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Identifying factors associated with lying behavior and mastitis in organic dairy cows

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I am submitting herewith a thesis written by Victoria Couture entitled "Identifying factors associated with lying behavior and mastitis in organic dairy cows." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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ABSTRACT

Dairy cow health and welfare recommendations based on lying behavior and udder health are standardized in systems that rely on confined housing. However, factors may influence mastitis and behavior differently under organic pasture systems, due to changes in time budgets and treatment methods. Our objectives were to 1) determine the association of lying behaviors with cow-level factors, including milk yield, DIM, and parity when cows are managed under two organic management systems 2) identify probability of subclinical mastitis on organic farms in the southeastern region of the US and 3) characterize frequency and probability of mastitis-causing organisms by season, parity, and stage of lactation in this region. For objective 1, farms were categorized based on housing and feeding management. Lying behavior was seasonal and primiparous cows were more active than multiparous cows on all farms. Differences in behavior also were observed relative to milk yield on high input farms. Relative to objective 2 and 3, the probability for subclinical mastitis on organic farms was greatest in the summer, in older cows, and in early and late lactation. However, specific organisms found in milk from cows identified as recently having subclinical mastitis only differed in probability by parity. This indicates that specific pathogens may be driving the increased probability of subclinical mastitis in older cows. The association with season and stage of lactation may be more widespread and related to a decline in immune function due to other stressors present during summer and early lactation. Overall, a loss in milk production was associated with subclinical mastitis. Further work should identify 24-h time budgets and the chronicity of mastitis in organic herds, as well as the effect of cumulative stressors on udder health. Overall, our work establishes similarities between factors associated with behavior and mastitis on organic farms as has previously been established on

conventional farms. However, our results indicate that the seasonal variation in lying behavior and mastitis may indicate a need for welfare recommendations specific to pasture-based cows.

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CHAPTER I

LYING BEHAVIOR AND MILK QUALITY OF PASTURE-BASED AND ORGANIC DAIRY COWS: A REVIEW

INTRODUCTION

Dairy cows on 59.9% of dairies in the US are allowed pasture access. By utilizing pasture as a feed source or housing for dairy cows, a producer can potentially minimize feed costs, utilize untillable land, and improve consumer perception of the dairy industry. The latter benefit is due to the perceptions that the quality of dairy products is dependent on the animal's quality of life (Grunert et al., 2000), and that living naturally, i.e. with open space, pasture access, and a grass-based diet, can improve the quality of life for a cow (Cardoso et al., 2016). Organic management under USDA certification targets this idea of natural living by requiring that cows must receive at least 30% dry matter intake (DMI) from pasture for the duration of the grazing season (NOP, 2010). Furthermore, organic guidelines prohibit the use of antibiotics, hormones, and synthetic products for animal health, nutrition, or production. Ideally, this type of management promotes the welfare of dairy cows.

Indeed, natural living is an important aspect of animal welfare (Fraser et al., 1997). In addition to natural living, Fraser et al. (1997) suggested that definitions of welfare should also consider the biological functioning and affective state of an animal. Under this definition, all three aspects of welfare are fundamentally interconnected and dependent on the other. Therefore, while natural living is targeted by organic standards, it is imperative that we also consider the effect of organic management on biological functioning and affective state.

All three aspects of welfare can be assessed by examining the lying behavior of dairy cows and deviations from recommended standards. Grant (2004) suggests that cows should lay for 10 – 12 h/d and for every 1 h/d loss in lying time, there is an associated 1 kg/d loss in milk yield. This reflects the relationship between time spent lying and the cow's ability to biologically function well. Furthermore, this recommendation is within the amount of time a cow would naturally spend lying in a conventional free-stall system, as Ito et al. (2009) reported mean lying time of 2,033 cows was 11 – 12 h/d. Additionally, preventing this behavior results in signs of discomfort and negative affective state, such as kicking or weight shifting (Cooper et al., 2007). Current recommendations suggest that a decrease in lying is an indicator of depressed welfare; however, previous research reports that cows on pasture lay less in comparison to a confinement system (Hernandez-Mendo et al., 2007; Sepúlveda-Varas et al., 2014). Further consideration of different management practices should be examined in order to determine the role of pasture management on lying behavior and its role as an indicator of welfare.

Furthermore, mastitis is a reflection of biological functioning, while also negatively influencing affective state and the ability for a cow to live naturally. Because organic protocols, such as the exclusion of antibiotics, have consequences on mastitis, the welfare and productivity of dairy cows managed under this system should be considered separately from conventional systems. Although conventional producers have access to many preventative and treatment options which organic producers are prohibited from using, Cicconi-Hogan et al. (2013) found no difference between organic and conventional bulk tank somatic cell count (SCC). However, examining pathogens on an individual cow level might provide insight into mastitis dynamics on organic dairy farms, in order to improve welfare and production on these farms.

By gaining a better understanding of lying behavior and mastitis on pasture-based organic farms, we can begin to examine the effects of natural living on biological functioning and overall welfare, in an effort to formulate recommendations to producers. We aim to provide a critical review of behavior and mastitis dynamics on pasture and organic farms, and propose areas of further research to gain a better understanding of the welfare and productivity in these systems.

PASTURE USAGE AND PREFERENCE

Cows are grazing animals and outdoor access allows them to express a range of behaviors not exhibited as frequently indoors (Boyle et al., 2008). Therefore, pasture access may improve welfare and understanding preferences for environment can aid in providing this resource adequately. However, previous research has reported a complex relationship between preference for pasture and other factors beyond comfort while lying. To illustrate, Smid et al. (2018) compared preference for freestalls, a sand pack outdoor area, and a pasture area. While percent of time lying was similar on the sand pack and pasture area when confined to each, time spent in each area varied greatly during the choice phase, with 90% and 0.8% of the evening hours from 2000 h to 0730 h spent on pasture and sand pack, respectively. This demonstrates a similar level of comfort in lying in either area. However, despite this similarity in the two areas, other factors drove a preference for pasture. These differences raise questions about which factors may influence preference for pasture, beyond it being a more natural housing system allowing more comfort while lying.

Influence of Experience on Pasture Use

One factor which has been found to influence environmental preference is previous experience (Fraser and Matthews, 1997) and this may be a key factor in explaining pasture usage. Charlton et al. (2011b) reported that cows with limited experience on pasture spent only 1.6 h/20h on pasture, with the majority of time spent within cubicle housing. In contrast, a similarly designed study observed that experienced pasture cows prefer to spend 71.1% of time on pasture rather than in freestall housing, despite the location of supplemented feed (Charlton et al., 2011a). Further studies show a similar pattern of preference when cows have experience with pasture, with time spent on pasture ranging from 58 – 71% of observed time (Krohn et al., 1992; Charlton et al., 2013; Motupalli et al., 2014). Legrand et al. (2009) observed cows that were reared and housed on pasture during the dry period and housed in freestalls during lactation. While these cows spent 54% of their time outdoors, this was not significantly different than no preference (50% of time) for pasture. Additionally, Shepley et al. (2017) observed that when cows had previous year-long grazing experience, the majority of cows spent time on pasture during the day. Therefore, considering a cow's prior experience may be beneficial when interpreting her use of pasture and providing this resource.

Motivation for Pasture

Beyond evaluating preference for a resource, assessing a cow's motivation can quantify the strength of the preference. Motivation is measured by factoring in the amount of work that must be performed to achieve use of the resource, such as the distance walked or the weight moved. To illustrate this difference in motivation and preference, Krohn et al. (1992) indicated that cows spend 3 h/d more consuming fresh forages on pasture than a mixed ration, and 17.2 h/d outdoors, indicating a preference to eat grass and be outdoors. However, von Keyserlingk et al. (2017) tested cows' motivation to reach pasture versus freestalls with a total mixed ration and observed that cows would push a similar amount of weight on a gate to reach either resource, suggesting a similar motivation for either resource. While feed quality and forage type factors into preference and motivation (Rutter, 2006), this demonstrates the difference in motivation and preference.

Furthermore, measuring the distance a cow will walk to pasture is another way to assess motivation for pasture. Charlton et al. (2013) reported that cows with prior pasture experience spent 58% of their time outdoors, indicating a partial preference. However, a difference in pasture usage was observed when evaluating distance traveled, with 62.7% of the day spent on pasture when cows were required to walk 60 m to reach pasture and 7.9% reduction when the distance was increased 200 m. Time of day also played a role in preference, and during the daylight hours, cows preferred to be indoors at all distances, with as little as 21.2% of time spent outdoors when cows had to walk 260 m to pasture. This reflects similar results reported by Motupalli et al. (2014), who reported that cows preferred to spend time throughout the 24 h day on pasture both 38 and 254 m away, but when examining just daylight hours, cows preferred cubicle housing over the distant pasture. This interaction between distance and time of day could be influencing pasture usage due to general diurnal behavioral patterns or because of environmental changes throughout the day. Because cows were allowed TMR throughout the day at the barn, and feeding usually occurs during the daytime (DeVries et al., 2003), this may have encouraged cows to remain within the barn during these hours. However, preference for barn over pasture has also been associated with environmental conditions, such as an increased temperature humidity index (THI) throughout daylight hours (Legrand et al., 2009). The interaction between distance, diurnal patterns, and environmental conditions indicates the complex array of factors which can influence preference and motivation for an environment.

