2020

Microbial Interactions Between Compost Bedded Pack Barn Bedding and Teat Exposure in Transition Dairy Cattle

Tanya Lynn France
University of Kentucky, tanya.france@ymail.com
Digital Object Identifier: https://doi.org/10.13023/etd.2020.077

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Tanya Lynn France, Student

Dr. Melissa Morgan, Major Professor

Dr. David Harmon, Director of Graduate Studies

MICROBIAL INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING AND TEAT EXPOSURE IN TRANSITION DAIRY CATTLE

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Tanya Lynn France

Lexington, Kentucky

Director: Dr. Melissa Morgan, Associate Professor of Animal and Food Sciences

Lexington, Kentucky

2020

ABSTRACT OF THESIS

MICROBIAL INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING AND TEAT EXPOSURE IN TRANSITION DAIRY CATTLE

Compost bedded pack barns utilize composting methods which provide a soft surface for dairy cows to lie on. This requires optimal microbial growth, which may increase the exposure of mastitis-causing pathogens to the teats of early lactation animals. Bedding characteristics, bedding bacteria, and bacterial counts on the teat skin, teat ends, and in the milk of early lactation dairy cows housed on a compost bedded pack were assessed over a 6-month time. The main objective was to determine the relationship between environmental effects (bedding characteristics and weather conditions) and cow-level (teat skin, teat end, milk) bacteria counts over time in transition cows. A secondary objective was to assess CBP characteristics across time and what environmental factors influence bedding bacteria counts. The final objective was to determine if various stages of the transition period (2-weeks prepartum, 72-hours postpartum, 60 days in milk) influenced the cow-level microbial populations.

KEYWORDS: Bacteria count, environmental mastitis, intramammary infection, housing

Tanya Lynn France

April 17th, 2020

MICROBIAL INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING AND TEAT EXPOSURE IN TRANSITION DAIRY CATTLE

By

Tanya Lynn France

Dr. Melissa C. Morgan
Director of Thesis

Dr. David L. Harmon
Director of Graduate Studies

April 17th, 2020

ACKNOWLEDGEMENTS

The first person I'd like to thank is my mother. I would not be who I am today nor would I have made it this far in life without your love, support and encouragement. You have pushed me to become the best version of myself (literally, you pushed me out of the car for tryouts my freshman year of high school because I needed to get out of my comfort zone!). Thank you for always being just a phone call away and listening to my hour-long stories about dairy cows. Thank you for always telling me how proud you are of me; you truly have no idea how much that means to me. You have given me the world, and I cannot wait to give it back to you. Look ma, I made it!!

Secondly, I'd like to thank my major advisor, Dr. Morgan, and second-in charge professor, Dr. Costa. Thank you both for taking me under your wings and always believing in me. Dr. Morgan, thank you for teaching me so much… not just about research and academics, but about life in general. You are truly a role model to me, and I will value that forever. Dr. Costa (aka Joao), thank you for pushing me to my fullest potential, as you have been one of my biggest supporters since starting graduate school. Thank you for your ridiculous jokes and ability to lighten the mood 90% of the time. I will forever be able to hear your laugh as soon as you are half a mile away. On a serious note, I truly admire you as a researcher and hope I am half as knowledgeable about statistics as you are when I (eventually) finish school. Thank you for being not only a great mentor, but a friend.

I would also like to thank Kelsey Lamb, for literally everything. From teaching me all the lab analysis for my project, to spending countless hours helping me plate samples, cleaning up popped stomacher bags, ordering thousands of supplies, and making

thousands of agar plates. Not only that, but also being the person who kept me sane when reaching hour 12 of being in the lab, or when life throws curve balls and I needed a little pick-me-up. My project would have taken 3x as long, with 3x more mess-ups if you were not a part of it. I am forever thankful for all your help.

To the friends I've made during my time at UK… thank you for not only showing me the ropes of graduate school, but being there for every part of my time as a Masters student. Derek Nolan, you have not only been a wonderful officemate, but the world's best designated van-driver, someone I have looked up to tremendously since arriving at Kentucky and cannot forget a fellow Iowa State University Cyclone Alum!! Thank you for teaching me so much and listening to my random rants and endless questions about stats (and those dang superscripts!). I cannot wait to see all your future success and am truly happy to be able to call you my friend. Anna Hawkins, I would not have made it through the past two years without you. Thank you for being you and brightening my days. Melissa Cantor, Megan Woodrum, Gustavo Mazon, Paige Cecil, Emily Rice, Lisa Miller, Jessica Ferrell, and Badri Jagannathan… thank you for helping make graduate school slightly less stressful; you are all wonderful.

I would also like to thank my committee members Dr. Harmon and Dr. Taraba for their advice, knowledge, and patience. Having the opportunity to work with them has been an absolute blessing. Dr. Harmon was so helpful with not only anything and everything regarding mastitis, but also advice on project ideas, sampling techniques and statistical analysis. Dr. Taraba was so helpful with bringing my ideas to a more producer-friendly endpoint, as well as teaching me so much about compost bedded pack barns.

Thank you also to the farm staff at University of Kentucky's Coldstream Dairy (Amelia Carter, Matt Collins, and Joey Clarke) for all your help during my project. Amelia, thank you so much for running all my (seemingly endless) milk samples, helping me bring the girls into the parlor (especially the heifers), and sharing a mutual love for all the farm cats (RIP Rocket). Joey and Matt, thank you for always keeping me updated on my cows, helping me with any sampling issues at 4:30 AM, keeping the pack near perfect during my study, and being all around wonderful people to work with.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

This literature review will discuss research to date on Compost Bedded Pack (CBP) barn management, mastitis, and the relationships found between bacteria in bedding, on teat skin and/or ends, and mastitis in dairy cattle. I will begin briefly with the history and key management factors associated with housing dairy cows in CBP barns. I will then review the literature on mastitis, with emphasis on environmental pathogens. Lastly, I will discuss literature on bacteria found in different bedding types and its associations with bacteria found on the teat skins, teat ends, and udder health parameters. Where possible I will point out gaps in the literature and include recommendations for future research.

1.2 Compost Bedded Pack Barns

In the U.S., the first compost bedded pack (CBP) barn was developed by Virginia dairy producers in the 1980s (Wagner, 2002). The goal was to increase cow comfort and longevity. Compost bedded pack barns are a loose housing system, similar to a traditional straw-bedded pack (without the usage of large amounts of straw bedding material). They are characterized by a large, open resting area without any stalls or partitions (Galama, 2011). Without stalls, the cows' resting and exercise areas are combined (Barberg et al., 2007b, Janni et al., 2007, Black et al., 2013). This combination of resting and exercise space for animals can reduce greenhouse gas emissions and cost compared with freestall barns while maintaining cow health and well-being (Galama, 2011). Through frequent tillage and addition of organic bedding material (such as sawdust), the system utilizes a semi-composting process to break down the organic material by aerobic microorganisms

(Black et al., 2013). The main goal is to provide a soft surface for cows to lie on which is obtainable through maintaining a well-managed pack. Today, CBP barns can be found in many states in the US, including Kentucky, Minnesota, Ohio, and New York. Additionally, other countries have adapted to this type of housing system, including Israel, Germany, the Netherlands, Denmark, Italy, Austria, South Korea, Brazil, Argentina, and Colombia (Bewley et al., 2017).

1.2.1 Pack management

It has been recommended that the composting areas of a CBP barns are tilled twice-daily with a roto-tiller or deep-tillage tool (Barberg et al., 2007b, Janni et al., 2007, Black et al., 2013). However, CBP barns in Israel and Denmark are generally only tilled once per day (Klaas et al., 2010), and some US producers till up to 3 times per day (Black et al., 2014). The depth of tilling varies by producer and the tillage tool used, but is recommended to reach depths of 18 to 30 cm (Barberg et al., 2007b, Janni et al., 2007) Aeration (tilling) incorporates manure and air (oxygen) into the pack which promotes aerobic microbiological activity, heating, and drying of the pack (Shane et al., 2010). Tilling also exposes increased pack surface area for quicker drying (Janni et al., 2007).

Frequency of bedding addition to the pack is influenced by multiple factors. Cow density, ambient weather conditions, and air flow are major factors that affect the need for new bedding addition (Barberg et al., 2007b, Janni et al., 2007). In Minnesota, for example, it was recommended that 14 to 16 metric tons should be added when moisture content enables bedding to stick to the cows (when bedding moisture percentage reaches >60%); this occurs every 1 to 5 weeks in those conditions (Barberg et al., 2007b, Endres and Barberg, 2007, Janni et al., 2007).

Research on stocking density recommendations in a CBP barn varies by region in the U.S. as well as countries outside the United States. Minnesota researchers suggested 7.4m^2/cow for a 540-kg Holstein cow (Janni et al., 2007). Kentucky researchers found that producers within the state (n= 47) had a mean stocking density of 9.0 ± 2.2 m^2 per cow (Black et al., 2014). Recent studies from the United States suggest a minimum of 9.3 m^2/cow because higher cow density on a CBP may increase pack compaction and cause excessive moisture (Leso et al., 2019). In Brazil, Favero et al. (2015) reported a range of 11 to 19 m^2 per cow, whereas Klaas et al. (2010) suggested 15 m^2 per cow for CBP barns in Israel. The differences seen for space per cow are due to several factors, all of which relate to the drying rate of the pack with the goal of provide a soft surface area of the bedding. One factor is the type of bedding material used which varies by cost and availability around the world. In CBP systems that utilize wood materials, such as in the US, the pack can reach relatively high temperatures, which facilitates evaporation, thus reducing the area need to keep the bedding dry (Leso et al., 2019). Another factor to consider is climate conditions. In warm, dry, and windy weather, rapid drying of the pack is likely to occur, resulting in the reduced space allowance per cow. On the other hand, cold and humid weather conditions limit water evaporation rate, and consequently pack drying rate, which may require a larger area per cow to reduce moisture (Smits and Aarnink, 2009). Regardless of climate, CBP barns require other management practices and the measurement of various characteristics that help ensure an effective housing system.

1.2.2 Bedding material.

The CBP barn managers use organic bedding material, such as fine wood shavings or sawdust, which improves mixing, aeration, and microbial activity from increased surface area-to-volume ratio compared with straw and woodchips (Janni et al., 2007). This increases the ability of microorganisms to breakdown manure and urine, while also preventing excessive compaction of the bedding between tillage (Janni et al., 2007). Inorganic bedding, such as sand or crushed limestone, typically hinders bacterial growth within bedding material through a lack of nutrients compared with organic bedding materials (Fairchild et al., 1982, Hogan et al., 1989, Zdanowicz et al., 2004, LeJeune and Kauffman, 2005). Because a CBP system utilizes the composting process, bacteria must proliferate which requires a carbon source (the organic bedding), thus making inorganic bedding an impractical choice for use in CBP barns (Black et al., 2014).

1.2.3 Temperature.

The internal temperature of the pack is one of the main bedding characteristics that must be monitored in order to maintain an effective compost bedding system. The optimal internal temperature for a CBP at a depth of 15-31 cm ranges from 43.3 to 65.0°C (Janni et al., 2007, Bewley et al., 2013). However, internal temperature ranges reported by researchers have varied significantly. Zhao et al. (2012) reported Ohio farms had internal pack temperatures from 32.2 to 48.9°C, whereas Galama (2011) found that compost barns in the Netherlands had ranges most commonly at 25 to 30°C. Increased internal temperatures have been linked to areas on the pack that visually appeared fluffy and loose (Shane et al., 2010). Areas of the barn that appeared to be chunky and compacted after stirring indicated pockets of anaerobic microbial activity and lower internal temperatures (Janni et al., 2007). Increased stirring depth, tilling frequency, and

space per cow have been shown to increase pack internal temperature (Black et al., 2013). Temperatures reaching above 65.0°C would typically result in bedding sanitation, or microbial death, however research has not reported temperatures above the recommended range. Measuring the internal temperature is just one tool producers can utilize to ensure their CBP barn is working properly.

1.2.4 Moisture.

Moisture content is another bedding characteristic that should be closely monitored to ensure effective composting. Bewley et al. (2013) recommended the ideal moisture content of a CBP should range between 40 to 60%. The more recent suggested moisture benchmark was 55% moisture (Eckelkamp et al., 2016a). The influx of moisture in compost barns comes from manure, urine, and drying rate of the pack (Janni et al., 2007). Increased stirring depth, space per cow, and drying rate of the CBP have been shown to decrease CBP moisture (Black et al., 2013). Ambient temperature has also been shown to influence the moisture content of a CBP. Barberg et al. (2007b) and Eckelkamp et al. (2016b) found that the moisture content increases over the cooler months of the year, likely due to a slower drying rate. Moisture content typically has an inverse relationship with internal temperature. Determining the moisture content and making the necessary management adjustments to get levels back within the recommended range of 40-60% is likely to increase internal pack temperature to its range as well. Moreover, specific bedding nutrients play a key role in adjustment of management strategies.

1.2.5 Carbon-to-Nitrogen (C:N) Ratio.

Calculating the C:N ratio is a critical component when determining the effectiveness of a composting system. This is because carbon and nitrogen are the most

important elements for microbial decomposition. Carbon provides both an energy source and the basic building block making up about 50% of the mass of microbial cells. Nitrogen is a crucial component of the proteins, nucleic acids, amino acids, enzymes and co-enzymes necessary for cell growth and function (Dickson et al., 1991). In a CBP barn housing system, the amount of carbon required for composting (the main source being organic bedding material added) is directly dependent on the amount of nitrogen present (the main source being cow urine and manure). The recommended range for optimal composting has been reported at 25:1 to 30:1 (Rynk et al., 1992). Minnesota farms had a mean C:N ratio of 19.5 ± 7.5 (Barberg et al., 2007a), whereas Kentucky farms had a higher mean C:N ratio of 26.7 ± 7.8 (Black et al., 2014). New York CBP barns exhibited a large range of C:N ratios, at 29.1, 21.5 to 45.1 (Petzen et al., 2009). Due to the various management factors associated with the amount of carbon and nitrogen in the pack, this is likely the reason for the large range of C:N ratio seen throughout the United States. However, carbon and nitrogen are key components for the proliferation of bacteria, which is how a composting system works in the first place.

1.2.6 Compost Bedding Bacteria.

It was originally suggested that maintaining high enough internal bedding temperature (54 to 65°C) in the composting system had the potential to inactivate mastitis-causing pathogens. However, research by Black et al. (2014) and Eckelkamp et al. (2016b) showed that samples from well-managed CBP barns had ample growth of Coliforms, *Staphylococcus* spp., *Streptococcus* spp., and *Bacillus* spp. in the pack. Similarly, Petzen et al. (2009) isolated *Streptococcus* spp., *Staphylococcus* spp., gram-

negative and gram positive-bacillus species, *Klebsiella* spp., and *Escherichia coli* from CBP samples.

It is evident that CBP barns maintain high microbial populations. Researchers wanted to investigate the quantity of those previously stated microbial species. When conducting laboratory analysis on microorganisms, the method of plating the sample on Plate Count Agar gives the total bacteria count (TBC) of the sample. The mean TBC observed on Minnesota farms was 7.0 ± 6.8 $\log_{10}$ cfu/g DM (Barberg et al., 2007a). In Kentucky, the mean TBC observed was at 8.2 ± 0.4 $\log_{10}$ cfu/g DM (Black et al., 2014). Like Kentucky, farms in Brazil were found to have a mean TBC of 8.7 ± 0.4 $\log_{10}$ cfu/g DM (Fávero et al., 2015).

Coliform is described as gram-negative, rod-shaped bacteria commonly found in the environment, such as *E. coli* and *Klebsiella* species. Coliform counts were found to be relatively stable over the year, with a mean count of 6.2 ± 0.6 $\log_{10}$ cfu/g DM (Eckelkamp et al., 2016b). Similarly, Fávero et al. (2015) reported a mean coliform count of 6.5 ± 0.7 $\log_{10}$ cfu/g DM and Black et al. (2014) reported a mean coliform count of 6.3 ± 0.6 $\log_{10}$ cfu/g DM. *Streptococcus* spp. and *Staphylococcus* spp. are other species of bacteria commonly found in the dairy environment. Additionally, Staphylococcus spp. microorganisms are common habitants to mammalian skin microbiota. *Streptococcus* spp. counts were found to have a mean count of 6.5 ± 0.8 $\log_{10}$ cfu/g DM (Fávero et al., 2015), whereas other researchers reported mean counts of 7.2 ± 0.7 $\log_{10}$ cfu/g DM (Eckelkamp et al., 2016b) and 7.2 ± 0.7 $\log_{10}$ cfu/g DM (Black et al., 2014). *Staphylococcus* spp. counts of CBP barns in Kentucky varied from 6.3 ± 0.5 $\log_{10}$ cfu/g DM (Eckelkamp et al., 2016b) to 7.9 ± 0.5 $\log_{10}$ cfu/g DM(Black et al., 2014). Italy CBP

barns reported *Staphylococcus* spp. counts of 5.6 ± 0.5 log10 cfu/g DM (Biasato et al., 2019). Black et al. (2014) and Eckelkamp et al. (2016b) reported similar *Bacillus* spp. counts of 7.6 ± 0.5 $\log_{10}$ cfu/g DM and 7.7 ± 0.6 $\log_{10}$ cfu/g DM, respectively. Overall, bacterial population quantity differences may be due to the environment and management differences not only by states in the U.S. but also compared to other countries.

It is important to consider different compost bedding characteristics and their influence on the bacterial population, specifically internal temperature and moisture content. Eckelkamp et al. (2016b) found that as compost internal temperature increased, coliform species growth increased. Black et al. (2014) reported similar findings, with internal temperature positively correlated with coliform count ($r = 0.42$, $P < 0.05$) and pack moisture negatively correlated with coliform count ($r = -0.34$, $P < 0.05$). Barberg et al. (2007b) described similar results with coliforms increasing in the summer (5.3 $\log_{10}$ cfu/g) compared to the winter (4.6 $\log_{10}$ cfu/g), consistent with warmer internal pack temperatures and lower moisture content in the summer. When discussing *E.coli* separately, Black et al. (2014) found that *E. coli* counts were strongly positively correlated with ambient temperature ($r = 0.46$; $P < 0.05$).

Additionally, Eckelkamp et al. (2016b) found that as compost internal temperature increased, *Staphylococcus spp.*, *Streptococcus spp.*, and *Bacillus spp.* growth in the pack area decreased. Regarding *Bacillus spp.,* Black et al. (2014) reported *Bacillus spp*. counts were reduced with increasing CBP moisture, C:N ratio, and ambient temperature. *Staphylococcus spp*. counts showed a strong positive correlation with ambient temperature ($r = 0.53$; $P < 0.05$) and strong negative correlations with moisture ($r = -0.44$; $P < 0.05$) and C:N ratio ($r = -0.52$; $P < 0.05$) (Black et al., 2014). Overall, lower

CBP moisture and high CBP temperature reduced bacteria concentrations in the bedding (Eckelkamp et al., 2016b). While compost bedding bacterial levels will not be eliminated, well-managed CBP barns optimize bedding characteristics (decreasing moisture and increasing internal temperature), consequently reducing bedding bacterial concentrations.

1.2.7 Udder Health/ Hygiene.

Udder hygiene is one of many useful ways to monitor animal health, as hygiene scores have been linked to Somatic Cell Count (SCC) and mastitis incidence in dairy cattle (Schreiner and Ruegg, 2003, Reneau et al., 2005). Because proper cow hygiene management can reduce mastitis risk (Neave et al., 1969, Philpot, 1979, Schreiner and Ruegg, 2003, Reneau et al., 2005), much research has been done to evaluate udder hygiene in relation to CBP barns and its bedding characteristics. Barberg et al. (2007b) observed a mean hygiene score of 2.66, where 1 = clean and 5 = very dirty (Reneau et al., 2005), for 12 CBP barns visited, whereas Shane et al. (2010) observed a mean hygiene score of 3.1 for 6 CBP barns. More recently, Eckelkamp et al. (2016a) observed no differences between mean herd hygiene score between CBP and sand-bedded freestalls. Similarly, Costa et al. (2018) reported no differences between udder hygiene scores for cows housed on a CBP compared to freestalls. Interestingly, Lobeck et al. (2011) found that increased hygiene score showed no effect on mastitis prevalence on compost, naturally ventilated, and cross-ventilated freestall, respectively. When well-managed CBP and well-managed sand freestall barns were compared, no differences were found between SCC, clinical mastitis incidence, or bulk tank SCC (Eckelkamp et al., 2016a). Research has also been conducted looking at mastitis prevalence when cows are moved from one type of housing system to another. Barberg et al. (2007b) found that the average

prevalence of mastitis and SCC decreased after moving into the CBP from previous housing facilities. This may be due to improved cow comfort, as seen by a low prevalence of lameness reported for cows housed in this type of housing system (Burgstaller et al., 2016, Costa et al., 2018). Improved cow comfort results in less stressed cows and likely an improved immune system which reduces the risk of mastitis.

Ideal management strategies have been developed to help maintain clean udders on cows housed in CBP barns. Black et al. (2013) found that drier CBP surface layers resulted in cleaner cow legs and udders, which was accomplished through a high drying rate, deep CBP stirring, and adequate space per cow. Barberg et al. (2007b) and Janni et al. (2007) suggested that tilling the pack area, drying the surface and incorporating manure could potentially improve cow comfort and decrease mastitis in CBP barns unlike conventional bedded pack barns.

Monitoring bulk tank SCC on farms has been a common approach when determining udder health. Researchers out of Minnesota found that the SCC values were below the state average in cows after they transitioned to a CBP barn (Barberg et al., 2007b). Additionally, the mean SCC of cows housed in CBP in Italy was at 51,510 cells/mL which was significantly lower than cows housed in free stalls (Biasato et al., 2019). Astiz et al. (2014) found positive effects of CBP-systems compared to sand freestalls on udder health [lower incidence of first mastitis-cases, 22.1 vs. 35% of second-mastitis cases, 6.8 vs. 15%]. However, no differences in relation to bulk tank SCC were found between CBP-housed animals and those in sand free stalls (Eckelkamp et al., 2016a). Klaas et al. (2010) reported a mean SCC of 192,000 cells/mL for 3 CBP barns in Denmark, whereas Black et al. (2014) observed a mean SCC of 252,860 cells/mL for

CBP barns in Kentucky. With the U.S. dairy industry's continuous goal of reducing bulk tank SCC, it's imperative to note that cows housed in well-managed CBP barns can maintain low SCC values and having healthy udders.

1.3 Mastitis

History reveals that cows have been milked since 3100 BC (Nemet-Nejat, 1998) and bovine mastitis has likely existed since that time (Ruegg, 2017). Mastitis is defined as the inflammation of the mammary gland and continues to be the costliest disease in the dairy industry. Murphy (1947) described it as a 3-phase process in which there is (1) invasion of an organism (with or without the establishment of infection), (2) infection (the bacteria become established in the gland), and (3) inflammation. The inflammatory response is responsible for destroying the invading organisms; however, it may also occur when there is physical damage or chemical irritation to that specific location. Bacterial agents likely enter the udder via the teat end and teat canal (Jain, 1979). Once inside the udder, these microorganisms multiply in the secretory tissue, resulting in toxin production and damage to the mammary gland (Bramley et al., 1996). The severity and outcome of mastitis varies tremendously, which is why it has deemed the title of a multifactorial disease.

Mastitis can be categorized as either a clinical or subclinical infection. Clinical mastitis is characterized by abnormal milk and swelling or pain in the udder and may be accompanied by systemic signs such as elevated rectal temperature, lethargy and anorexia (Harmon, 1994). Liang et al. (2017) found that the average cost per clinical mastitis case was \$325.76 ± 71.12 for primiparous (1st lactation) cows and \$426.50 ± 80.27 for multiparous (>1 lactations) cows. Subclinical mastitis is the form in which there is no

detectable change in the udder and there are no observable abnormalities in the milk. However, milk production decreases, bacteria are present in the secretion, and composition is altered (Harmon, 1994). Halasa et al. (2007) found that the average cost per subclinical mastitis case was $116 for primiparous cows and $325 for multiparous cows. The cost of milk loss and treatment are the 2 most expensive cost categories associated with total mastitis cost.

The National Mastitis Council (NMC) developed a mastitis control program known as the "5-Point Plan" that is the basis for controlling contagious mastitis and includes (1) effective post-milking teat dipping, (2) use of antibiotic dry cow therapy in every quarter at the end of each lactation, (3) appropriate treatment of clinical cases, (4) culling of chronically affected cows, and (5) maintenance of milk equipment to ensure stable teat end vacuum (Ruegg, 2017). However, with an emphasis on decreasing subclinical mastitis (largely due to contagious organisms), there has been a relative increase in the incidence of environmental organisms, showing an increase in clinical mastitis (Bradley, 2002).

1.3.1 Mastitis Detection: Somatic Cell Count (SCC)

Somatic cells are leukocytes whose purpose is to phagocytize and destroy microbes that are present in the body, as a result of an infection. In the case of dairy cows, the focus is on the presence of these microorganisms in the infected quarter. Milk SCC is often used to measure mammary inflammation and an increase in SCC is strongly correlated with increased probability of intramammary infection (IMI) (Eberhart et al., 1979, Dohoo and Leslie, 1991). Milk from healthy mammary glands contains <100,000 somatic cells/ml. If the SCC in milk is >200,000 cells/ml, it has been suggested that an

inflammatory response has been elicited and that a mammary quarter is infected or is recovering from an infection (Nickerson and Oliver, 2014). Thus, an increase in milk SCC is a good indicator of mastitis (Nickerson and Oliver, 2014). Dairy producers utilize bulk tank somatic cell counts (BTSCC) as an indicator of the prevalence of IMI within a dairy herd and as a key indicator of milk quality (Wenz et al., 2007). A lower BTSCC is likely indicative of a healthier herd and may also lead to economic benefits for producers. Ott and Novak (2001) reported herds attained significantly more profit per cow when their BTSCC was < 200,000 cells/mL compared to herds that had BTSCC ≥ 400,000 cells/mL. Many processors pay quality premiums for low-BTSCC milk because there is a negative relationship between SCC and casein composition and shelf life of processed fluid milk (Ali et al., 1980, Ma et al., 2000). In order to remain competitive in the global market, U.S. dairy producers must maintain a positive image while continuing to lower their BTSCC.

1.3.2 Mastitis Causing Pathogens

Although many bacteria are recognized as being able to cause an IMI, initial emphasis of mastitis control was directed at contagious pathogens, specifically *Streptococcus agalactiae* and *Staphylococcus aureus* (Ruegg, 2017). Contagious pathogens are transferred from cow to cow by contact with infected quarters, per example via contaminated milking machine inflations, the hands of milkers, or dirty towels. Between 1994 and 2001, isolation of *Streptococcus agalactiae* and *Staphylococcus aureus* from milk samples submitted to the Wisconsin Veterinary Diagnostic Laboratory declined dramatically (Makovec and Ruegg, 2003) and gram-negative pathogens (or culture-negative results) have become the predominant results of milk samples obtained

from cows (Oliveira et al., 2013). Today, a diversity of opportunistic pathogens (i.e., *Streptococcus* spp., coagulase-negative *Staphylococcus* spp. (CNS), *Prototheca* spp., and others) are responsible for a significant portion of IMI (Bradley and Green, 2001, Oliveira et al., 2013). Of importance is the group of microorganisms that fall under the term CNS. *Staphylococcus* spp. microorganisms are divided into two classifications according to laboratory identification as coagulase positive or coagulase negative. Coagulase positive species are predominantly *Staphylococcus aureus*, and the rest (majority) fall under this term CNS. They were once considered minor pathogens and are ubiquitous to the environment and to the skin microbiota; they are now recognized as the major cause of subclinical mastitis, can cause clinical mastitis, and are associated with elevated SCC (Piepers et al., 2007, Oliveira et al., 2013).

1.3.2.1 Environmental microorganisms.

As the name states, environmental pathogens are commonly found in the environment. Environmental risk factors include bacteria level, pathogen nature, environmental condition, and cow exposure (Jain, 1979, Bramley et al., 1996, Breen et al., 2009). Typical environmental microorganisms that are known to cause mastitis include *Streptococcus uberis, Streptococcus dysgalactiae*, and coliforms (which include *Klebsiella pneumoniae*, *Klebsiella oxytocia, Escherichia coli, Serratia spp*., and *Enterobacter aerogenes)* (Smith et al., 1985, Bramley et al., 1996). Both environmental streptococcal and coliform infection rates increased with increasing parity (from the 1^{st} to $\geq 6^{th}$ lactation; (Smith et al., 1985)). Additionally, both have been reported to cause subclinical mastitis, typically with no extensive damage or decrease in milk production (Smith et al., 1985). On the other hand, Hogan et al. (1989) found that herds with low

SCC (usually indicative of successful control of contagious mastitis) could experience serious mastitis problems, specifically higher rates of clinical cases.

1.3.2.2 Coagulase-negative Staphylococcus (CNS).

In mastitis diagnostics, staphylococci are divided into coagulase-positive staphylococci and coagulase-negative staphylococci (CNS) based on the ability to coagulate plasma. The major pathogen, *Staphylococcus aureus*, is generally coagulase-positive although coagulase-negative strains do occur (Fox et al., 1996). CNS have traditionally been considered normal skin microbiota that can cause mastitis as opportunistic bacteria (Devriese and De Keyser, 1980). Many studies have determined that the following four species of CNS were most frequently isolated in bovine milk samples: *Staphylococcus chromogenes*, *Staphylococcus epidermidis, Staphylococcus hyicus*, and *Staphylococcus simulans* (Devriese and De Keyser, 1980, Trinidad et al., 1990, Jarp, 1991, Waage et al., 1999, Taponen et al., 2006, Thorberg et al., 2006, Taponen and Pyörälä, 2009).

