cows begin to reject bedding materials for sand might differ from the threshold for sawdust.

The average number of lying bouts for summer and winter in the current study were 10.9 ± 0.4 bouts/d and 8.8 ± 0.6 bouts/d, respectively. Lying duration for each bout in the summer was 67.6 ± 3.0 min/d, and 86.5 ± 4.3 min/d in the winter. These averages were again consistent with reported values of 7 – 10 bouts/d and 65 – 112 min/bout by Ito et al. (2009). Although lying bouts were not affected within the current study, many factors have been established that do affect frequency of lying bouts and lying bout duration. Bedding quality was one factor that affected lying bout behavior. When cows were housed on deep sand bedded freestalls lying bout duration increased while frequency of lying bouts decreased (Ito et al., 2014), and the same was observed as dry matter increased in bedding material. Similarly, cows housed on sand had longer lying bout durations (92.0 ± 12.9 min/bout) when compared to foam mattresses at 47.9 ± 7.4 min/bout (van Gastelen et al., 2011). Amount of bedding has also affected lying bouts. When cows were given three different levels of sawdust on geotextile mattresses, cows had higher number of lying bouts when 7.5 kg of sawdust was provided (10.0 ± 0.6 bouts/d) compared to no sawdust provided (8.5 ± 0.6 bouts/d; (Tucker and Weary, 2004). Similarly for sand, as level of sand decreased within the stall, frequency of lying bouts decreased as well (Drissler et al., 2005). In the current study sand was added daily to ensure effects were representative of treatments and not depth of bedding.

Cows that started on recycled sand had a higher number of lying bouts (11.7 ± 0.6) compared to cows that started on control sand (10.0 ± 0.6). Previous studies reported similar results for cows housed on sand. Cook et al. (2004) and Gomez and Cook (2010) reported that cows had an average of 10.2 - 10.3 lying bouts/d when housed on sand, yet Drissler et al. (2005) reported that lying bout frequency was 11.4 ± 0.6 when bedding provided was sand. As there were differences in frequency of lying bouts of cows housed on the same bedding material, it was suggested that lying behaviors were cow specific and varied among them (Ito et al., 2009). The same could be suggested for the current study as the frequency of lying bouts were similar to other studies, and Ito et al. (2009) reported that individual cow lying bouts ranged between 1 – 28 bouts/d and averaged 7 – 10 bouts/d across farms. The frequency of lying bouts in the current study were within the normal ranges provided, and a difference of 1.7 number of lying bouts may not be significantly different.

Total steps taken for cows in the summer was 1521.4 ± 138.3 steps/d and 1649.6 ± 103.1 steps/d for cows in the winter; however cows on control sand had higher number of steps taken during the winter session (1728.6 ± 103.1). These results were similar to total steps taken of cows when housed on sand. During the summer months of June through September, cows took an average of 1657 ± 7.0 steps/d and while only taking 1437 ± 10.0 steps/d in the winter months from December through January when housed on sand (Brzozowska et al., 2014). Although total steps taken in the winter were higher in the current study, cows were likely not biologically affected from the extra 200 steps taken. For instance, cows on control sand in the winter in the current study had similar total steps taken to cows that were housed on a bedded-pack when temperatures were below 22°C (1718.4 ± 29.2 steps/d; (Endres and Barberg, 2007). When temperatures rose above 22°C cows stepped an average of 2899.2 ± 63.6 steps/d (Endres and Barberg, 2007); however in the current study, cows total steps did not exceed these high averages, and to help alleviate heat during the summer, fans were in place for air circulation for cows as previously reported cows experience heat stress during the summer (Frazzi et al., 2000). This indicates that sand in the current study may not have affected the increased number of steps, and the increased steps were not biologically significant.

Body temperatures were not affected by control or recycled sand. Body temperature for the summer averaged 38.5°C ± 0.1 and 38.0°C ± 0.3 in the winter. Previous studies reported that core body temperatures were higher during warmer months and that lying behaviors could be altered as temperature increased, standing time increased as well (Zahner et al., 2004). When a cow had a core body temperature of 38.9°C the likelihood of a cow standing was at 50% (Allen et al., 2015). As temperatures outside increased from 37.8°C to 40.5°C, standing bouts increased to 60 minutes while lying bouts decreased by 30 minutes for cows housed in freestalls and dry lots (Allen et al., 2015). Even though temperatures in the current study never reached 37.8°C, they did reach 23°C where cows decreased lying time by three hours and increased standing time by one hour (Cook et al., 2007); however, lying times were not affected during the warmer months in the current study suggesting that housing environment was sufficient. Although milk production data was not collected in the current study, temperatures and humidity affected milk production. When temperatures were 29°C and 40% humidity, cows' milk production was normal at 98%; however, when humidity increased to 90% milk production reduced to 69% (Bianca, 1965). It is unknown how bedding with recycled sand and increased humidity would affect cows' milk production.

Concentrations were higher for cows on control sand for the summer (0.75 ± 0.2 ng/mL) and higher for cows on recycled sand for the winter (1.3 ± 0.3 ng/mL). The average cortisol concentration for summer was 0.54 ± 0.2 ng/mL and 0.89 ± 0.2 ng/mL for winter. These average cortisol concentrations were much lower than the normal baseline of cortisol concentration of 8 – 10 ng/mL and cortisol concentrations of cows that were stressed (30 – 40 ng/mL; (Negrao et al., 2004). Many of the samples in the current study were below the detectable limit of the assay; however, variation among cortisol concentrations between cows occurred previously (Negrao et al., 2004), and could possibly explain some variation in the current study. Variation in cortisol concentrations occurred when cows were released from milking (Negrao et al., 2004), and when cows were restrained in chutes (Bertoni et al., 2010). This indicated that cows experience acute stress during normal procedures. There were unusual manipulations that also caused increased stress. For instance, when cows were deprived of lying time for roughly 14 h/d, cortisol concentrations tended to increase for lying deprived cows (Fisher et al., 2002). Also, when cows were either isolated from other cows or deprived of lying time, cows that had been deprived of lying time had higher cortisol concentrations than those that had been isolated (Munksgaard and Simonsen, 1996). Despite the differences in cortisol concentrations in the current study, the concentrations were very low and lying time was not affected, indicating that if cows did experience a stress response to recycled sand they were able to adapt quickly.

There were very little differences in dry matter samples for control and recycled sand for both summer and winter. Kristula et al. (2005) found similar dry matter percentages as those from the current study, for clean and recycled sand from different dairy facilities for sample d 0, but as sample days increased, dry matter increased. Previous work with dry matter has found that when sawdust bedding was sufficiently wet at 60% dry matter, lying time decreased by an hour (Reich et al., 2010). However, when dry matter was lowered to 26% cows reduced lying time by 5 h/d (Fregonesi et al., 2007).The reduction in these lying times were not present in the current study. As percentages of sand never dropped below 88% in the current study, lying time was not affected. There may be a threshold to wetness of sand bedding before differences in lying time occur.

Many variables were considered when assessing bacterial loads; among those were bacteria in bedding. Bacterial loads changed over time for *Staphylococcus* spp., but no differences were established for gram-negative bacteria or *Streptococcus* spp. The bacterial loads presented in the current study were similar to those previously with recycled sand. When bacterial loads of recycled sand and clean sand were compared for summer and winter, Kristula et al. (2005) reported that there were no differences between bacterial load in either sand, but did increase over the sample period. It was reported that coliforms and *Klebsiella* spp. were relatively low compared to *Streptococcus* spp., especially in clean sand (Kristula et al., 2005).

The highest bacterial load in the current study was 15 million colony forming units across both beddings compared to 33 million colony forming units found in clean sand reported by Kristula et al. (2005). However, it was reported that bedding bacteria normally exceed bacterial counts of $10^8 – 10^{10}$ per gram of bedding (Hogan et al., 1989), indicating that bacterial loads in the current study were well below the threshold accepted. Despite bacterial loads occurring below the threshold, proper milking procedures would need to be implemented.