BEHAVIOR ON PASTURE

The usage of pasture creates unique time budgets, which can vary from confinement systems and are influenced by a variety of factors, including feeding management, housing, and health status. Understanding these factors can aid in formulating appropriate welfare recommendations that can improve health and production of grazing dairy cows, including those under organic management.

Housing and Environmental Conditions

One of the factors that has the largest impact on the lying behavior of dairy cows is the housing environment. The lying area influences comfort in lying and changing positions, which in turn can impact overall amount of time spent lying. Krohn and Munksgaard (1993) observed that cows kept in a loose-housing system with access to pasture spent 10.1 h/d lying while cows in tiestalls spent 11.8 – 13.0 h/d lying. The variation in the lying time in tiestalls was a result of shallow or deep bedding, or the allowance of time in an exercise area. This demonstrates the impact of housing on lying time, but also reflects a trend in lying time seen throughout the literature, with cows with pasture access lying less than cows in a confinement system. Legrand et al. (2009) illustrates this pattern in a comparative study that reported that cows spent 1.6 h/d more laying while confined indoors than confined to pasture.

However, when stall availability is reduced, cows do not use outdoor pasture more or less often and total lying time is also similar (Falk et al., 2012). Cows laid 7 – 8 h on pasture alone, with a total lying time of 10 – 11 h, suggesting that cows hit a ceiling in lying time on pasture, and were already lying for a maximum amount of time before stall availability was reduced. Maybe they also get a higher quality of lying time and therefore, the need is fulfilled in smaller window of time. This can lead into lying bouts to suggest decreased lying bouts would indicate increased comfort.

Qualities of the pasture itself can also influence cows' behavior. Cows graze more and lay more outdoors when the pasture is closer to the barn than when it is distant (Charlton et al., 2013; Motupalli et al., 2014). Increasing environmental conditions can influence cows to spend more time indoors (Legrand et al., 2009), and decreases lying time in a confinement system (Cook et al., 2007). However, even when shade structures are utilized by intensively grazing cows, there is no variation in total lying time from 9 h/d, daytime lying time, or grazing time, suggesting that cows are meeting their behavioral requirements regardless of heat abatement (Tucker et al., 2008; Palacio et al., 2015).

Influence of Feeding Management

In addition to housing management, feeding management also influences the behavior of grazing dairy cows. Cows in a confinement system fed a TMR diet typically spend 3 – 5 h/d feeding, which allows for the majority of the day to fulfill other requirements, such as laying and drinking, as well as time spent out of the pen (Grant, 2004). Whenever feeding management is altered, so is time spent feeding and the overall time budget. For example, when cows are in a grazing system, 10.1 h/d is spent eating and 10.6 h/d when offered 4 kg of concentrates (Rook et al., 1994). Two notable conclusions from this are that cows relying on pasture spend much more time reaching their nutrient requirement and offering even minimal supplement can create significant alterations to the time budget.

While the direct relationship between supplementation provided and time spent lying is unclear, studies have found indirect associations between feeding time, supplementation, and lying behavior. Legrand et al. (2009) reported a 1.0 h/d decrease in time spent eating TMR when cows were allowed free access to a high quality pasture and ad libitum TMR, with a similar 1.0 –

1.5 h/d decrease in lying time. A similar relationship was observed by Krohn et al. (1992), who studied a group of cows with access to pasture and ad libitum TMR. Time spent laying was observed to increase over the winter season (approximately 10 h/d in January compared to 5 h/d in September), as time spent eating grass and overall time spent eating decreased (0.7 h/d eating grass and 2.9 h/d eating in total in winter vs 1.3 and 5.3 h/d). This decrease in time spent eating is likely due to an easier availability of feed when only TMR is easily available and therefore feeding time does not limit lying time. Furthermore, these results suggest that cows may be meeting their lying time demands, even when the potential for grazing is at a maximum. Otherwise, cows are giving up the opportunity to lay down in order to meet their nutritional requirements and this may be negatively affecting their welfare, as cows are highly motivated to lay down even when feed deprived (Munksgaard et al.). However, the direct relationship between supplementation to pasture and lying behavior is unstudied.

Additionally, impact of seasonality on grazing time has been observed by others (Charlton et al., 2011a). This may be due to a general decline in quality of forages as the grazing season progresses, or limited forage availability. Clark et al. (2018) evaluated the effect of pasture state on behavior and observed that cows that were in a previously grazed, high quality pasture spent more time eating compared to cows that were in a fresh pasture of the forages. Furthermore, a linear increase in grazing during the afternoons coincided with a linear decrease in lying during this time, indicating a relationship between these behaviors. Other studies that have examined the effect of restricting herbage mass or time grazing on behavior have found differences in grazing time, but this change in behavior was not reflected in lying time (Ketelaar-de Lauwere et al., 1999; Motupalli et al., 2014). These observations indicate that grazing behaviors are more directly effected by the quality or quantity of forages rather than lying behaviors, perhaps due to the short term length of previous studies. Long term studies are needed to determine if pasture state has an indirect effect on lying behavior.

While research has begun to evaluate the effect of feeding management in pasture systems on behavior, much of this is based on temperate regions where grazing management is very different than management in warmer climates, like in the Southeast, USA. Pasture is frequently used in this area, but producers may face two unique situations, which are not clearly addressed in the literature. Firstly, limited, low quality forage may be provided in a pasture used mainly for housing, and so the producer encourages most or all DMI from TMR. This situation would limit grazing behaviors, while allowing potential effects on lying behaviors. Secondly, the producer may have goals to maximize DMI from pasture by limiting TMR and concentrate supplementation. Yet, due to hot, dry climates in the Southeast, pasture may not be suitable either from a cow comfort or nutrition standpoint. While these are scenarios occurring on-farm, research has not addressed the full effects of these management practices on behavior, welfare, or productivity, but insights into these areas would be beneficial.

Influence of Biological Functioning

In addition to external management dynamics that shape behavior in a variety of ways, internal, cow-level factors also contribute to variations. While circadian rhythms are influenced by environmental conditions, they are also a result of biological functioning. In cows, circadian rhythms contribute to the establishment of diurnal patterns. These patterns are further promoted by on-farm practices, such as morning feed delivery in a confinement system and milking, which encourages activity (DeVries et al., 2003). Furthermore, grazing animals have been observed to eat in a similar diurnal pattern, with a large meal in the evening, thought to fill the gut before a fast overnight, and another large meal in the morning to refill the gut with smaller meals throughout the day (Rook et al., 1994). Because of the inherently active nature of grazing, we expect to see lying behavior as an inverse of grazing, with long lying bouts throughout the night and smaller lying bouts consisting of less lying time throughout the day.

The association of DIM and milk yield with behavior on pasture is likely an indirect effect of the mutually exclusive relationship between grazing and lying. As nutrient requirements decrease, or as these requirements can be fulfilled in shorter amount of time, cows have the opportunity to engage in more lying behaviors. This relationship has been established in a confinement system by Bewley et al. (2010), who found that cows later in lactation and those producing less laid more, hypothetically because these cows had a lower nutrient requirement. A similar relationship between DIM and lying time was observed by Olmos et al. (2009). Cows were observed over 3 periods where mean DIM was 33, 83 and 193, respectively. From period 1 to 2, pasture cows had an increasing lying time, but lying time was similar between period 2 and 3. However, Tucker et al. (2007) found that cows' lying time remained similar from peak to mid lactation (9.8 to 9.6 h/d). This suggests that the impact of DIM may be dependent on outside factors, such as feeding management, which would influence the amount of time needed to feed and consequently how much time could be spent laying. Further research is needed to clarify the effect of DIM on behavior when cows are housed on pasture.

In addition, health has been observed to have an effect on behavior on pasture. Sepúlveda-Varas et al. (2014) observed dairy cows on pasture and reported that when primiparous cows became clinically ill, lying time increased, as it did when cows were severely lame. Furthermore, primiparous cows laid 1 h/d less than multiparous cows after calving (7.5 h/d vs 8.5 h/d). This study establishes that cows on pasture exhibit classical signs of sickness behavior (Johnson, 2002). However, because greater time spent lying may indicate a decline in time spent grazing, the ability to fulfill nutritive needs may be impaired, leading to greater negative impacts of poor health on pasture than in a confinement system. Further research should explore the progression of illness on pasture and the relationship with behavior.

Currently, numerous comparative studies have reported that cows on pasture lay less than cows in confinement (Krohn and Munksgaard, 1993; Legrand et al., 2009), with some lying times falling well below the recommended 10 – 12 h/d needed to maintain welfare (Grant, 2004; Sepúlveda-Varas et al., 2014). Because greater lying time is typically preferred in healthy dairy cows, these results suggest that cows in confinement may have superior welfare in comparison to pasture. To determine if lying behavior of pasture dairy cows is an accurate indicator of welfare, the individual variation between cows must be better understood in the context of both health and production.

MASTITIS ON PASTURE AND ORGANIC DAIRY FARMS

Mastitis, defined as inflammation of the mammary gland, is the most common disease affecting dairy cows. The association of mastitis with pain and abnormal behaviors indicates negative impacts on cow welfare (Leslie and Petersson-Wolfe, 2012; Fogsgaard et al., 2015). Due to cost of treatment, labor, replacement cows, and veterinary services, as well as the loss in current and future milk production, milk quality, and fertility, mastitis has a widespread impact on farm profitability. Subclinical mastitis causes elevations in somatic cell count ($\geq$ 200,000 cells/mL), decreases in milk production and quality, and presence of bacteria in milk secretion

(Harmon, 1994). Visible signs such as abnormalities in the milk or swelling in the mammary gland are indications of clinical mastitis, which may also include elevated body temperature and behavioral abnormalities (des Roches et al., 2017).