Researchers have classified CNS as minor mastitis-causing pathogens, with many considering their importance to udder health very limited. Studies on CNS have given mixed results, thus now making this group of microorganisms more difficult to deem 'minor' pathogens. Hogan and Smith (1997) reported that CNS rarely caused clinical mastitis, but are the most frequent cause of an IMI in lactating cattle. Jarp (1991) found that CNS IMI's have been associated with an increase SCC of affected cows. Others have indicated that CNS infections provide a means of preventing an IMI from other major pathogens (White et al., 2001). Moreover, CNS infections have been studied in pre-partum treatment trials in heifers and bacteriological cure was associated with decreased

SCC (Borm et al., 2006). Additionally, Schukken et al. (2009) concluded that in herds with low BTSCC, CNS infections may be an important contributor to the total BTSCC. On the other hand, herds with milk quality problems would not attribute CNS infections to increased BTSCC. Interestingly, CNS are the most prevalent pathogens causing IMI in heifers (Taponen and Pyörälä, 2009), with up to 54% of quarters being infected by CNS at calving (Trinidad et al., 1990).

These conflicting results as to the importance of CNS have likely been due to the failure to acknowledge variations within and between species (Fry et al., 2014). With this idea coming to light, researchers have begun identifying effects of specific CNS species on udder health parameters. Researchers have reported that *Staphylococcus chromogenes*, *Staphylococcus haemolyticus, Staphylococcus epidermidis*, and *Staphylococcus simulans* have been associated with persistent infections (Piessens et al., 2011, Supré et al., 2011). Fry et al. (2014) reported similar findings, that *Staphylococcus chromogenes* and *Staphylococcus simulans* were associated with persistent IMI which suggested that those species were better host-adapted, whereas others may have an environmental reservoir. Nyman et al. (2018) found that there was a significant association between different CNS species and SCC of udder quarters, but different CNS species had no effect on milk yield. Similarly, Tomazi et al. (2015) reported *Staphylococcus chromogenes* as the most prevalent CNS species that caused an IMI, which showed an increase in SCC but had no effect on milk yield or composition at the quarter level. Identifying mechanisms to reduce exposure to IMI caused by opportunistic organisms, particularly CNS, while also finding suitable interventions for affected cows will continue to be a challenge (Ruegg, 2017).

1.4 Interaction Between Bedding Bacteria, Teat Exposure, and Mastitis

Intramammary infections (IMI) caused by environmental pathogens have likely occurred from bacteria invading the teat canal and multiplying in the gland (Hogan and Smith, 2012). Because cows lay for 12 to 14 hours a day, their teats are in direct contact with the bedding or other materials where they lay down. Populations of the bacteria in bedding have been reported to be related to the number of bacteria on teat ends and rates of clinical mastitis for cows housed in freestall barns (Hogan et al., 1989, Zdanowicz et al., 2004). Investigating and potentially reducing the number of bacteria in different bedding types has been the goal in hopes of decreasing environmental mastitis.

1.4.1 Bacterial species in various types of bedding material.

1.4.1.1 Organic bedding.

Bacteria proliferate more easily in organic bedding (gram-negatives: 7.1 $\log_{10}$ cfu/g; coliforms: 6.2 $\log_{10}$ cfu/g; *Klebsiella* spp.: 4.3 $\log_{10}$ cfu/g; *Streptococci* spp.: 7.5 $\log_{10}$ cfu/g) compared with inorganic bedding (gram-negatives: 6.41 $\log_{10}$ cfu/g; coliforms: 5.7 $\log_{10}$ cfu/g; *Klebsiella* spp.: 3.4 $\log_{10}$ cfu/g; *Streptococci* spp.: 6.8 $\log_{10}$ cfu/g) because organic bedding supplies the adequate amounts of nutrients, temperature, and moisture for microorganisms to survive (Hogan et al., 1989). It's been well researched that organic materials such as straw and sawdust, when used as bedding, often contain $>10^6$ cfu/g of coliform bacteria (Bramley and Neave, 1975). Additionally, bacteria counts differ within these organic bedding materials. Wood products are known to contain the greatest number of coliform bacteria (Rendos et al., 1975). Sawdust and shavings are known to have an increased amount of *Klebsiella* species (Newman, 1973). Manure solids bedding were found to be generally associated with higher bacteria counts compared with organic non-manure materials or sand bedding materials (Patel et al., 2019). *Streptococcus* spp. counts and coliform bacteria counts were found in the greatest

quantities in manure solids bedding material (2.1×10^8 cfu/g and 2.2×10^6 cfu/g, respectively) (Rowbotham and Ruegg, 2016). Additionally, recycled manure solids were found to have the greatest quantities of gram-negative bacteria (16.3 ln cfu/ml), followed by straw (at 13.8 ln cfu/ml) and wood (at 10.3 ln cfu/ml) (Robles et al., 2019).

1.4.1.2 Inorganic bedding.

Materials such as new sand or recycled sand are considered types of inorganic bedding material. Inorganic materials lack organic nutrients which are required for the growth of microorganisms. Since microbes are unable to proliferate in inorganic bedding materials, this results in the decreased exposure to environmental mastitis-causing pathogens. Rowbotham and Ruegg (2016) found that *Streptococcus* spp. and coliform bacteria counts were the least in new sand bedding material (8.6×10^6 cfu/g and 3.6×10^3 cfu/g, respectively). Robles et al. (2019) reported sand bedding was the driest (highest dry matter (DM%)) compared to straw and wood, and recycled manure solids; where higher DM% was associated with lower *Streptococcus* spp. counts, *Staphylococcus* spp. counts, and gram-negative bacteria counts. Additionally, some studies have reported that the use of inorganic bedding (vs. organic bedding) was associated with reduced clinical mastitis risk (Hogan et al., 1989) or lower SCC measures (Wenz et al., 2007, Rowbotham and Ruegg, 2015).

1.4.2 Relationship between Bedding Bacteria and Udder Health Parameters.

The teat canal is the first barrier that microorganisms face when invading the cow's mammary gland (Jain, 1979, Paulrud, 2005). Extensive research has been conducted to find relationships between bacterial counts in bedding and on the teat ends of dairy cattle. In turn, indirect conclusions have been made regarding the negative effect

on udder health, such as mastitis prevalence and incidence rates. Common mastitis-causing pathogens have been isolated from teat canals which included CNS, *Staphylococcus aureus*, *Streptococcus uberis* and coliform bacteria (Zecconi et al., 1992, Paduch et al., 2012, Quirk et al., 2012). A direct correlation has been found between bacterial counts in bedding and bacterial counts on the teat ends (Hogan and Smith, 1997, Zdanowicz et al., 2004) and clinical mastitis rates (Hogan et al., 1989). Paduch et al. (2013) reported associations between bacteria counts in sawdust bedding and bacteria counts in the teat canal; *Streptococcus uberis* had a strong correlation (r = 0.49), *E. coli* a moderate correlation (r = 0.33), and other coliform bacteria also were found to be associated. For *Staphylococcus aureus*, no associations were found. Researchers have also looked at relationships between bedding bacteria counts and bacteria counts on the teat skin rather than the teat ends/ teat canals. Positive correlations were found between bedding bacteria counts and bacterial counts on teat skin for Gram-negative bacteria, coliforms, *Klebsiella* spp., and *Streptococci* spp. (Hogan and Smith, 1997, Hogan et al., 1999, Zdanowicz et al., 2004). Another study reported that heifers with *Staphylococcus chromogenes*, *Staphylococcus simulans*, and *Staphylococcus xylosus* isolated from their teat skin prepartum were at increased odds of having an IMI with the same species postpartum (Adkins et al., 2018).

Interestingly, many studies have not found strong associations between teat end or teat skin bacteria counts and mastitis. Consequently, there is a lack of consistent evidence to support the widely held belief that high bedding bacteria counts are a risk factor for IMI and mastitis (Rowe et al., 2019). Recent studies, however, had the goal of determining associations between bedding bacteria counts and different udder health

outcomes. Patel et al. (2019) found that a 1-$\log_{10}$ increase in unused bedding *Staphylococcus* spp. counts were associated with an estimated (SE) increase in IMI (%) and chronic IMI of 0.62 (0.33) and 0.66 (0.21), respectively. Patel et al. (2019) also found that a 1-$\log_{10}$ increase in bedding *Streptococcus* spp. counts were associated with an estimated increase in IMI of 0.50 (0.23), and that a 1-$\log_{10}$ increase in bedding coliform counts were associated with an estimated increase in IMI and chronic IMI of 1.04 (0.30) and 0.48 (0.20), respectively. The same research group reported only modest differences in IMI prevalence observed between 4 bedding material types (manure solids, organic non-manure, new sand, and recycled sand) (Rowe et al., 2019). A positive association was observed for *Streptococcus* spp. counts in unused bedding and *Streptococcus*-IMI (Odds ratios = 1.09) (Rowe et al., 2019). In conclusion, it is evident that bedding bacteria counts influence the bacterial loads on the teat skins and ends of dairy cows, however more research is needed to investigate the relationship between bacteria in bedding and if that is a risk factor for mastitis.

1.5 Conclusions

Compost bedded pack barns require unique management practices for dairy cows to reap the many benefits associated with this type of housing system. Tilling twice-daily, addition of sawdust bedding as needed, and appropriate cow stocking density allows the pack to stay within the correct levels of moisture content (40-60%), internal temperature (43.4 – 65 °C), and C:N ratio (25:1 – 30:1). However, producers' question whether cows housed in a CBP are at a higher risk of getting an IMI due to the environmental bacteria exposure, since studies have shown that CBP barns have high amounts of bedding bacteria (Black et al., 2014). Additionally, bedding bacteria are found to be associated

with bacterial counts on the teat ends, and organic bedding materials provide an adequate environment for bacterial growth. With many studies conducted looking at these relationships, none have looked at CBP bedding and its relationship with milk quality and udder health.

CHAPTER 2. MICROBIAL INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING AND TEAT EXPOSURE IN TRANSITION DAIRY CATTLE: THE DATA COLLECTION AND LABORATORY ANALYSIS

T.L. France, J.H.C. Costa, and M.C. Morgan

Department of Animal and Food Sciences, University of Kentucky, Lexington Kentucky 40546

2.1 Introduction

Dairy cattle housing systems have been reported to widely influence all aspects of the animal, including animal welfare, health, and milk quality (Bewley et al., 2017). Because the dairy industry continues to strive for improved cow comfort and well-being, the compost bedded pack (CBP) barn was developed to achieve this. The large, combined resting and exercise area has yielded promising results, where cows have had a decreased somatic cell count (Biasato et al., 2019), lower prevalence of mastitis (Barberg et al., 2007b), and lower prevalence of lameness (Costa et al., 2018) when compared to freestall housing systems. A well-managed CBP barn provides a soft surface for dairy cows to lie on. Consequently, it provides the optimal environment for the proliferation of microorganisms in the compost bedding (Black et al., 2014). This is due to the ample levels of carbon and nitrogen present in compost bedding which are necessary for microbial growth and function, as well as moisture content suitable for microbes to proliferate more rapidly. Exposure to environmental pathogens found in bedding occurs when teats are exposed to the bedding and bacteria are transferred to the teat skin (Rowbotham and Ruegg, 2016). Of concern has been the potential increased exposure of these mastitis-causing pathogens to the teats of early lactation dairy cattle during the transition period (3 weeks pre- to 3 weeks postpartum) (Grummer, 1995). The transition period is a stressful time when dairy cows are at an increased risk for a myriad of health problems, including mastitis. Svensson et al. (2006) found that during the period of 7 days before, through the first 305 days of lactation, over 30% of mastitis cases occurred in the first week of lactation, and more than half of all cases occurred during the period of -7 to 30 days postpartum. Therefore, it is fundamental to provide these vulnerable animals with an environment that will allow them to thrive.

Because mastitis continues to be the costliest disease in the dairy industry, research has focused on environmental risk factors associated with this infection. Bacterial load relationships between different stall bedding types, teat skin samples, and teat end samples with the goal of reducing mastitis risk due to environmental pathogens have been investigated in depth (Paduch et al., 2013, Patel et al., 2019, Robles et al., 2019, Rowe et al., 2019). However, research is lacking when determining this relationship when cows are housed on CBP barns. Additionally, it has been reported that ambient temperature influences CBP characteristics, resulting in altered pack performance throughout the year (Shane et al., 2010, Eckelkamp et al., 2016b). Indeed, it could be valuable to producers of CBP barns who experience poorer pack performance in colder temperatures (Barberg et al., 2007b) for more specific research on various temperature conditions and its influence on both CBP bedding characteristics and bacterial levels.

The main objective of this study was to evaluate the change over time on the microbial population of the compost bedding, on teat skin, teat ends, and in the milk of dairy cows housed in CBP barns. Research has shown positive correlations between bedding bacterial load and bacteria counts on the teat ends (Hogan et al., 1999, Zdanowicz et al., 2004), with recent studies connecting clinical mastitis cases to bedding bacteria counts. Knowing this, a second objective was to identify potential interactions of the microbial populations between the bedding, teat skins, teat ends, and in the milk. The last research objective was to investigate the influence of various stages of the transition period (2-weeks prepartum, 72-hours postpartum, 60 days in milk (DIM)) on the microbial populations of the cow-level variables (teat skin, teat ends, milk). I

hypothesized that there would be a positive correlation between the bacterial counts found in compost bedding and the microflora found on the teat skin and teat ends. Additionally, I hypothesized that the bacteria found on the teat ends were strongly correlated to the bacteria counts found in the milk.

2.2 Materials and Methods

The study was conducted from December 2018 to May 2019 as a longitudinal observational study at the University of Kentucky's Coldstream dairy research farm (2810 Georgetown Road, Lexington, KY, USA, 40511). Twenty-six Holstein dairy cows were enrolled (parity: 2.08 ± 1.17 [mean ± SD]) during the study with no cow exclusion criteria. Cows were enrolled based on their expected calving dates. Close-up dry cows were moved to a smaller pen (155 m^2) of the CBP, which remained at 100% stocking density (16 cows at 9.7 m^2 per cow) for the duration of the study. Once a cow calved, she was moved to the adjacent pen (465 m^2), which also remained at 100% stocking density (48 cows at 9.7 m^2 per cow). Cows were fed a TMR formulated following the National Research Council guidelines (NRC, 2001) to meet or exceed the requirements of lactating dairy cows producing at least 39 kg of milk daily and 680 kg of body weight. Composition of the TMR as fed was 40.7% corn silage, 27.8% lactating cow grain mix, 23.6% alfalfa silage, 5.1% cottonseed, 1.8% alfalfa hay, and 1.0% mineral mix. Milking occurred twice daily (0430 and 1530 h.). Tilling of the pack occurred twice/d, at approximately 0430 and 1400 h, taking 30 minutes to till each side of the barn. The CBP was tilled using a 40-cm penetration capacity rototiller (LVI Bedded Pack Composter, model 750, Richland, PA, USA) with the addition of sawdust shavings when needed based on bedding moisture level. Each cow remained on the study until nominally 60

DIM (59.27 ± 2.01 DIM). All cows enrolled were housed on the left side of the barn, so CBP samples were only taken from that side.

2.2.1 Cow-level sample collection.

Animals were enrolled in the experiment during the dry period and a baseline teat skin and teat end sample was collected at nominally 14 d before parturition (11.96 ± 3.19 d). The second sampling occurred nominally 72 h after parturition (61.85 ± 11.86 h). Samples were collected biweekly from January 7, 2019 to May 14, 2019. To ensure every cow had a final sample of around 60 DIM, an additional sample was collected on an individual basis to meet that requirement. All samples were collected at the milking parlor and placed in a cooler (temperature remained < 4°C) until transportation to the University of Kentucky's Animal and Food Sciences microbiology laboratory for same-day bacterial analysis.

2.2.1.1Teat end condition scores.

The teat end condition score (scale 0-5) for all teats were determined. Teats were scored a "0" indicating a smooth teat with no ring, up to a "5" indicating a very raised, rough ring with cracks and bumps (Goldberg et al., 1994, Neijenhuis et al., 2000).

2.2.1.2 Teat skin sponges.

Teat skin sponges were aseptically collected for every teat using individually packaged pre-moistened sponges (Nasco Whirl-Pak 18-oz hydrated speci-sponge with glove, sterile bags, Fort Atkinson, WI, USA). Using a sterile glove, the sponge was removed from its bag and gently rubbed downwards on the teat skin surface. This was repeated multiple times to ensure the sponge collected contents from the entire teat skin

surface. The sponge was aseptically placed back in its original bag and placed in a cooler (temperature remained < 4°C) for temporary storage.

2.2.1.3 Teat end swabs.

Teat end swabs were collected for every teat using sterile transport swabs (3M Quick Swab, St. Paul, MN, USA). Each swab was packaged in its own container with 1ml buffer solution. The buffer solution was squeezed into the sterile container to moisten the cotton tip prior to sampling. The sterile swab was removed, and the cotton tip was used to touch the teat end, rotating the swab and pushing gently up to collect contents inside the teat orifice. Swabs were aseptically placed back inside the original container and placed in a cooler (temperature remained < 4°C) for temporary storage.

2.2.1.4 Milk samples.

Teats were sanitized according to National Mastitis Council (1999) guidelines. 1.0% iodine pre-dip (FS-103 II Teat Dip, Millbury, MA, USA) was applied to each teat. After 30 seconds, iodine was wiped off using a clean dry cloth towel. Using clean, nitrile gloves, each teat was scrubbed with a cotton ball soaked in 70% isopropanol. 3 streams of milk were discarded, and milk was aseptically collected into a 10ml sterile sample vial for each teat. Additionally, individual teat milk samples were collected for same-day somatic cell count analysis. Milk was collected into 40ml Bentley tubes, filling the tube about half-full. 2-3 drops of Azidiol preservative was added to each milk sample. Samples were stored at a temperature of < 4°C until analysis (< 2 wk.) using a Bentley Flow Cytometer (Bentley Instruments, Inc., Chaska, MN, USA).

2.2.2 Compost bedding collection.

Compost bedding samples were collected weekly for the duration of the study. The pen was divided into 9 evenly distributed sections (as described by Black et al. (2013). To explore the tilling effect, samples were obtained both before and after tilling, at approximately 1300 and 1500 h. Temperature was collected for each section at 20.3-cm deep using an immersion thermocouple-based thermometer (accuracy of ±2.2°C; Model 87; Fluke Inc., Everett, WA, USA), and the CBP surface using an infrared thermometer (accuracy of ± 1°C; Model 62; Fluke Inc., Everett, WA, USA). Compost bedding samples were collected individually from each of the 9 sections. A composite sample was obtained from each of the 9 sections. Each composite sample was formed by a combination of 6 subset bedding samples collected randomly at the top 2 inches of bedding material from the inside of each section (shown as green stars in Figure 2.1) using a 115 cm^3 measuring cup, for an approximated 700 cm^3 sample collected in a 3.8-L plastic bag (Ziploc, S. C. Johnson & Son Inc., Racine, WI, USA). All bedding samples were mixed manually to homogenize contents. Samples were divided in to two equal parts and placed in a cooler (temperature remained < 4°C) for transportation. One sample was transported to the University of Kentucky's Animal and Food Sciences microbiology laboratory and stored in a -20ºC freezer for later microbiological analysis.

The duplicate sample was taken to the University of Kentucky Division of Regulatory Services Soil Research Laboratory for bedding nutrient analysis. Samples were oven dried at 75°C, ground to pass a 2 mm screen, and stored at room temperature prior to analysis. Samples were analyzed following the standard Animal Waste methods described by Peters et al. (2003). Briefly, nitrogen was analyzed using a LECO Nitrogen Analyzer (LECO, St. Joseph, MI, USA). Two grams of sample was digested with a

combination of HCl and H_2SO_4 acids. The acid digest was analyzed for P, K, Ca, Mg, Zn, Cu, Mn, and Fe concentrations using an ICP (Inductive Coupled Plasma Mass Spectrometer (ICP-MS)). Moisture content was determined by weight difference of sample before and after it was oven dried (at 75°C for 6 hours). The carbon-to-nitrogen (C:N) ratio was calculated for all samples.

Weather data was monitored (Hobo, Onset Computer Corp., Bourne, MA, USA) from inside the barn every day which included ambient temperature (ºC), relative humidity (RH) (%) and dew point (ºC).

2.2.3 Cow sample microbial analysis.

2.2.3.1 Teat skin sponges.

Once in the laboratory, teat skin sponges were placed in a temperature controlled (4 °C) walk-in cooler until same-day analysis. Sponges were immediately removed from the cooler and diluted in 15-ml of phosphate buffer solution (stock phosphate buffer solution was made with 24 mL of PO_4 stock, 95 mL $MgCl_2$ stock, and 19 L double deionized water; pH of 7.4-7.5) for enumeration. To obtain total bacteria count (TBC), 50 µl of the solution was added in duplicate on Plate Count Agar (PCA) (Difco™ Plate Count Agar. Becton, Dickinson and Company. Sparks, MD, USA) and spiral plated (Eddy Jet 2W; Neutec Group Inc., IUL Instruments I.K.S., Leerdam, the Netherlands) onto each plate. Coliforms were enumerated by pipetting 1-ml onto a 3M Petrifilm E. coli/Coliform Count Plate (3M Microbiology Products, St. Paul, MN, USA). To enumerate *Staphylococcus* spp., 0.1ml was plated on the selective media Mannitol Salt Agar (MSA) (Mannitol Salt Agar, Criterion, Hardy Diagnostics. Santa Maria, CA, USA) using the surface spread plate method. Similarly, 0.1ml of the same sample was plated on non-

selective media, 5% Sheep Blood Agar (BD BBL Stacker Plate, Becton, Dickinson and Company, Sparks, MD, USA) to account for *Streptococcus* spp. growth. All medium was incubated at 35ºC for 24h. The TBC was counted automatically using a colony plate reader (Flash & Go plate reader; Neutec Group Inc., IUL Instruments I.K.S., Leerdam, the Netherlands) as colony-forming units (cfu) per gram. Coliforms were counted manually and reported as cfu/ml. *Staphylococcus* spp. colonies and *Streptococcus* spp. colonies were counted manually, reported as cfu/ml, and the genus verified using the Vitek 2 Compact analyzer (Biomerieux, Hampshire, UK; Vitek 2 Gram Positive card kit, 20 cards; Vitek 2 Gram Negative card kit, 20 cards).

2.2.3.2 Teat end swabs.

Once in the laboratory, teat end swabs were placed in a temperature controlled (4 °C) walk-in cooler until same-day analysis. Once removed from the cooler, the cotton tip used to collect the sample was placed into a sterile tube that contained 9ml phosphate buffer, thus creating a 1:10 dilution. Tubes were vortexed to homogenize the solution with the sample contents. The procedure described for teat skin sponges was followed to enumerate TBC, coliform, *Staphylococcus spp.*, and *Streptococcus* spp. for all teat end swabs.

2.2.3.3 Milk samples.

Once in the laboratory, milk samples were placed in a temperature controlled (4 °C) walk-in cooler until same-day analysis. Each milk sample was analyzed in duplicates for TBC and coliform counts using previously described enumeration methods. Briefly, 50 µl was directly taken from each milk sample and plated on PCA using the spiral plater. Coliforms were enumerated by pipetting 1-ml on 3M Petrifilm E. coli/ coliform Count

Plates. Non-selective 5% Sheep Blood Agar was quartered and using a 0.01ml sterile loop, milk samples were streaked onto the blood agar with one sample per quarter plate (1 blood plate per cow). All plates were incubated at 35ºC for 24h. TBC was automatically counted using a colony plate reader (Flash & Go plate reader). Coliforms were manually counted, and colony morphology was determined from the blood agar.

Milk samples were classified as contaminated as described by Parker et al. (2008), where >2 distinct colony types present on any plate of the same sample was considered contaminated and discarded. An intramammary infection (IMI) was defined as the isolation of 100 cfu/mL of identical colonies on the same plate. For all IMI, a colony was picked using a sterile 0.01ml sterile loop and placed into Brain Heart Infusion (BHI) agar. Isolates were incubated at 35ºC for 24h, gram stained, and bacterial identification to the species level was determined using a Vitek 2 Compact Analyzer (Biomerieux, Hampshire, UK; Vitek 2 Gram Positive card kit, 20 cards; Vitek 2 Gram Negative card kit, 20 cards)

2.2.4 Compost bedding microbial analysis.

Bedding samples were moved from a -20ºC freezer to a 4 °C cooler the day before analysis to allow for gradual thawing. Samples were mixed by hand and a subsample of 25 g was added aseptically to a sterile stomacher bag (Standard bags; Homogenizers; Atkinson, NH, USA) followed by 225 g of 0.1% peptone solution (1g/1L Sigma Peptone from Animal Tissue powder (autoclave media cycle: 15-min at 121°C to be made sterile); pH of 7.4). Contents of the bag were hand mixed for 1min until bedding was thoroughly suspended in the peptone solution, creating a 1:10 dilution. The pH of each sample was recorded from the original 1:10 dilution bag using a pH meter (Accumet

AB150 pHmV. Fisher Scientific Company, Ottawa, Ontario, K2E 7L6). A subsample of 1ml was added to a test tube containing 9ml 0.1% peptone and vortexed creating a 10^2 dilution. A subsample of 1ml of solution from the 10^2 tube was added to a new test tube containing 9ml 0.1% peptone and vortexed creating a 10^3 dilution. All bedding samples were diluted to 10^3 for enumeration.

2.2.4.1 Coliform counts.

Bedding coliforms were enumerated by pipetting 1ml of the 10^3 sample in duplicate on 3M Petrifilm E. coli/Coliform Count Plates and incubated at 35°C for 24h. Colonies were counted manually to obtain coliform counts as cfu per gram.

2.2.4.2 TBC, Streptococcus spp., and Streptococcus spp.

To enumerate TBC, 50 µL of the appropriate dilution of bedding sample was added in duplicate on PCA. The same procedure was followed to enumerate *Staphylococcus* spp. on the selective media MSA and for *Streptococcus* spp. on non-selective 5% Sheep Blood agar. All bedding samples were spiral plated onto each plate and incubated at 35°C for 24h. Colony-forming units per gram were counted automatically using a colony plate reader. All bacterial counts were converted to a DM basis by dividing the wet matter basis count by the moisture percentage of the sample, resulting in cfu/g of DM.

2.3 Conclusions

For the ease of analyzing data and interpreting results, the results of this study are divided into two chapters. Chapter 3 discusses the results of CBP bedding characteristics, nutrient analysis, and bacterial counts, as well as weather data. Chapter 4 discusses the results of all cow-level variables. Additionally, chapter 4 includes the results of the

interactions between cow-level variables and environmental (i.e. bedding and weather) variables.

Figure 2.1 Diagram of the 9 even-distributed sections of the compost bedded pack barn used for bedding sampling, adapted from Black et al., (2013). The green stars represent the 6 superficial samples collected to create a composite sample of each of the 9 locations.

CHAPTER 3. INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING CHARACTERISTICS AND BACTERIAL COUNTS OVER TIME

T.L. France, J.H.C. Costa, J.L. Taraba, R.J Harmon, and M.C. Morgan

Department of Animal and Food Sciences, University of Kentucky, Lexington Kentucky 40546

3.1 Introduction

Improvement of dairy cattle housing management to increase cow comfort has led producers to develop a unique housing system, the compost bedded pack (CBP) barn. Without stalls, the cows' resting and exercise areas are combined such that cows have free access to a large, open space that helps to maintain cow health and well-being (Galama, 2011). Twice-daily tilling of the pack and addition of sawdust bedding as needed are essential practices to maintain a well-managed pack. Ideal bedding characteristics have been determined to help promote microbial growth through the increase in bedding internal temperature and a decrease in moisture content. It is recommended to keep CBP internal temperature at depths of 15-31 cm between 43.3 – 65.0 °C (Janni et al., 2007, Bewley et al., 2013), bedding moisture content between 40 – 60% (Bewley et al., 2013, Eckelkamp et al., 2016b), and a carbon-to-nitrogen (C:N) ratio between 25:1 to 30:1 (Rynk et al., 1992). In turn, this ensures the cows have a soft, comfortable semi-composted material to lie on.

Potential environmental risk factors associated with mastitis have been studied extensively, as research has shown that mastitis, a complex multifactorial disease, continues to be the costliest disease in the dairy industry. One area to consider is the bedding bacteria, as dairy cows spend 40 to 65% of their time lying down where teats come in direct contact with those environmental microbes (Hogan and Smith, 2012). Research is needed to determine what environmental factors contribute to bacterial growth and population over time in CBP barns. Additionally, it has been reported that ambient temperature influences CBP characteristics, resulting in altered pack performance throughout the year (Shane et al., 2010, Eckelkamp et al., 2016b). It could be valuable to producers of CBP barns who experience poorer pack performance in

colder temperatures (Barberg et al., 2007b) for more specific research on the influence of ambient temperature on both CBP bedding characteristics and bacterial counts.

To the author's knowledge, no study that investigates the change over time of CBP barn bedding characteristics and bacterial counts currently exists. It's important to identify specific bedding characteristics that influence bacterial load more than others, as well as pinpoint specific timeframes over the winter to spring seasonal change that may attribute to changes in the CBP. This in turn may help producers manage their CBP barn more effectively throughout various seasonal changes. The objectives of the study were to (1) evaluate changes in the CBP bedding characteristics and bedding bacteria over time (from Winter to Spring), (2) determine the effects of compost bedding characteristics on the different groups of bedding bacteria, and (3) the effects of tillage and sample location within the pack have on the bedding characteristics and bedding bacteria. I hypothesized that the main CBP bedding characteristics (internal temperature, moisture, and C:N ratio) and ambient temperature conditions would strongly influence bedding bacteria counts.