Organic bedding materials provided an optimal environment for bacteria growth more than inorganic bedding materials. Bacterial counts for gram-negative bacteria, coliforms, *Klebsiella* spp., and *Streptococcus* spp. were higher in organic bedding materials than inorganic bedding materials (Hogan et al., 1989). The growth of *Staphylococcus* spp. in the current study particularly increased through sample days. However, bacteria growth changes overtime. For instance, gram-negative and coliform bacteria were higher during the summer and fall months compared to winter and spring months (Hogan et al., 1989). Some variation in bacterial counts in the current study may have been contributed to the additional bedding added every day. Although the amounts added to each stall were relatively small, bedding was stored in an uncovered area and exposed to the elements. Although milk production data was not collected for the current study, cows that had clinical mastitis caused by *Staphylococcus* spp. in previous studies reported that milk yield was not different between clinical mastitic cows and non-clinical mastitic cows (Gröhn et al., 2004). It was also reported that *Staphylococcus* spp. had a small impact on somatic cell count and occurrence of clinical mastitis (Lam et al., 1997). This is important as these bacteria are in close proximity with teat ends and increase exposure that lead to potential mastitic problems.

Teat end bacterial counts for gram-negative bacteria and *Staphylococcus* spp. were higher for cows on recycled sand compared to control sand. The higher bacterial loads on teat ends were a result from the bedding material as gram-negative bacteria were known for growth in manure, water, and soil (Hogan and Smith, 2003). The higher number of bacterial populations in recycled sand was likely due to the flushing system where sand was in contact with water and manure. The results from this study for gram-negative bacteria were much lower when compared to bacteria on teat ends of cows housed on sand and sawdust. In the current study gram-negative bacteria was similar for recycled sand compared to *Klebsiella* spp. in sand, but coliform bacteria was much higher in sand reported by Zdanowicz et al. (2004). However, bacterial counts were higher on teat ends for cows housed on sawdust compared to sand (Zdanowicz et al., 2004). The similarity between these studies may have been the dry matter for sand within each study. The current study averaged 91.7% dry matter while Zdanowicz et al. (2004) reported dry matter for sand at 94.7%. As dry matter was higher, bedding material may not have adhered to teat ends as much bedding with a lower dry matter; however the threshold for sand dry matter is still unknown. There could also be the likelihood of an increase in coliform bacteria with each additional reclaiming of recycled sand.

Bacterial counts in milk samples were not different between treatments for summer or winter, but did change across time for *Streptococcus* spp. in the summer. The bacterial loads from these milk samples were below the general expectance of <1,000 bacteria/mL (Kurweil and Busse, 1973). However, *Streptococcus spp.* is a major bacteria in the cause of mastitis (Wilson et al., 1997). These results were similar with previous studies determining bacteria in milk samples. When bulk milk tank samples were analyzed, *Streptococcus* spp. made up 69% of the bacteria observed with *Streptococcus uberis* accounting for 81% (Zadoks et al., 2004). Similarly for isolation of bacteria for clinical mastitic cows, *Streptococcus* spp. were most often isolated directly after *Escherichia coli* (Gröhn et al., 2004). It was reported that cows with clinical mastitis had high somatic cell counts prior to and after occurrence of the infection (de Haas et al., 2002). Although milk production was not observed for the current study and previous research reported a decrease in milk production when cows had mastitis (Gröhn et al., 2004), cows did alter lying behaviors when they had mastitis. When cows had naturally occurring mastitis, lying time was decreased by 40 minutes and movement, steps, kicks, and leg lifts, within the parlor increased (Medrano-Galarza et al., 2012); however, when experimentally induced with mastitis lying time decreased by 73 min/d (Cyples et al., 2012). Within the current study, if cows experienced signs of mastitis it was likely not due to treatment as duration on treatment was not long enough and lying behaviors of cows were not affected. Hygiene scores were higher for cows on recycled sand during the winter. However, score averages were below a score of 1 for both treatments, and few cows were scored higher than a zero for both seasons. The cows in the current study were classified as clean based upon Schreiner and Ruegg (2003) which suggested there was a lower risk for subclinical mastitis. Although the scoring system was numbered differently in the current study, scores of 2 and 4 had an association with isolating major mastitis causing pathogens (Schreiner and Ruegg, 2003). There have been contradictory results for cow's hygiene when bedding with sand. The current study along with Norring et al. (2008), reported cows were cleaner when housed on sand. Zdanowicz et al. (2004) reported that cows were cleaner when housed on sawdust compared to sand, but udder cleanliness did not change on either bedding throughout the study. The differences in these studies may have contributed from factors within the pen. For instance, udder hygiene declined when neck-rail position was least restrictive at 190 cm from the rear curb (Fregonesi et al., 2009a). When alleyways were scraped by a hydraulic automatic scrapper 12 times daily, udder and teat hygiene improved (Magnusson et al., 2008). In the current study a flushing system was used two times daily to clean alleyways, this indicates that flushing of the alleyways was sufficient in clearing the alleyways of debris and keeping cows clean.

There was no preference between control and recycled sand. However, results have been contradictory when comparing preference of sand bedding for dairy cows. Cows preferred sand and mattresses for stall surfaces over concrete and rubber mats (Wagner-Storch et al., 2003), but preferred soft rubber mats over straw and sand (Manninen et al., 2002). Cows preferred straw bedding compared to sand; however cows had previous exposure to straw and not sand within this study (Norring et al., 2008). This indicated that previous exposure to bedding materials affected preferences. Cows within the current study were housed on sand prior to the beginning of the study and there were little differences between control and recycled sand. Other factors also affected preferences of bedding, such as bedding dry matter. Fregonesi et al. (2007a) reported that cows spent 12 h/d lying on dry sawdust bedding at 86% dry matter compared to one h/d on wet sawdust bedding at 26% dry matter. The dry matter in the current study may not have affected the choice of bedding as there were small percentage differences between dry matter and both were above 86%.

In conclusion, recycled sand presented many favorable qualities as bedding. It was indicated that recycled sand was a comfortable bedding material as cows did not reduce lying time and there were no preferences. Bacterial loads within bedding could become a concern.

Maintenance of recycled sand bedding would be required often. It is unknown what the threshold of bacterial load is in recycled sand and how many times the bedding can be reclaimed and safely used.

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APPENDIX

Table 1. Mean and standard error of lying time, number of lying bouts, lying bout length, and total steps taken for cows while on control and recycled sand bedding during no-choice phase in the summer session[1].

	Treatment			
Variable	Control	Recycled	SE	*P*-value
Lying time, h/d	11.8	11.5	0.34	0.28
Number of lying bouts, d	10.8	11.0	0.42	0.46
Lying bout length, min/d	69.2	66.0	3.0	0.15
Total steps	1493.4	1549.4	138.3	0.78

[1]Values shown are for each lying behavior within each treatment (n = 4 groups of 8 cows, each treatment tested within each group). Cows were on each treatment for one week, and then switched treatments the following week for the no-choice phase of the study.

Table 2. Mean and standard error of lying time, number of lying bouts, lying bout length, and total steps taken for cows while on control and recycled sand bedding during no-choice phase in the winter session[1].

	Treatment			
Variable	Control	Recycled	SE	*P*-value
Lying time, h/d	11.9	11.9	0.40	0.40
Number of lying bouts, d	8.8	8.8	0.55	0.89
Lying bout length, min/d	86.1	86.8	4.3	0.89
Total steps	1728.6	1570.6	103.1	0.03

[1]Values shown are for each lying behavior within each treatment (n = 4 groups of 8 cows, each treatment tested within each group). Cows were on each treatment for one week, and then switched treatments the following week for the no-choice phase of the study.

Table 3. Means and least-square SE for bedding DM (%) for used control (control), new control added (new control), recycled used (recycled), and new recycled sand added (new recycled) for the duration of the summer session.

Bedding type	Dry Matter (%)	SE
Control	94.9^{a}	0.9
Control New	94.8^{ab}	1.7
Recycled	92.3^{ab}	0.9
Recycled New	90.2^{a}	1.7

$^{a-b}$ Means with different superscripts tended to differ (P = 0.07).