Bacteria, yeast, and mold can cause intramammary infections (IMI) resulting in mastitis. Bacterial pathogens are categorized as contagious or environmental. Spread of contagious pathogens occurs through exposure of an uninfected mammary quarter to infected milk, most commonly during the milking procedure. *Staphylococcus aureus* and *Streptococcus agalactiae* are common major contagious pathogen (USDA, 2016b), while Corynebacterium spp. are typically categorized as a minor contagious pathogen (Hogan et al., 1989); however, others consider it an opportunistic organism (Busato et al., 2000). In contrast to contagious pathogens, environmental pathogens are spread when the animal comes in direct contact with the pathogen, usually as it grows in the bedding, feed, or pasture. Although environmental pathogens, specifically Streptococci spp. are the most common major pathogen on dairy farms in the US (USDA, 2016b), several studies report that coagulase negative Staphylococci (CNS) spp. are the leading cause of mastitis (Hogan et al., 1989; Busato et al., 2000; Levison et al., 2016). Only recently have CNS spp. been studied in-depth (Sampimon et al., 2009); therefore, data are limited regarding pathogenesis, but they are commonly considered opportunistic pathogens part of normal skin flora. Characterizing mastitis-causing organisms aids in improving prevention and treatment mechanisms for mastitis on dairy operations.

Many effective treatment methods are available to control most pathogens on conventional dairy farms, including antibiotics and anti-inflammatories. However, organic dairies are faced with the additional challenge of managing mastitis without these resources, or any synthesized products (USDA, 2013). With more than 2,500 farms and 260,000 milk cows managed under USDA organic certifications, understanding mastitis dynamics on organic dairies is important. As there are no effective organic treatments for mastitis, there is a concern that treatment is withheld, although this is in disagreement with organic requirements (USDA, 2013). Because of this concern, comparative studies have aimed to identify any differences in mastitis and milk quality between conventional and organic management. Levison et al. (2016) reported that incidence rate of clinical mastitis was higher on conventional farms in Canada, although organic farms tended to have a higher bulk tank SCC (BTSCC; 222k vs. 272K). This effect on BTSCC may be related to feeding management, as organic dairies rely on pasture for > 30% dry matter intake (DMI), decreasing grain fed, yet an increased amount of grain provided is negatively associated with BTSCC (Cicconi-Hogan et al., 2013). In contrast, SCS was similar between conventional and organic dairies in North Carolina, as well as the proportion of cows with subclinical mastitis and pathogens identified through microbiological analysis (Mullen et al., 2013). In a review of mastitis on organic and conventional farms, Ruegg (2009) concluded that there was little difference between milk quality on these farms, despite differences in management.

Although differences in management have not been consistently reported to effect mastitis and milk quality between organic and conventional herds, other external factors can influence mastitis, like season of the year. Hogan et al. (1989) reported increased prevalence of clinical mastitis rates during the summer months, while rates were lowest during the spring. This may be because the climate at this time of year promotes the growth of environmental organisms, putting cows at greater risk for intramammary infections caused by these pathogens (Smith et al., 1985). Increased individual cow SCC also contributes to increased BTSCC during the summer in organic herds in Wisconsin, New York, and Oregon (Cicconi-Hogan et al., 2013), contributing to lower quality milk entering the supply chain at this time of year. This is particularly a challenge in areas of high heat and humidity, such as southeastern USA.

In addition, rates of subclinical and clinical mastitis varies as cows progress in lactation. Hogan et al. (1989) reported that the highest rate of clinical mastitis occurred in the first 90 d of lactation. Elevated SCC not associated with an IMI is commonly observed within the first 2 – 4 weeks postpartum (Dohoo, 1993), which suggests subclinical mastitis at this time may be a result of inflammation stimulated by parturition. In addition, increased SCC in late lactation is also observed (Busato et al., 2000). While this may be a dilution effect as milk production declines in late lactation, exposure to pathogens, particularly contagious pathogens, also accumulates as cows progress in lactation and this may drive increased rates of IMI at this stage of lactation (Breen et al., 2009). On conventional dairies, antibiotic dry cow therapy aids in relieving the effects of mastitis in late lactation, as it can be treated during the dry period. However, organic dairies can not use this resource and therefore, mastitis in late lactation may have carryover effects to the next lactation.

For reasons similar to those related to increased rates of mastitis in late lactation, cows of a greater parity are observed to experience increased rates of mastitis. Cows in later lactations accumulate exposure to both contagious and environmental pathogens, increasing their risk for mastitis on both organic and conventional farms (Hardeng and Edge, 2001; Breen et al., 2009). In addition, the external immune defenses, such as teat sphincters and skin, may also decline as cows progress in age and allow the mammary gland to become more susceptible to IMI. Because rate of culling for mastitis is lower for herds managed on pasture, organic dairies may have older cows, which would increase overall herd prevalence and make prevention in older cows even more necessary.

Many comparative studies have determined that mastitis dynamics are similar between conventional and organic dairies, suggesting that factors influencing mastitis would be similar. However, previous studies examine herds during a limited time frame and do not address chronicity of mastitis over a span of time on organic dairies. Because there may be a lack of treatment on organic dairies, mastitis may become chronic and influence associations between mastitis and stage of lactation, parity, and season. In addition, there are a limited number of studies examining mastitis dynamics on organic or pasture based operation in hot or humid climates, like in the southeastern region of the US, where maintaining milk quality can be a challenge (Mullen et al., 2013). Therefore, there is a need to examine chronicity of mastitis on organic dairy farms, as well as associations between mastitis and stage of lactation, parity, and season on organic dairies in the southeastern region of the US.

CHAPTER II

FACTORS AFFECTING THE LYING BEHAVIOR OF GRAZING DAIRY COWS UNDER TWO ORGANIC MANAGEMENT SYSTEMS

ABSTRACT

Dairy cow welfare recommendations based on lying behavior are standardized in systems that rely on confined housing. However, time budgets differ when cows are grazing and factors may influence behavior differently in pasture systems. Our objective was to determine the association of lying behaviors with cow-level factors, including milk yield, DIM, and parity when cows are managed under two different types of organic housing and feeding management systems. To do this, 5 USDA-certified organic dairy farms were enrolled and farms were categorized based on housing and feeding management. Low input (n = 3) systems utilized loose housing and relied on pasture for > 50% DMI. High input (n = 2) system managed cows in tiestall housing and relied on pasture for 30 – 50% DMI. Production and cow data for a random selection of focal cows (n = 15/farm/sampling period for 4 farms; n = 30/farm/sampling period for 1 farm) were accessed through DHI records. Lying behavior of focal cows was measured with an accelerometer during 28-d sampling periods conducted in spring, summer, and fall on low input farms and during only spring and fall on high input farms. Associations were analyzed using separate mixed model analyses of variance for low and high input systems to test the categorical fixed effects of level of milk production (high, low), stage of lactation (early, mid, late), and parity (1, 2, 3, $\geq$ 4) on lying time (h/d), lying bouts (n/d), lying bout duration (min/bout), and steps (n/d). Cows became less active from spring to fall on low and high input farms. Early lactation cows were more active than mid or late lactation cows managed under both systems as represented by decreased lying time. High producing cows on high input farms modified their lying bouts in comparison to low producing cows. These results indicate that factors influence behavior similarly on organic farms as has previously been established on

conventional farms. However, the seasonal variation in lying behavior may indicate a need for welfare recommendations specific to pasture-based cows.

INTRODUCTION

Currently, welfare recommendations for dairy cows are standardized in systems that primarily rely on confined housing facilities. A common welfare indicator is lying duration, with the recommendation that cows spend 12 h/d lying down (NFACC, 2009). Deviations in lying time suggest that a health or management event is disrupting lying behavior. Cows are highly motivated to engage in lying behaviors and therefore these disruptions affect the welfare of the animal (Munksgaard et al., 2005). Identifying natural variations in lying behavior influenced by physiological factors is critical to understand the welfare and management implications of lying behavior. Cows on 60% of dairies in the US are allowed pasture access, 7.5% of which are managed under organic standards (USDA, 2016a), yet current welfare recommendations do not account for differences in lying behavior stimulated by management on pasture.

Reported daily lying times on pasture-based dairies vary from 7.5 h/d (Sepúlveda-Varas et al., 2014) to 10.9 h/d (Hernandez-Mendo et al., 2007), with many factors, like housing and feeding management, which may contribute to this variation in lying time. Legrand et al. (2009) observed that when cows were confined to pasture they laid for 1.6 h/d less than when confined to freestalls. This may be due in part to the increased distance travelled to pasture which is associated with decreased lying time (Motupalli et al., 2014). Furthermore, feeding strategy can impact time budget, as cows meeting their nutritional requirement on pasture alone spend an additional 7.6 min/kg DMI eating compared to cows eating only harvested forages and concentrates (Oshita et al., 2008). Consequently, because of changes in the time budget, grazing cows spend less time lying than cows provided a harvested feed source (Dohme-Meier et al., 2014). Therefore, management strategies are key sources of differences in lying time.