3.2 Materials and Methods

Detailed bedding sampling protocols and laboratory analysis for this study can be found in Chapter 2. Briefly, surface layer CBP bedding samples were collected weekly from December 2018 – May 2019. Samples were collected individually from each of the 9 evenly distributed sections of the pack (Black et al., 2013), both before and after tilling. Surface (infrared thermometer; Model 62; Fluke, Inc., Everett, WA, USA) and internal (immersion thermocouple-based thermometer; Model 87; Fluke Inc., Everett, WA, USA) bedding temperature was measured for each section at the time of collection. All bedding samples (n = 396) were mixed manually to homogenize the content, then divided into two equal parts. One sample was transported to the University of Kentucky Division of

Regulatory Services Soil Research Laboratory for bedding nutrient analysis and moisture content. The duplicate sample was taken to the University of Kentucky's Animal and Food Sciences microbiology laboratory and stored in a -20°C freezer until microbiological analysis was conducted. Microbial analysis (total bacteria count (TBC), coliform count, *Staphylococcus* spp. (Staph.) counts, and *Streptococcus* spp. (Strep.) counts) for all bedding samples required serial dilutions to 10^3 for bacterial enumeration. All samples were spiral plated (Eddy Jet 2W; Neutec Group Inc., IUL Instruments I.K.S., Leerdam, the Netherlands) onto each plate and incubated at 35°C for 24h. Colony-forming units (cfu) per gram were counted automatically using a colony plate reader (Flash & Go plate reader; Neutec Group Inc., IUL Instruments I.K.S., Leerdam, the Netherlands). All bacteria counts were converted to a DM basis by dividing the wet matter basis count by the moisture percentage of the sample, resulting in cfu/g of DM. Additionally, weather data was monitored (Hobo, Onset Computer Corp., Bourne, MA, USA) from inside the barn every day.

3.3 Statistical Analysis.

Compost bedding TBC, coliforms, Strep. counts, and Staph. counts were logarithmically transformed ($\log_{10}$ cfu/g DM) to produce normally distributed values. The SUMMARY procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA) was used to determine the mean (± SD) categorized by week (0-24) of the following variables: bedding moisture (%), internal temperature (at depths of 20 cm), surface temperature, pH, carbon-to-nitrogen (C:N) ratio, phosphorous (%), potassium (%), calcium (%), magnesium (%), zinc (ppm), copper (ppm), manganese (ppm), iron (ppm), bedding TBC, coliform count, Staph. counts, Strep. counts, ambient temperature, dew point, and relative humidity (RH). The variable "Week" was described by the values 0-

24, where each number indicating one week of the study. Week 0 was the week of December 2, 2018 – December 8, 2018. Week 1 was the week of December 9, 2018 – December 15, 2018…, etc. Week 24 was the week of May 19, 2019 – May 25, 2019.

Pearson correlation coefficients were calculated to find simple correlations among all continuous variables (ambient temperature, RH, dew point, bedding moisture, surface temperature, internal temperature, C:N ratio, pH, TBC, coliforms, Staph. counts, and Strep. counts) using the CORR procedure of SAS. Relationships were considered statistically significant at $p < 0.05$. Correlations were used as a guideline for what explanatory variables to include in the regression models to avoid confounding variables.

Regression models (MIXED procedure of SAS) were performed to determine the effect of time on each of the following response variables: bedding moisture, internal temperature, C:N ratio, pH, TBC, coliforms, Staph. counts, and Strep counts. Regression models were then constructed to determine what effect does both time and bedding characteristics have on the response variables (bedding TBC, coliforms, Staph. counts, and Strep. counts). The variables included in the model were week, surface temperature, internal temperature, moisture, C:N ratio, pH, P, K, Ca, Mg, Zn, Cu, Mn, and Fe. Variables were subject to removal using a stepwise backward elimination process if $p > 0.10$. Week remained in the model regardless of significance. Overall statistical significance for main effects was declared at $p < 0.05$.

3.3.1 Effects of tillage and sample location.

The TTEST procedure of SAS was used to determine if there was a true difference between means of all variables from before versus after tillage. Due to no

statistical significance ($p < 0.05$) for any bedding characteristic or bacteria variable, the effects of tillage were deemed insignificant and removed from all further analysis.

The MIXED procedure of SAS was used to determine the relationships between the explanatory variables (week, sample location (sections 1-9), and the interaction between week and sample location) and the following bedding dependent variables: moisture, internal temperature, C:N ratio, pH, TBC, coliforms, Staph. counts, and Strep. counts. Overall statistical significance for main effects was declared at $p < 0.05$. The LSMeans (± SE) of each of the 9 sample locations were compared, and the Bonferroni correction factor was used to adjust the p-value to perform multiple contrasts among the 9 sample locations.

3.4 Results and Discussion

3.4.1 Description of Bedding Characteristics, Bedding Bacteria, and Weather.

The mean CBP bedding characteristics and bacteria data were stratified by week, as shown in Table 3.1. Each week comprised of n = 18 bedding samples (with the total being n = 396). The bedding internal temperature at a depth of 20.3-cm remained below the recommended range of 43.3 to 65.0°C (Bewley et al., 2013) from December through the middle of March, where the mean (± SD) ranged from 31.27 ± 2.63 °C to 40.43 ± 3.60°C. The internal temperature reached the optimal range in the middle of March (week 15) at 46.23 ± 6.00°C and remained within the recommended range through the end of May. The CBP internal temperature reached its highest at the beginning of May (week 22), at 53.21 ± 5.02 °C. Overall, 36.11% (n = 143 out of 396) of all bedding samples collected were within the recommended range. The moisture content of the CBP, on average, stayed within the recommended range of 40 to 60% (Bewley et al., 2013,

Eckelkamp et al., 2016b), except for the months of January and February where it consistently remained above 60%. The highest moisture recorded was 63.06 ± 1.63% which occurred in the middle of February (week 11). Overall, 70.45% (n = 279 out of 396) of all bedding samples collected were within the recommended range. As shown in previous studies, CBP moisture and internal temperature have an inverse relationship because moisture percentage largely depends on the drying rate of the pack. The quicker the drying rate, influenced by higher internal temperature (among other management practices such as tilling frequency and bedding addition), the lower the moisture content will be. However, these numbers must remain within their recommended ranges for optimal composting. The inverse relationship, at the recommended ranges for both internal temperature and moisture content, was seen from April (week 17) through the end of the study. At week 17, the internal temperature was 45.34 ± 3.24°C and the moisture content was 51.94 ± 4.19%. Interestingly, most of February (weeks 8-11) not only had the highest moisture content observed (62.00 ± 3.97 to 63.06 ± 1.63%), but also experienced low internal temperatures (33.80 ± 4.42°C to 40.43 ± 3.60°C). During this time, it was likely that additional bedding was needed to help increase the drying rate of the pack. The mean C:N ratio fluctuated without much of a trend compared with the previous bedding characteristics. From February through March, the C:N ratio remained within the recommended range of 25:1 to 30:1 (Rynk et al., 1992). Interestingly, the C:N ratio was at its lowest from December through January, as well as May, which ranged from 22.70 ± 1.60 to 24.92 ± 1.36. Moreover, C:N ratio above 30:1 was observed in April, with the highest being 35.25 ± 5.03. Overall, 46.45% (n = 184 out of 396) of the bedding samples had a C:N ratio within the recommended range. The ratio is affected by

the amount of manure and urine output (nitrogen source), the addition of sawdust bedding (carbon source), and the effectiveness of the microbes to proliferate. The mean pH of the CBP by each week ranged from 9.12 ± 0.14 in March to 9.62 ± 0.26 in May. The pH levels were exceedingly alkaline compared to the recommended range of 6.5 – 8.0 (Bewley and Taraba, 2009). The pH should decrease when the C:N ratio of the CBP was higher, which was only observed at week 14 (mid-March). When the C:N ratio is higher, there is less ammonia present in the bedding, thus the environment is more acidic and less alkaline. Since the pH remained consistent for the duration of the study, it is not entirely understood why it was much higher than normal CBP ranges. Weather data was stratified by week, where week 0 = December 2-8, 2018 through Week 24 = May 19-25, 2019 (Table 3.2). January (weeks 4-8) had the coldest mean ambient temperature of 4.42 ± 3.89°C and the highest mean relative humidity at 80.52 ± 8.05%. Additionally, Figure 3.1. depicts a visual representation of the weather data over time. As expected, ambient temperature and dew point followed a similar trend, whereas relative humidity fluctuated between 60 to 89% but a drastic change was not observed during this study.

Overall, the mean surface bedding TBC, coliforms, Staph., and Strep. counts were 6.58 ± 0.20 $\log_{10}$ cfu/g DM, 5.05 ± 0.39 $\log_{10}$ cfu/g DM, 5.56 ± 0.27 $\log_{10}$ cfu/g DM, and 6.06 ± 0.36 $\log_{10}$ cfu/g DM, respectively. Bedding TBC were reported in highest amounts in May, from 7.13 ± 0.29 to 7.20 ± 0.28 $\log_{10}$ cfu/g DM (Table 3.1). Similarly, bedding coliforms and Staph. counts were reported in highest amounts in May, from 5.51 ± 0.49 to 5.50 ± 0.49 $\log_{10}$ cfu/g DM for coliforms, and 5.79 ± 0.21 to 6.56 ± 0.24 $\log_{10}$ cfu/g DM for Staph. counts. On average, bedding Strep. counts were the highest each week, followed by Staph. counts and coliforms (Figure 3.2). Similar trends were observed in

previous studies, where coliform counts were the lowest in compost bedding compared to Staph. counts, Strep. counts, and *Bacillus* spp. counts. (Barberg et al., 2007a, Black et al., 2014, Eckelkamp et al., 2016b). Interestingly, most have reported CBP bedding TBC ranging from 7.0 to 8.2 $\log_{10}$ cfu/g DM (Barberg et al., 2007a, Black et al., 2014). Additionally, reported mean CBP bedding coliforms ranged from 6.0 to 7.0 $\log_{10}$ cfu/g DM (Barberg et al., 2007b, Shane et al., 2010). Results from this study show that surface bedding bacteria counts were relatively lower than previously reported levels, with the lowest count reported in February (TBC of 6.06 ± 0.15 $\log_{10}$ cfu/g DM and coliforms at 4.67 ± 0.42 $\log_{10}$ cfu/g DM). Organic bedding generally has a coliform count of 10^6 cfu/g DM (Bramley and Neave, 1975), which this study continuously stays below. Furthermore, bedding containing $>10^6$ cfu/g is believed to increase IMI risk (Jasper, 1980). The results from this study show that CBP bedding, while require high bacteria counts to achieve effective composting and degradation of the material, are not at counts at the surface level that exceedingly contribute to an increase IMI risk. It is important to note that CBP bacteria population changes with depth. That change is largely driven by the internal temperature gradient, where the deeper parts of CBP bedding would have much higher internal temperature levels, which would favor more thermotolerant and thermophilic microbes. However, since dairy cows are exposed to the top-layer of the CBP, that is what this study focused on. Compost bedded pack barns must be well-managed to keep internal temperature, moisture, and C:N ratio within recommended ranges. This is achieved through twice-daily tilling, the addition of sawdust bedding, and proper stocking density, all of which improve drying rate of the pack, increase microbial populations and result in soft, comfortable bedding material. Compost bedding

characteristics, however, have been shown to interact with one another and the ambient weather conditions, which can complicate this housing system. Knowing the general trends of all individual variables, it was essential to determine relationships between all measured variables.

3.4.2 Relationships Between Bedding Characteristics and Bacteria Over Time.

Pearson correlations between the bedding characteristics (surface temperature, internal temperature, moisture, C:N ratio, and pH) and the 4 bacteria groups are presented in Table 3.3. Besides Strep. counts, bedding moisture content had a moderately to strongly negative correlation with bedding TBC, coliforms, and Staph. counts ($r = -0.61$, -0.30 and -0.43 at $p < 0.001$, respectively). Similar correlations were observed by Black et al. (2014), where moisture had a strong negative relationship with Staph. counts and coliforms. Additionally, compost internal temperature had a moderate to strong positive correlation with bedding TBC, coliforms, and Staph. counts ($r = 0.41$, 0.23 and 0.23 at $p < 0.001$, respectively). The bedding C:N ratio was found to have a moderate to weak negative relationship with bedding TBC, coliforms, and Staph. counts ($r = -0.26$, -0.16, and -0.14, respectively). Similarly, bedding pH was found to have moderate to weak positive correlations with bedding TBC, coliforms, and Staph. counts ($r = 0.36$, 0.11, and 0.26, respectively).

Table 3.4 depicts the Pearson correlation coefficients between weather data and the previously described bedding characteristics/bacteria. As expected, correlations between all variables with both ambient temperature and dew point followed the same trend due to the strong positive relationship between ambient temperature and dew point ($r = 0.98$, $p < 0.001$). Ambient temperature had a strong positive correlation with bedding

TBC ($r = 0.44$; $p < 0.001$), coliforms ($r = 0.21$; $p < 0.001$), and Staph. counts ($r = 0.38$; $p < 0.001$), but weak with Strep. counts ($r = 0.10$; $p = 0.04$). Black et al. (2014) reported similar relationships between ambient temperature and bedding Staph. counts ($r = 0.53$). Relationships between ambient temperature and the following bedding characteristics (moisture, surface temperature, internal temperature, and pH) were the strongest, at $r = -0.70$, 0.78, 0.74, and 0.53 at $p < 0.001$, respectively. At the simplest level, the results suggest that as ambient temperature increased, moisture content decreases and surface temperature, internal temperature, pH, and all bedding bacteria increased. Relative humidity (RH) had a weak positive relationship with bedding TBC, at $r = 0.13$ ($p < 0.001$). Additionally, The RH had a moderate to strong negative relationship with compost surface temperature, internal temperature, and C:N ratio ($r = -0.23$, -0.37, and -0.41 at $p < 0.001$, respectively). Conversely, RH has a moderate positive relationship with bedding moisture, at $r = 0.29$ ($p < 0.001$).

Additionally, correlations were determined between each of the previously described bedding characteristics (Table 3.5). Bedding surface and internal temperature had a strong positive correlation ($r = 0.60$, $p < 0.001$). Bedding internal temperature had a strong negative relationship with moisture ($r = -0.61$, $p < 0.001$), a weak positive relationship with C:N ratio ($r = 0.10$, $p = 0.05$), and a strong positive relationship with pH ($r = 0.48$, $p < 0.001$). Interestingly, bedding pH had a moderately negative correlation with moisture ($r = -0.37$, $p < 0.001$) and C:N ratio ($r = -0.34$, $p < 0.001$). Results validated what was to be expected for bedding characteristic relationships. The bedding pH and C:N ratio had an inverse relationship, where the higher the pH (more alkaline) the lower the C:N ratio, or the more ammonia present. Moreover, as the bedding internal

temperature at 20.3-cm increased, the moisture content decreased. This is likely due to an increased drying rate of the pack which would decrease moisture content. This was observed by Eckelkamp et al. (2016b), where an increase in compost internal temperature was related to an increase in ambient temperature (also observed in this study). This ultimately drove the drying rate of the pack, which reduced bedding moisture.

All described correlations were used as a guide for what explanatory variables to include in the mixed models, as strongly correlated explanatory variables may be confounding. For that reason, the ambient temperature was the only weather variable that was used in all mixed models (RH and dew point were not included). It is important to note that relative humidity is an important component of the drying rate of the pack (Black et al., 2013). However, for the purpose of this study, only ambient temperature was included, as that variable had more significant correlations with all other CBP variables. Results from the regression models indicated that the following bedding variables were influenced by week: bedding moisture, internal temperature, C:N ratio, pH, TBC, coliforms, Staph. counts, and Strep. counts (Tables 3.6 and 3.7). In December, bedding moisture was reported at 55% and internal temperature at 20.3-cm depth was at 31.27°C. In February, bedding moisture reached a peak of 63%, whereas internal temperature was at 40.43°C. At the end of May, moisture decreased to 48% and internal temperature increased to 48.83°C. The bedding C:N ratio fluctuated over time, at 25.60 in December, up to 31.66 in March, and down to 23.95 at the end of May. Additionally, bedding TBC fluctuated around 6.76 to 6.06 $\log_{10}$ cfu/g DM from December through April and increased to 7.18 $\log_{10}$ cfu/g DM in May. Boxplots were constructed for the dependent variables' moisture (Figure 3.3), internal temperature (Figure 3.4), C:N ratio

(Figure 3.5), bedding TBC (Figure 3.6), and pH (Figure 3.7) which provided visual representations of the univariate models.

Compost bedded pack moisture, internal temperature, C:N ratio, pH, bedding TBC, coliforms, Staph. counts, and Strep. counts all changed over time. As ambient temperature increased, bedding moisture decreased and internal temperature at 20.3-cm depth increased. All bedding bacteria counts increased as seasons changed from winter to spring, which was also seen in previous studies (Black et al., 2014). These results suggest that when seasons begin to shift (in this case, winter to spring), producers should pay particular importance to their management strategies to ensure compost bedding effectiveness, because the environment which we cannot control, strongly influences these bedding characteristics.

Results from the final mixed models indicated what bedding characteristics influence each bedding bacteria group (Table 3.8). Moisture content was the only bedding characteristic that significantly influenced all bedding bacteria groups. Regression coefficients were reported, such that as bedding moisture increased by 1%, TBC, coliforms, and Staph. counts decreased by 2.35 (0.30 (SE); $p < 0.001$) $\log_{10}$ cfu/g DM, 1.68 (0.55; $p < 0.001$) $\log_{10}$ cfu/g DM, and 1.89 (0.65; $p < 0.001$) $\log_{10}$ cfu/g DM, respectively whereas Strep. counts increased by 3.07 (0.58; $p < 0.001$) $\log_{10}$ cfu/g DM. These results were confirmed from the Pearson correlations reported between moisture and bedding bacteria groups. Interestingly, bedding internal temperature only influenced Staph. counts. As the bedding internal temperature increased by 1°C, Staph. counts had the tendency to decrease by 0.01 (0.01, $p = 0.07$) $\log_{10}$ cfu/g DM. However, while these values were statistically significant, a bacterial count change > 1-log would not be

considered practically significant. Eckelkamp et al. (2016b) reported similar findings, that internal temperature influenced Staph. counts ($p = 0.01$). Conversely, Black et al. (2014) noted that internal temperature did not remain in the final model, indicating that internal temperature did not influence Staph. counts when other variables were involved. For the current study, while bedding internal temperature had relatively strong correlations with bedding TBC and coliforms, these relationships were not maintained when other variables were accounted for in the final models. This may be due to the confounding variables of internal temperature and moisture (correlation coefficient of $r = -0.61$). As both bedding characteristics have been shown to affect overall pack performance, both needed to remain in all models originally to determine their overall influence on the bedding bacteria population. Furthermore, bedding pH influenced coliforms and Strep. counts. As the bedding pH increased by 1.0, coliforms and Strep. counts decreased by 0.51 (0.18, $p = 0.004$) $\log_{10}$ cfu/g DM and 0.64 (0.19, $p = 0.001$) $\log_{10}$ cfu/g DM, respectively. Similarly, C:N ratio only coliforms, such that as the C:N ratio increased by 1.0, coliforms decreased by 0.03 (0.01, $p = 0.004$) $\log_{10}$ cfu/g DM. Results of the effect of pH and C:N ratio on bacteria counts are as expected, as a higher C:N ratio is indicative of low nitrogen levels (which would also result in a higher pH value, as observed in this study). When there is insufficient nitrogen, this does not allow for optimal microbial growth, so the degradation rate would slow down, ultimately resulted in poorer pack performance.

Similar to previous studies, CBP moisture, internal temperature, and C:N ratio levels, when other variables were included, did not all equally contribute to a change in the bedding bacteria levels. Moisture was the only bedding characteristic that

significantly influenced all bedding bacteria levels. As moisture content increased, coliforms and Staph. counts decreased, whereas Strep. counts increased. It is not entirely understood why moisture affected the bacteria groups in different ways. Moisture content does not necessarily tell researchers much regarding ideal environmental conditions for microbial growth or degradation. Water activity (Aw) of the bedding, however, may be a more effective measurement of the contributions that moisture would have on microbial growth. This is because Aw explains how exactly the water content would react with microorganisms. The higher the Aw (closest to 1.0), the faster microorganisms can proliferate, given other environmental characteristics are within optimal conditions for those microbes (such as temperature). For future research, it may be useful to measure the water activity of bedding rather than using moisture, if looking specifically at microbial growth. Compost bedded pack pH has not been largely reported, nor has it been considered a key bedding characteristic for CBP barns (where internal temperature, moisture, and C:N ratio are considered key CPB characteristics). However, results from this study indicated that pH was influential, where an increase in pH (more alkaline environment) resulted in a reduction of bedding coliforms and Strep. counts. Similarly, Hogan et al. (1999) reported a reduction in coliforms and *Streptococcus* spp. counts due to an alkaline conditioner in manure solids bedding material. Moreover, the internal temperature only slightly decreased *Staphylococcus* spp. counts and C:N ratio only slightly decreased coliforms. Knowing the effect of moisture on bacterial counts, it may be of benefit to ensure the moisture content is within the recommended 40-60% when seasons are changing, as that is when the moisture fluctuated the greatest.

3.4.3 Effects of Sample Location on Bedding Characteristics and Bedding Bacteria.

For all variables, the sample location remained statistically significant in the models. The LS means (SE) of all dependent variables by sample location are displayed in Table 3.9. Sample locations 1, 4 and 7 represented the bedding area at the 3 pack entrances from the feed alley. Internal temperature (LS means (SE)) was the lowest at the entrances at 36.98 (0.49) °C, 39.07 (0.48) °C, and 36.36 (0.49) °C for locations 1, 4, and 7, respectively. The remaining six locations had an internal temperature of > 41.0°C. Bedding moisture followed a similar trend to internal temperature, with the pack entrances (locations 1, 4, and 7) having the highest moisture content at 59.52 (0.35) %, 59.52 (0.35) %, and 55.22 (0.34) % for locations 1, 4, and 7, respectively. This may be due to the increased amount of cow traffic at those locations and the proximity to the feed alley. Interestingly, bedding TBC was the highest in locations 3 and 9, which were the outer corners of the CBP, at 6.66 (0.02) $\log_{10}$ cfu/g DM and 6.74 $\log_{10}$ cfu/g DM, respectively. Results suggest that the biggest problem-areas throughout the CBP were the entrances from the feed alleys and the two outer corners. Producers should be aware of these problem-areas and adjust accordingly, such as the addition of bedding material more specifically to those locations and ensuring the pack is being adequately tilled in those locations.

Results showed that week and the interaction between the week and sample location influenced all the dependent bedding variables. This interaction took into consideration not only the sample location within the pack but also the effect of the season, such as differences seen between December and May. Mean bedding moisture of the 9 sample locations over time (Figure 3.3) showed there was variation among each of the 9 locations, but overall they followed a general trend of increased levels from

December 2018 – mid-February 2019, then decreased as weeks progressed into Springtime. Similar graphs were constructed for internal temperature, C:N ratio, bedding TBC, and pH (Figures 3.4, 3.5, 3.6, and 3.7), where each colored dot indicated the sample location of that given week. Interestingly, the variation among the 9 locations for internal temperature and C:N ratio over time was much greater than moisture. However, the bedding TBC and pH did not have as much variation when compared to internal temperature and the C:N ratio. The bedding TBC at sample location 9 (shown as a yellow dot in Figure 3.6), however, had the highest bacteria counts for nearly every week of the study. It is not entirely understood why that location had the highest bacteria levels.

Tilling of the pack, while an important tool for incorporating oxygen throughout the bedding, thus ensuring an effective composting system, did not have any effect on bedding moisture, internal temperature, C:N ratio, or any bedding bacteria counts. However, location within the pack strongly affected all bedding characteristics and bacteria counts. As expected, bedding located at the entrances from the feed alley had the highest moisture content and lowest internal temperature. Additionally, corner locations of the pack had increased bacteria counts and moisture. These results conclude that not every surface that dairy cows have access to lay on are equal. Producers should be aware of this and adjust management strategies as needed, such as increased addition of bedding material at entrance locations and ensure tilling of the pack occurs in those specific locations. Tilling improves pack performance by increasing the drying rate of the pack, which would result in a decrease in moisture content and an increase in internal temperature. Specifically, in colder or rainy weather conditions, the incorporation of side curtains to the barn may be an effective tool to help keep out excess moisture and

increase the pack drying rate. This would likely reduce the large variation seen in bedding internal temperature, moisture, and C:N ratio based on bedding location.

3.5 Conclusions

Compost bedded pack barns require well-executed management techniques throughout the year to produce a soft surface for cows to lie on. The goal of these management techniques (which include twice-daily tilling, the addition of new bedding as needed, and proper stocking density) is to increase the bedding internal temperature, reduce moisture content, and provide the correct C:N ratio (25:1 to 30:1) throughout the entire year. However, results from this study suggest that there should be more emphasis on management strategies during colder weather conditions and when seasons begin to change (in this case, from winter to spring) because weather conditions strongly influence the compost bedding characteristics. Results found that there were large fluctuations seen in the moisture content and internal temperature during March in Kentucky (i.e. the time when seasons were shifting from winter to spring), which may contribute to poorer pack performance. Interestingly, results indicated that bedding moisture had the strongest effect on all bedding bacterial counts compared to other bedding characteristics. Additionally, bedding pH influenced the microbial population more than expected, where a higher pH decreased bacterial counts. Keeping this in mind, however, producers should not look at one specific bedding characteristic to determine the effectiveness of their CBP barn, but rather look at the whole environmental picture.

This study also concluded that not every surface that the cows have access to lie on is equal, in that the entrances to the pack and the outer corners had the highest moisture and lowest internal temperature. Producers should ensure adequate tilling has

occurred in those locations and simply add more bedding material in those specific areas. Both management adjustments would help reduce bedding moisture and increase internal temperature while promoting microbial growth. Furthermore, the addition of side curtains to the barn design may be a more permanent but effective tool to help improve overall pack performance, especially during colder and rainy weather conditions. This is because the side curtains would act as a barrier to prevent excess moisture in the pack but also aid in reducing the temperature difference between bedding temperature and ambient temperature. Reducing the temperature difference would increase the drying rate of the pack, which results in an overall improved pack performance.

Compost bedded pack barn bedding characteristics and weather conditions are all inter-related and the complex interaction ultimately drives bedding bacterial population. Further research is needed to investigate these interactions during other seasonal changes (fall to winter) and in different regions to consider climate effects (i.e. in very high or low humidity regions to see the effects of drying rate and microbial population). Research is also needed to determine the microbiome of CBP barn bedding, through the enumeration of other common environmental microorganisms found in compost bedding (such as *Bacillus* spp. or *Klebsiella* spp.). While CBP barns are an effective alternative housing system for dairy cows, they must be consistently well-managed year-round to achieve a very soft, comfortable bedding surface for dairy cows to lie on.

Table 3.1. Mean ± standard deviation of compost bedded pack barn bedding characteristics, bacterial counts, and nutrient content stratified by week, with each week had n = 18.