Table 4. Means and least-square SE for bedding DM (%) for used control (control), new control added (new control), recycled used (recycled), and new recycled sand added (new recycled) for the duration of the winter session.

Bedding type	Dry Matter (%)	SE
Control	93.9^{ab}	1.6
Control New	94.0^{a}	1.3
Recycled	88.4^{c}	1.3
Recycled New	90.5^{bc}	1.3

$^{a\text{-}c}$ Means with different superscripts differ ($P < 0.05$).

Table 5. Change in body temperatures (°C) over sample day for cows on each treatment during the summer session. Each sample day has a representation of cows on control sand that started on control sand (Control - C/R) and cows that started on recycled sand (Control - R/C). Each sample day also has a representation of cows on recycled sand that started on control (Recycled – C/R) and cows that started on recycled sand (Recycled – R/C).

	Day (°C)			
Treatment	0	3	7	SE
Control – C/R	38.4^{cde}	38.5^{bcde}	38.5^{bcd}	0.13
Control – R/C		38.3^{de}	38.7^{b}	0.13
Recycled – C/R		38.4^{cde}	38.3^{e}	0.13
Recycled – R/C	38.9^{a}	38.5^{bc}	38.6^{bc}	0.13

$^{a\text{-}e}$ Within treatment and sequence, numbers with different letters differ ($P < 0.0001$).

Table 6. Mean body temperature (°C) changed across treatment (control; recycled) and sequence (C/R; R/C) during the summer session.

	Treatment		
Sequence	Control	Recycled	SE
C/R	38.5^{b}	38.4^{b}	0.09
R/C	38.5^{b}	38.7^{a}	0.09

$^{a-b}$ Means with different superscripts differ ($P < 0.0001$).

Table 7. Change in body temperatures (°C) over sample day for cows on each treatment during the winter session. Each sample day has a representation of cows on control sand that started on control sand (Control - C/R) and cows that started on recycled sand (Control - R/C). Each sample day also has a representation of cows on recycled sand that started on control (Recycled – C/R) and cows that started on recycled sand (Recycled – R/C).

	Day (°C)			
Treatment	0	3	7	SE
Control – C/R	37.3^{d}	37.7^{bd}	38.1^{ac}	0.30
Control – R/C		38.3^{a}	38.2^{a}	0.30
Recycled – C/R		38.2^{a}	38.1^{abc}	0.30
Recycled – R/C	38.3^{a}	37.7^{cd}	38.2^{a}	0.30

[a-d] Within treatment and sequence, numbers with different letters differ ($P < 0.0001$).

Table 8. Mean body temperature (°C) changed across treatment (control; recycled) and sequence (C/R; R/C) during the winter session.

	Treatment		
Sequence	Control	Recycled	SE
C/R	37.7^{b}	38.1^{a}	0.09
R/C	38.2^{a}	38.1^{ab}	0.09

$^{a\text{-}b}$ Means with different superscripts differ ($P < 0.0001$).

Table 9. Bacterial counts (cfu/mL) did not differ between treatments for composite milk samples during the winter.

	Treatment			
Media	Control	Recycled	SE	*P*-value
MacConkey	0.01	0.0	0.009	0.43
Baird-Parker	0.46	0.42	0.07	0.46
Modified Edwards	0.17	0.21	0.06	0.52

Groups	C	R	C	R	C	R	C	R
1/2	R	C	R	C	R	C	R	C

Figure 1. Set-up of freestalls for the choice phase. Cows (n = 8) were given the option between control (C) and recycled sand (R). Stall availability remained at 2 stalls (n = 16) per cows to ensure there was always a treatment available.

Figure 2. The change in cortisol concentration over sample days when cows (n = 4 groups of 8 cows) were on each treatment for a week (control and recycled sand) during the summer session. Each sample day has a representation of cows while on control and recycled sand and each treatment the cow began with. The lowest cortisol concentrations occurred on sample day 3 for cows on recycled sand that also started on recycled sand. The highest cortisol concentrations occurred on sample day 3 for cows on clean sand that had started on recycled sand. To adjust for skewed data, means are presented as back-transformed (A) and log-transformed (B). Columns within sample day with different letter designations (a - d) differ ($P < 0.001$).