Additionally, physiological factors impact lying time. Sepúlveda-Varas et al. (2014) reported that primiparous cows on pasture spent an hour less lying than multiparous cows during the postpartum period. Illnesses, such as lameness, also altered lying behavior. A comparable relationship has been observed in cows housed in a confinement system (Neave et al., 2017), but this relationship between parity and lying time on pasture has not been examined throughout the rest of lactation. Furthermore, in confinement systems, cows later in lactation or producing less milk spend more time lying, as a lower nutrient requirement allows for less time spent feeding (Bewley et al., 2010; Norring et al., 2012; Løvendahl and Munksgaard, 2016). Similarly, pasture-based cows in early lactation also spend less time lying than when in mid or late lactation (Olmos et al., 2009). However, the influence of milk yield on lying behavior on pasture is unknown.

Because management practices impact lying behavior, physiological factors may influence behavior differently under pasture-based systems compared to confinement systems, creating a need to identify these variations under differing management in order to understand welfare implications of lying behavior. Therefore, the objective was to determine the association of lying behaviors with cow-level factors, including milk yield, DIM, and parity when cows are managed under two different types of organic feeding and housing management systems.

MATERIALS AND METHODS

All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. The study was conducted from April to November 2017 on 4 USDA certified organic dairy farms located in Kentucky and one in Tennessee. Farms were recruited through the University of Tennessee and University of Kentucky Extension Cooperative with the requirement that all herds participate in regular Dairy Herd Information Association (DHIA) testing programs (Tennessee DHIA, Knoxville, TN; Mid-South Dairy Records, Springfield, MO).

Farm Categorization by Management System

Farms were categorized based on feeding and housing management into low and high input farms (Table 1; all tables and figures located in the appendix). Low input farms (**LI**) relied on pasture for > 50% of estimated DMI and utilized loose housing systems. Three farms met this criteria, with specific housing systems comprising of compost bedded pack barns (n = 2) or a concrete-based pen (n = 1) that was used primarily when weather restricted pasture access. Herd size for LI farms ranged throughout the year from 30 to 85 lactating cows with a mean of 55 ± 18 cows. Annual production was 5,208 ± 1,447 kg (mean ± SD). The dominant breeds on LI farms were Jerseys (n = 1), Holsteins (n = 1), and crossbred cows (n = 1). Cows were milked twice a day beginning between 0600 – 0700 h and 1800 – 1900 h in a herringbone (n = 2) or parallel parlor (n = 1).

The remaining 2 farms maintained a high input system **(HI)**, defined as relying on pasture for 30 – 50% of estimated DMI, with the majority of nutrient requirements being met by harvested forages and concentrates, and utilizing tie-stall housing. Herd size ranged throughout the year from 26 to 50 lactating cows with a mean of 39 ± 6 cows. Annual production was 8,941 ± 1,060 kg. All cows on both farms were Holsteins. Cows were milked twice a day beginning between 0500 – 0600 h and 1700 – 1800 h with bucket milkers in the tie-stall barn. Cows were restricted to the barn for 3 – 4 h/d around the time of milkings.

Producers, in conjunction with their organic certifier, estimated DMI from pasture as required through the USDA organic certification process (USDA, 2011). To do this, dry matter demand (DMD) was first estimated based on milk production and body weight. Then DMI from supplemented feeds such as harvested forages and concentrates was calculated. The DMI from pasture was the difference of DMD and DMI from supplemented feed.

Feeding and Management

Pasture was assessed for dry matter, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) throughout each season at every farm (Table 2). Briefly, 0.09 m^2 clippings (n = 5) were collected randomly throughout pastures. Samples then were measured for wet and dry weight to calculate dry matter and ground at the University of Tennessee forage laboratory and near-infrared spectroscopy (NIRS) was conducted to assess CP, ADF, and NDF. All farms utilized intensive rotational grazing management, where animals were allowed fresh pasture every 12 to 24 h. Silage, haylage, and concentrated feed was provided as supplementation to pasture and was delivered either directly before or after milkings.

Animals and Data Collection

Seasonal sampling periods (28 d) were conducted at each LI farm once each in spring (April to June), summer (July to August), and fall (September to November) and in spring and fall on HI farms due to producer preference and availability. Data on environmental conditions were accessed through online databases (Kentucky Mesonet at WKU, www.kymesonet.org; Weather Underground, www.wunderground.com). The daily temperature humidity index (THI) was calculated following Ravagnolo et al. (2000): THI = (1.8T + 32) – [(0.55 – 0.0055RH) × (1.8T – 26)]; where T = air temperature (°C) and RH = relative humidity (%).

Fifteen focal cows were randomly selected at the start of each sampling period at four farms, while 30 cows were randomly selected on the fifth farm for each season. These cows were followed until the end of the sampling period. On LI farms, mean DIM was 189.5 ± 93.1 (range: 4 – 605 DIM), mean parity was 3.1 ± 1.6 (range: 1 – 7 parity), and mean milk yield was 15.2 ± 5.8 kg (range: 4.1 – 30.8 kg) across the three sampling periods. On HI farms, mean DIM was 197.9 ± 90.5 (range: 3 – 433 DIM), mean parity was 3.5 ± 1.8 (range: 1 – 10 parity), and mean milk yield was 29.7 ± 7.2 kg (range: 17.2 – 50.8 kg) across the two sampling periods.

Behavior Data Collection. Accelerometers (IceTag, IceRobotics, Inc., Edinburgh, Scotland; (McGowan et al., 2007) were attached to focal cows at each farm to collect lying time (h/d), lying bouts (n/d), bout duration (min/bout per d), and steps (n/d). Data were recorded at 1-min intervals and summarized by 24 h. The procedure for attaching loggers was to visit farms on d 0 of each sampling period and attach to the rear fetlock of cows during milking and remove loggers on d 28. Technological difficulties delayed attachment during spring for LI farms until d 11 for two farms and d 20 for the third farm. This allowed for greater than the minimum of a 3-d sampling period needed to accurately estimate lying behavior (Ito et al., 2009). All other attachments occurred on d 0. The first 2 d of each sampling period were removed from analysis to account for an adaptation period (MacKay et al., 2012).

Production Measurements. Data from DHIA was accessed through PCDART (Dairy Records Management Systems, Raleigh, NC) to record individual focal cow data, including milk yield, parity, DIM, and SCC. Milk yield and SCC from the test date closest in time to behavioral data collection was used (mean difference: 9.4 ± 6.3 d). Milk yield then was categorized as either low, if milk yield was below the mean for the management system (LI or HI) or high, if milk yield was above the mean. Stage of lactation was categorized based on DIM (early: ≤ 100 DIM; mid: 101 – 200 DIM; late > 200 DIM).

Health Indicators. On d 0 and 28 of each sampling period, focal cows were assessed for body condition on a 5-point scale with quarter increments (Ferguson et al., 1994) with 1 being severely under-conditioned and 5 being severely over-conditioned. Locomotion was assessed on a 3-point scale, with 1 being normal and 3 being severely lame (NAHMS, 2014). Udder health was measured using somatic cell score (SCS) information accessed through DHI. Subclinical mastitis was diagnosed at SCS ≥ 4.

Statistical Analyses

Separate mixed model analyses of variance were performed for LI and HI systems using the MIXED procedure in SAS v 9.4 (SAS Institute Inc., Cary NC) to test the categorical fixed effects of level of milk production (high, low), stage of lactation (early, mid, late), and parity (1, 2, 3, ≥4) on behavioral measures. Behavioral outcomes of interest were lying time, lying bouts, lying bout duration, and steps. Seasonal sampling period (spring, summer, fall) was included as an additional fixed variable that may influence behavior. Cow was included as a random effect within farm and day was included as a repeated measure for each cow subject. Least square mean separation was performed using the LSMEANS option with Tukey adjustment. Reported are LS means with SE. Significance was determined at $P \leq 0.05$.

RESULTS

Health indicators were not included in the final analysis due to lack of variation across BCS, SCS, and locomotion scores (Table 3). During spring, THI ranged from 41 to 89 (mean ± SD; 63 ± 7), 59 to 93 (78 ± 3) during summer and 42 to 87 (66 ± 8) during fall.

Associations with Behavior on LI Farms

The daily lying time on LI farms followed a diurnal pattern with mean lying time in between morning and evening milking less than 15 min/h, while lying time peaked just before morning milking at 53 min/h (Figure 1). Season was associated with differences in steps, lying time, lying bout duration, and number of lying bouts ($P < 0.01$; Figure 2). As cows progressed into lactation, steps decreased while lying time and lying bout duration increased ($P < 0.01$; Table 4). Number of lying bouts also varied by stage of lactation ($P < 0.01$), with the fewest bouts taken during mid-lactation. First parity cows engaged in more bouts than third parity cows ($P = 0.03$) and bouts were of shorter duration in first and second parity ($P = 0.04$). There was no association of any behaviors with milk yield ($P > 0.05$).

Associations with Behavior on HI Farms

On HI farms, cows laid 20 – 40 min/h throughout the day, outside of milking times (Figure 1). Season was associated with steps, number of lying bouts, and lying bout duration on HI farms ($P < 0.01$; Figure 3). As stage of lactation progressed, lying time and lying bout duration increased ($P < 0.01$; Table 5). Parity ($P = 0.04$) and milk yield ($P < 0.01$) also were associated with differences in lying bout duration. High producing cows had an increased lying bout duration (113.5 ± 6.8 min/bout per d; mean ± SEM) compared to low producing cows (92.0 ± 6.4 min/bout per d; $P < 0.01$). High producing cows also engaged in fewer lying bouts than low producing cows (10.6 vs 11.7 n/d; $P < 0.01$). Lying time and steps were similar between high and low producing cows ($P > 0.05$).