Week	CST[1] Mean ± SD	CIT[2] Mean ± SD	Moisture Mean ± SD	C:N[3] Mean ± SD	pH Mean ± SD	TBC[*4] Mean ± SD	Coliforms[*] Mean ± SD	Staph.[*5] Mean ± SD	Strep.[*6] Mean ± SD
0	3.76 ± 7.63	31.27 ± 2.63	55.33 ± 2.74	25.60 ± 2.04	9.21 ± 0.09	6.76 ± 0.20	5.11 ± 0.29	5.35 ± 0.22	5.35 ± 0.38
1	8.88 ± 4.51	33.58 ± 1.57	57.28 ± 2.52	22.70 ± 1.60	9.32 ± 0.11	6.73 ± 0.29	5.01 ± 0.54	5.79 ± 0.14	6.04 ± 0.26
2	13.68 ± 3.58	36.67 ± 1.28	56.72 ± 2.87	23.48 ± 1.70	9.28 ± 0.07	6.60 ± 0.13	5.14 ± 0.33	5.74 ± 0.17	5.69 ± 0.38
4	8.67 ± 4.50	32.31 ± 3.24	61.50 ± 2.28	23.25 ± 2.21	9.32 ± 0.09	6.53 ± 0.13	4.84 ± 0.33	5.75 ± 0.12	6.26 ± 0.23
5	-1.44 ± 3.37	35.93 ± 2.58	58.06 ± 1.98	24.22 ± 1.34	9.32 ± 0.09	6.45 ± 0.09	4.94 ± 0.29	5.50 ± 0.16	5.75 ± 0.15
6	8.94 ± 9.34	33.46 ± 3.24	62.06 ± 1.59	24.92 ± 1.36	9.27 ± 0.04	6.54 ± 0.16	4.91 ± 0.28	5.38 ± 0.32	5.94 ± 0.32
7	9.65 ± 1.92	34.57 ± 2.57	61.11 ± 2.61	26.83 ± 1.55	9.33 ± 0.08	6.40 ± 0.09	4.93 ± 0.29	5.58 ± 0.15	6.20 ± 0.23
8	-8.91 ± 5.72	33.80 ± 4.42	62.22 ± 2.05	26.95 ± 1.58	9.22 ± 0.10	6.34 ± 0.21	4.76 ± 0.22	5.47 ± 0.14	6.18 ± 0.21
10	8.42 ± 5.44	37.25 ± 8.02	62.00 ± 3.97	27.30 ± 1.70	9.29 ± 0.06	6.30 ± 0.13	4.89 ± 0.16	5.33 ± 0.10	6.20 ± 0.28
11	12.57 ± 4.53	40.43 ± 3.60	63.06 ± 1.63	25.96 ± 1.52	9.33 ± 0.11	6.16 ± 0.20	4.96 ± 0.34	4.28 ± 1.89	5.54 ± 0.40
12	12.52 ± 2.71	32.56 ± 5.05	57.89 ± 2.47	27.29 ± 1.66	9.33 ± 0.08	6.06 ± 0.15	4.67 ± 0.42	5.32 ± 0.20	6.20 ± 0.19
13	3.83 ± 5.60	35.4 ± 6.46	59.33 ± 2.20	29.20 ± 1.95	9.19 ± 0.08	6.85 ± 0.47	5.53 ± 0.35	5.51 ± 0.21	6.87 ± 0.38
14	16.04 ± 1.77	39.85 ± 5.61	57.28 ± 3.69	31.66 ± 4.93	9.12 ± 0.14	6.19 ± 0.22	4.96 ± 0.44	5.40 ± 0.16	6.28 ± 0.44
15	8.87 ± 4.07	46.23 ± 6.00	60.44 ± 2.53	25.69 ± 1.68	9.31 ± 0.11	6.43 ± 0.17	5.19 ± 0.24	5.36 ± 0.20	5.62 ± 0.34
16	13.91 ± 2.43	38.36 ± 6.76	56.83 ± 3.33	30.59 ± 2.96	9.29 ± 0.08	6.17 ± 0.14	4.33 ± 0.40	5.31 ± 0.20	5.62 ± 0.37
17	15.10 ± 2.98	45.34 ± 3.24	51.94 ± 4.19	35.25 ± 5.03	9.24 ± 0.11	6.51 ± 0.16	5.17 ± 0.29	5.45 ± 0.17	6.07 ± 0.32
18	16.79 ± 2.90	51.79 ± 3.19	51.83 ± 3.31	30.24 ± 1.99	9.42 ± 0.11	6.52 ± 0.13	4.82 ± 0.35	5.49 ± 0.21	5.40 ± 0.49
19	21.09 ± 1.99	46.79 ± 3.86	52.78 ± 6.01	23.22 ± 1.78	9.43 ± 0.09	6.83 ± 0.28	5.45 ± 0.33	5.57 ± 0.21	6.14 ± 0.37
21	22.47 ± 1.00	50.43 ± 2.41	50.56 ± 5.25	23.62 ± 1.71	9.46 ± 0.13	6.78 ± 0.17	4.79 ± 0.49	6.18 ± 0.21	6.64 ± 0.61
22	24.23 ± 2.57	53.21 ± 5.02	48.06 ± 5.95	26.45 ± 2.65	9.42 ± 0.07	7.20 ± 0.28	5.59 ± 0.49	6.56 ± 0.24	6.54 ± 0.75
23	21.04 ± 1.27	50.31 ± 3.66	50.61 ± 5.10	23.65 ± 1.06	9.62 ± 0.26	7.13 ± 0.29	5.51 ± 0.49	5.76 ± 0.21	6.62 ± 0.26
24	25.00 ± 1.40	48.83 ± 4.37	47.61 ± 5.39	23.95 ± 1.61	9.59 ± 0.12	7.18 ± 0.25	5.54 ± 0.49	6.18 ± 0.27	6.13 ± 0.64
Overall	12.05 ± 3.69	40.38 ± 4.03	56.57 ± 3.35	26.46 ± 2.07	9.33 ± 0.10	6.58 ± 0.20	5.05 ± 0.39	5.56 ± 0.27	6.06 ± 0.36

Week	P (%) Mean ± SD	K (%) Mean ± SD	Ca (%) Mean ± SD	Mg (%) Mean ± SD	Zn (ppm) Mean ± SD	Cu (ppm) Mean ± SD	Mn (ppm) Mean ± SD	Fe (ppm) Mean ± SD
0	0.55 ± 0.11	1.20 ± 0.24	1.61 ± 0.49	0.54 ± 0.12	144.52 ± 115.16	28.22 ± 10.63	283.67 ± 70.85	3457.64 ± 1532.89
1	0.65 ± 0.07	1.46 ± 0.16	1.87 ± 0.29	0.66 ± 0.07	161.81 ± 103.22	37.70 ± 7.96	461.94 ± 232.12	5652.47 ± 4154.21
2	0.59 ± 0.06	1.38 ± 0.10	1.62 ± 0.14	0.63 ± 0.06	111.46 ± 19.46	34.17 ± 9.90	344.05 ± 109.03	3719.27 ± 2128.57
4	0.63 ± 0.05	1.52 ± 0.12	1.83 ± 0.21	0.67 ± 0.07	135.55 ± 47.71	45.34 ± 13.99	356.84 ± 92.37	4152.78 ± 2047.04
5	0.58 ± 0.05	1.37 ± 0.10	1.82 ± 0.23	0.62 ± 0.05	151.84 ± 104.02	39.26 ± 10.07	324.15 ± 95.78	3828.57 ± 1910.43
6	0.62 ± 0.05	1.57 ± 0.13	1.95 ± 0.50	0.66 ± 0.05	117.50 ± 15.54	46.73 ± 12.02	325.35 ± 50.88	3530.66 ± 925.50
7	0.56 ± 0.05	1.40 ± 0.09	1.59 ± 0.22	0.60 ± 0.06	104.98 ± 10.66	42.93 ± 7.05	280.75 ± 45.83	2974.88 ± 681.83
8	0.57 ± 0.05	1.41 ± 0.10	1.75 ± 0.52	0.61 ± 0.05	127.52 ± 89.65	36.45 ± 10.00	293.94 ± 92.52	3254.26 ± 1281.04
10	0.52 ± 0.04	1.41 ± 0.10	1.56 ± 0.21	0.57 ± 0.07	99.24 ± 10.79	34.53 ± 7.87	282.95 ± 67.52	2931.51 ± 714.05
11	0.57 ± 0.04	1.52 ± 0.08	1.68 ± 0.14	0.61 ± 0.03	154.97 ± 69.64	38.72 ± 7.00	277.14 ± 50.63	2966.20 ± 1120.66
12	0.54 ± 0.04	1.22 ± 0.09	1.57 ± 0.46	0.55 ± 0.04	130.24 ± 90.93	30.69 ± 8.00	272.09 ± 108.71	2794.99 ± 1305.71
13	0.48 ± 0.04	1.11 ± 0.08	1.37 ± 0.24	0.47 ± 0.04	128.43 ± 112.48	26.19 ± 7.53	223.30 ± 46.89	2159.21 ± 800.41
14	0.47 ± 0.07	1.30 ± 0.20	1.42 ± 0.27	0.51 ± 0.07	173.47 ± 94.97	31.28 ± 7.05	223.70 ± 47.01	2068.22 ± 708.99
15	0.55 ± 0.04	1.40 ± 0.11	1.62 ± 0.13	0.60 ± 0.04	145.83 ± 68.19	40.12 ± 8.83	251.07 ± 58.25	2205.66 ± 767.31
16	0.52 ± 0.07	1.25 ± 0.15	1.44 ± 0.20	0.55 ± 0.07	126.41 ± 64.11	31.84 ± 6.77	222.50 ± 36.35	1819.88 ± 462.50
17	0.43 ± 0.07	1.20 ± 0.17	1.14 ± 0.19	0.47 ± 0.08	88.27 ± 17.57	28.59 ± 8.56	189.68 ± 40.04	1422.02 ± 404.46
18	0.53 ± 0.07	1.44 ± 0.14	1.47 ± 0.46	0.58 ± 0.08	113.87 ± 24.63	41.07 ± 9.20	219.37 ± 41.43	1723.11 ± 423.40
19	0.59 ± 0.03	1.70 ± 0.09	1.71 ± 0.20	0.69 ± 0.04	134.49 ± 38.10	46.34 ± 11.35	234.58 ± 18.35	1811.97 ± 238.75
21	0.66 ± 0.04	1.83 ± 0.08	2.09 ± 0.29	0.79 ± 0.04	182.92 ± 72.89	53.68 ± 7.72	260.34 ± 22.18	2098.95 ± 359.04
22	0.64 ± 0.06	1.93 ± 0.16	1.76 ± 0.22	0.73 ± 0.07	139.71 ± 25.56	53.38 ± 12.49	249.43 ± 28.14	1880.26 ± 270.17
23	0.64 ± 0.08	1.88 ± 0.23	1.95 ± 0.54	0.70 ± 0.10	174.74 ± 73.09	52.53 ± 11.44	239.81 ± 36.87	1931.75 ± 516.80
24	0.74 ± 0.04	2.03 ± 0.09	1.91 ± 0.34	0.78 ± 0.05	188.22 ± 56.21	57.97 ± 10.15	266.50 ± 32.41	2166.56 ± 477.90
Overall	0.57 ± 0.05	1.48 ± 0.13	1.67 ± 0.30	0.62 ± 0.06	130.00 ± 59.75	39.90 ± 9.34	276.51 ± 64.73	2752.31 ± 1055.98

*$\log_{10}$ cfu/g DM

[1] CST = Compost surface temperature (°C)

[2] CIT = Compost internal temperature (°C) at depth of 20 cm.

[3] C:N = Carbon-to-nitrogen ratio

[4] TBC = Total bacteria count

[5] Staph. = *Staphylococcus* spp. counts

[6] Strep. = *Streptococcus* spp. counts

Table 3.2. Descriptive statistics of weather data (ambient temperature, relative humidity, and dew point) stratified by week, with Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019.

	Ambient Temperature [°C]				Relative Humidity [%]				Dew Point [°C]			
Week	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S. D.	Min.	Max.
0	2.22	2.59	-2.73	8.15	78.97	8.78	54.57	89.4	-1.14	2.38	-5.43	3.74
1	3.5	5.36	-5.36	14.1	73.54	9.7	45.57	90.5	-0.93	4.8	-7.53	9.08
2	6.73	3.16	-0.47	13.63	81.44	9.4	48.43	90.97	3.6	2.89	-2.27	9.87
3	5.23	4.46	-1.37	15.32	83.22	6.34	62.53	91.07	2.56	4.26	-2.71	13.25
4	6.4	3.84	0.54	18.25	85.16	5.49	61.7	91.6	4.06	3.82	-1.61	16.15
5	5.16	6.83	-6.01	18.28	74.19	11.28	40.97	90.6	0.77	6.6	-9.9	12.83
6	1.74	2.15	-3.51	6.7	89.31	2.17	71.5	92.17	0.15	2.17	-4.81	5.08
7	-0.91	7.18	-12.74	12.6	78.04	11.46	46.93	91.4	-4.4	7.53	-14.95	10.44
8	-3.42	6.59	-15.68	11.42	75.87	9.84	53.57	90.87	-7.14	7.14	-21.39	4.59
9	10.77	6.98	-5.25	21.6	80.24	10.36	49.4	92.17	7.36	7.2	-9.19	18.54
10	4.59	5.85	-8.09	15.28	73.41	13.97	30.9	91.7	-0.07	5.82	-10.5	11.76
11	3.3	3.6	-3.33	13.53	80.78	7.64	57.73	91.5	0.25	3.75	-5.56	10.89
12	5.78	5.06	-2.34	17.86	72.21	15.76	38.4	91.8	0.8	5.25	-9.82	13.68
13	-1.29	4.58	-11.65	6.96	79.01	11.93	49.7	91.37	-4.59	5.56	-14.4	4.83
14	10.66	5.64	1.46	23.94	66.94	14.49	37.93	90.53	4.4	5.09	-2.73	16.67
15	6.62	3.78	-0.74	15.49	64.1	17.01	32.27	89	-0.23	2.78	-4.95	7.44
16	10.28	5.59	-1.24	20.74	60.78	17.22	28.77	90.4	2.44	5.21	-5.39	12.96
17	10.26	6.53	-2.45	22.6	63.62	16.43	30.57	91.03	3.16	6.03	-5.94	14.71
18	17.66	3.59	8.43	27.87	70.97	15.45	37.23	89.6	11.92	3.39	3.33	18.28
19	15.32	6.41	3.59	26.34	68.13	14.79	36.3	90.4	9.02	4.23	-0.67	18.26
20	15.63	5.94	4.26	26.59	73.24	13.12	44.9	88.57	10.53	4.51	1.83	18.73

21	18.5	5.48	6.4	28	73.95	11.4	42.47	90.33	13.54	4.71	3.37	21.28
22	18.94	3.93	10.34	28.31	75.24	10.5	48.63	86.27	14.22	2.78	7.44	19.23
23	16.43	4.79	8.46	29.69	75.36	10.28	47.87	87.83	11.83	3.79	5.48	22.25
24	21.81	4.77	11.21	30.59	71.55	11.75	46.57	87.33	16.17	2.71	9.11	20.91
Overall	8.48	4.99	-1.13	18.95	74.77	11.46	45.82	90.34	3.93	4.58	-4.37	13.42

Table 3.3. Pearson correlation coefficients between compost bedding characteristics (moisture, surface temperature, internal temperature, C:N ratio, and pH) and bedding bacteria groups (total bacteria count, coliforms, Staphylococcus spp. counts, and Streptococcus spp. counts) where n = 396. Relationships were deemed statistically significant at $p < 0.001$. Correlations were considered (±) strong when $r \geq 0.40$ and (±) moderate at $r \leq 0.39$ - ≥ 0.20.

		Bedding Characteristics					
		Moisture	CST[d]	CIT[e]	C:N[f]	pH	
Bedding Bacteria*	TBC[a]	-0.61	0.39	0.41	-0.26	0.36	*r*
		<0.001	<0.001	<0.001	<0.001	<0.001	*p-value*
	Coliforms	-0.30	0.20	0.23	-0.16	0.11	*r*
		<0.001	<0.001	<0.001	0.001	0.04	*p-value*
	Staph.[b]	-0.43	0.30	0.23	-0.14	0.26	*r*
		<0.001	<0.001	<0.001	0.005	<0.001	*p-value*
	Strep.[c]	0.04	0.09	0.05	-0.09	0.06	*r*
		0.42	0.07	0.34	0.07	0.21	*p-value*

*$\log_{10}$ cfu/g DM
[a] TBC = Total bacteria count
[b] Staph. = *Staphylococcus* spp. counts
[c] Strep. = *Streptococcus* spp. counts
[d] CST = Compost surface temperature [°C]
[e] CIT = Compost internal temperature [°C]
[f] C:N = Carbon-to-nitrogen ratio

Table 3.4. Pearson correlation coefficients between weather data (ambient temperature, relative humidity, and dew point) and compost bedding characteristics/ bacteria groups (moisture, surface temperature, internal temperature, C:N ratio, pH, total bacteria count, coliforms, Staphylococcus spp. counts, and Streptococcus spp. counts) where n = 396. Relationships were deemed statistically significant at $p < 0.001$. Correlations were considered (±) strong when $r \geq 0.40$ and (±) moderate at $r \leq 0.39$ - ≥ 0.20.

		Weather			
		Amb. Temp[g]	RH[h]	Dew Point	
Bedding Bacteria*	TBC[a]	0.44	0.13	0.50	*r*
		<0.001	<0.001	<0.001	*p-value*
	Coliforms	0.21	0.06	0.24	*r*
		<0.001	0.27	<0.001	*p-value*
	Staph.[b]	0.38	-0.01	0.41	*r*
		<0.001	0.78	<0.001	*p-value*
	Strep.[c]	0.10	0.08	0.13	*r*
		0.04	0.13	0.01	*p-value*
Bedding Characteristics	Moisture	-0.70	0.29	-0.69	*r*
		<0.001	<0.001	<0.001	*p-value*
	CST[d]	0.78	-0.23	0.77	*r*
		<0.001	<0.001	<0.001	*p-value*
	CIT[e]	0.74	-0.37	0.71	*r*
		<0.001	<0.001	<0.001	*p-value*
	C:N[f]	-0.04	-0.41	-0.14	*r*
		0.49	<0.001	0.01	*p-value*
	pH	0.53	-0.03	0.56	*r*
		<0.001	0.57	<0.001	*p-value*

*$\log_{10}$ cfu/g DM
[a] TBC = Total bacteria count
[b] Staph. = *Staphylococcus* spp. counts
[c] Strep. = *Streptococcus* spp. counts
[d] CST = Compost surface temperature [°C]
[e] CIT = Compost internal temperature [°C]
[f] C:N = Carbon-to-nitrogen ratio
[g] Amb. Temp. = Ambient temperature [°C]
[h] RH = Relative humidity [%]

Table 3.5. Pearson correlation coefficients between compost bedding characteristics (moisture, surface temperature, internal temperature, C:N ratio, and pH) where n = 396. Relationships were deemed statistically significant at $p < 0.001$. Correlations were considered (±) strong when $r \geq 0.40$ and (±) moderate at $r \leq 0.39 - \geq 0.20$.

	Bedding Characteristics				
	CST[a]	**CIT**[b]	**C:N**[c]	**pH**	
Moisture	-0.58	-0.61	-0.13	-0.37	*r*
	<0.001	<0.001	0.01	<0.001	*p-value*
CST[a]	1.00	0.60	0.003	0.41	*r*
		<0.001	0.96	<0.001	*p-value*
CIT[b]		1.00	0.10	0.48	*r*
			0.05	<0.001	*p-value*
C:N[c]			1.00	-0.34	*r*
				<0.001	*p-value*

[a] CST = Compost surface temperature [°C]
[b] CIT = Compost internal temperature [°C]
[c] C:N = Carbon-to-nitrogen ratio

Table 3.6. Least squares means (SE) for the effect of week on CBP bedding characteristics (moisture, internal temperature [°C], carbon-to-nitrogen [C:N] ratio, and pH), where n = 396. All values were significant at $p < 0.001$. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019.

Week	Moisture (%) Estimate (SE)	Internal Temperature (°C) Estimate (SE)	C:N Ratio Estimate (SE)	pH Estimate (SE)
0	55.0 (1.0)	31.27 (1.03)	25.60 (0.54)	9.21 (0.03)
1	57.0 (1.0)	33.58 (1.03)	22.70 (0.54)	9.32 (0.03)
2	57.0 (1.0)	36.67 (1.03)	23.48 (0.54)	9.28 (0.03)
4	62.0 (1.0)	32.32 (1.03)	23.25 (0.54)	9.32 (0.03)
5	58.0 (1.0)	35.93 (1.03)	24.22 (0.54)	9.32 (0.03)
6	62.0 (1.0)	33.46 (1.03	24.92 (0.54)	9.27 (0.03)
7	61.0 (1.0)	34.57 (1.03)	26.83 (0.54)	9.33 (0.03)
8	62.0 (1.0)	33.80 (1.03)	26.95 (0.54)	9.22 (0.03)
10	62.0 (1.0)	37.25 (1.03)	27.30 (0.54)	9.29 (0.03)
11	63.0 (1.0)	40.43 (1.03)	25.96 (0.54)	9.33 (0.03)
12	58.0 (1.0)	32.56 (1.03)	27.29 (0.54)	9.33 (0.03)
13	59.0 (1.0)	35.40 (1.03)	29.20 (0.54)	9.19 (0.03)
14	57.0 (1.0)	39.85 (1.03)	31.66 (0.54)	9.12 (0.03)
15	60.0 (1.0)	46.24 (1.03)	25.69 (0.54)	9.31 (0.03)
16	57.0 (1.0)	38.36 (1.03)	30.59 (0.54)	9.29 (0.03)
17	52.0 (1.0)	45.34 (1.03)	35.25 (0.54)	9.24 (0.03)
18	52.0 (1.0)	51.79 (1.03)	30.24 (0.54)	9.42 (0.03)
19	53.0 (1.0)	46.79 (1.03)	23.22 (0.54)	9.43 (0.03)
21	51.0 (1.0)	50.43 (1.03)	23.62 (0.54)	9.46 (0.03)
22	48.0 (1.0)	53.21 (1.03)	26.45 (0.54)	9.42 (0.03)
23	51.0 (1.0)	50.31 (1.03)	23.65 (0.54)	9.62 (0.03)
24	48.0 (1.0)	48.83 (1.03)	23.95 (0.54)	9.59 (0.03)

Table 3.7. Least squares means (SE) for the effect of week on bedding bacteria (total bacteria count, coliforms, Staphylococcus spp. counts, and Streptococcus spp. counts). All values were significant at $p < 0.001$. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019.

Week	Total bacteria count* Estimate (SE)	Coliforms* Estimate (SE)	*Staphylococcus* spp.* Estimate (SE)	*Streptococcus* spp.* Estimate (SE)
0	6.76 (0.05)	5.12 (0.09)	5.35 (0.11)	5.35 (0.09)
1	6.73 (0.05)	5.01 (0.09)	5.79 (0.11)	6.04 (0.09)
2	6.60 (0.05)	5.14 (0.09)	5.74 (0.11)	5.69 (0.09)
4	6.53 (0.05)	4.84 (0.09)	5.75 (0.11)	6.26 (0.09)
5	6.45 (0.05)	4.94 (0.09)	5.50 (0.11)	5.75 (0.09)
6	6.54 (0.05)	4.91 (0.09)	5.38 (0.11)	5.94 (0.09)
7	6.40 (0.05)	4.93 (0.09)	5.58 (0.11)	6.20 (0.09)
8	6.34 (0.05)	4.76 (0.09)	5.47 (0.11)	6.18 (0.09)
10	6.30 (0.05)	4.89 (0.09)	5.33 (0.11)	6.21 (0.09)
11	6.16 (0.05)	4.96 (0.09)	4.28 (0.11)	5.54 (0.09)
12	6.06 (0.05)	4.67 (0.09)	5.32 (0.11)	6.20 (0.09)
13	6.85 (0.05)	5.53 (0.09)	5.51 (0.11)	6.87 (0.09)
14	6.19 (0.05)	4.96 (0.09)	5.40 (0.11)	6.28 (0.09)
15	6.43 (0.05)	5.19 (0.09)	5.36 (0.11)	5.62 (0.09)
16	6.17 (0.05)	4.33 (0.09)	5.31 (0.11)	5.62 (0.09)
17	6.52 (0.05)	5.17 (0.09)	5.45 (0.11)	6.07 (0.09)
18	6.52 (0.05)	4.82 (0.09)	5.49 (0.11)	5.40 (0.09)
19	6.83 (0.05)	5.45 (0.09)	5.57 (0.11)	6.14 (0.09)
21	6.78 (0.05)	4.79 (0.09)	6.18 (0.11)	6.64 (0.09)
22	7.20 (0.05)	5.59 (0.09)	6.56 (0.11)	6.54 (0.09)
23	7.13 (0.05)	5.51 (0.09)	5.76 (0.11)	6.62 (0.09)
24	7.18 (0.05)	5.54 (0.09)	6.18 (0.11)	6.13 (0.09)

*$\log_{10}$ cfu/g DM

Table 3.8. Final mixed models of relationships found between the explanatory bedding characteristics (moisture, surface temperature (°C), internal temperature (°C), C:N ratio, pH, P, K, Ca, Mg, Zn, Cu, Mn, and Fe) with the response variables (bedding total bacteria count, coliforms, Staphylococcus spp. counts, and Streptococcus spp. counts), where n = 396 and values were statistically significant at $p < 0.05$. Week was included in the model as a fixed effect.

	Total Bacteria Count*		Coliforms*		*Staphylococcus* spp.*		*Streptococcus* spp.*	
Variables	β (SE)[1]	*P*-value	β (SE)[1]	*P*-value	β (SE)[1]	*P*-value	β (SE)[1]	*P*-value
Moisture	-2.35 (0.30)	<0.001	-1.68 (0.55)	0.002	-1.89 (0.65)	0.004	3.07 (0.58)	<0.001
CST[a]	0.005 (0.003)	0.06[d]	NS		NS		NS	
CIT[b]	NS[e]		NS		-0.01 (0.01)	0.07[d]	NS	
C:N[c]	NS		-0.03 (0.01)	0.004	NS		NS	
pH	NS		-0.51 (0.18)	0.004	NS		-0.64 (0.19)	0.001
Phosphorus	-0.81 (0.36)	0.02	NS		NS		-1.21 (0.67)	0.07[d]
Potassium	0.44 (0.14)	0.002	NS		NS		0.46 (0.26)	0.07[d]
Calcium	NS		NS		NS		NS	
Magnesium	NS		NS		NS		NS	
Zinc	NS		NS		0.001 (0.0003)	0.07	NS	
Copper	NS		NS		NS		0.01 (0.003)	<0.001
Manganese	NS		0.001 (0.0002)	0.03	-0.001 (0.001)	0.02	NS	
Iron	NS		NS		0.0001 (0.00004)	0.03	0.0001 (0.00002)	0.002

*$\log_{10}$ cfu/g DM

β (SE)[1] = Regression coefficients (standard error)

[a] CST = Compost surface temperature (°C)

[b] CIT = Compost internal temperature (°C)

[c] C:N = Carbon-to-nitrogen ratio

[d] Variables showed a tendency with statistical significance of $0.10 \leq p > 0.05$

NS[e] = not significant.

Table 3.9. LS means (SE) of the dependent bedding variables (moisture, internal temperature, C:N ratio, TBC, coliforms, Staphylococcus spp. counts, and Streptococcus spp. counts) with the explanatory variables week, sample location, and week*sample location interaction included in all models, where n = 396. The sample location within the CBP barn was statistically significant ($p < 0.05$) for all dependent variables.

Sample Location	Moisture	CIT[1]	C:N[2]	pH	TBC*[3]	Coliforms*	Staph.*[4]	*Strep.*[5]
1	59.52 (0.35)	36.98 (0.49)	24.91 (0.28)	9.33 (0.01)	6.49 (0.03)	5.07 (0.05)	5.44 (0.07)	6.19 (0.04)
2	58.25 (0.34)	41.06 (0.49)	25.62 (0.28)	9.36 (0.01)	6.51 (0.03)	5.03 (0.05)	5.43 (0.07)	6.19 (0.04)
3	56.90 (0.34)	41.21 (0.49)	25.12 (0.28)	9.40 (0.01)	6.66 (0.02)	5.13 (0.05)	5.62 (0.06)	6.26 (0.04)
4	59.52 (0.35)	39.07 (0.48)	26.99 (0.28)	9.33 (0.01)	6.47 (0.03)	4.93 (0.05)	5.52 (0.06)	6.08 (0.04)
5	57.80 (0.34)	41.12 (0.49)	27.95 (0.28)	9.35 (0.01)	6.45 (0.03)	4.84 (0.04)	5.38 (0.07)	6.12 (0.04)
6	56.30 (0.34)	42.60 (0.49)	26.49 (0.28)	9.39 (0.01)	6.59 (0.03)	4.90 (0.04)	5.60 (0.07)	6.00 (0.04)
7	55.22 (0.34)	36.36 (0.49)	26.41 (0.28)	9.20 (0.01)	6.67 (0.02)	5.29 (0.05)	5.69 (0.07)	6.10 (0.04)
8	54.19 (0.34)	41.25 (0.49)	27.07 (0.28)	9.25 (0.01)	6.60 (0.02)	5.21 (0.05)	5.59 (0.07)	5.91 (0.04)
9	51.41 (0.35)	43.76 (0.49)	27.54 (0.28)	9.38 (0.01)	6.74 (0.03)	5.02 (0.05)	5.75 (0.07)	5.69 (0.04)

*$\log_{10}$ cfu/g DM

[1] CIT = Compost internal temperature (°C)

[2] C:N = Carbon-to-nitrogen ratio

[3] TBC = Total bacteria count

[4] Staph. = *Staphylococcus* spp. counts

[5] Strep. = *Streptococcus* spp. counts

Figure 3.1. Mean weather data (ambient temperature [°C], dew point [°C], and relative humidity [%]) stratified by week, where Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019. Relative humidity was divided by 5 in order to visually compare on the same graph.