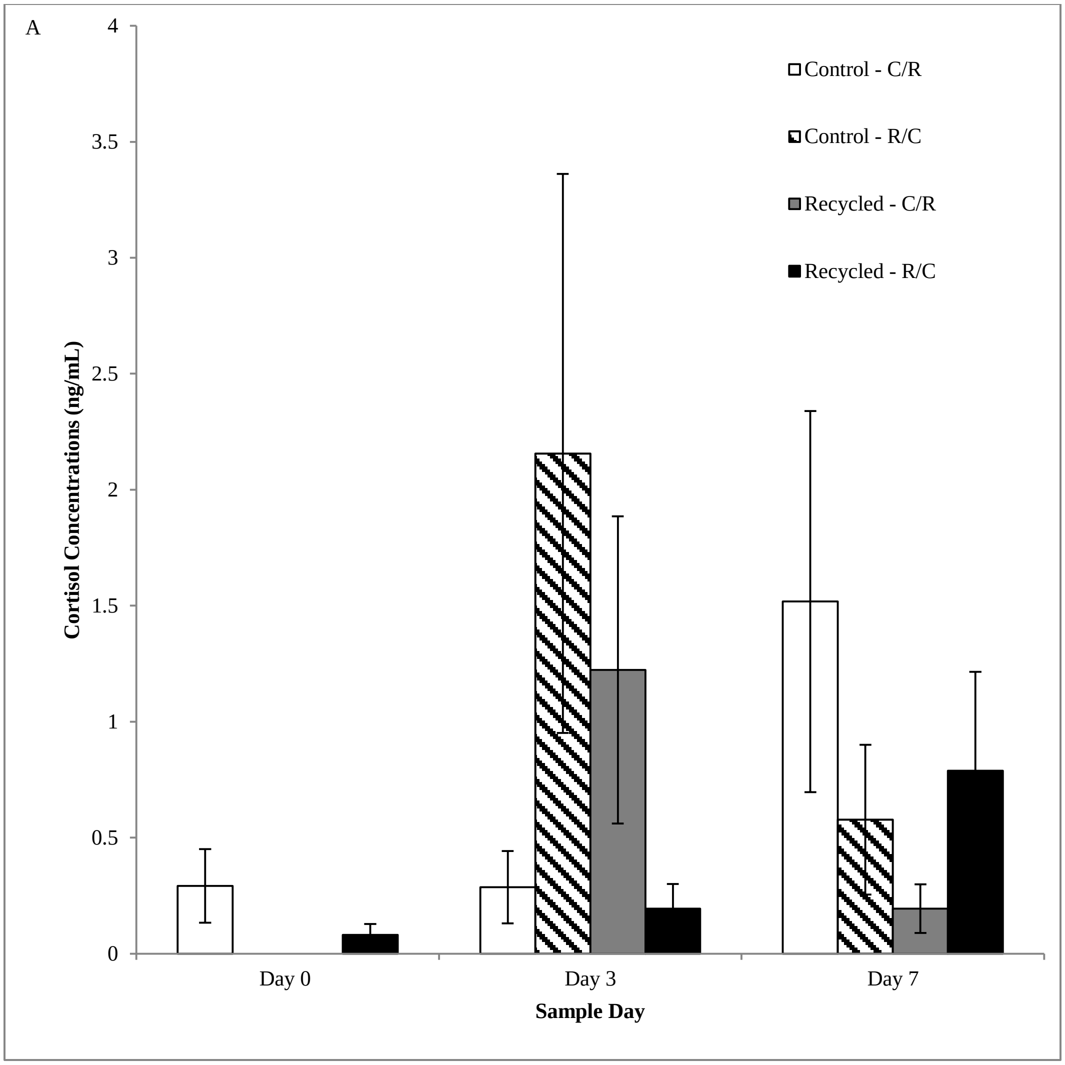


Figure 2. Continued

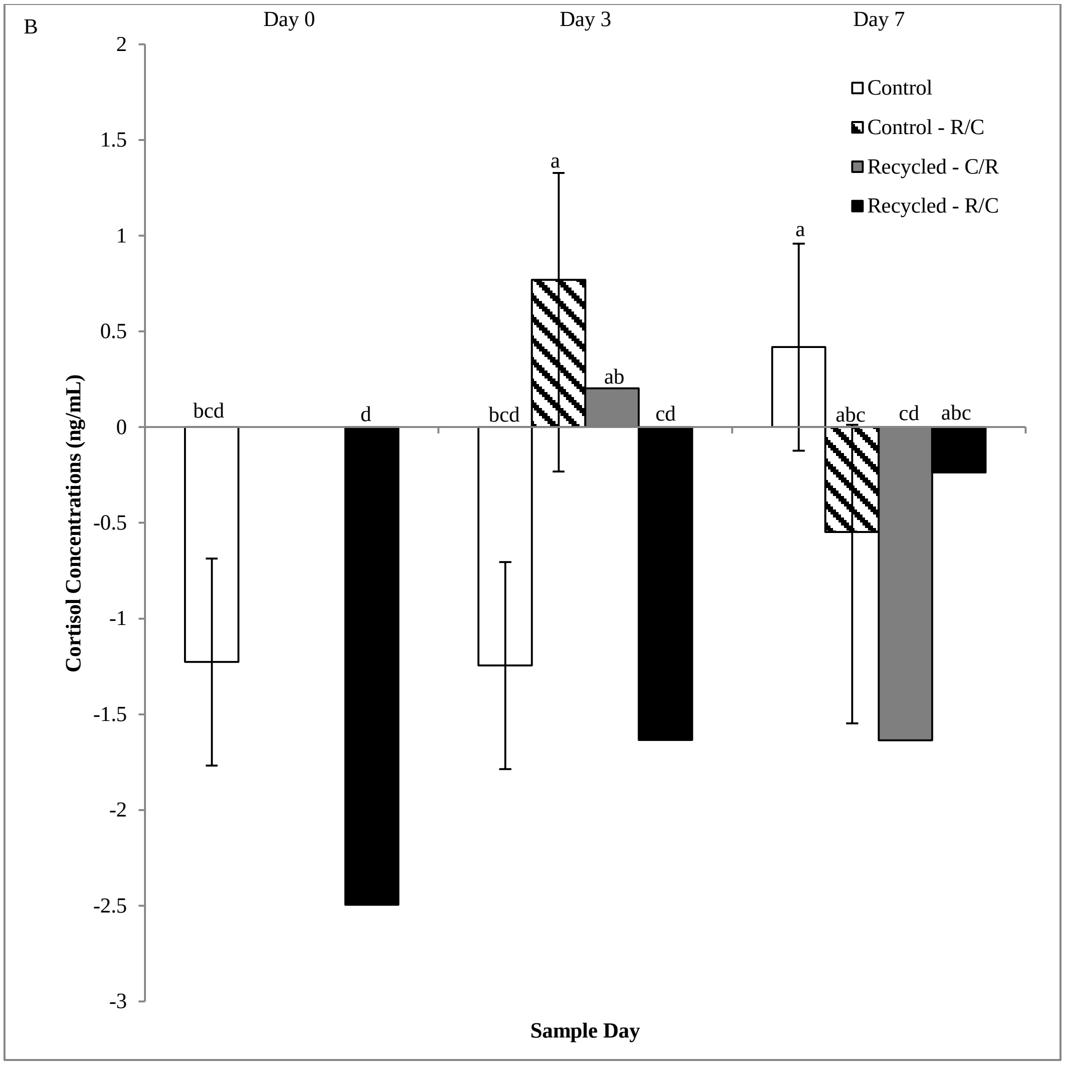


Figure 2. Continued.

Figure 3. The interaction between treatment and sequence of events for cortisol concentrations in the summer session where cows started on control or recycled sand and switched treatments. Cows that started on control are represented as C/R and cows that started on recycled sand are represented as R/C. Cortisol concentrations increased when cows started on recycled sand and switched treatments. Means and SE are presented in back-transformed (A) and log-transformed (B) due to skewed data. Columns within sequence of events with different letter designations (a - b) differ ($P < 0.05$).

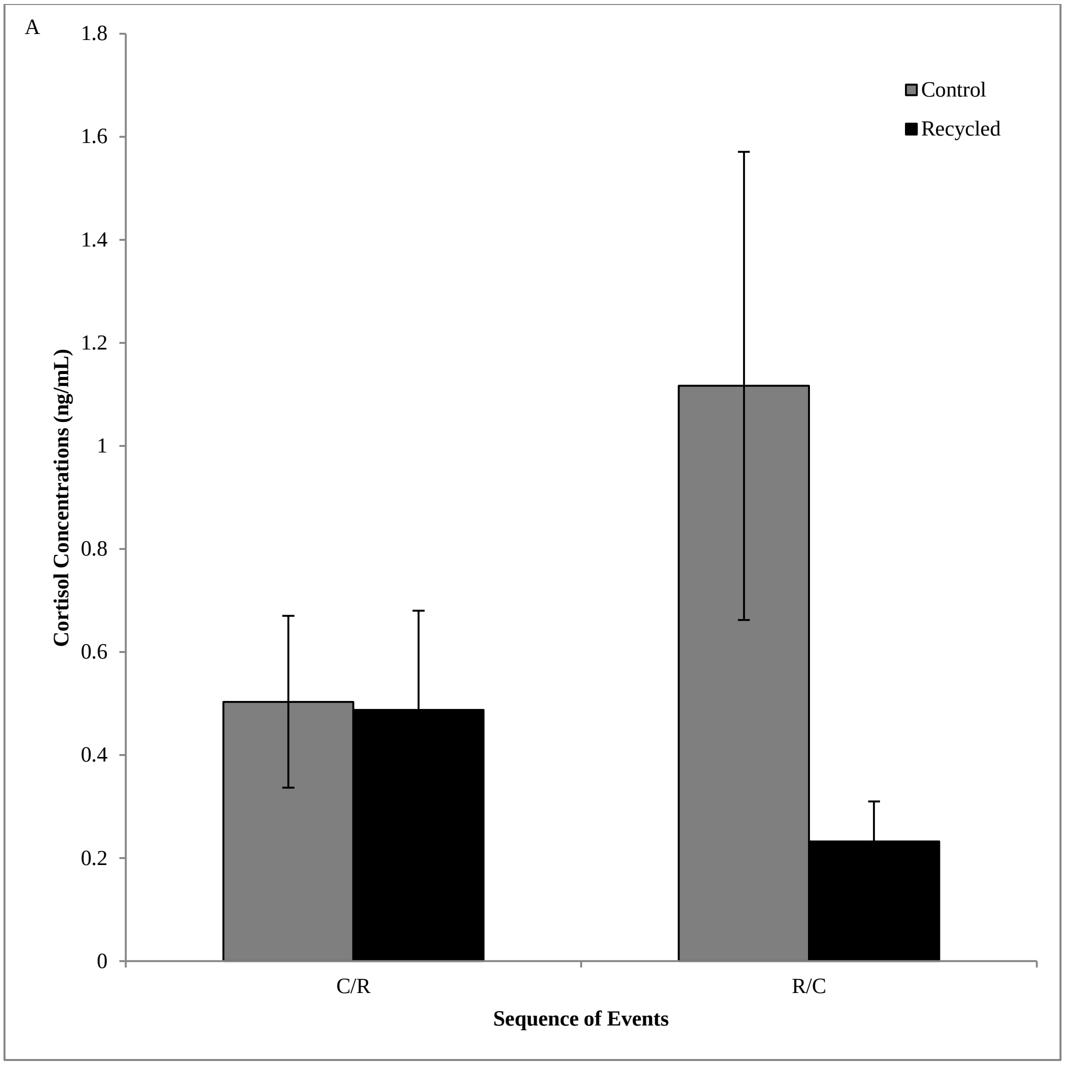


Figure 3. Continued.