DISCUSSION

Although previous studies have identified external causes of variation in lying behavior of dairy cows on pasture, the present study examined the impact of cow-level, physiological factors including stage of lactation, parity, and milk yield in the context of management. All of these factors were associated with aspects of lying behavior on pasture; however, the influence of factors were unique within each management system. Increased milk yield was associated with less lying bouts of greater duration on HI farms, but there was no association with milk yield on LI farms. Lying behavior of cows under both management systems differed relative to season, parity, and stage of lactation. Our research establishes the influence of physiological factors on lying behavior of cows under management systems that vary in housing and feeding strategies.

Milk yield did not influence behaviors on LI farms. Yet, on HI farms, cows producing > 29.7 kg/d engaged in less lying bouts for increased duration compared to cows producing < 29.7 kg/d, suggesting that the high producing cows were less active. Because increased milk production is associated with higher energy requirements, the energy conservation resulting from decreased activity may have promoted higher milk yield. However, previous reported observed that increased lying duration was associated with cows of lower production in both tie-stall and freestall housing and milking systems (Norring et al., 2012; Deming et al., 2013). In addition, an increase in milk yield decreased lying and increased feeding behavior before and after milkings in tie-stall housing (Norring et al., 2012). These differences in behavior may be an indirect effect of the increased energy requirement associated with higher production levels, requiring more time spent feeding and allowing less time to lay down (Fregonesi and Leaver, 2001). Specifically, primiparous cows require an additional 2.67 – 4.83 min feeding per kg of ECM yield (Løvendahl and Munksgaard, 2016). In the current study, production may not have been high enough to result in significant variations in lying time, as previous studies that reported an association between lying time and milk yield observed cows with a mean yield of 38.3 ± 7.8 and 35.1 ± 0.4 kg/d, respectively (Norring et al., 2012; Deming et al., 2013). This might especially contribute to the lack of association between milk yield and lying behaviors in LI herds, as mean milk yield was 15.2 kg/d in these systems. Understanding incremental changes in behavior as milk production increases would aid in management recommendations based on milk production levels.

Nutritional requirements also may be indirectly promoting the relationship between stage of lactation and lying behavior in both management systems. Early lactation cows spent 0.8 – 1.8 h/d less time lying than cows in other stages of lactation under both management systems. Similarly, Olmos et al. (2009) recorded lying behavior of cows on pasture over 3 periods, which were aligned with 33, 83, and 193 DIM and reported that lying time increased after the first sampling period. Furthermore, Løvendahl and Munksgaard (2016) studied primiparous cows housed in freestalls and reported that lying time was 1.07 h/d less when cows were 50 to 123 DIM comparted to 152 to 248 DIM, while feeding time tended to decrease at 152 to 248 DIM. This suggests that as nutritional requirements lessened throughout lactation, so did time dedicated to DMI and, therefore, increased time available to spend lying down. Our results indicate that a similar relationship between lying time and stage of lactation occurs on pasture as has previously been found in confinement systems.

Primiparous cows engaged in more lying bouts of shorter duration than third parity cows on LI farms, but bout duration was greater for primiparous cows compared to multiparous cows on HI farms. In agreeance with the observations from LI farms, Sepúlveda-Varas et al. (2014) reported that postpartum primiparous cows engaged in 1.3 more bouts per d in lesser duration with overall less time spent lying than multiparous cows on pasture. Similar observations related to variations in lying behavior between parities have been reported in a freestall system, as well as differences in feeding behavior between parities (Neave et al., 2017). Primiparous cows visited the feed bins more frequently and fed at a slower rate than multiparous cows, which was related to differences in body weight and milk production (Neave et al., 2017). This indicates that the relationship between lying behavior and parity may be driven by nutritional requirements. However, our results also may relate to housing system. Cows on LI farms were housed on pasture or in loose housing, which may allow for older, larger cows to lay more comfortably, resulting in less position changes. In contrast, cows in HI systems were managed in tiestalls, which may have been more restricting and less comfortable for older cows in comparison to primiparous cows, leading to shorter lying bouts for older cows. Observations on HI farms may be confounded with season, as primiparous cows were only observed in the fall on these farms. However, examining variations in lying behavior across parities in different housing systems would contribute to management recommendations.

Cows were less active on LI and HI farms in the spring compared to the fall, with only lying time on HI farms remaining similar between periods while all other behavior measures differed. This may be an effect of THI and environmental conditions. As heat stress increases, cows prefer to be in a barn (Legrand et al., 2009) and spend less time lying down (Cook et al., 2007). In the current study, mean THI peaked during summer and was lowest during fall, which does not follow the linear changes in steps and lying time on LI farms. While this may be influencing the shorter bout duration in the summer, this suggests other factors may be influencing other changes in behavior across season. Potentially, the quality or quantity of forages may be influencing behavior as time spent eating increases and time spent lying decreases when cows are grazing depleted or lower quality pastures (Clark et al., 2018). Furthermore, level of supplementation at every farm varied throughout the year to support pasture state and because cows fed harvested feeds spend more time lying (Dohme-Meier et al., 2014), this may be influencing the relationship between lying behavior and season. While the current study establishes relationships between seasonality of lying behavior on pasture-based dairies, future studies should examine the individual and cumulative effects of THI, pasture state, and feed supplementation on lying behavior of cows on pasture.

The present study aimed to quantify relationships between lying behavior and physiological factors within a management style to account for underlying differences in feeding and housing strategies. All enrolled farms were USDA-certified organic with ≥ 30% of DMI received from pasture during the grazing season. However, specific farm management techniques differed based on producers' goals. The LI farms aimed to reduce resource input, while accepting a similarly reduced output in the form of milk yield. In contrast, the HI farms aimed to maximize output, while increasing input of supplemented feed. Within that context, more behavioral measures varied by stage of lactation and parity on LI farms than on HI farms, whereas milk yield was influential on HI farms and not on LI farms. The differing relationships between management systems may be due to feeding and housing strategies. HI farms relied less on pasture for nutrition and housed cows in tie-stalls. Because cows spend less time grazing when supplemented with concentrated feeds compared to grazing alone (Rook et al., 1994), HI cows may have had more flexibility in time spent on other required activities, such as lying down, relative to LI farms. In addition, cows spend more time lying when housed in tie-stalls like on HI farms compared to loose-housing systems such as that utilized on LI farms (Krohn and Munksgaard, 1993), a difference potentially stemming from decreased time engaging in other behaviors like socializing and walking in tie-stall systems. This suggests that cows on HI farms may have been able to reach a ceiling in lying time because of feeding and housing management and therefore physiological differences between cows made less impact on lying behavior than on LI farms. In relation, LI feeding management may have restricted flexibility in time budgets, as there was greater reliance on pasture to reach DMI. This is supported by numerically lesser lying time on these farms and a daily lying pattern that reflects diurnal grazing patterns (Rook et al., 1994). Although implications related to overall time budget are limited as feeding time was not observed and management systems were not compared directly, the current study indicates the impact of management on the relationship between physiological factors and lying behavior.

CONCLUSIONS

Our findings establish the relationship between the physiological factors of stage of lactation, parity, and milk yield within the context of management differing by feeding and housing strategies on organic, pasture-based farms. Stage of lactation and parity was associated with differences in lying behavior on LI farms, as well as on some aspects of behavior on HI farms. In addition, behavioral differences were observed relative to milk yield on HI farms. Cows on all farms became less active from spring to fall. Complete time budgets of cows under varying management systems are needed to further understand the welfare implications of lying behavior of organic cows on pasture.

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CHAPTER III

PROBABILITY OF SUBCLINICAL MASTITIS AND MASITIS-CAUSING ORGANISMS IN ORGANIC DAIRY HERDS IN SOUTHEASTERN, USA

ABSTRACT

Organic farms face the challenge of managing mastitis without the use of antibiotics or synthetic products. Understanding factors that contribute to the probability of mastitis on organic dairies will aid with preventative strategies that promote cow welfare and farm profitability. The objectives were twofold: 1) identify probability of subclinical mastitis on organic farms in the southeastern region of the US and 2) characterize frequency and probability of mastitis-causing organisms by season, parity, and stage of lactation in this region. Five organic dairies using Dairy Herd Information (DHI) testing were enrolled. The DHI tests for 2017 were accessed for stage of lactation, parity, somatic cell score (SCS), and milk yield. A SCS > 4 was defined as positive for subclinical mastitis. Cows with subclinical mastitis were then aseptically milk sampled during farm visits (4 – 6/farm) and microbiological identification was conducted on milk samples. Logistic regression within generalized linear mixed models were utilized to test factors associated with the probability of subclinical mastitis and specific organisms within milk samples. The probability for subclinical mastitis on organic farms was greatest in the summer, in older cows, and in early and late lactation. However, specific organisms only differed in probability by parity. Staphylococci spp. had a greater probability in younger cows, whereas the probability of Corynebacterium spp. was highest in fourth or greater parities. Overall, a loss in milk production was associated with subclinical mastitis. These results indicate that specific pathogens may be driving the increased probability of subclinical mastitis in older cows. The association with season and stage of lactation may be more widespread and related to a decline in immune function due to other stressors present during summer and early lactation. Decreasing stress during these times may decrease probability for subclinical mastitis in organic herds.