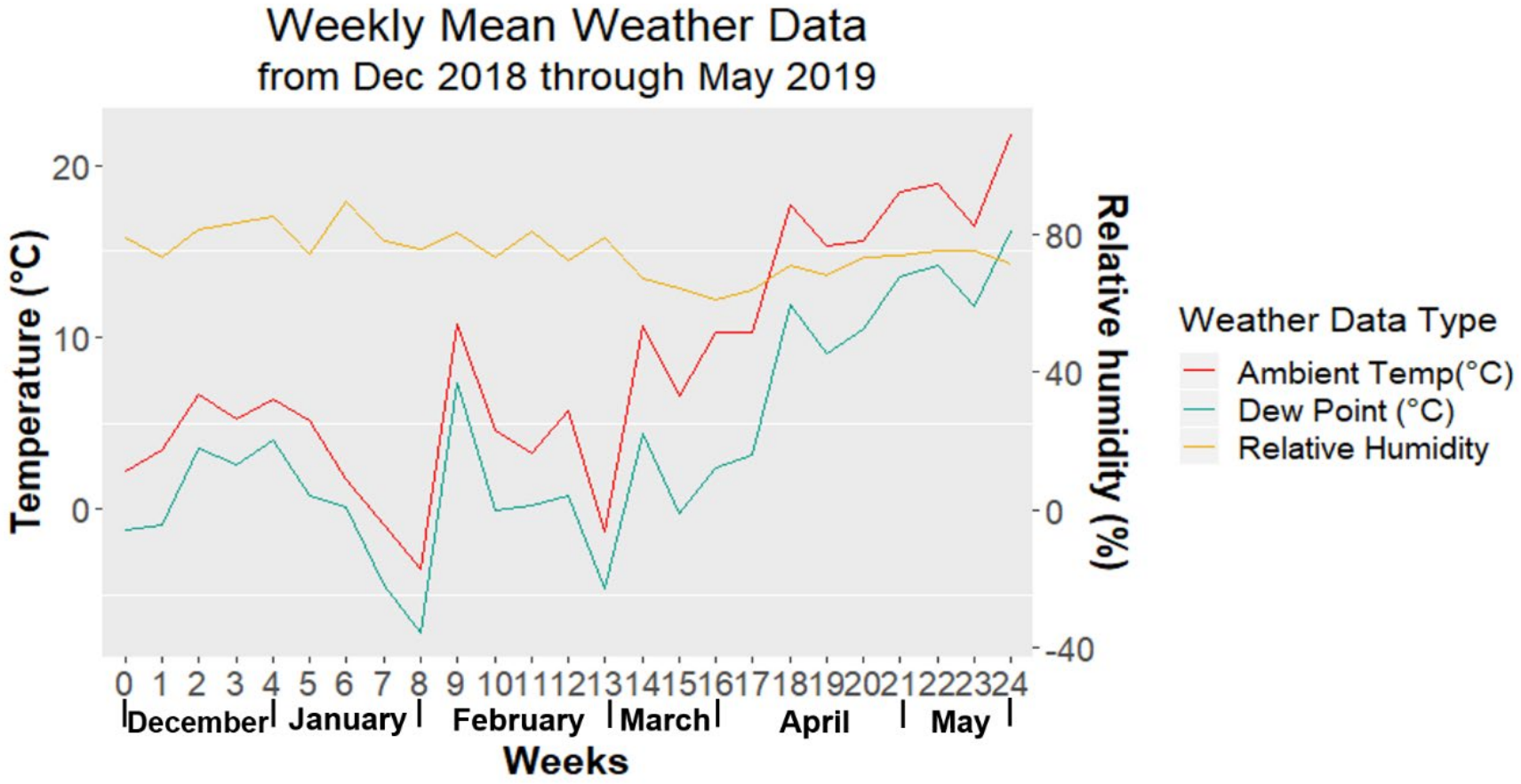


Figure 3.2 Mean bedding bacteria counts separated by bacteria group (coliforms, Staphylococcus (Staph.) spp., and Streptococcus (Strep.) spp.) stratified by week, where Week 0 = December 2-8, 2018 and Week 24 = May 19- 25, 2019.

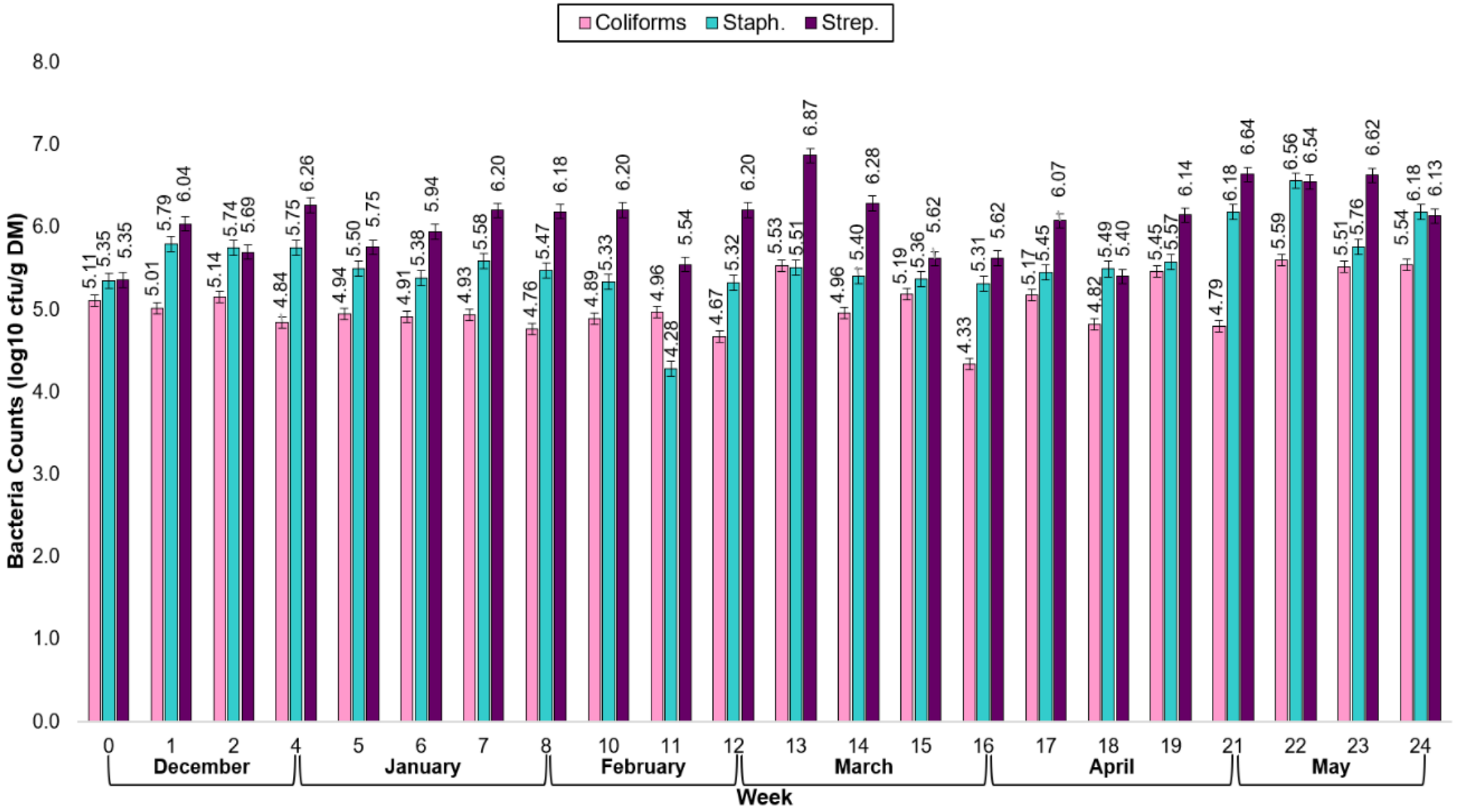


Figure 3.3. Bedding moisture percent over time, where the boxplots indicate the mean moisture by week and the colored dots indicate the moisture percentage for each of the 9 individual sample locations by week. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 20 19. The yellow shaded area indicates the recommended CBP moisture (40-60%).

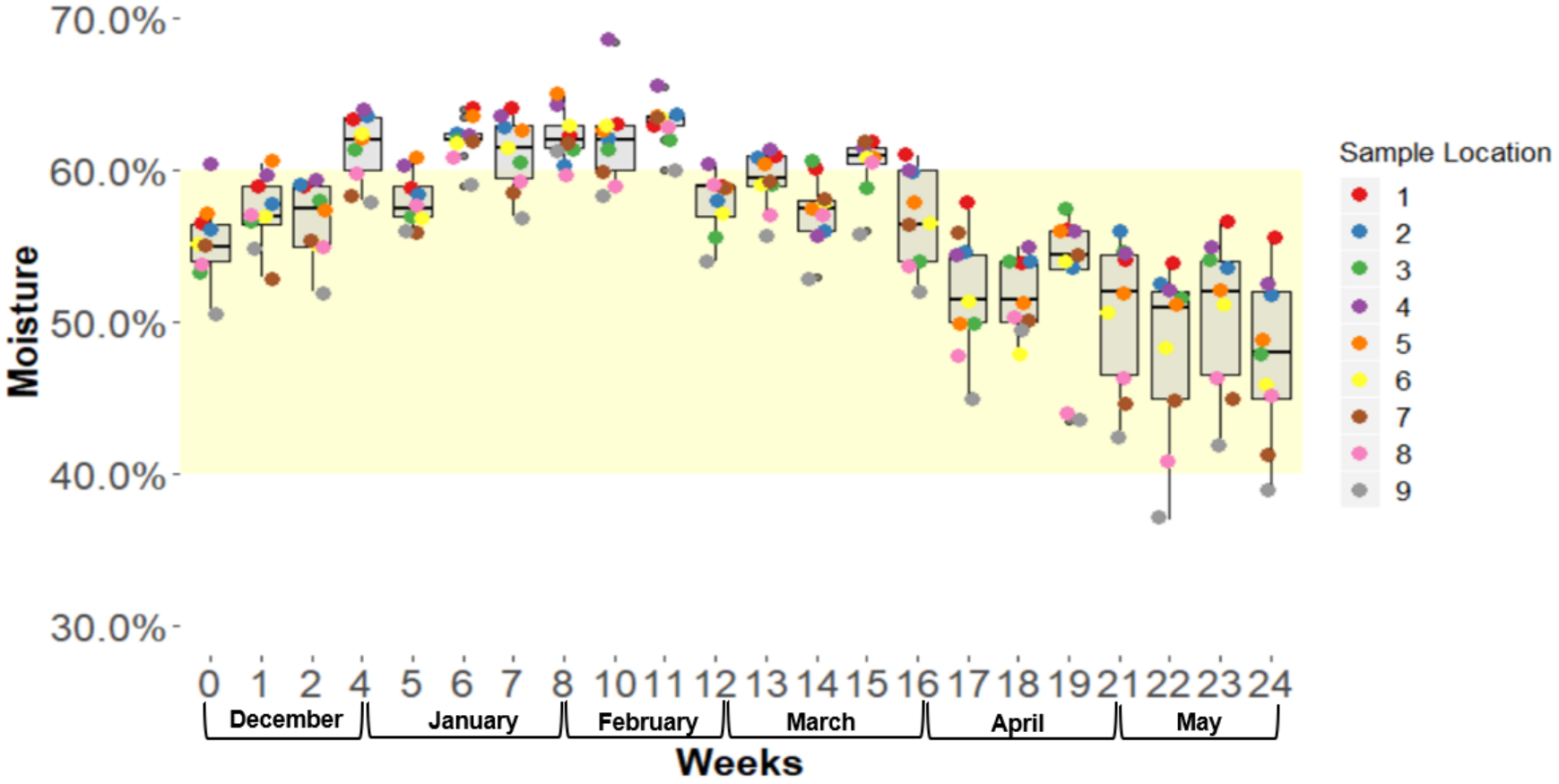


Figure 3.4. Bedding internal temperature (°C) over time, where the boxplots indicate the mean internal temperature by week and the colored dots indicate the internal temperature for each of the 9 individual sample locations by week. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019. The green shaded area indicates the recommended CBP internal temperature (43.3-65.0°C).

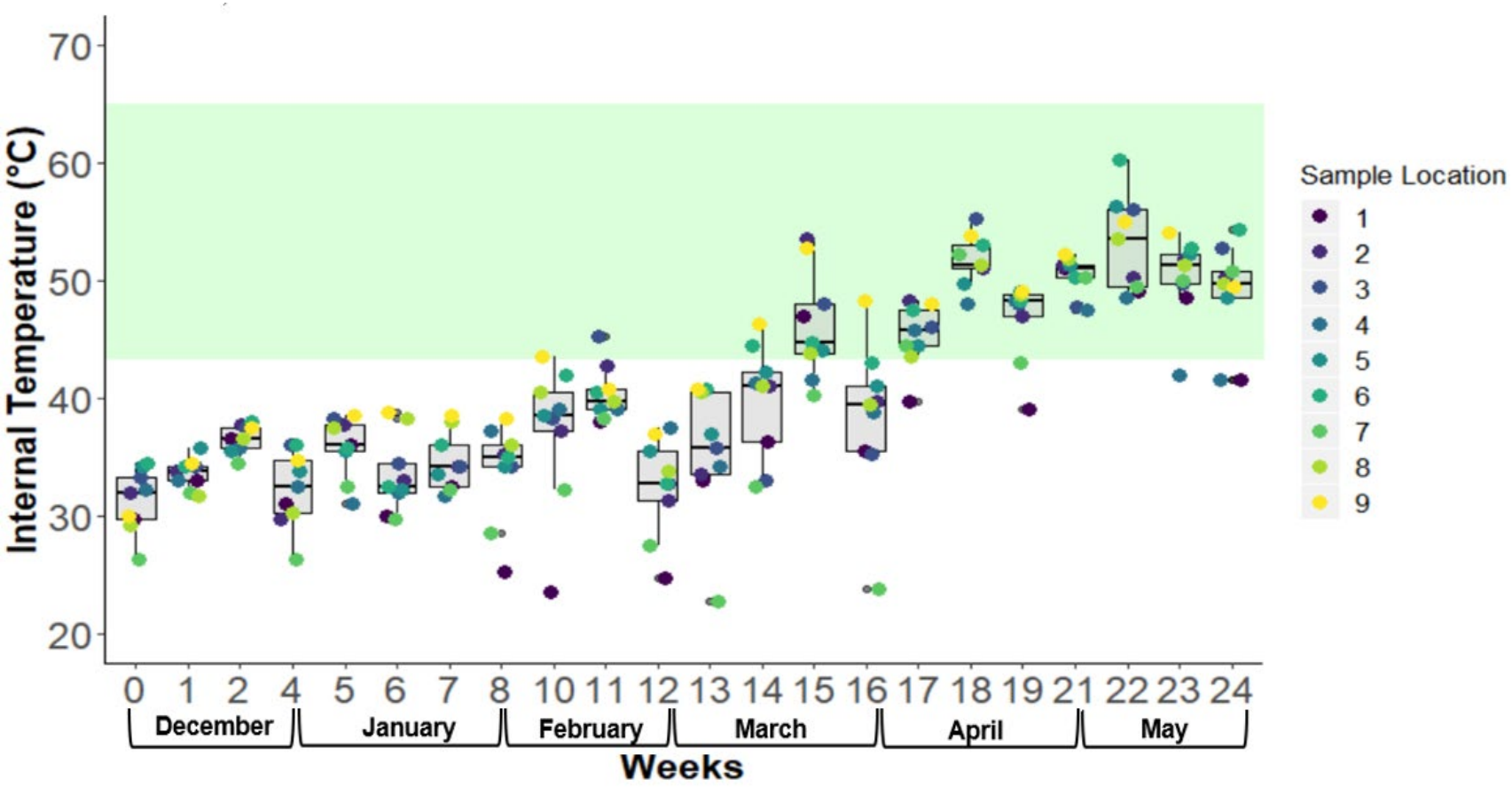


Figure 3.5. Bedding carbon-to-nitrogen (C:N) ratio over time, where the boxplots indicate the mean C:N ratio by week and the colored dots indicate the C:N ratio for each of the 9 individual sample locations by week. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019. The blue shaded area indicates the recommended CBP C:N ratio (25:1 – 30:1).

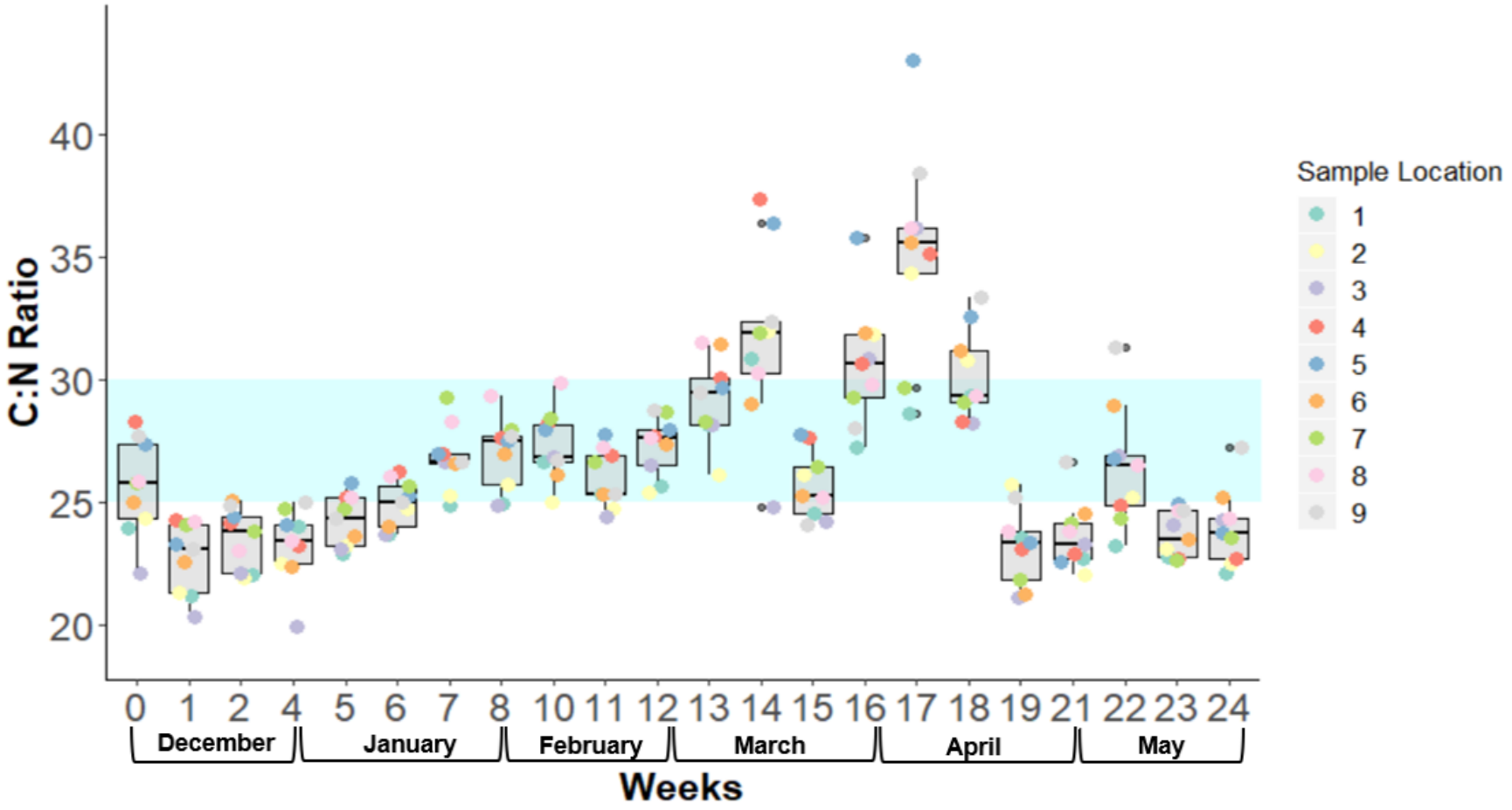


Figure 3.6. Bedding total bacteria count (TBC) over time, where the boxplots indicate the mean TBC by week and the colored dots indicate the TBC for each of the 9 individual sample locations by week. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019.

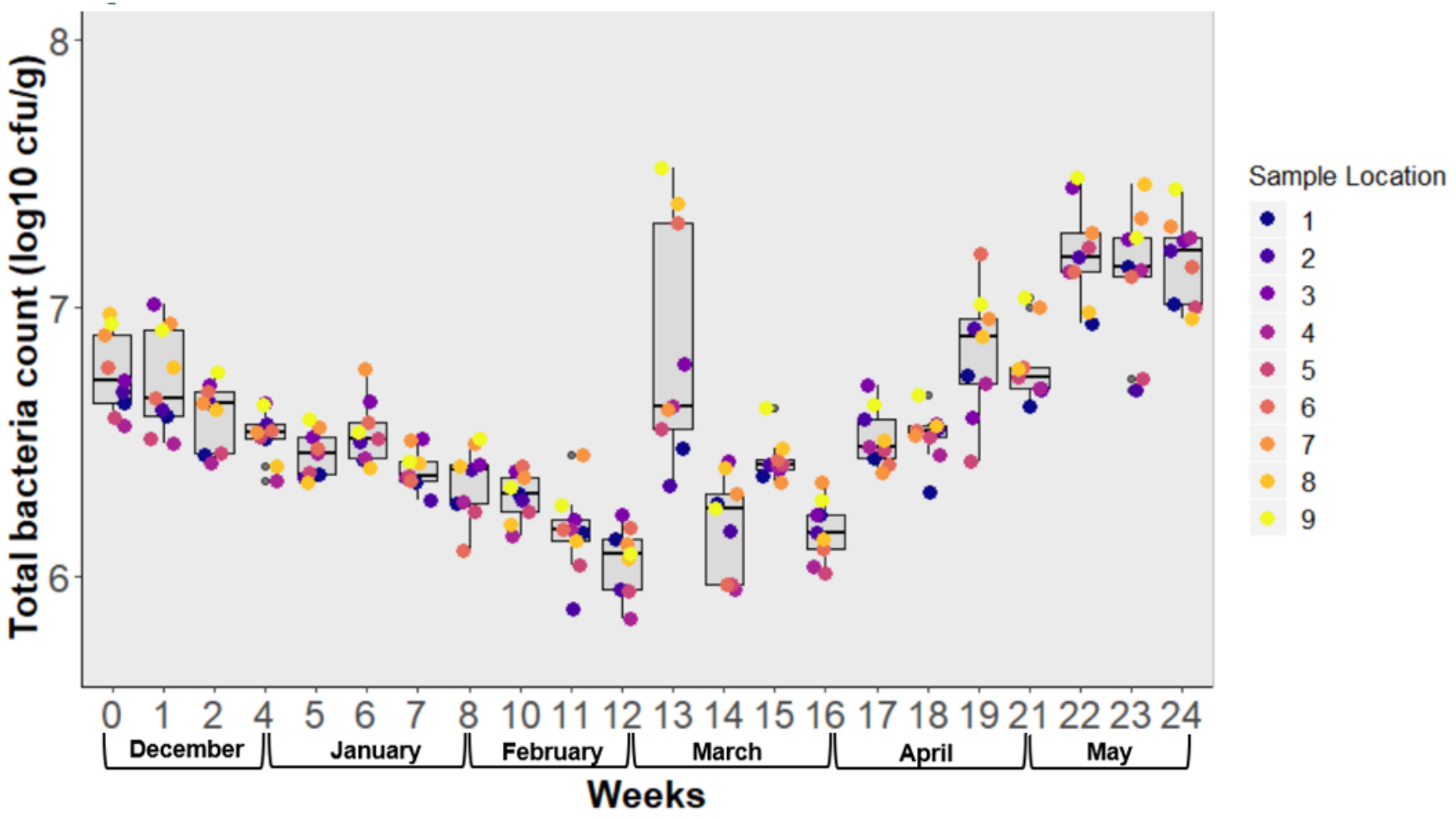
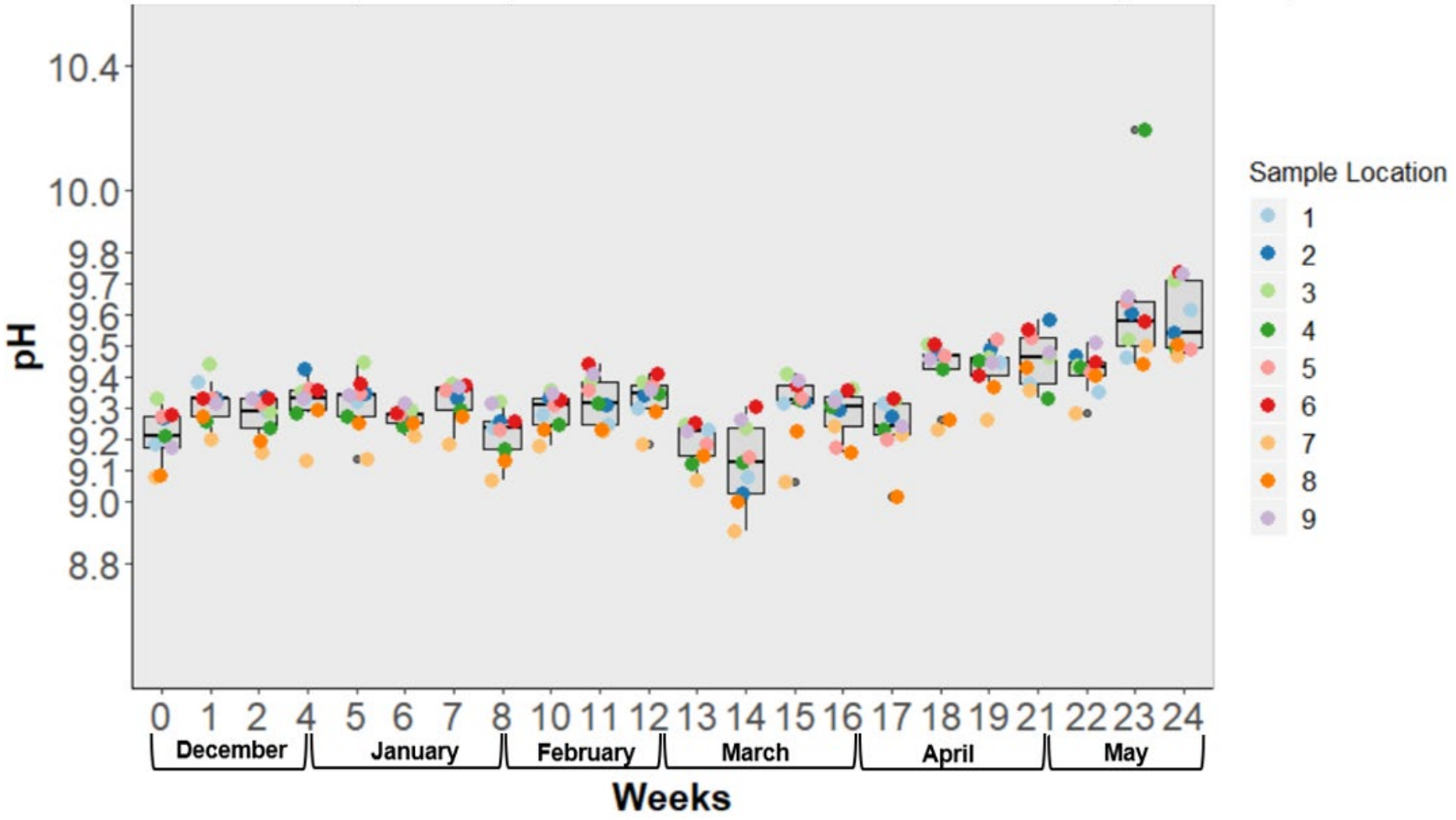


Figure 3.7. Bedding pH over time, where the boxplots indicate the mean pH by week and the colored dots indicate the pH for each of the 9 individual sample locations by week. Week 0 = December 2-8, 2018 and Week 24 = May 19-25, 2019.



CHAPTER 4. MICROBIAL INTERACTIONS BETWEEN COMPOST BEDDED PACK BARN BEDDING AND TEAT EXPOSURE IN TRANSITION DAIRY CATTLE

T.L. France, J.H.C. Costa, R.J. Harmon, J.L. Taraba, and M.C. Morgan

Department of Animal and Food Sciences, University of Kentucky, Lexington Kentucky 40546

4.1 Introduction

The detrimental effects of mastitis in the dairy industry have prompted continued research into how it can be controlled and prevented. One area to consider is the cow's environment. Improvement of dairy cattle housing management to increase cow comfort has led producers to develop a unique housing system, the compost bedded pack (CBP) barn. Key management practices such as twice-daily tilling of the pack and addition of organic bedding material are necessary for microbial growth. Optimal microbial activity is achieved by maintaining a CBP environment that has a moisture content of 40 – 60%, an internal temperature of 43.3°C – 65.0°C, and a carbon-to-nitrogen (C:N) ratio of 25:1 to 30:1. This environment ultimately produces a soft, comfortable material for dairy cows to lie on. However, Black et al. (2014) found that there are high amounts of mastitis-causing pathogens in compost bedding. Exposure to environmental pathogens found in bedding occurs when teats are exposed to the bedding and bacteria are transferred to the teat skin (Rowbotham and Ruegg, 2016). In turn, extensive research has been conducted to look at various bedding material and the bacteria associated with it as possible risk factors for mastitis. Studies have reported positive correlations between bacterial populations in bedding and on teat ends (Hogan et al., 1999, Zdanowicz et al., 2004).

However, many studies have not found strong relationships between teat end or teat skin bacteria counts and mastitis. Consequently, there is a lack of evidence to support the widely held belief that high bedding bacteria counts are a risk factor for IMI and mastitis (Rowe et al., 2019). More recently, researcher have begun attempting to prove or disprove that belief. Several research groups have investigated bacteria counts in common bedding

types such as new sand, recycled sand, recycled manure solids, and wood bedding (sawdust, shavings), and their impact on udder health parameters (Patel et al., 2019, Robles et al., 2019, Rowe et al., 2019). Rowe et al. (2019) reported that the IMI prevalence in late-lactation dairy cows was low in the US, indicating that high bedding bacteria may not be a risk factor for IMI prevalence in late lactation. Research is needed that investigates the effects of CBP barn bedding bacteria counts on udder health parameters, since the compost bedding to which cows are exposed to is home to an increased microbial population. Additionally, research is needed that specifically looks at bedding bacteria and its effects on transition cow health, which is the time period of 3 weeks pre- through 3 weeks postpartum (Grummer, 1995). During this time, cows are at a higher risk for mastitis due to a compromised immune system such that it's imperative to reduce exposure to environmental factors that would add to that risk.

To the author's knowledge, no study that investigates the relationships between CBP barn bedding characteristics, bacterial counts, and seasonal change (winter to spring) on the microbial population change of teat skin, teat ends, and milk samples currently exist. The objectives of this study were to (1) investigate the direct relationships between bacteria counts on the teat skin, teat ends, and in the milk, and if different stages within the transition period effect these bacteria counts. Objective (2) was to determine what environmental factors (previously described in Chapter 3) effect cow-level bacterial counts when both time and the transition period time points were considered. I hypothesized that there would be a positive correlation between the bacterial counts found in compost bedding and the microflora found on the teat skin and teat ends.

Additionally, I hypothesized that the bacterial counts on the teat ends were strongly correlated to the bacteria found in the milk.