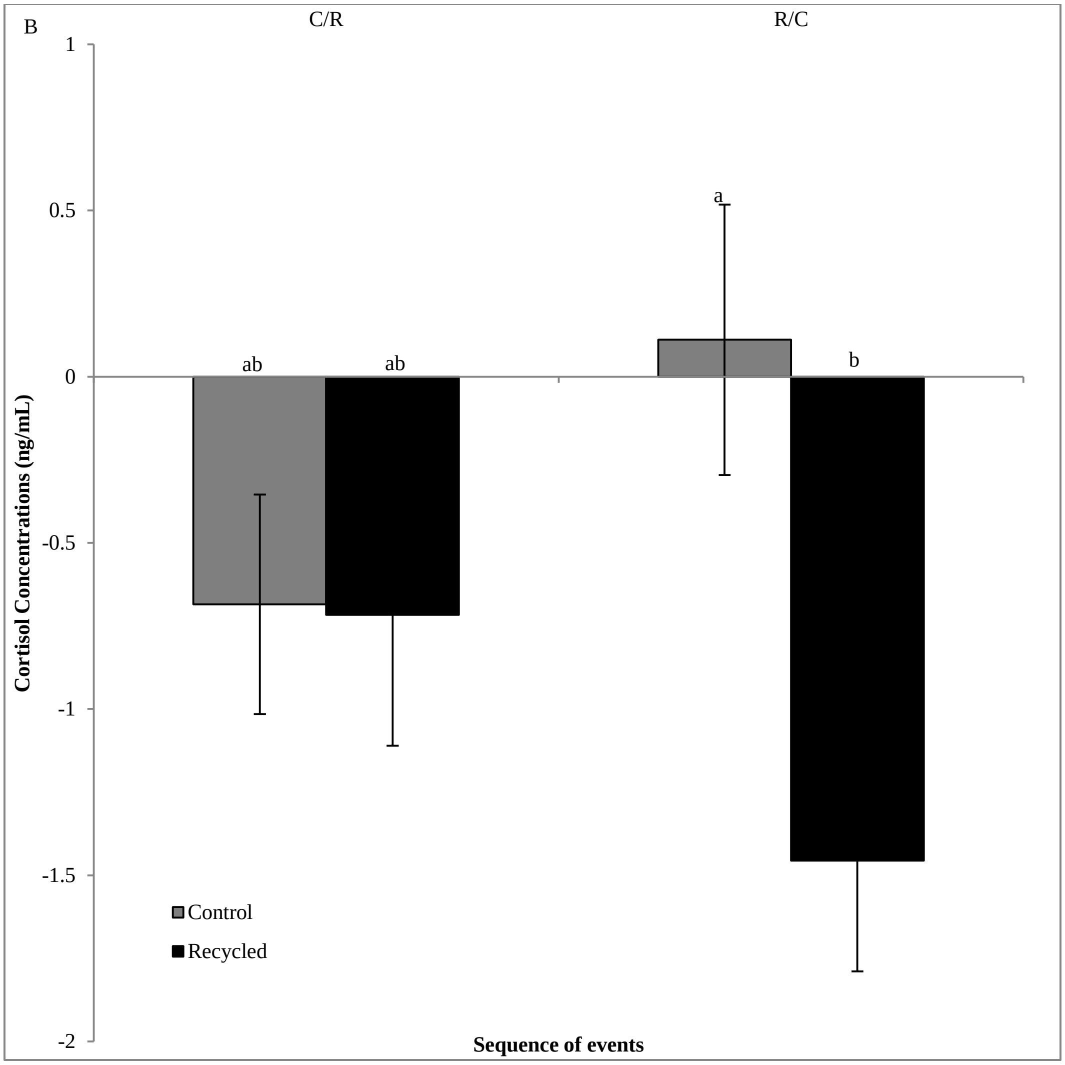
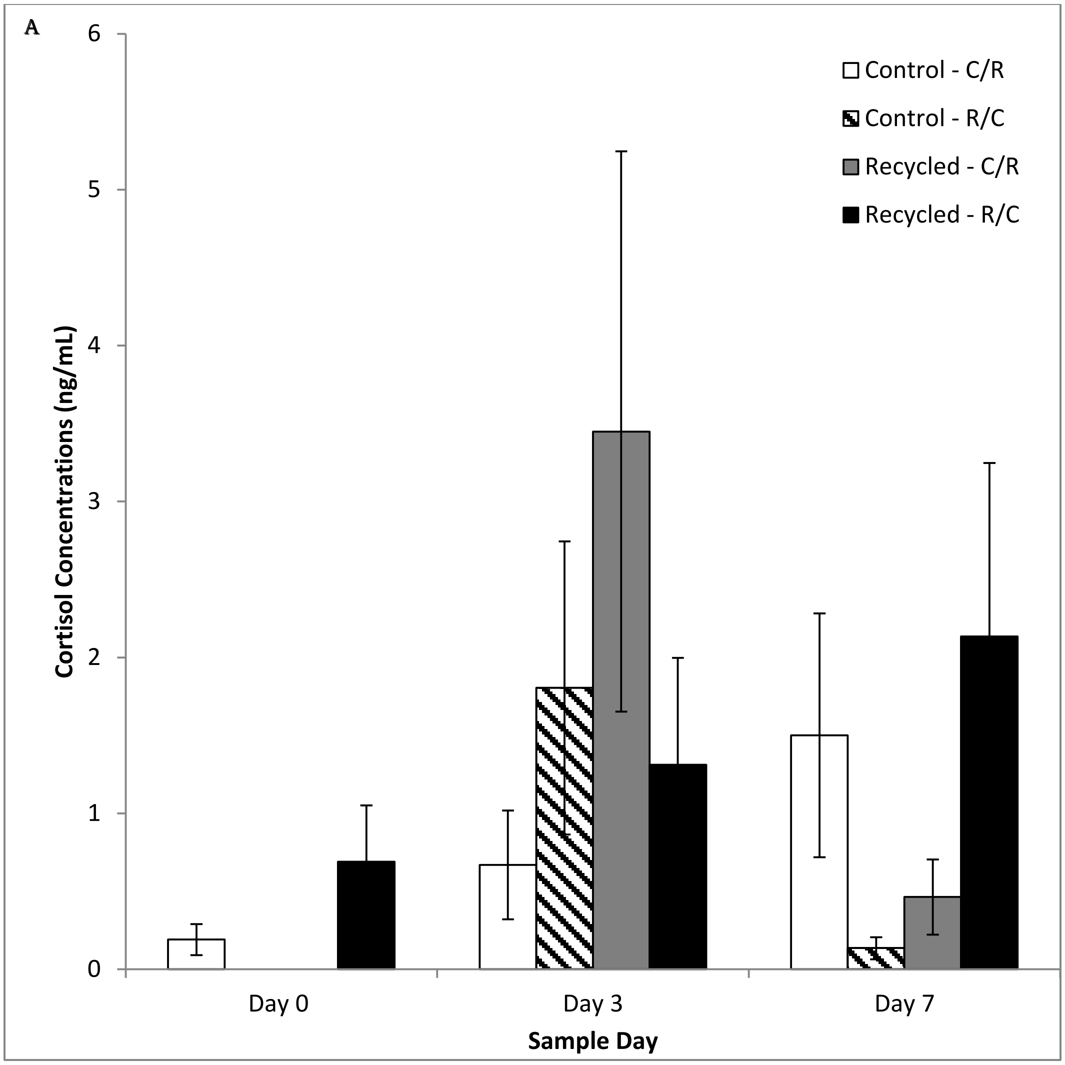


Figure 3. Continued.

Figure 4. The change in cortisol concentration over sample days when cows (n = 4 groups of 8 cows) were on each treatment for a week (control and recycled sand) during the winter session. Cortisol concentrations steadily increased for cows that started on recycled sand and the highest cortisol concentration occurred on d 3. To adjust for skewed data, means are presented as back-transformed (A) and log-transformed (B). Columns within sample day with different letter designations (a - d) differ ($P < 0.001$).