INTRODUCTION

Mastitis, defined as inflammation of the mammary gland, is the most common disease affecting dairy cows in the United States (USDA, 2016). Organic farms face the challenge of managing this disease without the use of antibiotics or synthetic products (USDA, 2013). The lack of approved treatment poses a significant concern for cow welfare and profitability of organic farms (Bar et al., 2008; Leslie and Petersson-Wolfe, 2012). The proportion of cows with subclinical mastitis in organic herds in the US has been observed at 23.3% (Mullen et al., 2013). While this is similar to conventional farms, there are limited organic treatments for mastitis, making it a particular challenge for organic systems.

Understanding factors that contribute to mastitis incidence will aid with management decisions on organic dairies. One of these factors observed in conventional herds is season. Hogan et al. (1989) reported the rate of clinical mastitis during the summer was 0.58 ± 0.8 compared to 0.36 ± 0.07 at the lowest point during the spring. Similarly, there is an increase in bulk tank SCC during the summer on organic farms (Cicconi-Hogan et al., 2013). The climate at this time of year promotes bacterial loads in the environment, contributing to increased rates of environmental organisms isolated in relation to mastitis in conventional herds (Smith et al., 1985; Hogan et al., 1989). The heat and humidity during the summer in the southeastern region of the US may increase this effect on mastitis in organic herds.

Furthermore, differences in the rate of mastitis is associated with stage of lactation. Hogan et al. (1989) reported that the highest rate of clinical mastitis occurred in the first 90 d of lactation. During this time, cows are undergoing stress resulting from parturition and peak production, both of which are associated with increased SCC (Dohoo, 1993; Gröhn et al., 1995).

While Olde Riekerink et al. (2007) reported that incidence rate for clinical mastitis was greatest in early lactation, the likelihood of increased SCC was greatest for late lactation cows. Accumulated exposure to pathogens, a dilution effect of SCC as milk yield declines, and effect of chronic infections may contribute to increased likelihood of late lactation mastitis. Similar risks, including accumulated exposure and persisting infection, are present for cows of a greater parity and may be associated with the reported increased risk of mastitis seen in older cows (Hardeng and Edge, 2001; Breen et al., 2009).

The dynamics of subclinical mastitis in organic herds may differ from conventional dairies, as there is a lack of effective treatment and preventative measures during lactation and the dry period. This may allow for progression of the disease and may influence probability during certain times, especially across stage of lactation and parity. Additionally, the climate of the southeastern region of the US heightens challenges of summer heat and humidity. Therefore, the objectives of the current study were twofold: 1) identify probability of subclinical mastitis on organic farms in the southeastern region of the US and 2) characterize frequency and probability of mastitis-causing organisms by season, parity, and stage of lactation in this region.

MATERIALS AND METHODS

All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. The study was conducted on five USDA certified organic dairy farms located in Kentucky and Tennessee. Farms were recruited through the University of Tennessee and University of Kentucky Extension Cooperative with the requirement that all herds participate in regular Dairy Herd Information (DHI) testing programs (Tennessee DHIA, Knoxville, TN; Mid-South Dairy Records, Springfield, MO).

Participating Herds and Management

Production information for participating dairies was collected from DHI (Table 6). Farm A was utilizing DHI testing < 1 year at the time sampling began and therefore rolling herd average was not calculated. Mean DHI test period was 36.5 d across all farms. Cows on all farms were milked twice daily. Morning milking began between 0500 and 0700h and evening milking began between 1700 and 1900 h. Either iodine (n = 3) or hydrogen peroxide (n = 2) based products were used as a pre-disinfectant and iodine was used as a post-disinfectant. Peppermint-based udder cream was used to minimize the effects of clinical mastitis on farms A and B. Besides this, treatments were not administered to cows with subclinical or clinical mastitis within any herds.

Housing of lactating cows comprised of tiestalls, compost bedded packs, or concrete-based pens (Table 1). As required by USDA organic regulations, all herds had access to pasture and relied on pasture for > 30% of dry matter intake during the grazing season, which was at minimum through the months of April through October on these farms. Dry cows were managed on pasture.

Data Collection

Subclinical mastitis. To identify probability of subclinical mastitis within organic herds, all DHI tests from 2017 were accessed for individual cow SCS, milk weight (kg), days in milk (DIM), and lactation number. Stage of lactation was determined from DIM: > 100 DIM = early lactation; 100 – 200 DIM = mid lactation; and > 200 DIM = late lactation. The DHI test date was categorized by season according to the astronomical definition (Spring: March 20 – June 20; Summer: June 21 – September 21; Fall: September 22 – December 20; Winter: December 21 –

March 19). Cows were tested various numbers of times within a given season and these values were combined to give an overall proportion of subclinical mastitis events that occurred for an individual cow within a season. Cows were considered positive for subclinical mastitis when SCS was ≥ 4 (SCC = 200k cells/mL) or negative if SCS was < 4.

Mastitis-causing organisms. To characterize frequency and probability of mastitis-causing organisms, aseptic milk sampling was conducted during visits to the farms (n = 4 - 6/farm). Farm visits took place twice each during three sampling periods (period 1- April to June; period 2- July to September; period 3- October to November) for farms A, B and E and during period 1 and 3 for farms C and D. Within a period, visits to a single farm were 28-d apart. Following NMC guidelines (Oliver et al., 2004), aseptic milk samples were collected from each productive mammary quarter of cows that were positive for subclinical mastitis (SCS ≥ 4) on the DHI test date directly prior to the farm visit. Mean difference between sample date and DHI test date was 23.5 ± 17 d (mean ± SD). DHI records were retained for sampled cows, including SCS, milk weight, DIM, and lactation number from the test date prior to the visit.

Milk samples were frozen awaiting microbiological identification at the Tennessee Quality Milk Laboratory. Microbiological identification followed National Mastitis Council guidelines (Oliver et al., 2004). Briefly, 10 μL of milk from each quarter sample was plated on a quadrant of Trypticase soy agar with 5% sheep blood (BD, Sparks, MD). Plates were incubated at 37°C and growth was observed at 24-h intervals for 3 d. Bacteria were identified tentatively according to morphologic features, catalase test, and gram stain. Staphylococci spp. were further tested for coagulase by the tube coagulase method. The API Staph System (bioMerieux Inc., Hazelwood, MO, USA) was used to identify species of coagulase negative Staphylococci (CNS) isolates. The API Strep System (bioMerieux Inc.) was used to distinguish Streptococci species and the API 20E System (bioMerieux Inc.) was used to identify gram negative species. Samples with 1 or 2 organisms isolated were considered positive for IMI and samples with ≥ 3 organisms isolated or with *Bacillus* identified were considered contaminated. If an organisms was isolated in ≥ 1 quarter sample, a cow was considered positive for that pathogen on the sample date, with the possibility that a cow would be positive for > 1 organism.

Statistical Analyses

Probability of subclinical mastitis. To test differences occurred in daily milk weight (kg) between cows that were infected with subclinical mastitis and cows that were not, mixed model analysis of variance (ANOVA) was performed using SAS v 9.4 (SAS Institute Inc., Cary NC). The GLIMMIX procedure was utilized with the fixed effect of mastitis (presence or absence) and the random effects of cow within herd and season within cow and herd. The difference in least square means was determined using mean separation.

In addition, logistic regression within a generalized linear mixed model was used to test factors associated with the probability for subclinical mastitis. A binomial distribution in the form of events divided by trials was specified, where the number of subclinical cases detected equaled the events and the number of individual observations equaled the trials. Factors tested included season (spring, summer, fall, winter), stage of lactation on test date (early, mid, late), and parity (1, 2, 3, 4+). Cow was included as a random effect. The repeated measures over time were accounted for using a random residual of season, the subject of cow within herd, and an autoregressive covariance structure.

Probability of mastitis-causing organisms. Descriptive analyses were used to observe the distribution of mastitis-causing organisms at a mammary quarter-level using the frequency procedure of SAS. Logistic regression within generalized linear mixed models was used to test factors associated with the probability for a cow to be positive for specific mastitis-causing organisms. A binomial distribution in the form of events divided by trials was specified, where the observations positive for the organism equaled the events and the number of cow-level observations equaled the trials. Factors tested included season (spring, summer, fall, winter), stage of lactation on sample date (early, mid, late), and parity (1, 2, 3, 4+). Herd was included as a random effect. All factors of interest were forced into initial models. If convergence criteria were not met, single variables were removed until convergence was reached.

Reported is the model adjusted probability of subclinical mastitis and specific mastitis-causing organisms. Significance was determined at $P < 0.05$.

RESULTS

Probability of Subclinical Mastitis

A difference was observed in milk weight between cows negative for subclinical mastitis and cows positive for subclinical mastitis ($P = 0.02$). Mean test date milk weight was 21.1 ± 0.5 kg for cows negative for subclinical mastitis, while cows positive for subclinical mastitis had test date milk weight of 20.3 ± 0.5 kg.