4.2 Materials and Methods

Detailed cow-level sampling protocols and laboratory analysis for this study can be found in Chapter 2. Briefly, the observational study occurred from December 2018 to May 2019 at the University of Kentucky's Coldstream Dairy research farm. Twenty-six Holstein dairy cows were enrolled (parity: 2.08 ± 1.17 [mean ± SD]) during the study with no cow exclusion criteria. All cows were housed on a twice-daily tilled CBP barn and were enrolled based on their expected calving dates. Samples were collected at specific time points within the transition period for each cow, which was deemed the term Experimental Week, "Expt week". Each cow had samples collected for Expt weeks -1, 0, and 1-8. Experimental week -1 represented the sample collection time at about 2 weeks prepartum, which included teat end scores, teat skin total bacteria count (TBC), teat end TBC, teat skin coliforms, teat end coliforms, teat skin *Staphylococcus* spp. (Staph.) counts, teat end Staph. counts, teat skin *Streptococcus* spp. (Strep.) counts, and teat end Strep. counts. The Expt week 0 represented samples collected at 48-72h postpartum, which included the previously stated samples as well as milk TBC, milk coliform count, and somatic cell count (SCC). Expt week 1 represented the first biweekly set of samples that were collected, followed by Expt week 2-8 which were the continuation of biweekly sampling. Expt week 8 represented the last set of samples collected, which was at 60 ± 7 days in milk (DIM) for each individual cow.

All teat skin sponges, teat end swabs, and milk samples were collected aseptically. All samples were transported in a cooler (remained at < 4°C) to the University of Kentucky's Animal and Food Science microbiology laboratory where microbial analysis was conducted within 24h of collection. Previously described enumeration methods were used on the teat skin sponges and teat end swabs to obtain TBC, coliforms, Staph. counts, and Strep counts. Milk samples were analyzed for the presence or absence of bacteria and enumeration methods were used to determine TBC and coliform count in each sample. Furthermore, milk samples were classified as contaminated as described by Parker et al. (2008), where >2 distinct colony types present on any plate of the same sample was considered contaminated and discarded. An intramammary infection (IMI) was defined as the isolation of 100 cfu/mL of identical colonies on the same plate. For all IMI, bacterial identification to the species level was determined using a Vitek 2 Compact Analyzer (Biomerieux, Hampshire, UK; Vitek 2 Gram Positive card kit, 20 cards; Vitek 2 Gram Negative card kit, 20 cards)

4.3 Statistical Analysis

4.3.1 Cow-level information.

The MEANS procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA) was used to determine the mean (± SD) of the following cow variables: parity, teat end scores, milk TBC, milk coliforms, SCC, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph. counts, teat end. Staph. counts, teat skin Strep. counts, teat end Strep. counts. Every sample was analyzed on an individual, quarter-level basis, with n = 532 for all milk-related samples and n = 639 for all teat skin & teat end samples.

Pearson correlation coefficients were calculated to find associations among the continuous variables (parity, milk TBC, milk coliforms, SCC, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph. counts, teat end Staph. counts, teat skin Strep. counts, and teat end Strep. counts) using the CORR procedure of SAS. Relationships were considered statistically significant at $p < 0.05$. Univariate mixed models (proc MIXED of SAS) were used to investigate the direct relationship of the effect of experimental week on the response variables: Milk TBC, SCC, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph. counts, teat end Staph. counts, teat skin Strep. counts, and teat end Strep. counts.

Mastitis-causing pathogens isolated from milk samples. Due to the large portion of milk samples that came back as culture-negative (no growth), the analysis of pathogens isolated in the milk samples was limited. The SUMMARY procedure of SAS was used to determine the mean SCC of each of the mastitis-causing pathogens isolated from the milk samples. Mastitis prevalence by each experimental week was calculated by dividing the number of culture-positive milk samples by the total number of milk samples within each time point.

4.3.2 Interactions between environmental factors and cow over time.

Pearson correlation coefficients were calculated to find relationships between the continuous cow variables and environmental variables (ambient temperature, relative humidity, dew point, bedding moisture, internal temperature, surface temperature, carbon-to-nitrogen (C:N) ratio, pH, bedding TBC, bedding coliforms, bedding Staph. counts, and bedding Strep. counts). Relationships were considered statistically significant

at $p < 0.05$. Correlations were used as a guideline for what explanatory variables to include in the regression models to avoid confounding variables.

The MIXED procedure of SAS was used to investigate how the explanatory variables (week, experimental week, ambient temperature, bedding moisture, internal temperature, surface temperature, C:N ratio, pH, bedding TBC, parity, and teat end scores) effect the cow response variables (milk TBC, SCC, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph. counts, teat end Staph. counts, teat skin Strep. counts, and teat end Strep. counts). The explanatory variables week, experimental week, and the week*experimental week interaction remained in every mixed model regardless of significance. All other explanatory variables were subject for removal using backward stepwise elimination process if $p > 0.10$. Overall statistical significance for main effects was declared at $p \leq 0.05$. The experimental week variable was included as a repeated measure. Quarter nested within cow was included as the subject. Least squares means (SE) of the experimental weeks were compared, and the Bonferroni correction factor was used to adjust the p-value to perform multiple contrasts among the experimental weeks. Similarly, the LS means of parity were compared, but only on the response variables that kept parity in their final mixed model.

The final mixed model results were interpreted in two specific ways: (1) the explanatory variables were statistically significant in that the p-value < 0.05. This follows the same interpretation as any other study that utilizes statistical analysis. However, (2) was to interpret the numerical change observed by the response variables due to the

statistically significant explanatory variables, which we called practically significant. Because the response variables were microbiological bacteria counts, while the change in the bacteria counts would have $p < 0.05$, the numerical change was considered negligible if it was lower than 1-log difference. This was simply due to what we were measuring. For example, if the bacteria counts only changed by 0.07 log10 cfu/g due to one of the explanatory variables at $p < 0.05$; we would conclude that this was statistically significant but not practically significant. This interpretation will be used for the results of this chapter.

4.4 Results and Discussion

4.4.1 Cow-level information.

Mean cow-level variables (parity, milk TBC, milk coliforms, SCC, teat end scores, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph., teat end Staph., teat skin Strep., and teat end Strep. counts) were described in Table 4.1, where n = 532 for milk-related samples, n = 639 for teat skin/end samples, and n = 26 for parity. Cows in the study ranged from first lactation heifers (parity = 1) to sixth lactation cows (parity = 6), with a mean of 2.13 ± 1.24.

For all bacteria types, the teat skin samples had nearly double the bacteria load compared to the teat end swabs. The mean teat skin TBC was at 5.93 ± 0.51 $\log_{10}$ cfu/g, whereas the mean teat end TBC was at 3.46 ± 1.26 $\log_{10}$ cfu/g. Moreover, the mean teat skin Staph. counts were at 5.07 ± 1.64 $\log_{10}$ cfu/g compared to the mean teat end Staph. counts at 2.63 ± 1.20 $\log_{10}$ cfu/g. The large difference was likely due to the surface area between teat skin samples and teat end swabs.

The mean milk TBC was 0.54 ± 1.15 $\log_{10}$ cfu/ml. This value can be explained due to the large portion of milk samples with no growth (0.00 $\log_{10}$ cfu/ml) at 81.58% (434 out of 532 milk samples). Results showed that many culture-positive milk samples occurred at the 48-72h sampling time, with the mastitis prevalence at 27.88% (Table 4.2). For the remainder of the study, mastitis prevalence fluctuated between 14.29% to 33.33% with no apparent trend. Of the milk samples that had bacterial growth, the following microorganisms were those identified as the source of infection: *Escherichia coli, Streptococcus* spp., *Staphylococcus aureus*, *Bacillus licheniformis*, *Staphylococcus chromogenes*, *Staphylococcus warneri*, *Staphylococcus hyicus, Staphylococcus epidermidis*, and *Staphylococcus lugdunensis*. *Staphylococcus chromogenes* was isolated the most frequently (n = 60), followed by *Staphylococcus aureus* (n =15). The mean SCC for CNS species (all *Staphylococcus* species identified except *Staphylococcus aureus*) was 4.76 ($\log_{10}$), or 57,544 somatic cells/ml compared with a SCC of 3.38 ($\log_{10}$) or 2,399 cells/ml for culture negative quarters (**Figure 4.1**). Of the CNS species, *Staphylococcus chromogenes* was the most prevalent and had a mean SCC of 4.40 ($\log_{10}$) or 25,119 cells/ml. Interestingly, *Staphylococcus warneri*, another CNS species, had a mean SCC of 5.64 ($\log_{10}$) or 436,516 cells/ml. Previous research has provided mixed results on the effects of CNS species on milk SCC (Jarp, 1991, Borm et al., 2006) , which was also seen in this study when CNS species were identified to the genus level. Results suggest that the usage of the umbrella term "CNS" mastitis may be an inaccurate interpretation, as the severity of each Staphylococcus species varies significantly. Conversely, *Staphylococcus aureus* had a slightly higher mean SCC at 5.06 ($\log_{10}$), or

114,815 somatic cells/ml and *E. coli* had a mean SCC of 5.74 (log_{10}), or 549,541 cells/ml. These results were expected due to the pathogenic nature of both *Staphylococcus aureus* and *E. coli* infections, likely resulting in more severe inflammation as seen by an elevated milk SCC.

Pearson correlation coefficients of all continuous cow variables helped determine relationships between bacteria counts on the teat skin, teat end, and in the milk samples (Table 4.3). Milk coliforms were excluded from the table due to no statistical significance with any other variable. Milk TBC had a weak positive relationship with teat end TBC ($r = 0.18$; $p < 0.001$), teat end Staph. counts ($r = 0.24$; $p < 0.001$), and teat end Strep. counts ($r = 0.16$; $p = 0.001$). Interestingly, the relationship between milk TBC and teat skin TBC was very weak ($r = 0.10$; $p = 0.02$). Moreover, milk TBC had a moderately positive correlation with SCC (log_{10}) ($r = 0.27$; $p < 0.001$). The correlation between milk bacteria count and SCC may be thought to have a stronger relationship, however, depending on the pathogen and the immune response of each individual cow, the somatic cells present in the udder may have a slight delay or may fluctuate over time. Thus, the SCC reported at the same time the milk sample was collected may not be as correlated as one would believe. As expected, teat skin TBC had a strong positive correlation with teat end TBC ($r = 0.42$; $p < 0.001$). Similarly, teat skin and teat end coliform counts had a strong positive correlation ($r = 0.46$; $p < 0.001$), teat skin and teat end Staph. counts had a moderately positive correlation ($r = 0.22$; $p < 0.001$), and teat skin and teat end Strep. counts had a moderately positive correlation ($r = 0.22$; $p < 0.001$). Interestingly, relationships were found between various bacteria types (coliforms, Staph., and Strep.

counts) within each sample type (teat skin or teat ends). Teat skin coliforms had a positive relationship with teat skin Staph. counts ($r = 0.21$; $p < 0.001$) and with teat skin Strep. counts ($r = 0.38$; $p < 0.001$). Similarly, teat end coliforms had a positive relationship with teat end Staph. counts ($r = 0.14$; $p = 0.001$) and with teat end Strep. counts ($r = 0.22$; $p < 0.001$). It is not entirely understood why these correlations were found, because coliforms do not normally inhabit the skin, environment whereas *Staphylococcus* spp. and *Streptococcus* spp. do. This relationship may also be possible if the sample sites were covered in feces, for example. This would lead to an overall increase in bacterial population. However, correlation coefficients do not account for other factors that may contribute to the relationship or lack-there-of. In turn, these results were used a guideline for expected relationships that should be seen in later statistical analyses.

4.4.2 Effect of experimental weeks on cow variables.

Results from the univariate models indicated that most cow variables changed ($p < 0.05$) depending on the time within the transition period (expt week), with the exception of milk TBC which had a tendency ($p = 0.06$) to change (Table 4.4). Interestingly, milk SCC was reported highest immediately postpartum, with the LS means (SE) of 5.31 (0.17) $\log_{10}$. Over the following week, the SCC significantly decreased to 4.13 (0.24) $\log_{10}$ and continued a slow decrease with time. The high SCC observed at 48-72h postpartum may be due to some milk samples still containing colostrum. Since colostrum is comprised of many immune cells, the SCC would reflect that which would result in a higher SCC. This could make the results potentially

inaccurate if SCC was the only udder health parameter measured. In turn, it was important to interpret results by the milk TBC and not the SCC alone.

Effect of experimental week on teat skin bacterial counts. The least squares means (SE) of teat skin coliforms and teat skin Staph. counts were statistically significant ($p < 0.05$) from expt week -1 (prepartum) to expt week 0 (48-72h postpartum). When cows calved, teat skin coliform counts increased from 2.09 (0.07) to 2.75 (0.07) $\log_{10}$ cfu/g and teat skin Staph. counts decreased from 5.67 (0.16) to 4.53 (0.16) $\log_{10}$ cfu/g. However, as cows progressed through early lactation to 60 DIM (expt weeks 1-8), the mean teat skin coliforms and Staph. counts fluctuated slightly but stabilized with time (Figure 4.2). Moreover, teat skin Strep. counts were not significantly affected by expt week. Post-dip teat disinfectant is implemented once cows calve and aid in the prevention of new infections in the udder. While the target of teat disinfectant is primarily the teat end, results suggest that it may also contribute to the change seen in bacterial load at the teat skin.

Effect of experimental week on teat end bacterial counts. The least squares means (SE) of teat end coliforms, Staph., and Strep. counts were statistically significant ($p < 0.05$) from prepartum to postpartum (expt weeks -1 to 0). When cows calved, teat end coliforms increased from 1.19 (0.04) to 1.46 (0.04) $\log_{10}$ cfu/g. However, at the same time, Staph. counts decreased from 4.08 (0.09) to 2.63 (0.09) $\log_{10}$ cfu/g, and Strep. counts decreased from 2.85 (0.06) to 2.46 (0.06) $\log_{10}$ cfu/g. Similar to what was observed for teat skin bacteria counts, as cows progressed through early lactation to 60

DIM (expt weeks 1-8), there was no significant changes for any of the teat end bacteria groups, and with little fluctuation, as shown in Figure 4.3. This suggests that the cow's microflora at the teat ends stabilize once her body gets past the first few stressful days immediately after calving. Additionally, teat end disinfectant post-milking is implemented immediately postpartum. This proper milking procedure step is likely a major contributor to the decrease in total bacteria counts at the teat ends, which decreased from 4.54 (0.11) to 3.65 (0.11) log10 cfu/g (Table 4.4). These results provide evidence that post-dipping is an effective management tool in reducing bacterial load at the teat end level.

Relationships were found between milk bacteria counts and teat end bacteria counts, suggesting that the bacterial load and bacteria species found on the teat ends may contribute to the presence or absence of an IMI. Additionally, strong correlations between teat skin and teat end bacteria counts were evident, such that as the bacterial load on the teat skin increased, the bacterial load on the teat end increased. These relationships, however, may vary significantly when environmental factors are taken into consideration. Results from this study also indicated that various stages in the transition period affected all bacteria counts. The transition period, or the time period 3 weeks pre- to 3 weeks postpartum (Grummer, 1995), has been noted to increase oxidative stress in dairy cows, resulting in increased risk of metabolic diseases and mastitis. Significant changes were observed from prepartum to 72 postpartum, where teat skin and teat end coliforms increased and Staph. counts decreased. It is not entirely understood why the changes varied by bacteria species. It does suggest that the heightened oxidative stress at time of

calving may affect the microbial population and quantity found on the teat skin and teat end. Moreover, the observed stabilization of teat skin and teat end bacterial counts after 1-2 weeks postpartum may be a consequence of effective teat disinfectant during the milking procedure. However, it may take >3 days of daily disinfectant use (i.e. the first 3+ days of lactation) to see noticeable teat bacteria changes. Future research is needed to measure stress at parturition and determine what mechanism may contribute to alterations of teat microbial population.

4.4.3 Interactions between cow and environmental measures.

Pearson correlation coefficients were measured to determine associations between the cow and environmental (bedding and weather) variables (Tables 4.5 and 4.6). Not all variables were listed in either table; only relationships that were statistically significant ($p < 0.05$). Bedding internal temperature at a depth of 20.3-cm had moderately negative relationships ($p < 0.001$) with the following variables: milk TBC ($r = -0.19$), SCC ($r = -0.30$), teat skin TBC ($r = -0.24$), teat end TBC ($r = -0.25$), teat end Staph. counts ($r = -0.26$), and teat end Strep. counts ($r = -0.23$). Similarly, bedding surface temperature had a moderately negative relationship SCC ($r = -0.27$), teat skin TBC ($r = -0.32$), teat end TBC ($r = -0.20$), teat end Staph. counts ($r = -0.26$), and teat end Strep. counts ($r = -0.18$). Milk TBC, however, had no correlation with bedding surface temperature. Bedding moisture had significant ($p < 0.001$) weak relationships with the following variables: milk TBC ($r = 0.13$), teat skin TBC ($r = 0.18$), teat end TBC ($r = 0.15$), teat end Staph. counts ($r = 0.16$), and teat end Strep. counts ($r = 0.18$). Moreover, bedding moisture had a moderately positive correlation with SCC ($r = 0.32$; $p < 0.001$).

The bedding pH was negatively associated with SCC ($r = -0.25$; $p < 0.001$) and teat end Staph. counts ($r = -0.13$; $p < 0.001$). Interestingly, no relationships were found between any of the cow variables and the following environmental measures: bedding C:N ratio, bedding coliform counts, and bedding Strep. counts. Additionally, bedding TBC and bedding Staph. counts were only moderately correlated with SCC, at $r = -0.27$ and $r = -0.20$; $p < 0.001$, respectively. There were no statistically significant relationships found between bedding bacterial counts and the cow-level bacterial counts. These results contradict previous statements that a direct correlation exists between bedding bacteria counts and bacteria counts on the teat ends (Hogan and Smith, 1997, Zdanowicz et al., 2004).

Correlations between weather variables and the cow variables were reported in Table 4.6. Ambient temperature had a moderately negative relationship (at $p < 0.001$) for with the following variables: SCC ($r = -0.29$), teat skin TBC ($r = -0.23$), teat end TBC ($r = -0.21$), teat end Staph. counts ($r = -0.25$), and teat end Strep. counts ($r = -0.18$). The relative humidity (RH) had a moderate to weak association ($p < 0.001$) with the following variables: teat skin TBC ($r = 0.25$), teat end TBC ($r = 0.24$), teat end Staph. counts ($r = 0.16$), teat end coliforms ($r = 0.19$), and teat end Strep. counts ($r = 0.22$).

Pearson correlations were used as a guide for what explanatory variables to include in the final mixed models to avoid confounding variables. This became difficult to determine what environmental (both bedding and weather) variables to include or exclude, as many were strongly correlated with each other. For weather variables, RH

was excluded due to the strongly association with ambient temperature. For bedding bacteria, only the bedding TBC was included in the final models, thus bedding coliforms, Staph. counts, and Strep. counts were excluded. Results from the previous chapter indicated strong associations with the remaining bedding characteristics (moisture, internal temperature, surface temperature, C:N ratio, and pH), such that when bedding internal temperature increased, moisture decreased (at r = -0.61), for example. This would inevitably result in confounding variables in the final mixed models; however, none were excluded. Results of the final mixed models described the environmental effects on cow bacterial counts, shown in Table 4.7.

4.4.3.1 Final mixed models of udder health measures.

Interestingly, the only variables that effected milk TBC were teat end scores ($p = 0.004$) parity ($p < 0.001$), and bedding C:N ratio had the tendency to influence milk TBC ($p = 0.09$) (Table 4.7). Since parity remained in the final model, the differences between least squares means by parity were compared (Table 4.8). Results showed that the milk TBC LSMeans (SE) of parity 1 cows was 0.73 (0.19) $\log_{10}$ cfu/ml, whereas parity 2 cows had a milk TBC of 0.06 (0.24) $\log_{10}$ cfu/ml. Moreover, there was a significant ($p < 0.001$) difference between milk TBC of parity 1 cows compared to parity 2, 3, and 6, but no differences between the latter 3 parities. The low milk bacterial counts observed were due to the regression model which included all milk samples in the study, most of which were culture negative. To verify the drastic difference between milk TBC of parity 2 versus the others, the mean milk TBC by parity was determined for only milk samples that had a milk TBC of > 0.00 $\log_{10}$ cfu/ml (Figure 4.4). Results were confirmed that parity 1 had a

higher mean milk TBC of infected quarters at 2.83 $\log_{10}$ cfu/ml compared to parity 2 at 1.94 $\log_{10}$ cfu/ml, parity 3 at 2.09 $\log_{10}$ cfu/ml, and parity 6 at 1.41 $\log_{10}$ cfu/ml. This suggests that first lactation heifers experienced more IMI than 2+ parity cows. These findings contradict previous research that higher parity (older cows) were at greater risk of getting an IMI compared to primiparous cows (Hertl et al., 2011, Jamali et al., 2018) . The other udder health parameter measured was the milk SCC. Results indicated that the SCC increased by 4.93 (1.60) $\log_{10}$ at $p = 0.002$ as bedding moisture increased by 1% and decreased by 1.59 (0.56) $\log_{10}$ at $p = 0.01$ as bedding pH increased by 1.0. It is not entirely understood why bedding pH had such a large influence on SCC, but more research is needed to investigate that specific relationship. The bedding moisture influence on SCC may be explained in that a CBP environment with high moisture tends to adhere to the cows' udders more which would increase udder hygiene score, an indirect measure of an IMI (Lobeck et al., 2011, Eckelkamp et al., 2016b). Additionally, the milk SCC was significantly influenced by the week * expt week interaction ($p = 0.001$). The week * expt week interaction explained the effect of both the stage within the transition period and the time when the cows were enrolled, which could have been January – February, March – April, etc. Since both variables were previously shown to separately influence all other variables, it was important to keep the interaction between the two in all final mixed models.

4.4.3.2 Final mixed models of teat skin bacterial counts.

Environmental variables influenced the teat skin bacterial counts differently, which varied by bacteria species (Table 4.7). Regression coefficients (SE) indicated that

as bedding moisture increased by 1%, teat skin TBC had a tendency ($p = 0.07$) to decrease by 1.06 (0.59) $\log_{10}$ cfu/g. Bedding internal temperature at a depth of 20.3-cm only influenced teat skin Staph. counts ($p = 0.01$), in that as the internal temperature increased by 1°C, teat skin Staph. counts decreased by 0.03 (0.01) $\log_{10}$ cfu/g. The impact of CBP moisture and internal temperature characteristics on the teat skin bacterial load was not expected, as both variables had relatively strong correlations with teat skin bacteria counts. Bedding C:N ratio was the only other bedding characteristic that influenced teat skin TBC, teat skin coliforms, and teat skin Strep. counts. As the C:N ratio increased by 1.0, teat skin TBC, coliforms, and Strep. counts decreased by 0.02 (0.01) $\log_{10}$ cfu/g, 0.02 (0.01) $\log_{10}$ cfu/g, and 0.04 (0.02) $\log_{10}$ cfu/g, respectively (at $p = 0.02$, $p < 0.001$, and $p = 0.01$, respectively). This suggests that bedding C:N ratio was a major factor that influences teat skin bacterial load. However, the changes observed were so negligible that while statistically significant, the bacteria change was not practically significant. Results determined that bedding TBC was statistically significant in the final mixed models of teat skin TBC and teat skin coliforms. As bedding TBC increased by 1.0 $\log_{10}$ cfu/g DM, teat skin TBC and teat skin coliforms increased by 0.14 (0.06) $\log_{10}$ cfu/g and 0.34 (0.08) $\log_{10}$ cfu/g, respectively (at $p = 0.02$ and $p < 0.001$). Interestingly, while Pearson correlations reported no significant relationship between teat skin bacterial counts and bedding bacteria counts, the final models suggested that when all variables were taken into consideration, bedding TBC did affect the teat skin TBC and coliform count. The effect of bedding TBC specifically on teat skin coliforms was likely due to the similar environmental origins of the bedding bacteria and teat skin coliforms, as

coliforms are not considered natural skin microflora (whereas Staph. species are). Because of this, the environmental bedding bacteria specifically effected the environmental-type bacteria species that were observed on the teat skins of the cows. Moreover, similar results were reported by Hogan et al. (1999) in that there were positive relationships between bedding bacteria counts and teat skin coliform counts. Moreover, ambient temperature influenced teat skin TBC and Staph. counts. As the ambient temperature increased by 1°C, teat skin TBC decreased by 0.02 (0.01) $\log_{10}$ cfu/g and teat skin Staph. counts decreased by 0.07 (0.02) $\log_{10}$ cfu/g. Similar to the C:N ratio results, the bacteria change was statistically significant but not practically significant. This suggests that ambient temperature does effect teat skin bacterial counts, but to a level of degree that should be of concern is debatable.

Interestingly, the week * expt week interaction significantly influenced teat skin TBC ($p = 0.03$), teat skin coliforms ($p = 0.04$), teat skin Staph. counts ($p < 0.001$), and teat skin Strep. counts ($p = 0.001$). Additionally, the effect of parity remained significant in all teat skin bacteria counts. The differences between least squares means by parity were compared (Table 4.8). Results showed that in general, all teat skin bacteria counts were at the lowest in first lactation cows (parity 1) and increased as parity increased. Specifically, the LSMeans of the teat skin TBC for parity 1 cows was 5.70 (0.05) $\log_{10}$ cfu/g compared to parity 6 cows at 6.23 (0.13) $\log_{10}$ cfu/g. This suggests that first lactation cows have a lower bacterial population on the teat skins compared to second and greater lactation cows. This was the opposite of what was observed for milk TBC by parity. These findings suggest that first lactation cows had higher IMI but lower teat skin

bacterial counts, whereas parity 2+ cows had lower IMI but higher teat skin bacterial counts.

4.4.3.3 Final mixed models of teat end bacterial counts.

Results indicated that no environmental variables significantly affected teat end TBC, coliform count, or Strep counts (Table 4.7). The ambient temperature did impact teat end Staph. counts ($p = 0.003$), in that as the ambient temperature increased by 1°C, teat end Staph. counts decreased by 0.04 (0.01) $\log_{10}$ cfu/g. The change observed for Staph. counts, however, was not practically significant. Additionally, the bedding internal temperature at a depth of 20.3-cm had the tendency ($p = 0.10$) to increase teat end Staph. counts by 0.01 (0.01) $\log_{10}$ cfu/g for every 1°C increase. The week * expt week interaction only influenced teat end TBC ($p < 0.001$) and teat end Staph. counts ($p = 0.01$). Results suggest that environmental factors don't seem to have much influence on the bacterial load at the teat ends. These results contradicted the Pearson correlations previously described, as bedding internal temperature and moisture had, in fact, no effect on teat end bacteria counts when all other environmental factors were included.

Different environmental measures influenced the various bacteria species on the teat skins and teat ends. We determined that all bedding characteristics changed with time; we also determined that cow-level bacterial counts changed based on the stage of the transition period. The interaction between the week and stage of the transition period, which remained in all final models, allowed for a more accurate representation of what bedding and weather characteristics influenced cow bacterial counts.

4.5 Conclusions

The environmental measures (bedding and weather) effected teat skin bacteria species differently and had limited influence on the teat end microbial population. Specifically, the CBP bedding bacteria counts were not practically significant in effecting any cow-related measurements. This suggests that while the compost bedding had relatively high bacteria counts, that was not a major factor effecting the microbial population on the teat skins, teat ends, or in the milk. These findings suggest that when a CBP barn is well-managed, the complex environmental interactions produce a soft, comfortable bedding material that does not increase the risk of mastitis in dairy cows. Effective bedding management practices must occur to prevent bedding characteristics, such as moisture, to get out of the recommended range. When drastic changes begin occurring in the compost bedding, that is likely when cows would have a higher risk of mastitis.

The teat skin and teat end bacteria counts were more influenced by specific times during the transition period than environmental factors. From pre- to postpartum, teat skin Staph. counts decreased and coliforms increased. This was likely due to proper post-dipping management practices in the parlor which seemed to effectively decrease natural skin microflora bacteria species (such as Staph. species). Additionally, parity was a much greater factor that contributed to teat skin bacteria counts and milk bacteria counts. Older cows had more bacteria species present on the teat skin but fewer IMI, compared to first lactation heifers which had much fewer bacteria counts on the teat skin but experienced much more IMI. Results also showed moderately positive relationship between teat skin

and teat end bacteria counts, and a weak positive relationship between teat end and milk bacteria counts. It's important to note the relationships found between the cow-level variables were only simple associations, thus more research is needed to determine the complex microbial relationship and causations. To conclude, the microbial population transfer from bedding to the teat skins does not occur when a CBP barn is well-managed. The microbial population transfer from the teat skin to the teat end was relatively strong, as expected. However, the teat end bacteria counts were not strongly associated with milk bacterial counts. Thus, there is enough evidence to state that high CBP bedding bacteria counts are not a risk factor for mastitis when the CBP barn is well-managed.

4.6 Acknowledgements

The authors would like to thank the farm staff at the University of Kentucky's Coldstream Dairy for their assistance and cooperation on this project. We would also like to thank the University of Kentucky's Division of Regulatory Services for all their time and effort put into running nutrient analyses for the bedding samples. Finally, we would like to thank the graduate, undergraduate, and staff members who assisted in laboratory analysis and data collection.

Table 4.1 Descriptive statistics of cow variables (parity, milk TBC, milk coliforms, somatic cell count, teat end scores (0-5; 0.5 scoring system), teat skin TBC, coliforms, Staph. and Strep counts, and teat end TBC, coliforms, Staph. and Strep. counts), where n = 352 for milk-related samples, n = 639 for teat skin/end-related samples and n = 26 for parity.