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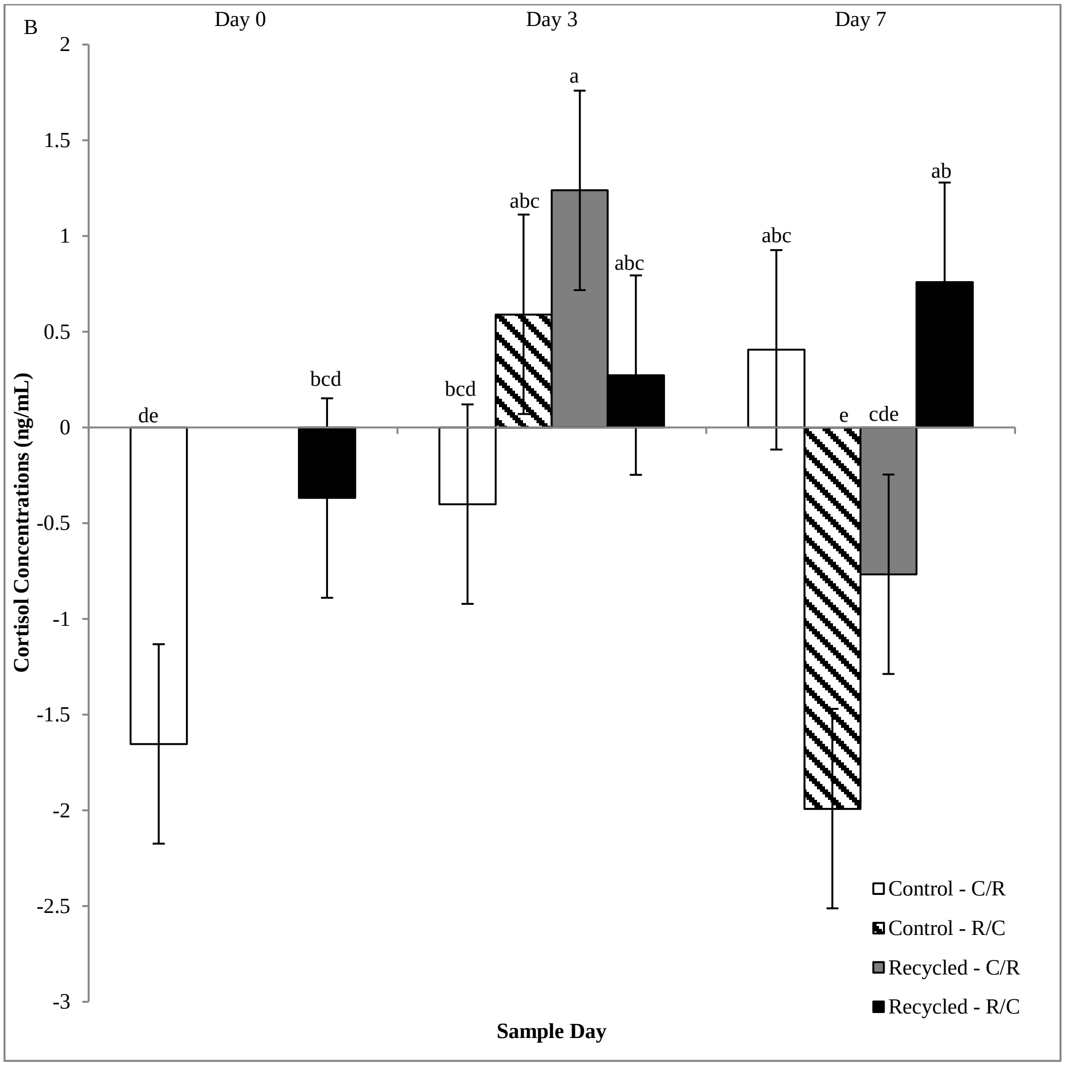

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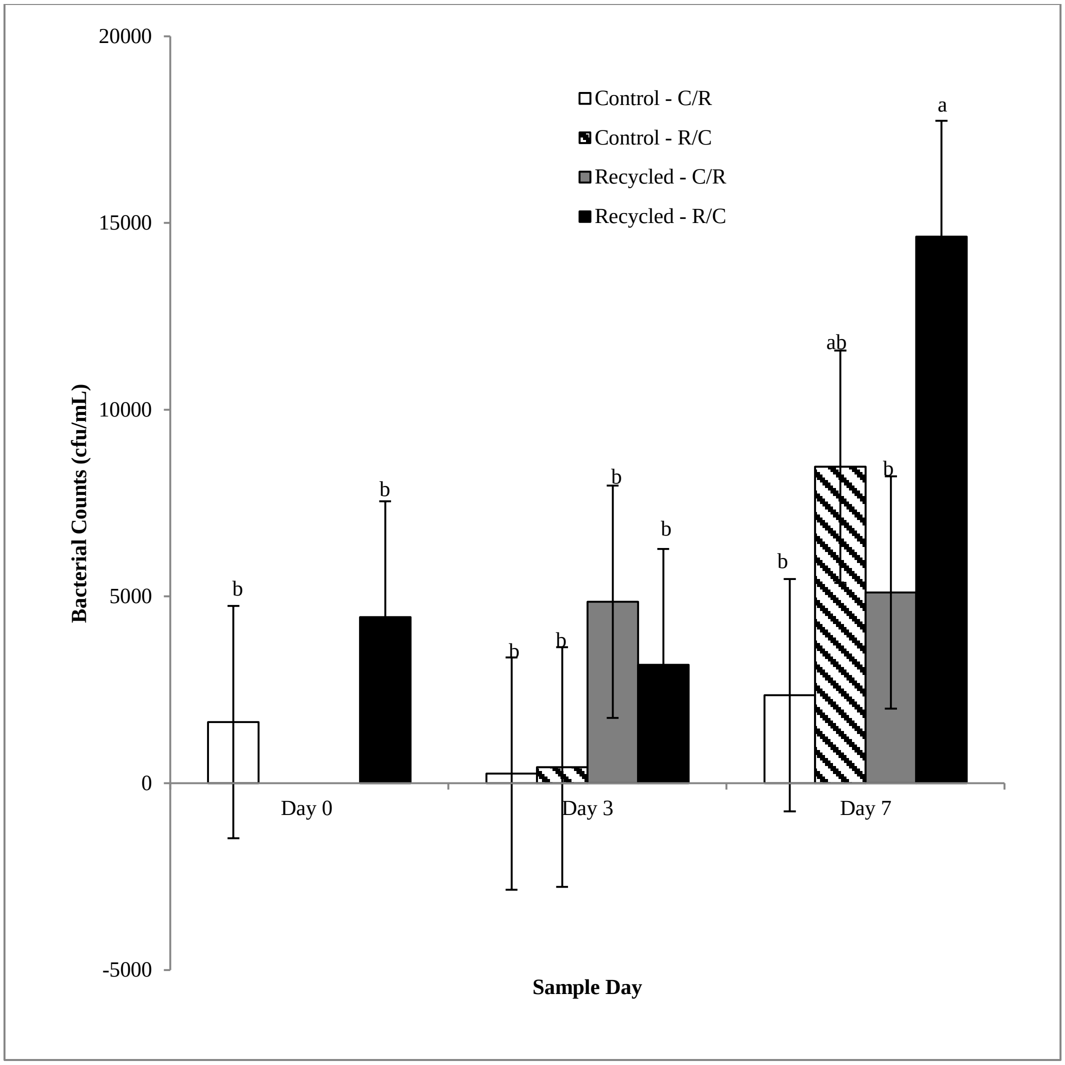

Figure 5. Bacterial counts on Baird-Parker agar for teat-swabs during the winter session changed over time. Bacterial counts increased throughout the study, and peaked on d 7 for each treatment. Columns within sample day with different letter designations (a – b) differ (*P* < 0.01).