Season was associated with the probability of subclinical mastitis ($P < 0.01$; Figure 4). In the summer, cows had 1.4 times the odds for mastitis compared to fall (OR = 1.4; 95% CI: 1.1, 1.8), 2.3 times the odds compared to winter (OR = 2.3; 95% CI: 1.3, 3.8), and 1.5 times the odds compared to spring (OR = 1.5; 95% CI: 1.2, 1.9). Probability of mastitis increased with parity (*P*

= 0.2; Figure 5). Cows in fourth or greater parities had 1.9 times the odds for subclinical mastitis compared to cows in first parity (OR = 1.9; 95% CI: 1.2, 3.0) and 1.7 times the odds compared to cows in second parity (OR = 1.7; 95% CI: 1.1, 2.6). Cows in third parity had 1.8 times the odds for subclinical mastitis compared to cows in first parity (OR = 1.8; 95% CI: 1.1, 2.9). Stage of lactation was associated with the probability of subclinical mastitis (*P* = 0.01; Figure 6). Cows in early lactation were 1.4 times more likely to have subclinical mastitis compared to cows in mid lactation (OR = 1.4; 95% CI: 1.1, 1.9), while cows in late lactation were 1.3 times more likely to have subclinical mastitis compared to cows in mid lactation (OR = 1.3; 95% CI: 1.0, 1.6).

Probability of Mastitis-Causing Organisms

A total of 128 cows were sampled at least once, with 65 cows meeting sampling requirements at more than one visit. This resulted in a total of 248 cow-level samples (n = 83 during period 1; n = 78 during period 2; n = 87 during period 3). A total of 992 quarters were sampled (n = 332 during period 1; n = 313 during period 2; n = 345 during period 3). No sample was collected from 48 non-productive quarters. Of the quarters sampled, 2% (n = 20) were considered contaminated. No growth was observed in 50.6% of samples (n = 501) and were considered negative for IMI, while 42.7% of samples (n = 423) were considered positive for IMI.

Two pathogens were isolated in 3.5% of quarter samples (n = 35). Of these samples, CNS spp. and *Streptococcus uberis* were most commonly observed with a second spp. (n = 15; 42.8%, n = 16; 45.7%). The combination of a CNS spp. with *S. uberis* was isolated in 17.1% (n = 6) of samples with 2 pathogens.

Only 1 pathogen was isolated in 39.2% (n = 388) of samples and these samples were used for further analyses. Of these samples, CNS spp. were most frequently isolated (n = 103), with

Staphylococcus chromogenes making up the majority of CNS samples (n = 56). Other isolated pathogens included *Staphylococcus aureus* (n = 74), *Staphylococcus hyicus* (n = 70), Corynebacterium spp. including *C. bovis* (n = 64), and *S. uberis* (n = 41). Other pathogens, including *Streptococcus dysgalactiae, Streptococcus equinus*, gram positive rod bacterium, *Enterococcus faecium, Escherichia coli, Citrobacter koseri, Aerococcus viridans*, and *Arcanobacterium pyogenes*, individually comprised ≤ 3% of total samples and were therefore removed from further analysis.

Parity was removed from the *S. uberis* model, due to failure to meet convergence criterion due to sample size (first parity, n = 0; second parity, n = 1; third parity, n = 2; fourth or greater parity, n = 31). Parity, stage of lactation, and season remained in all other models, including the CNS, *S. chromogenes*, Corynebacterium spp., *S. aureus*, and *S. hyicus* models. Parity was associated with the probability of *S. chromogenes*, Corynebacterium spp., *S. aureus*, and *S. hyicus* mastitis ($P < 0.05$; Figure 7). Cows in first parity had 4.2 times the odds for *S. chromogenes* mastitis compared to cows in fourth or greater parities (OR = 4.2; 95% CI: 1.6, 10.7), while cows in third parity had 4.1 times the odds for *S. chromogenes* mastitis compared to cows in fourth or greater parities (OR = 4.1; 95% CI: 1.4, 11.1). First parity cows had 5.1 and 2.8 times the odds of *S. aureus* mastitis compared to third (OR = 5.1; 95% CI: 1.4, 18.2) and fourth or greater parities (OR = 2.8; 95% CI: 1.2, 6.6), respectively. Relative to *S. hyicus*, first parity cows had 3.6 and 7.0 times the odds compared to third (OR = 3.6; 95% CI: 1.1, 11.9) and fourth or greater parities (OR = 7.0; 95% CI: 2.7, 18.4), respectively. Second parity cows had 3.0 and 5.9 times the odds for *S. hyicus* compared to third parity (OR = 3.0; 95% CI: 1.0, 9.0) and fourth or greater parities (OR = 5.9; 95% CI: 2.5, 13.8), respectively. In contrast, there was less likelihood for first parity cows to have Corynebacterium spp. compared to fourth parity (OR = 0.1; 95% CI: 0.01, 0.7) and second parity compared to fourth parity (OR = 0.1; 95% CI: 0.03, 0.6). The probability of *S. uberis* and CNS organisms was similar across all factors and the probability of all organisms was similar across stage of lactation and season ($P > 0.05$).

DISCUSSION

While other studies have examined the prevalence of mastitis by season, stage of lactation, and parity in conventional herds, the current study identified the probability of subclinical mastitis and mastitis-causing organisms in USDA-certified organic herds. The probability of subclinical mastitis was greatest during the summer, in third and fourth or greater parities, and in early and late lactation. Additionally, parity effected the probability of specific organisms, while season and stage of lactation did not. This relationship improves the understanding of the epidemiology of organisms associated with mastitis and contributes to management recommendations for subclinical mastitis on organic dairy farms.

The probability of subclinical mastitis in organic dairy herds peaked during the summer with decreased likelihood in the spring, fall, and winter. This followed a similar pattern to rate of clinical mastitis on conventional farms, where rate was 1.2 – 1.6 times greater in the summer compared to other seasons (Hogan et al., 1989). Additionally, bulk tank SCC in organic and conventional herds increased during the summer (Olde Riekerink et al., 2007; Cicconi-Hogan et al., 2013). Summer heat and humidity increases bacterial loads in the environment, which has been suggested to cause increased events of mastitis during this time (Smith et al., 1985). In support of the environmental effects on summer mastitis, the rate of environmental pathogens increases during the summer months, particularly coliforms (Hogan et al., 1989; Olde Riekerink et al., 2007). Coliforms such as *E. coli* are commonly identified in low SCC herds and when clinical cases are being studied, as in Hogan et al. (1989). Because samples of subclinical mastitis were collected in the present study, this may have contributed to the low frequency of *E. coli* identified and overall lack of association between season and probability for specific organisms. This suggests that in organic herds, mastitis during the summer is a widespread issue not specific to environmental features. Therefore, increased probability for mastitis in the current study may be associated with immunosuppression related to heat stress (Lacetera et al., 2005). As cows were on pasture during the summer with limited opportunities for heat abatement, the effect of heat stress may have been pronounced. This indicates that decreasing heat loads with heat abatement systems may decrease probability for subclinical mastitis during the summer on organic farms.

There was no association between season and probability of specific organisms identified in relation to subclinical mastitis. In contrast, previous reports from conventional herds have found associations between season and pathogens (Østerås et al., 2006; Olde Riekerink et al., 2007). As sampling was random or based on producer-identified clinical mastitis in previous studies, methodological differences in sample collection make it difficult to compare across studies. Additionally, the current study did not determine the first incidence of pathogen-specific mastitis and certain pathogens, such as *S. aureus* and *S. uberis*, have a high persistency in the udder (Barkema et al., 2006; Tamilselvam et al., 2006). Therefore, pathogens acquired in previous seasons contributed to probability in later seasons if not self-cured. This may have diluted significant associations between season and pathogens. Future studies should distinguish first observations of subclinical mastitis in organic herds to identify seasonal risk for acquiring new pathogens.

Cows in greater parities had a higher likelihood of subclinical mastitis. A similar relationship exists in conventional herds (Olde Riekerink et al., 2007; Breen et al., 2009). Many factors may contribute to the relationship. A primary contributor may be the decline in immune function in older cows (Gilbert et al., 1993), particularly as the oldest cow included in the current study was in her thirteenth lactation. Additionally, cows in greater parity experience accumulated exposure to pathogens, increasing the risk for mastitis. Previous infections with persistent organisms may also contribute to increased probability for subclinical mastitis in older cows (Zadoks et al., 2001). Although prevention in earlier parities may improve probability in older parities, culling older cows may be necessary to maintain milk quality, cow welfare, and farm profitability in organic herds where effective treatment is unavailable.

Parity also was associated with the probability for specific organisms. Cows in earlier parities had a higher probability for isolation of Staphylococci spp. in comparison to fourth or greater parities. In contrast, increased incidence of *S. aureus* has been associated with older cows in Dutch herds (Zadoks et al., 2001), likely due to the chronic nature of *S. aureus*. Potentially, producers involved in the current study were culling young cows that appeared to have chronic infections, leaving those cows that were more resistant to remain in the herd through greater parities with a decreased probability for Staphylococci spp. (Wall et al., 2005). Furthermore, Corynebacterium spp. were associated with greater probability in fourth or greater parities. The mammary gland of older cows which may be resistant to other pathogens may become colonized with this opportunistic pathogen and remain within the herd as Corynebacterium spp. are related to minor increases in SCC and damage to secretory function (LeVan et al., 1985; Sordillo et al., 1989). While parity was removed from the *S. uberis* model and probability was not estimated, this pathogen was isolated in 21.3% of samples from fourth or greater parity cows, making it the second most common organism in this group of cows. As *S. uberis* is an environmental pathogen found in the soil (Lopez-Benavides et al., 2007), managing pastures may decrease probability of mastitis in older organically managed cows. Increased sample sizes would allow for greater conclusions related to probability of organism-specific mastitis on organic farms.