	Mean	Std Dev	Min	Q1	Median	Q3	Max
Parity	2.13	1.24	1.00	1.00	2.00	3.00	6.00
Milk TBC[1] [a]	0.54	1.15	0.00	0.00	0.00	0.00	4.79
Milk coliforms[1]	0.03	0.19	0.00	0.00	0.00	0.00	2.40
SCC[2] (log_{10})	3.61	1.95	0.00	3.30	4.08	4.89	6.88
Teat End Scores	1.23	0.64	0.00	1.00	1.50	1.50	3.50
Teat Skin TBC *	5.93	0.51	4.24	5.55	5.95	6.28	7.16
Teat End TBC *	3.46	1.26	1.00	3.01	3.61	4.09	6.53
Teat Skin coliforms *	2.38	0.74	1.00	1.90	2.42	2.94	3.40
Teat End coliforms *	1.28	0.43	1.00	1.00	1.00	1.30	3.40
Teat Skin Strep.[b] *	4.44	1.69	2.00	3.18	3.70	6.76	6.76
Teat End Strep. *	2.37	0.68	2.00	2.00	2.00	2.48	6.76
Teat Skin Staph.[c] *	5.07	1.64	2.00	3.57	4.03	6.76	6.76
Teat End Staph. *	2.63	1.20	2.00	2.00	2.00	2.74	6.76

[1] log_{10} cfu/ml

* log_{10} cfu/g

[2] SCC = somatic cell count

[a] TBC = total bacteria count

[b] Strep. = *Streptococcus* spp. counts

[c] Staph. = *Staphylococcus* spp. counts

Table 4.2 Mastitis prevalence (percentage of culture-positive quarters) by experimental week, where Experimental Week 0 = 48-72h postpartum and Experimental Week 8 = 60 ± 7 DIM

Experimental Week	Mastitis Prevalence
0	27.88%
1	20.93%
2	25.93%
3	15.87%
4	23.08%
5	14.29%
6	33.33%
7	18.60%
8	17.48%

Table 4.3 Pearson correlation coefficients between cow variables (parity, milk TBC, somatic cell count (SCC), teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph. counts, teat end Staph. counts, teat skin Strep. counts, and teat end Strep. counts) where n = 352 for milk samples, n = 639 for teat skin/end samples and n = 26 for parity. Relationships were deemed significant at $p < 0.05$. Correlations were considered (±) strong when $r \geq 0.40$ and (±) moderate at $r \leq 0.39$ - ≥ 0.20.

	Milk TBC[1 a]	SCC[2] (log_{10})	Skin TBC*	End TBC*	Skin coliforms*	End coliforms*	Skin Staph.[b]*	End Staph.*	Skin Strep.[c]*	End Strep.*	
Parity	-0.23	-0.09	0.25	-0.02	0.21	0.08	0.15	-0.05	0.05	-0.05	r
	<0.001	0.03	<0.001	0.65	<0.001	0.05	<0.001	0.25	0.23	0.30	*p-value*
Milk TBC[1 a]	1.00	0.27	0.10	0.18	0.08	0.07	0.02	0.24	0.06	0.16	r
		<0.001	0.02	<0.001	0.08	0.09	0.72	<0.001	0.17	0.001	*p-value*
SCC (log_{10})		1.00	0.07	0.08	0.11	0.14	-0.05	0.09	0.03	0.07	r
			0.11	0.06	0.01	0.001	0.29	0.03	0.47	0.08	*p-value*
Skin TBC*			1.00	0.42	0.53	0.28	0.47	0.25	0.37	0.21	r
				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*p-value*
End TBC*				1.00	0.15	0.17	0.24	0.54	0.22	0.45	r
					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	*p-value*
Skin coliforms*					1.00	0.46	0.21	0.03	0.38	0.11	r
						<0.001	<0.001	0.50	<0.001	0.005	*p-value*
End coliforms*						1.00	0.13	0.14	0.19	0.22	r
							0.001	0.006	<0.001	<0.001	*p-value*
Skin Staph.*							1.00	0.22	0.24	0.16	r
								<0.001	<0.001	<0.001	*p-value*
End Staph.*								1.00	0.16	0.57	r
									<0.001	<0.001	*p-value*
Skin Strep.*									1.00	0.22	r
										<0.001	*p-value*

[1] log_{10} cfu/ml

*$\log_{10}$ cfu/g
[2] SCC = Somatic cell count
[a] TBC = Total bacteria count
[b] Staph. = *Staphylococcus* spp. counts
[c] Strep. = *Streptococcus* spp. counts

Table 4.4 Univariate mixed models of the cow variables (milk TBC, SCC, teat (T.) skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph., teat end Staph., teat skin Strep., and teat end Strep. counts) where Estimate (Est.) is the LSMeans of each variable by experimental week (-1 = prepartum; 0 = 48-72h postpartum, 1-8 = biweekly samples through 60DIM).

Expt Wk[3]	Milk TBC[1, A] Est.(SE)	SCC[2] (log_{10}) Est.(SE)	T. Skin TBC[*A] Est.(SE)	T. End TBC[*] Est.(SE)	T. Skin coliforms[*] Est.(SE)	T. End coliforms[*] Est.(SE)	T. Skin Staph.[*C] Est.(SE)	T. End Staph.[*] Est.(SE)	T. Skin Strep.[*D] Est.(SE)	T. End Strep.[*] Est.(SE)
-1	.	.	6.07 (0.05)^{a}	4.54 (0.11)^{b}	2.09 (0.07)^{a}	1.19 (0.04)^{a}	5.67 (0.16)^{a}	4.08 (0.09)^{b}	4.72 (0.16)^{a}	2.85 (0.06)^{b}
0	0.76 (0.11)^{a}	5.31 (0.17)^{a}	6.11 (0.05)^{a}	3.65 (0.11)^{a}	2.75 (0.07)^{b}	1.46 (0.04)^{b}	4.53 (0.16)^{b}	2.63 (0.09)^{a}	4.66 (0.16)^{a}	2.46 (0.06)^{a}
1	0.59 (0.15)^{a}	4.13 (0.24)^{cd}	5.89 (0.07)^{ac}	3.15 (0.17)^{ac}	2.32 (0.11)^{a}	1.30 (0.06)^{ab}	5.06 (0.24)^{ab}	2.28 (0.15)^{a}	3.81 (0.25)^{a}	2.25 (0.09)^{a}
2	0.50 (0.19)^{a}	4.01 (0.30)^{cd}	6.02 (0.09)^{ac}	3.46 (0.22)^{ac}	2.32 (0.13)^{ab}	1.39 (0.08)^{ab}	5.62 (0.30)^{a}	2.45 (0.19)^{a}	5.01 (0.32)^{a}	2.39 (0.12)^{a}
3	0.48 (0.14)^{a}	3.26 (0.21)^{bcd}	5.72 (0.06)^{bc}	3.03 (0.14)^{c}	2.23 (0.09)^{a}	1.25 (0.05)^{ab}	4.65 (0.19)^{b}	2.33 (0.13)^{a}	4.37 (0.21)^{a}	2.18 (0.08)^{a}
4	0.42 (0.16)^{a}	3.37 (0.25)^{bcd}	5.88 (0.08)^{ac}	3.07 (0.18)^{ac}	2.55 (0.11)^{b}	1.28 (0.07)^{ab}	4.82 (0.25)^{ab}	2.19 (0.16)^{a}	4.59 (0.26)	2.32 (0.10)^{a}
5	0.43 (0.14)^{a}	2.91 (0.21)^{bd}	5.89 (0.06)^{ac}	3.19 (0.14)^{ac}	2.41 (0.09)^{ab}	1.26 (0.05)^{ab}	5.43 (0.20)^{a}	2.32 (0.13)^{a}	4.16 (0.21)^{a}	2.18 (0.08)^{a}
6	0.78 (0.16)^{a}	3.08 (0.25)^{bcd}	5.84 (0.08)^{ac}	3.04 (0.18)^{ac}	2.46 (0.11)^{ab}	1.20 (0.07)^{ab}	5.53 (0.25)^{a}	2.19 (0.16)^{a}	3.99 (0.26)^{a}	2.16 (0.10)^{a}
7	0.46 (0.16)^{a}	2.40 (0.25)^{b}	5.96 (0.08)^{ac}	3.26 (0.18)^{ac}	2.55 (0.11)^{b}	1.25 (0.07)^{ab}	4.98 (0.25)^{ab}	2.24 (0.16)^{a}	4.48 (0.26)^{a}	2.33 (0.10)^{a}
8	0.43 (0.11)^{a}	2.94 (0.17)^{b}	5.82 (0.05)^{bc}	3.23 (0.11)^{ac}	2.27 (0.07)^{a}	1.20 (0.04)^{a}	4.94 (0.16)^{b}	2.26 (0.09)^{a}	4.40 (0.17)^{a}	2.23 (0.06)^{a}

$^{a-d:}$ LS means (SE) within a column with a different superscript are statistically different at $p \leq 0.05$

[1] log_{10} cfu/ml

*log_{10} cfu/g

[2] SCC = Somatic cell count

[3] Expt Wk = Experimental Week (-1 = prepartum; 0 = 48-72h postpartum; 1-8 = biweekly samples through 60DIM)
[A] TBC = Total bacteria count
[B] Staph. = *Staphylococcus* spp. counts
[C] Strep. = *Streptococcus* spp. counts

Table 4.5 Pearson correlation coefficients between cow variables (milk TBC, SCC, teat skin TBC, teat end TBC, teat end Staph. counts, teat end Strep. counts) and bedding variables (internal temperature, surface temperature, moisture, pH, bedding TBC, bedding Staph. counts). All relationships displayed were statistically significant at $p < 0.05$. Variables were excluded from table that had no significant relationships. Correlations were considered (±) strong when $r \geq 0.40$, (±) moderate at $r \leq 0.39$ - ≥ 0.20 and (±) weak at $r \leq 0.19$ - > 0.12.

		Bedding characteristics					
		Internal temp.(°C)	Surface temp.(°C)	Moisture (%)	pH	Bed TBC* [a]	Bed Staph.* [c]
Cow-level variables	Milk TBC[1] [a]	r = -0.19	NS[2]	r = 0.13	NS[2]	NS[2]	NS[2]
	SCC[b] (log_{10})	r = -0.30	r = -0.27	r = 0.32	r = -0.25	r = -0.27	r = -0.20
	Teat Skin TBC* [a]	r = -0.24	r = -0.32	r = 0.18	NS[2]	NS[2]	NS[2]
	Teat End TBC* [a]	r = -0.25	r = -0.20	r = 0.15	NS[2]	NS[2]	NS[2]
	Teat End Staph.* [c]	r = -0.26	r = -0.26	r = 0.16	r = -0.13	NS[2]	NS[2]
	Teat End Strep.* [d]	r = -0.24	r = -0.18	r = 0.15	NS[2]	NS[2]	NS[2]

*log_{10} cfu/g

[1] log_{10} cfu/ml

[2] NS = Not statistically significant; values not displayed.

[a] TBC = Total bacteria count

[b] SCC = Somatic cell count

[c] Staph. = *Staphylococcus* spp. counts

[d] Strep. = *Streptococcus* spp. counts

Table 4.6 Pearson correlation coefficients between cow variables (SCC, teat skin TBC, teat end TBC, teat end Staph. counts, teat end coliforms, teat end Strep. counts) and weather variables (ambient temperature, relative humidity). All relationships displayed were statistically significant at $p < 0.05$. Variables were excluded from table that had no significant relationships. Correlations were considered (±) strong when $r \geq 0.40$, (±) moderate at $r \leq 0.39 - \geq 0.20$ and (±) weak at $r \leq 0.19 - > 0.12$.

		Weather variables	
		Ambient temperature (°C)	Relative Humidity (%)
Cow-level variables	SCC[1] (log_{10})	r = -0.26	NS[2]
	Teat Skin TBC* [a]	r = -0.23	r = 0.25
	Teat End TBC* [a]	r = -0.21	r = 0.24
	Teat End Staph.* [b]	r = -0.25	r = 0.16
	Teat End coliforms*	NS[2]	r = 0.19
	Teat End Strep.* [c]	r = -0.18	r = 0.22

[1] SCC = Somatic cell count

[2] NS = Not statistically significant; values not displayed.

*log_{10} cfu/g

[a] TBC = Total bacteria count

[b] Staph. = *Staphylococcus* spp. counts

[c] Strep. = *Streptococcus* spp. counts

Table 4.7 Final mixed model results of the relationship between the environmental variables (bedding moisture, compost internal temperature, C:N ratio, pH, bedding TBC, ambient temperature, and the week*experimental week interaction (Wk*ExptWk)) with the following cow response variables (Milk TBC, teat skin TBC, teat end TBC, teat skin coliforms, teat end coliforms, teat skin Staph., teat end Staph., teat skin Strep., teat end Strep, and SCC). Explanatory variables were removed from the models at $p > 0.10$. Week, experimental week, and Wk*ExptWk were included in all models regardless of significance.

Variables	Milk TBC[*] [c]		Teat Skin TBC[*] [a]		Teat End TBC[*] [a]		Teat Skin coliforms[*]		Teat End coliforms[*]	
	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value
Amb. Temp.[2]	NS		-0.02 (0.01)	0.001	NS		NS		NS	
Moisture[3]	NS		-1.06 (0.59)	0.07[g]	NS		NS		NS	
CIT[4]	NS		NS		NS		NS		NS	
C:N[5]	-0.02 (0.01)	0.09[g]	-0.02 (0.01)	0.001	NS		-0.02 (0.01)	0.004	NS	
pH	NS		NS		NS		NS		NS	
Bed TBC[*] [b]	NS		0.14 (0.06)	0.02	NS		0.34 (0.08)	<0.001	NS	
*Wk*ExptWk*[7]	*NS*	*0.25*	*Yes*	*0.03*	*Yes*	*<0.001*	*Yes*	*0.04*	*NS*	*0.31*

β (SE) = regression coefficients, where NS = not significant, and were not included in the final mixed models.
The last italicized row (Wk*ExptWk) represents a categorial variable, where "Yes" indicates it was statistically significant at $p < 0.05$.

Table 4.7 Continued.

Variables	Teat Skin Staph.[*d]		Teat End Staph.[*d]		Teat Skin Strep.[*e]		Teat End Strep.[*e]		SCC[f] (log_{10})	
	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value	β[1](SE)	*P*-value
Amb. Temp.[2]	-0.07 (0.02)	0.003	-0.04 (0.01)	0.003	NS		NS		NS	
Moisture[3]	NS		NS		NS		NS		4.93 (1.60)	0.002
CIT[4]	0.03 (0.01)	0.01	0.01 (0.01)	0.10[g]	NS		NS		NS	
C:N[5]	NS		NS		-0.04 (0.02)	0.01	NS		NS	
pH	NS		NS		NS		NS		-1.59 (0.56)	0.01
Bed TBC[*b]	NS		NS		NS		NS		NS	
*Wk*ExptWk*[6]	*Yes*	*<0.001*	*Yes*	*0.01*	*Yes*	*0.001*	*NS*	*0.80*	*Yes*	*0.001*

[*] log_{10} cfu/g

[1] β = Regression coefficients

[2] Amb. Temp. = Ambient temperature (°C)

[3] Moisture = Bedding moisture (%)

[4] CIT = Compost internal temperature (°C)

[5] C:N = Bedding carbon-to-nitrogen ratio

[6] Wk*ExptWk = Interaction between the week and experimental weeks

[a] TBC = Total bacteria count

[b] Bed TBC = Bedding total bacteria count at log_{10} cfu/g of DM

[c] log_{10} cfu/ml

[d] Staph. = *Staphylococcus* spp. counts

[e] Strep. = *Streptococcus* spp. counts

[f] SCC = Somatic cell count

[g] Variables showed a tendency with statistical significance at $0.10 \leq p > 0.05$

Table 4.8 Least square means of the cow variables (milk TBC, teat skin TBC, teat skin coliforms, teat skin Staph. counts, and teat skin Strep. counts) by parity. The response variables that had the explanatory variable "parity" in the final mixed models were further analyzed to determine the difference between least squares means by parity.

	Milk TBC*[1]		Teat skin TBC*[1]		Teat skin coliforms*		Teat skin Staph.*[2]		Teat skin Strep.*[3]	
Parity	LSMeans (SE)	P-value	LSMeans (SE)	P-value	LSMeans (SE)	P-value	LSMeans (SE)	P-value	LSMeans (SE)	P-value
1	0.73 (0.19)[a]	0.001	5.70 (0.05)[a]	< 0.001	2.32 (0.06)[a]	< 0.001	4.59 (0.15)[a]	< 0.001	4.15 (0.14)[a]	< 0.001
2	0.06 (0.24)[b]	0.81	6.02 (0.07)[b]	< 0.001	2.43 (0.09)[ac]	< 0.001	5.61 (0.21)[b]	< 0.001	4.05 (0.21)[a]	< 0.001
3	0.02 (0.20)[b]	0.91	5.91 (0.05)[b]	< 0.001	2.58 (0.07)[bc]	< 0.001	5.27 (0.16)[b]	< 0.001	4.58 (0.16)[a]	< 0.001
6	0.14 (0.46)[b]	0.76	6.23 (0.13)[b]	< 0.001	2.99 (0.19)[b]	< 0.001	5.66 (0.42)[ab]	< 0.001	4.42 (0.42)[a]	< 0.001

[a-c] = Difference between the LSMeans (SE) in a column with a different superscript are statistically different at $p \leq 0.05$

* log10 cfu/ml (milk) or log10 cfu/g (teat skin samples)

[1] TBC = Total bacteria count

[2] Staph. = *Staphylococcus* spp. counts

[3] Strep. = *Streptococcus* spp. counts

Figure 4.1 Mean somatic cell count (SCC) (log_{10}) of milk samples (n = 532) by each pathogen identified and culture-negative (no growth), where the n-value at the end of each bar indicates the number of samples in each group. The blue vertical line is at the SCC of 5.30 log_{10} (equal to 200,000 cells/ml), where >200,000 cells/ml is considered an intramammary infection.

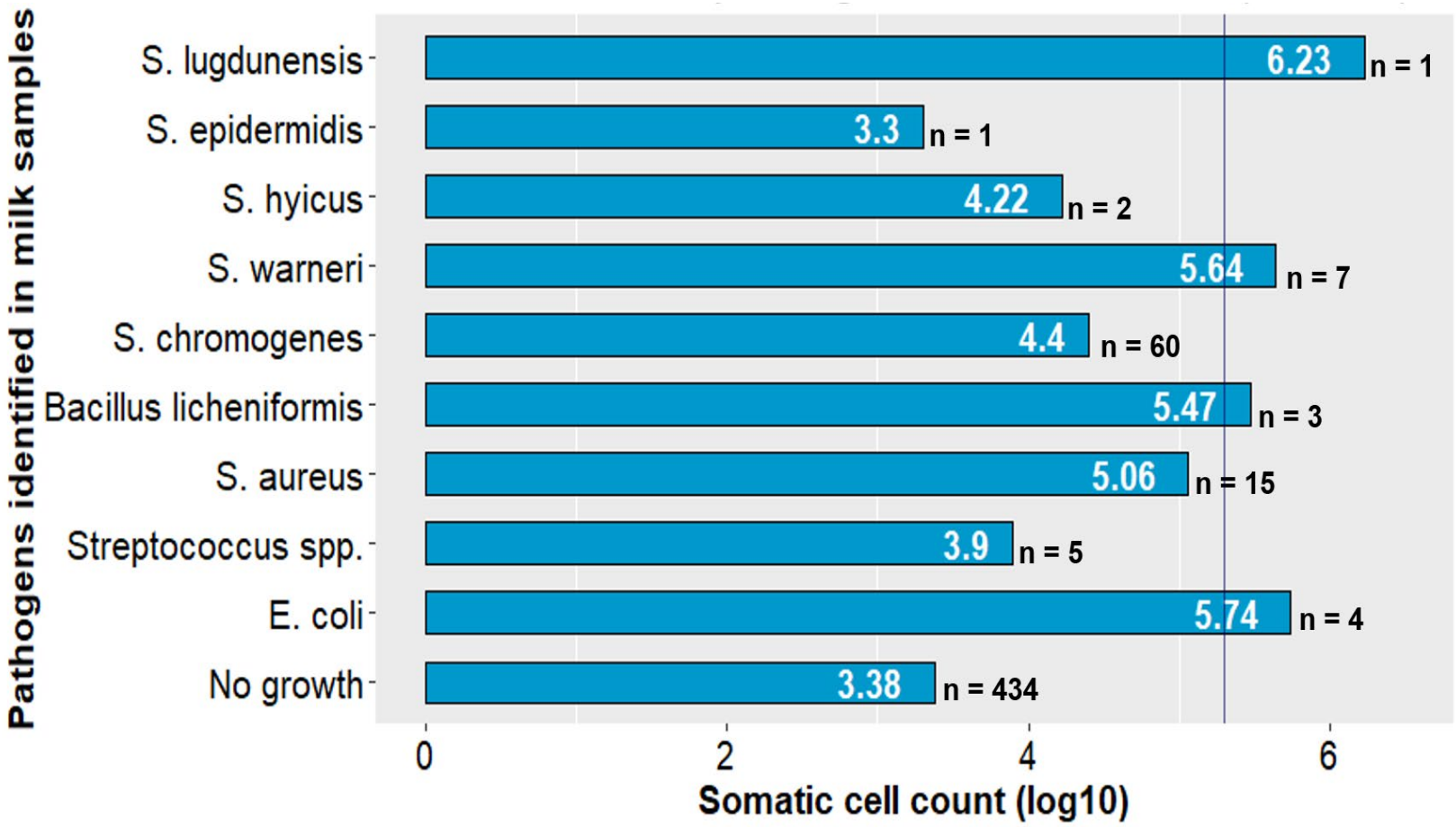


Figure 4.2 Mean teat skin bacteria counts of the three types of bacteria measured (coliforms, Staphylococcus spp. (Staph.), and Streptococcus spp. (Strep.)) by the experimental weeks (stages within the transition period/early lactation), where Week -1 = two weeks prepartum, Week 0 = 48-72h postpartum, and Weeks 1-8 = samples through 60 DIM.

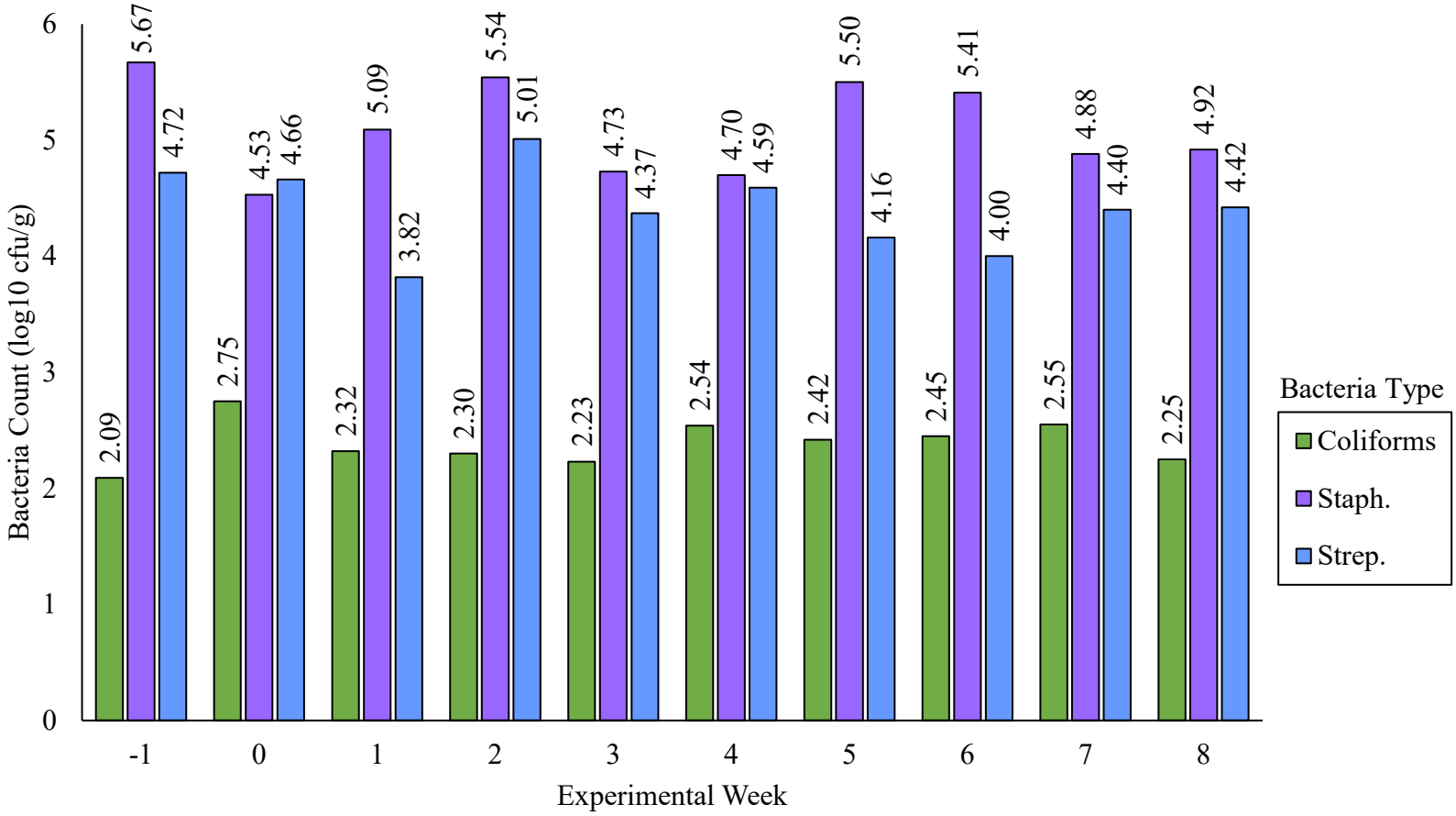


Figure 4.3 Mean teat end bacteria counts of the three types of bacteria measured (coliforms, Staphylococcus spp. (Staph.), and Streptococcus spp. (Strep.)) by the experimental weeks (stages within the transition period/early lactation), where Week -1 = two weeks prepartum, Week 0 = 48-72h postpartum, and Weeks 1-8 = samples through 60 DIM.