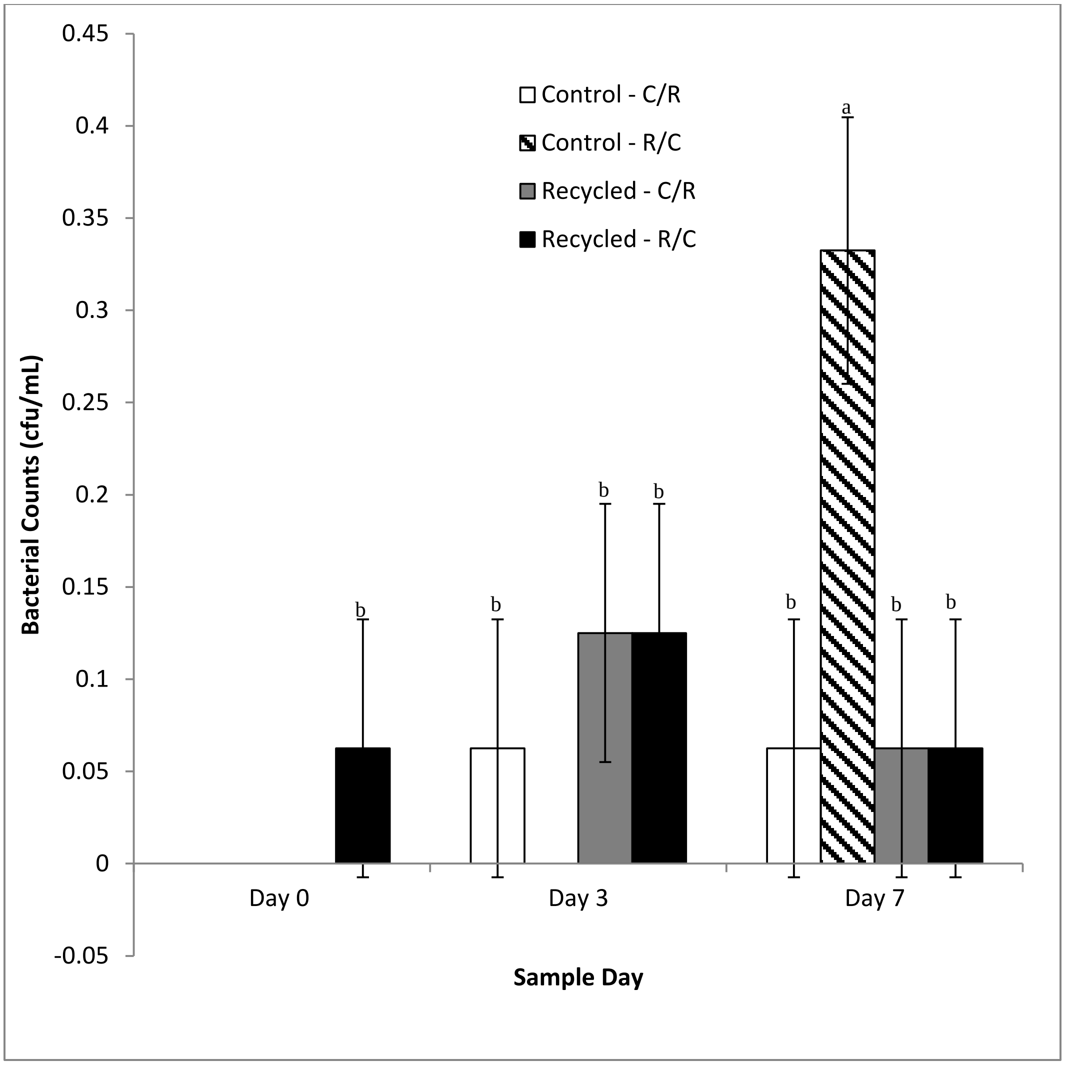

Figure 6. Bacterial counts on Modified Edwards medium for summer composite milk samples over time. Bacterial counts were highest on sample d 3 while cows were on clean sand; however, it is important to note that most counts were zero which contributed to the low numbers presented. Columns within sample day with different letter designations (a – b) differ ($P < 0.05$).

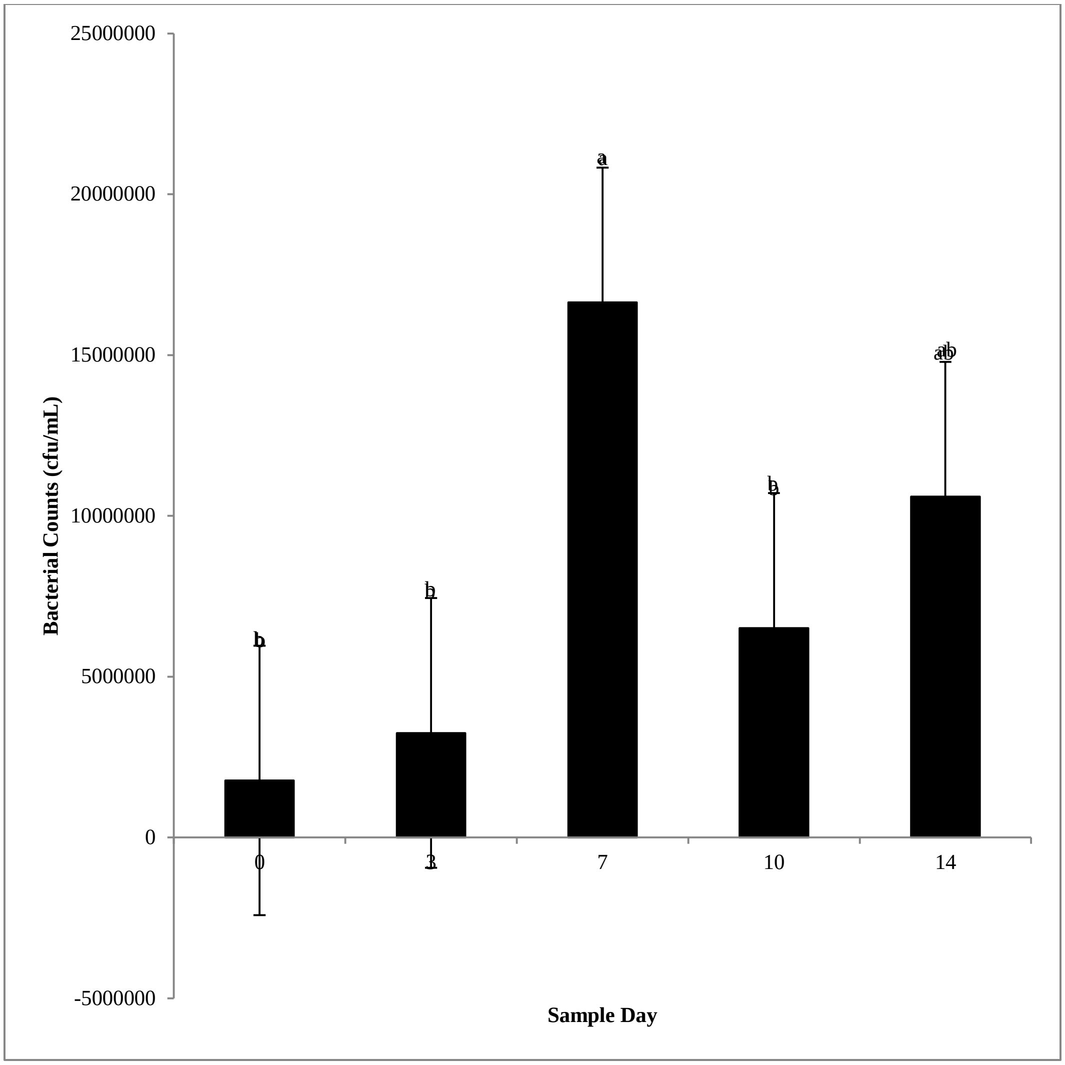

Figure 7. Bacterial counts changed over time for winter sand samples on Baird-Parker agar. Samples were collected the same day as all other collections, but treatment did not change within pen for these samples. Samples are representative across sample days. Means and SE are presented for sample day sand samples were collected. Columns with different letter designations (a – b) differ ($P < 0.05$).