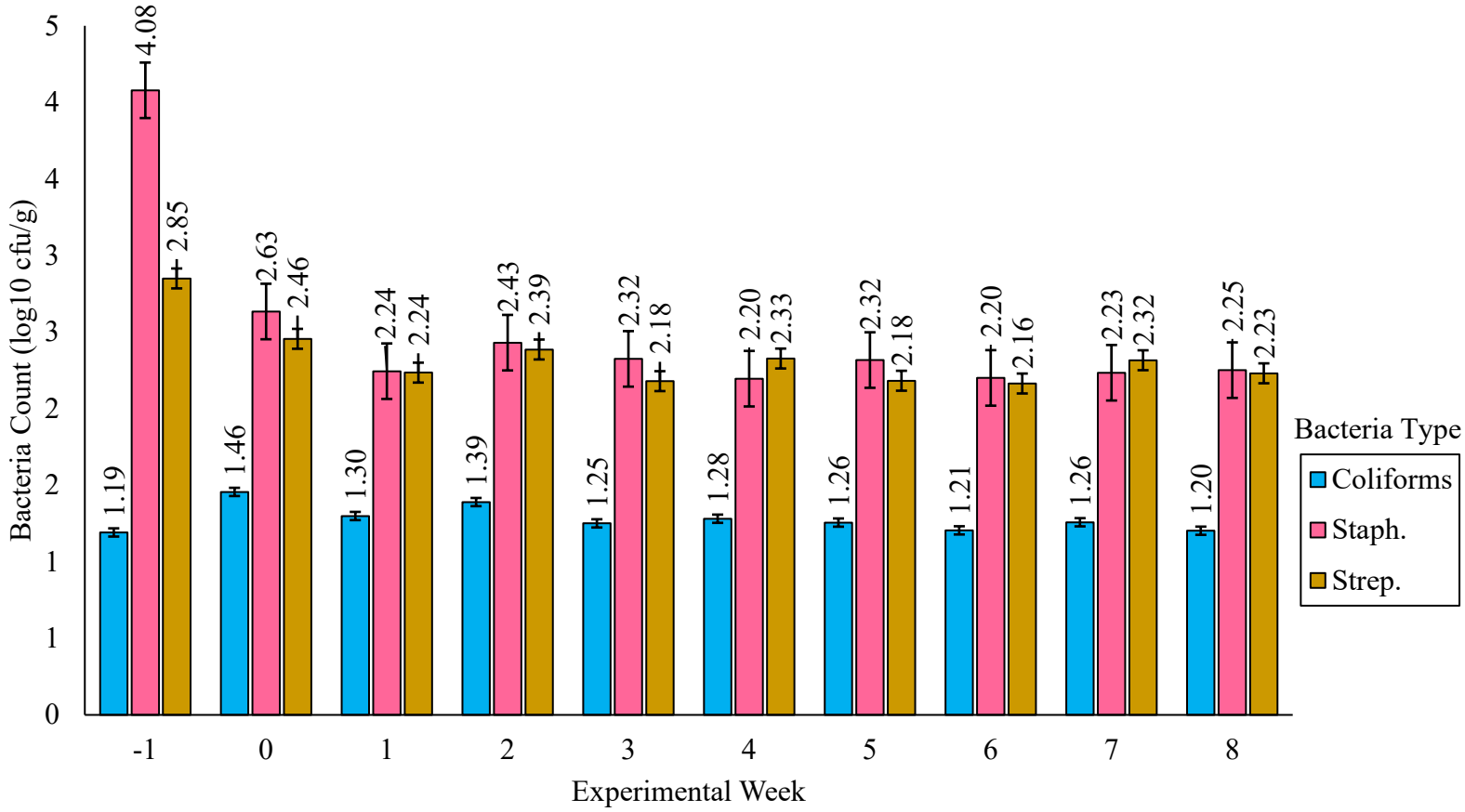
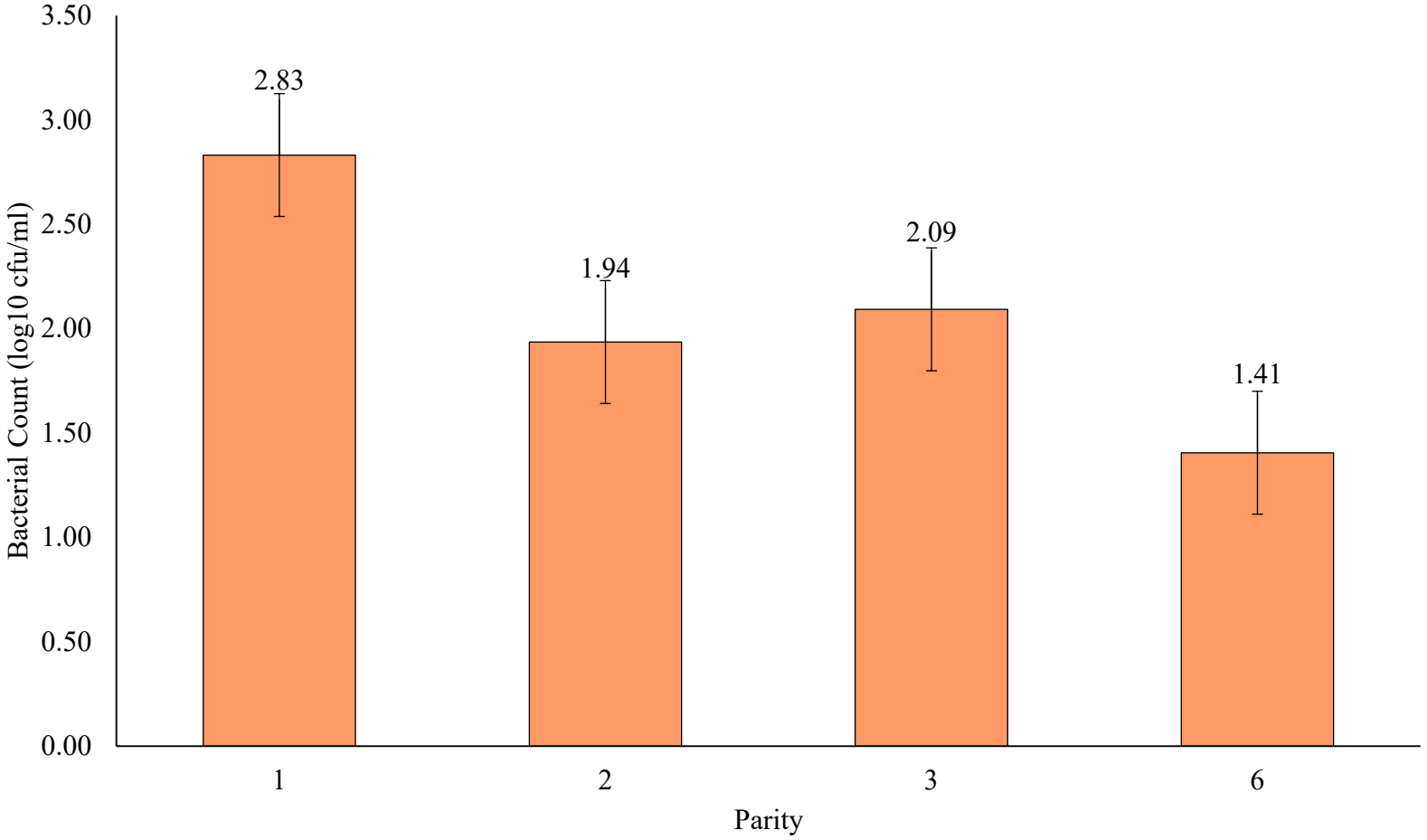


Figure 4.4. Mean milk TBC by parity of only culture-positive samples, where culture-positive was indicative of an intramammary infection. Samples with a milk TBC of 0 $\log_{10}$ cfu/ml were not included.



CHAPTER 5. OVERALL STUDY CONCLUSIONS, RECOMMENDATIONS, AND FUTURE RESEARCH

T.L. France, J.H.C. Costa, and M.C. Morgan

Department of Animal and Food Sciences, University of Kentucky, Lexington Kentucky 40546

The purpose of this study was to begin understanding the transfer of microbial populations from the bedding environment into the milk of dairy cows, which would indicate an intramammary infection. It is well understood that compost bedded pack (CBP) barns require microbial growth to achieve desired bedding characteristics, and cows' teats are exposed to that environment since they spend much of their time laying down. So, at the microbial level, what is occurring (or not occurring) that would lead to the conclusion that this type of housing system does not impose an increased risk of mastitis for cows living this way? To answer this, it was important to determine a "pathway" by which the microbes could transfer from bedding to milk, which was decided as the following: surface bedding samples, teat skin sponge samples, teat end swabs, and milk samples. Since this was an observational study, additional measurements were taken to account for environmental variation. This specifically included weather conditions and various time points within the transition and early lactation period. Ultimately, the results showed promise that a well-managed compost bedded pack barn poses no increased risk of mastitis in transition dairy cows.

One of the key take-aways from this study is the emphasis on good CBP management practices, specifically during colder weather conditions and when seasons begin to change (in this case, from winter to spring). This is because weather conditions strongly influence the compost bedding characteristics and can result in adverse pack performance. During March (i.e. the time when seasons were shifting from winter to spring), there were large fluctuations seen in the moisture content and bedding internal temperature. Additionally, the bedding characteristic variation observed was not only

ambient temperature-sensitive but also varied on the location within the pack. Specifically, the entrances to the pack and the outer corners had the highest moisture and lowest internal temperature. Keeping both in mind, producers should ensure adequate tilling throughout the entire pack, especially those locations. Moreover, adding more bedding material to those specific areas would be an effective way to increase internal temperature and decrease moisture. These strategies should be a top priority to producers during the winter and seasonal-shift timeframes, as uncontrollable environmental factors may attribute to poorer pack performance. CBP barn bedding characteristics and weather conditions are all inter-related and the complex interaction ultimately drives bedding bacterial population.

The environmental measures (bedding and weather) affected teat skin bacteria species differently and had limited influence on the teat end microbial population. Specifically, the CBP bedding bacteria counts were not practically significant in effecting any cow-related measurements. This suggests that while the compost bedding had relatively high bacteria counts, that was not a major factor affecting the microbial population on the teat skins, teat ends, or in the milk. When a CBP barn is well-managed, the complex environmental interactions produce a soft bedding material that does not increase the risk of mastitis in dairy cows.

Finally, the teat skin and teat end bacteria counts were more influenced by specific times during the transition period than environmental factors. From pre- to postpartum, teat skin Staph. counts decreased and coliforms increased. This was likely

due to proper post-dipping management practices in the parlor which seemed to effectively decrease natural skin microflora species (such as Staph. species). Additionally, parity was a much greater factor that contributed to teat skin bacteria counts and milk bacteria counts. Older cows had more bacteria present on the teat skin but fewer IMI, compared to first lactation heifers which had much fewer bacteria counts on the teat skin but experienced more IMI. Moreover, the microbial population transfer from the teat skin to the teat end had a relatively strong relationship, as expected. However, the teat end bacteria counts were not strongly associated with milk bacterial counts. Thus, there is enough evidence to state that high CBP bedding bacteria counts are not a risk factor for mastitis when the CBP barn is well-managed.

A major study limitation was that the relationships found between the cow-level variables were only simple associations, thus more research is needed to determine the microbial interactions and causations. This was largely due to the number of confounding variables that limited the statistical analysis and made the results difficult to interpret. Confounding variables were a common theme with all the statistical analyses, so interpretation of the data was not simply cause and effect. Additional research is needed to determine the microbiome of CBP barn bedding, with the inclusion of other common environmental microorganisms (such as *Bacillus* spp. or *Klebsiella* spp.). Research is also needed that looks deeper into the influence of bedding bacteria on mastitis through culture-positive, microbial identification measures rather than herd-level SCC or udder hygiene. It is clear that those parameters don't tell the whole story and that bedding bacteria load may not be as large of a risk factor for mastitis as previously suggested.

REFERENCES

Adkins, P. R. F., S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton. 2018. Molecular characterization of non-aureus Staphylococcus spp. from heifer intramammary infections and body sites. J. Dairy Sci. 101(6):5388-5403.

Ali, A. E., A. T. Andrews, and G. C. Cheeseman. 1980. Influence of elevated somatic cell count on casein distribution and cheese-making. J. Dairy Res. 47(3):393-400.

Astiz, S., F. Sebastian, O. Fargas, M. Fernández, and E. Calvet. 2014. Enhanced udder health and milk yield of dairy cattle on compost bedding systems during the dry period: A comparative study. Livestock Science 159(Supplement C):161-164.

Barberg, A. E., M. I. Endres, and K. A. Janni. 2007a. Compost dairy barns in Minnesota: a descriptive study. Appl. Eng. Agric. 23(2):231-238.

Barberg, A. E., M. I. Endres, J. A. Salfer, and J. K. Reneau. 2007b. Performance and Welfare of Dairy Cows in an Alternative Housing System in Minnesota. J. Dairy Sci. 90(3):1575-1583.

Bewley, J., L. Robertson, and E. Eckelkamp. 2017. A 100-Year Review: Lactating dairy cattle housing management. J. Dairy Sci. 100(12):10418-10431.

Bewley, J. and J. Taraba. 2009. Compost-bedded pack barns in Kentucky. University of Kentucky College of Agriculture Extension Factsheet. ID-178. Lexington, KY.

Bewley, J. M., J. L. Taraba, D. McFarland, P. Garrett, R. Graves, B. Holmes, D. Kammel, J. Porter, J. Tyson, S. Weeks, and P. Wright. 2013. Guidelines for Managing Compost Bedded-Pack Barns. The Dairy Practices Council.

Biasato, I., A. D'Angelo, I. Bertone, R. Odore, and C. J. I. J. o. A. S. Bellino. 2019. Compost bedded-pack barn as an alternative housing system for dairy cattle in Italy: effects on animal health and welfare and milk and milk product quality. 18(1):1142-1153.

Black, R. A., J. L. Taraba, G. B. Day, F. A. Damasceno, and J. M. Bewley. 2013. Compost bedded pack dairy barn management, performance, and producer satisfaction. J. Dairy Sci. 96(12):8060-8074.

Black, R. A., J. L. Taraba, G. B. Day, F. A. Damasceno, M. C. Newman, K. A. Akers, C. L. Wood, K. J. McQuerry, and J. M. Bewley. 2014. The relationship between compost bedded pack performance, management, and bacterial counts. J. Dairy Sci. 97(5):2669-2679.

Borm, A., L. Fox, K. Leslie, J. S. Hogan, S. Andrew, K. Moyes, S. Oliver, Y. Schukken, D. Hancock, and C. Gaskins. 2006. Effects of prepartum intramammary antibiotic

therapy on udder health, milk production, and reproductive performance in dairy heifers. J. Dairy Sci. 89(6):2090-2098.

Bradley, A. and M. J. V. R. Green. 2001. Aetiology of clinical mastitis in six Somerset dairy herds. 148(22):683-686.

Bradley, A. J. 2002. Bovine Mastitis: An Evolving Disease. The Veterinary Journal 164(2):116-128.

Bramley, A., L. Cullor, R. Erskine, L. Fox, R. Harmon, J. Hogan, S. Nickerson, S. Oliver, and L. Sordillo. 1996. Current concepts of bovine mastitis. National Mastitis Council.. 4ª. Inc., Madison, WI.

Bramley, A. J. and F. K. Neave. 1975. Studies on the Control of Coliform Mastitis in Dairy Cows. Br. Vet. J. 131(2):160-169.

Breen, J. E., M. J. Green, and A. J. Bradley. 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J. Dairy Sci. 92(6):2551-2561.

Burgstaller, J., J. Raith, S. Kuchling, V. Mandl, A. Hund, and J. Kofler. 2016. Claw health and prevalence of lameness in cows from compost bedded and cubicle freestall dairy barns in Austria. The Veterinary Journal 216:81-86.

Costa, J. H., T. A. Burnett, M. A. von Keyserlingk, and M. J. J. J. o. d. s. Hötzel. 2018. Prevalence of lameness and leg lesions of lactating dairy cows housed in southern Brazil: Effects of housing systems. 101(3):2395-2405.

Devriese, L. A. and H. De Keyser. 1980. Prevalence of different species of coagulase-negative staphylococci on teats and in milk samples from dairy cows. J. Dairy Res. 47(1):155-158.

Dickson, N., T. Richard, and R. Kozlowski. 1991. Composting to reduce the waste stream-A guide to small scale food and yard waste composting. Northeast regional agricultural engineering service.

Dohoo, I. R. and K. E. Leslie. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Preventive Veterinary Medicine 10(3):225-237.

Eberhart, R., H. Gilmore, L. Hutchinson, and S. Spencer. 1979. Somatic cell counts in DHI samples. in Proc. Annual meeting-National Mastitis Council, Inc.

Eckelkamp, E. A., J. L. Taraba, K. A. Akers, R. J. Harmon, and J. M. Bewley. 2016a. Sand bedded freestall and compost bedded pack effects on cow hygiene, locomotion, and mastitis indicators. Livestock Science 190:48-57.

Eckelkamp, E. A., J. L. Taraba, K. A. Akers, R. J. Harmon, and J. M. Bewley. 2016b. Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health. Livestock Science 190:35-42.

Endres, M. I. and A. E. Barberg. 2007. Behavior of Dairy Cows in an Alternative Bedded-Pack Housing System. J. Dairy Sci. 90(9):4192-4200.

Fairchild, T., B. McArthur, J. Moore, and W. Hylton. 1982. Coliform counts in various bedding materials. J. Dairy Sci. 65(6):1029-1035.

Favero, S., F. V. Portilho, A. C. Oliveira, H. Langoni, and J. C. Pantoja. 2015. Longitudinal trends and associations between compost bedding characteristics and bedding bacterial concentrations. J. Agric. Sci. 7(10):58.

Fávero, S., F. V. R. Portilho, A. C. R. Oliveira, H. Langoni, and J. C. F. Pantoja. 2015. Factors associated with mastitis epidemiologic indexes, animal hygiene, and bulk milk bacterial concentrations in dairy herds housed on compost bedding. Livestock Science 181(Supplement C):220-230.

Fox, L., T. Besser, and S. Jackson. 1996. Evaluation of a coagulase-negative variant of Staphylococcus aureus as a cause of intramammary infections in a herd of dairy cattle. J. Am. Vet. Med. Assoc. 209(6):1143-1146.

Fry, P. R., J. R. Middleton, S. Dufour, J. Perry, D. Scholl, and I. Dohoo. 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. J. Dairy Sci. 97(8):4876-4885.

Galama, P. 2011. Prospects for bedded pack barns for dairy cattle. Wageningen UR Livestock Research, Lelystad.

Goldberg, J. J., P. A. Murdough, A. B. Howard, P. A. Drechsler, J. W. Pankey, G. A. Ledbetter, L. L. Day, and J. D. Day. 1994. Winter Evaluation of a Postmilking Powdered Teat Dip. J. Dairy Sci. 77(3):748-758.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73(9):2820-2833.

Halasa, T., K. Huijps, O. Østerås, and H. J. V. q. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: a review. 29(1):18-31.

Harmon, R. J. 1994. Physiology of Mastitis and Factors Affecting Somatic Cell Counts1. J. Dairy Sci. 77(7):2103-2112.

Hertl, J., Y. Schukken, D. Bar, G. Bennett, R. González, B. Rauch, F. Welcome, L. Tauer, and Y. Gröhn. 2011. The effect of recurrent episodes of clinical mastitis caused by

gram-positive and gram-negative bacteria and other organisms on mortality and culling in Holstein dairy cows. J. Dairy Sci. 94(10):4863-4877.

Hogan, J., V. Bogacz, L. Thompson, S. Romig, P. Schoenberger, W. Weiss, and K. Smith. 1999. Bacterial Counts Associated with Sawdust and Recycled Manure Bedding Treated with Commercial Conditioners1. J. Dairy Sci. 82(8):1690-1695.

Hogan, J. and K. Smith. 1997. Bacteria counts in sawdust bedding1. J. Dairy Sci. 80(8):1600-1605.

Hogan, J. and K. L. Smith. 2012. Managing Environmental Mastitis. Veterinary Clinics of North America: Food Animal Practice 28(2):217-224.

Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conrad. 1989. Bacterial Counts in Bedding Materials Used on Nine Commercial Dairies1. J. Dairy Sci. 72(1):250-258.

Jain, N. C. 1979. Common Mammary Pathogens and Factors in Infection and Mastitis. J. Dairy Sci. 62(1):128-134.

Jamali, H., H. W. Barkema, M. Jacques, E.-M. Lavallée-Bourget, F. Malouin, V. Saini, H. Stryhn, and S. Dufour. 2018. Invited review: Incidence, risk factors, and effects of clinical mastitis recurrence in dairy cows. J. Dairy Sci. 101(6):4729-4746.

Janni, K., M. Endres, J. Reneau, and W. Schoper. 2007. Compost dairy barn layout and management recommendations. Appl. Eng. Agric. 23(1):97-102.

Jarp, J. 1991. Classification of coagulase-negative staphylococci isolated from bovine clinical and subclinical mastitis. Vet. Microbiol. 27(2):151-158.

Jasper, D. 1980. The coliform mastitis enigma. Pages 23-24 in Proc. 11th International Congress on Diseases of Cattle, Tel-Aviv, 20-23 October 1980. Israel Association for Buiatrics.

Klaas, I. C., B. Bjerg, S. Friedmann, and D. Bar. 2010. Cultivated barns for dairy cows-an option to promote cattle welfare and environmental protection in Denmark? Dansk Veterinærtidsskrift 93(9):20-29.

LeJeune, J. T. and M. D. Kauffman. 2005. Effect of sand and sawdust bedding materials on the fecal prevalence of Escherichia coli O157: H7 in dairy cows. Appl. Environ. Microbiol. 71(1):326-330.

Leso, L., M. Barbari, M. Lopes, F. Damasceno, P. Galama, J. Taraba, and A. J. J. o. D. S. Kuipers. 2019. Invited review: Compost-bedded pack barns for dairy cows.

Liang, D., L. Arnold, C. Stowe, R. Harmon, and J. J. J. o. d. s. Bewley. 2017. Estimating US dairy clinical disease costs with a stochastic simulation model. J. Dairy Sci. 100(2):1472-1486.

Lobeck, K., M. Endres, E. Shane, S. Godden, and J. Fetrow. 2011. Animal welfare in cross-ventilated, compost-bedded pack, and naturally ventilated dairy barns in the upper Midwest. J. Dairy Sci. 94(11):5469-5479.

Ma, Y., C. Ryan, D. Barbano, D. Galton, M. Rudan, and K. Boor. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83(2):264-274.

Makovec, J. and P. J. J. o. d. s. Ruegg. 2003. Results of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. 86(11):3466-3472.

Murphy, J. M. J. A. j. o. v. r. 1947. The genesis of bovine udder infection and mastitis; the occurrence of streptococcal infection in a cow population during a seven-year period and its relationship to age. 8(26):29-42.

Neave, F., F. Dodd, R. Kingwill, and D. Westgarth. 1969. Control of mastitis in the dairy herd by hygiene and management. J. Dairy Sci. 52(5):696-707.

Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. T. M. Noordhuizen. 2000. Classification and Longitudinal Examination of Callused Teat Ends in Dairy Cows. J. Dairy Sci. 83(12):2795-2804.

Nemet-Nejat, K. R. 1998. Daily life in ancient Mesopotamia. Greenwood Publishing Group.

Newman, L. E. 1973. Fresh sawdust bedding-a possible source of Klebsiella organisms. American Journal of Veterinary Research (EUA) no. 34 p. 979-980.

Nickerson, S. and S. Oliver. 2014. How well have United States dairy producers adopted mastitis-control technologies for reducing herd somatic cell counts improving milk quality? The Professional Animal Scientist 30(2):115-124.

NRC. 2001. National Research Council 2001. Nutrient requirements of dairy cattle 7:381.

Nyman, A. K., C. Fasth, and K. P. Waller. 2018. Intramammary infections with different non-aureus staphylococci in dairy cows. J. Dairy Sci. 101(2):1403-1418.

Oliveira, L., C. Hulland, and P. L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J. Dairy Sci. 96(12):7538-7549.

Ott, S. L. and P. R. Novak. 2001. Association of herd productivity and bulk-tank somatic cell counts in US dairy herds in 1996. J. Am. Vet. Med. Assoc. 218(8):1325-1330.

Paduch, J.-H., E. Mohr, and V. Krömker. 2012. The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. Vet. Microbiol. 158(3-4):353-359.

Paduch, J.-H., E. Mohr, and V. Krömker. 2013. The association between bedding material and the bacterial counts of Staphylococcus aureus, Streptococcus uberis and coliform bacteria on teat skin and in teat canals in lactating dairy cattle. J. Dairy Res. 80(2):159-164.

Parker, K., C. Compton, F. Anniss, C. Heuer, and S. J. J. o. D. S. McDougall. 2008. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic, or both, precalving. 91(1):169-181.

Patel, K., S. Godden, E. Royster, B. Crooker, J. Timmerman, and L. Fox. 2019. Relationships among bedding materials, bedding bacteria counts, udder hygiene, milk quality, and udder health in US dairy herds. J. Dairy Sci.

Paulrud, C. O. 2005. Basic concepts of the bovine teat canal. Vet. Res. Commun. 29(3):215-245.

Peters, J., S. Combs, B. Hoskins, J. Jarman, J. Kovar, M. Watson, A. Wolf, and N. Wolf. 2003. Recommended methods of manure analysis (A3769). University of Wisconsin, Madison, WI.

Petzen, J., C. Wolfanger, J. Bonhotal, M. Schwarz, T. Terry, and N. Youngers. 2009. Case Study: Eagleview Compost Dairy Barn. Cornell Cooperative Extension of Wyoming County, Warsaw, NY.

Philpot, W. 1979. Control of mastitis by hygiene and therapy. J. Dairy Sci. 62(1):168-176.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H. W. Barkema, and S. De Vliegher. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res. 74(4):478-483.

Piessens, V., E. Van Coillie, B. Verbist, K. Supré, G. Braem, A. Van Nuffel, L. De Vuyst, M. Heyndrickx, and S. De Vliegher. 2011. Distribution of coagulase-negative Staphylococcus species from milk and environment of dairy cows differs between herds. J. Dairy Sci. 94(6):2933-2944.

Quirk, T., L. Fox, D. Hancock, J. Capper, J. Wenz, and J. Park. 2012. Intramammary infections and teat canal colonization with coagulase-negative staphylococci after postmilking teat disinfection: Species-specific responses. J. Dairy Sci. 95(4):1906-1912.

Rendos, J., R. Eberhart, and E. Kesler. 1975. Microbial Populations of Teat Ends of Dairy Cows, and Bedding Materials1. J. Dairy Sci. 58(10):1492-1500.

Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Association between hygiene scores and somatic cell scores in dairy cattle. J. Am. Vet. Med. Assoc. 227(8):1297-1301.

Robles, I., D. Kelton, H. Barkema, G. Keefe, J. Roy, M. von Keyserlingk, and T. J. a. DeVries. 2019. Bacterial concentrations in bedding and their association with dairy cow hygiene and milk quality.1-15.

Rowbotham, R. and P. Ruegg. 2016. Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls. J. Dairy Sci. 99(8):6594-6608.

Rowbotham, R. F. and P. L. Ruegg. 2015. Association of bedding types with management practices and indicators of milk quality on larger Wisconsin dairy farms. J. Dairy Sci. 98(11):7865-7885.

Rowe, S., S. Godden, E. Royster, J. Timmerman, B. Crooker, and M. J. J. o. D. S. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows.

Ruegg, P. L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. J. Dairy Sci. 100(12):10381-10397.

Rynk, R., M. Van de Kamp, G. B. Willson, M. E. Singley, T. L. Richard, J. J. Kolega, F. R. Gouin, L. Laliberty, D. Kay, and D. Murphy. 1992. On-Farm Composting Handbook (NRAES 54). Northeast Regional Agricultural Engineering Service (NRAES).

Schreiner, D. and P. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci. 86(11):3460-3465.

Schukken, Y. H., R. N. González, L. L. Tikofsky, H. F. Schulte, C. G. Santisteban, F. L. Welcome, G. J. Bennett, M. J. Zurakowski, and R. N. Zadoks. 2009. CNS mastitis: Nothing to worry about? Vet. Microbiol. 134(1):9-14.

Shane, E., M. Endres, and K. Janni. 2010. Alternative bedding materials for compost bedded pack barns in Minnesota: a descriptive study. Appl. Eng. Agric. 26(3):465-473.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental Mastitis: Cause, Prevalence, Prevention1,2. J. Dairy Sci. 68(6):1531-1553.

Smits, M. and A. J. A. Aarnink. 2009. Verdamping uit ligbodems van vrijloopstallen; oriënterende modelberekeningen. Animal Sciences Group, Wageningen UR.

Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De Vliegher. 2011. Some coagulase-negative Staphylococcus species affect udder health more than others. J. Dairy Sci. 94(5):2329-2340.

Svensson, C., A.-K. Nyman, K. P. Waller, and U. Emanuelson. 2006. Effects of housing, management, and health of dairy heifers on first-lactation udder health in southwest Sweden. J. Dairy Sci. 89(6):1990-1999.

Taponen, S. and S. Pyörälä. 2009. Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from Staphylococcus aureus? Vet. Microbiol. 134(1):29-36.

Taponen, S., H. Simojoki, M. Haveri, H. D. Larsen, and S. Pyörälä. 2006. Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Vet. Microbiol. 115(1):199-207.

Thorberg, B.-M., I. Kühn, F. M. Aarestrup, B. Brändström, P. Jonsson, and M.-L. Danielsson-Tham. 2006. Pheno-and genotyping of Staphylococcus epidermidis isolated from bovine milk and human skin. Vet. Microbiol. 115(1-3):163-172.

Tomazi, T., J. L. Gonçalves, J. R. Barreiro, M. A. Arcari, and M. V. dos Santos. 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. J. Dairy Sci. 98(5):3071-3078.

Trinidad, P., S. Nickerson, and T. Alley. 1990. Prevalence of intramammary infection and teat canal colonization in unbred and primigravid dairy heifers. J. Dairy Sci. 73(1):107-114.

Waage, S., T. Mørk, A. Røros, D. Aasland, A. Hunshamar, and S. A. Ødegaard. 1999. Bacteria Associated with Clinical Mastitis in Dairy Heifers. J. Dairy Sci. 82(4):712-719.

Wagner, P. E. 2002. Bedded pack shelters. Lancaster Farming:47:36.

Wenz, J., S. Jensen, J. Lombard, B. Wagner, and R. J. J. o. d. s. Dinsmore. 2007. Herd management practices and their association with bulk tank somatic cell count on United States dairy operations. 90(8):3652-3659.

White, L., Y. Schukken, T. Lam, G. Medley, and M. Chappell. 2001. A multispecies model for the transmission and control of mastitis in dairy cows. Epidemiology & Infection 127(3):567-576.

Zdanowicz, M., J. Shelford, C. Tucker, D. Weary, and M. Von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. J. Dairy Sci. 87(6):1694-1701.

Zecconi, A., J. Hamann, V. Bronzo, and G. Ruffo. 1992. Machine-induced teat tissue reactions and infection risk in a dairy herd free from contagious mastitis pathogens. J. Dairy Res. 59(3):265-271.

Zhao, L., H. Keener, M. Wicks, M. Brugger, S. Wang, J. Rausch, A. Meddles, M. Klingman, R. Manuzon, and J. Upadhyay. 2012. Indoor environment of compost bedded dairy barns in Ohio. in Proc. Proceedings of multi-state workshop: composting bedded pack dairy barns. The Ohio State University Extension.

VITA

Tanya France was born in Highland Park, Illinois. She grew up in Lindenhurst, Illinois and graduated from Lakes Community High School in 2012. She obtained a Bachelor of Science degree in Animal Science from Iowa State University in May of 2017. During her time at Iowa State University, Tanya was a member of the Beta Kappa Chapter of Delta Zeta sorority. In Spring 2015, she had the opportunity to study abroad at University College Cork in Cork, Ireland. Following her semester abroad, Tanya interned at the Animal Rescue League of Iowa's animal behavior department, worked as an undergraduate research assistant for the Applied Swine Nutrition Laboratory, and was a summer intern for the Animal Nutrition Department at Omaha's Henry Doorly Zoo and Aquarium. She began studying for her Master's of Science in July of 2017. Her research focused on the influence of time and environmental factors on compost bedded pack barn bedding characteristics and bedding bacteria, bacterial load on the teat skin and ends and if this influences mastitis in dairy cattle.

Tanya acted as a teaching assistant for the Dairy Cattle Management course in Fall 2017, as well as for the Cow Signals course in Spring 2019. She had been a member of the University of Kentucky's Animal and Food Science Graduate Association for the duration of her studies at Kentucky. Additionally, Tanya has been a member of the American Dairy Science Association and National Mastitis Council since starting graduate school.