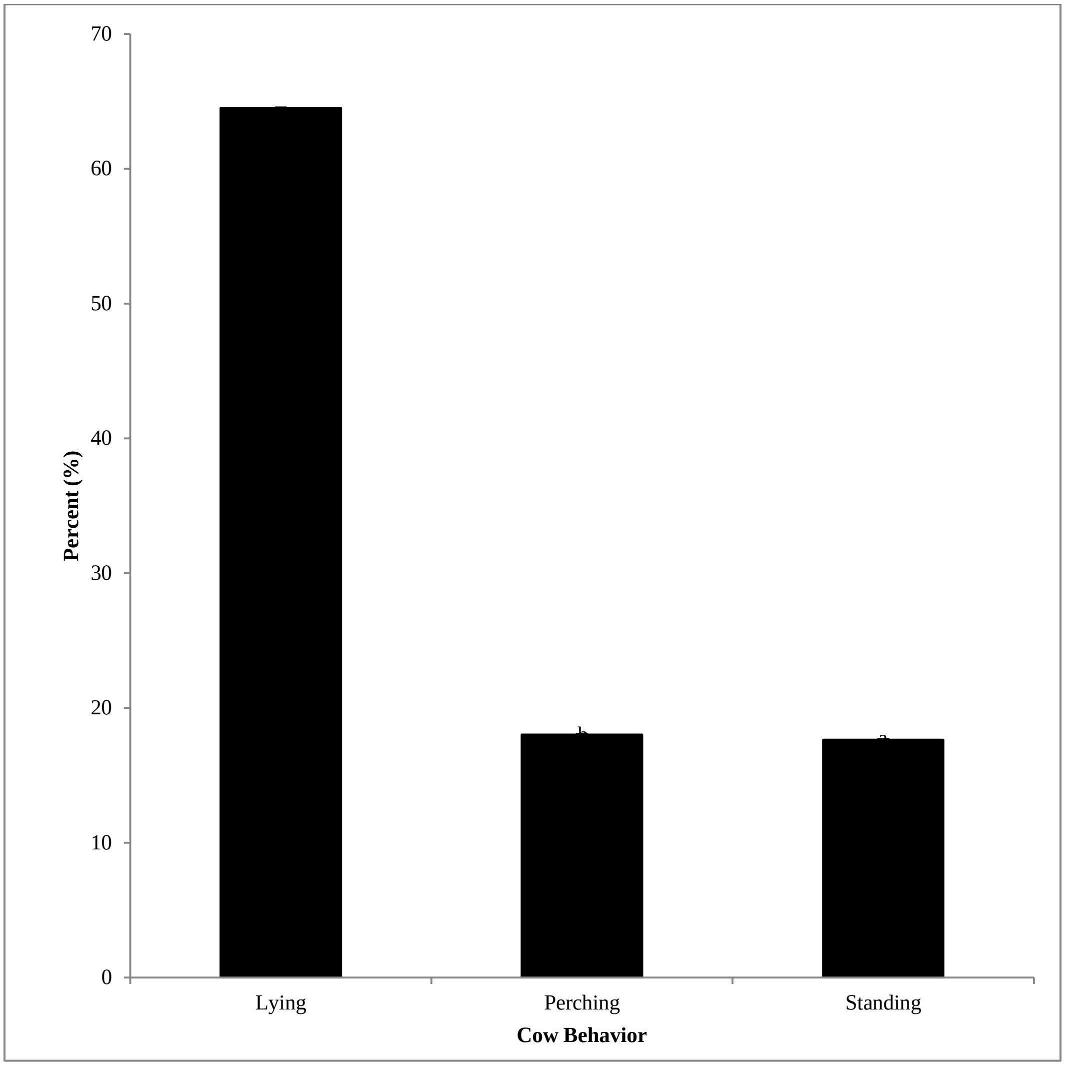

Figure 8. During the summer portion cows spent more time lying in stalls than standing (all four hooves in stall) or perching (front two hooves in stall). Cows were given the choice between control or recycled sand, and no preferences were observed. Means and SE are presented as percentages for each 10-min interval of video data. Columns within behavior with different letter designations (a – b) differ ($P < 0.05$).

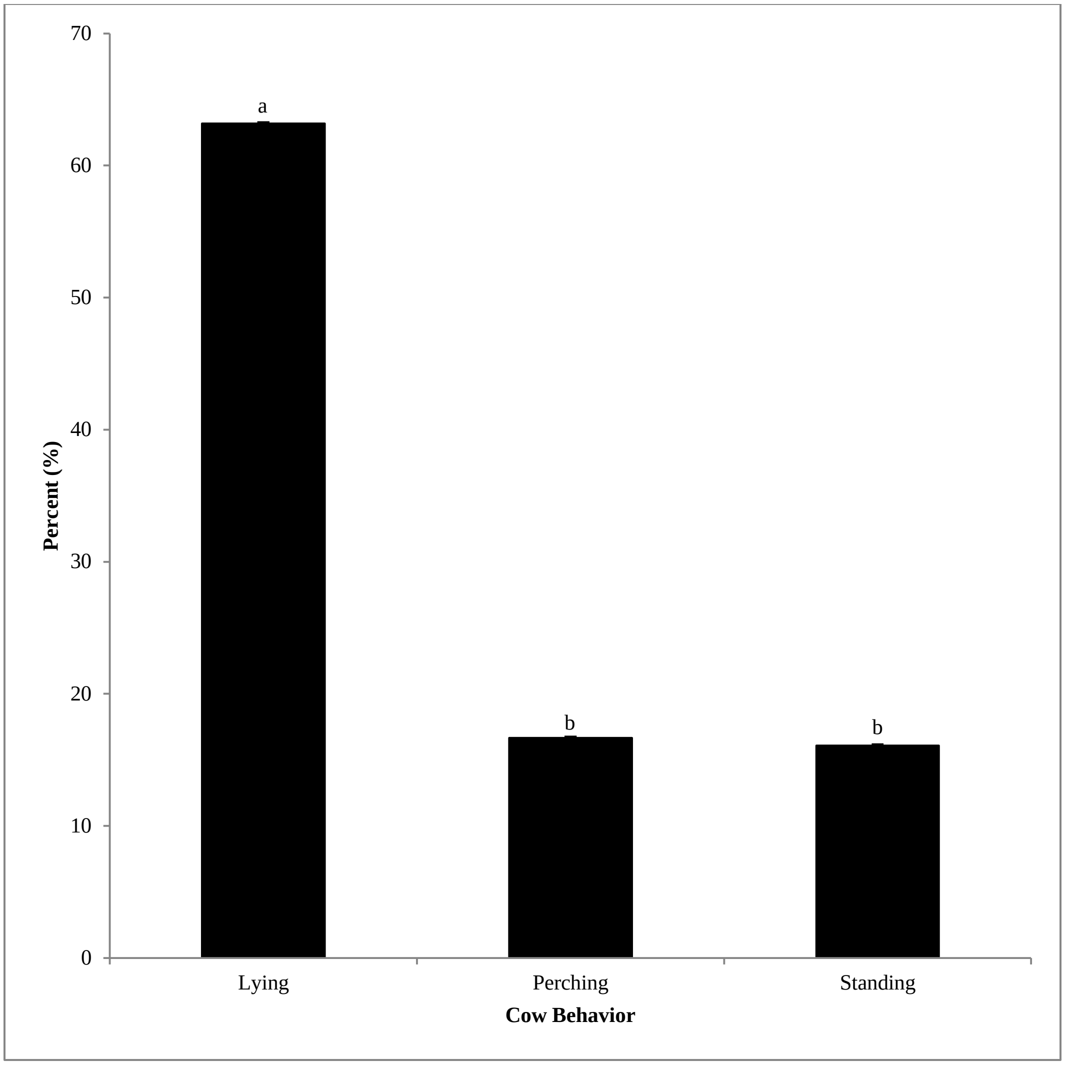

Figure 9. Cows spent more time lying in stalls than standing (all four hooves in stall) and perching (front two hooves in stall) during the winter choice phase. No preferences were observed for the winter session between control and recycled sand. Means and SE are presented as percentages for each 10-min interval of video data. Columns within behavior with different letter designations (a – b) differ ($P < 0.05$).

VITA

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