Cows in early and lactation had the greatest probability for subclinical mastitis. Increased rates of mastitis have been reported in conventional herds (Dohoo, 1993; Olde Riekerink et al., 2008; Breen et al., 2009) as early lactation cows experience stress from parturition and negative energy balance, which effects the inflammatory response (Esposito et al., 2014). Additionally, Olde Riekerink et al. (2007) reported that late-lactation cows were more likely have an increased SCC, reflecting results from organic herds in the current study. Cows in late lactation may have experienced a dilution effect, where somatic cells are concentrated as milk yield declines. While Busato et al. (2000) reported differences in frequency of organisms present in milk from early lactation cows compared to late lactation cows, we found to no association of specific organisms with stage of lactation. This suggests that the increased probability of subclinical mastitis in early and late lactation was widespread without a singular causal factor.

Overall frequency of pathogens on organic farms in the current study offers insight into the management challenges on these farms. Our results reflect prior reports, in that CNS spp. and *S. aureus* were the most common organisms on organic and conventional farms (Busato et al., 2000; Mullen et al., 2013; Levison et al., 2016). Limited data is available on CNS spp., particularly on *S. chromogenes,* which made up 26 and 14% of observations, respectively, in the herds sampled. Previous work has established that *S. chromogenes* can be misidentified as a coagulase-positive Staphylococci spp., which may have underestimated the prevalence of *S. chromogenes* in the current study (dos Santos et al., 2016). *S. chromogenes* is the most common CNS spp. and causes persistent subclinical infections with increases in SCC similar to *S. aureus* (Sampimon et al., 2009; Supré et al., 2011). There are associations between isolation of CNS spp. and heifers, particularly those with a low SCC, as well as environmental features; yet, causal factors are still unclear (De Vliegher et al., 2003; Sampimon et al., 2009). A better understanding of *S. chromogenes* is needed in order to control this organism and subclinical mastitis in dairy herds.

A difference of 0.8 kg milk yield per DHI test date was observed between cows with and without subclinical mastitis. The relationship between milk losses and increase in SCC has been previously established on conventional farms, with losses of 1.6 kg/d between cows with a SCC of 250,000 compared to those with a SCC of 50,000 (Potter et al., 2018). While our results indicate less of a loss in milk per d, mastitis in organic herds may be more chronic due to lack of approved treatment methods. Additionally, production levels differ between organic and conventional farms and milk losses differ between pathogens (Levison et al., 2016; Heikkilä et al., 2018). Understanding chronicity of subclinical mastitis and pathogen-specific milk losses in organic herds would allow for improved economic assessment and management decisions on these farms.

CONCLUSIONS

The probability for subclinical mastitis on organic farms in the southeastern region of the US was greatest in the summer, in older cows, and in early and late lactation. However, specific organisms found in milk from cows identified as recently having subclinical mastitis only differed in probability by parity. Staphylococci spp. had a greater probability in younger cows, whereas the probability of Corynebacterium spp. was highest in fourth or greater parities. This indicates that specific pathogens may be driving the increased probability of subclinical mastitis in older cows. The association with season and stage of lactation may be more widespread and related to a decline in immune function due to other stressors present during summer and early lactation. Overall, a loss in milk production was associated with subclinical mastitis. While our work establishes similarities between factors associated with mastitis on conventional and organic farms, further work should identify the chronicity of mastitis in organic herds, as well as the effect of cumulative stressors on udder health.

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CHAPTER IV

CONCLUSIONS

Consumer perception is in favor of cows raised on pasture and organic production maximizes this management practice. Research leading to scientific-based recommendations has not kept up with the growth in the organic market, leaving producers with limited resources. The lack in research is particularly clear when examining lying behavior and time budget recommendations, as well as treatment strategies offered for mastitis. While the literature focuses on comparisons between organic and conventional, or pasture-based and confinement, long-term studies focused within varying organic systems is limited. Therefore, the current study aimed to 1) determine the association of lying behaviors with milk yield, DIM, and parity when cows are managed under two different organic feeding and housing management systems 2) identify probability of subclinical mastitis on organic farms in the southeastern region of the US and 3) characterize frequency and probability of mastitis-causing organisms by season, parity, and stage of lactation in this region.

Lying behavior was associated with differences in season, stage of lactation, and parity on LI and HI farms; yet, milk yield was only associated with differences in lying behavior on HI farms. Because our analyses focused on differences within management system, it is difficult to draw conclusions between systems. However, the low daily lying duration on LI farms suggests these cows were not meeting welfare requirements as established in confinement systems, which may be limiting milk production. While a complete 24-h time budget is necessary before establishing welfare recommendations for pasture-based farms, this is likely a result of a limited time allowance for lying created by increased time spent reaching energy requirements through grazing. Although grazing is considered a natural behavior for dairy cows, research should consider if cows with today's high-production genetics can meet energy requirements through grazing while sustaining a healthy time budget. Additionally, in conventional systems cows will prioritize lying over feeding, as cows are highly motivated to spend time lying. Behavioral priorities of cows on pasture are not identified, but understanding the relationship between lying time and feeding time on pasture during times of limited nutrient availability would aid in welfare recommendations and management decisions. Overall, our study indicates that time may be a limited resource for grazing cows and therefore, farm design and management should consider methods to improve time availability of cows.

While our results established similar associations between parity and stage of lactation with behavior, overall lying time on pasture contributed to the variation currently reported in the literature. This indicates a need to examine causes for variation between studies. As our observational study was conducted on farms where management practices were not disrupted. While this did not allow for control of all aspects of management, it ensured cows were reacting to current management practices. However, in previous studies where a treatment is implemented, there is the potential that the observed behavior is reflecting behavior under previous management and diluting the effect of the treatment. Additionally, cows are pasture have been observed to have increased synchrony of behavior compared to confinement systems. When treatment groups are housed near control groups, there is the potential that behavior is again diluted as cows try to act as a herd and not in relation to imposed treatment. While the wash out periods are established to acclimate cows to treatment, there is the potential that previous management or social facilitation is diluting the effects of previous controlled studies. While controlled studies are necessary to establish causal relationships, a strength of the current study was its ability to control for these effects and understand behavior under commercial management strategies.

The current study established that factors, including season, parity, and stage of lactation, affected subclinical mastitis similarly in organic systems as previously reported in conventional systems. Previous studies have methodological differences in sampling as some focused on clinical mastitis, sampled the entire herd, or randomly sampled cows. However, the similarity in results suggests the risks for mastitis during certain periods of time, such as during the summer or in early lactation, are present despite management techniques. Identifying first incidence of subclinical mastitis on these farms would be informative to determine the cause of mastitis during the observed time periods, as well as chronicity and duration of infection. Our results suggest that cumulative stressors may be contributing to the probability of mastitis, as specific organisms were not isolated in different probabilities between seasons or stages of parities, indicating a single causal factor is not driving this relationship. Further work should establish the controlled effect of cumulative stressors on mastitis in order to make science-based recommendations related to the prevention of mastitis.

Although some previous recommendations state that it is not cost effective to treat subclinical mastitis, clinical mastitis was rarely observed within participating herds and therefore, not included within our analyses. However, we observed milk loss resulting from subclinical mastitis that would impact financial decisions, especially as organic milk is priced higher than conventional milk. As milk buyers look to purchase higher quality milk and technology is able to detect SCC more efficiently, subclinical mastitis is going to become more important for producers to monitor. In order for recommendations related to milk quality and mastitis to stay current, research needs to focus more on understanding the cause, progression, and effect of subclinical mastitis. A large contributor to subclinical mastitis appears to be CNS spp., particularly S. chromogenes. Although this organism is not associated with clinical symptoms, the increase in SCC alone, as well as the prevalence of the organisms makes further research on this specific organism necessary.

Our research aimed to answer questions that would contribute to the understanding of lying behavior and mastitis on organic farms, with the greater objective of aiding current knowledge regarding welfare on these farms. While our study was not designed to test welfare directly, our results suggest that some cows may not be spending enough time lying as time may be limited. However, results related to mastitis indicate that probability follows similar patterns as reported on conventional farms, although it is difficult to compare rates or prevalence between studies due to methodological differences. Our results should suggest that organic producers should consider time as a valuable resource for grazing cows, particularly during the spring and early lactation, while probability of mastitis may be decreased by limiting cumulative stress.

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APPENDIX

Table 1- Management practices on participating farms with production data retrieved from Dairy Herd Information Association

Farm	Management System	Herd Size	Rolling herd average (kg)	Dominant Breed	Housing	Milking System
A	Low input	36 ± 4; 30 – 41	--	Crossbred	Compost bedded pack	Parallel parlor
B	Low input	77 ± 6; 69 – 85	3643.4 ± 67.5; 3583.8 – 3782.1	Jersey	Compost bedded pack	Herringbone swing parlor
C	Low input	56 ± 6; 46 – 63	6460.4 ± 180.5; 6155.2 – 6665.5	Holstein	Concrete-based pen	Herringbone parlor
D	High input	40 ± 8; 26 – 50	7938.4 ± 154.8; 7709.7 – 8135.6	Holstein	Tiestall	Bucket milking system
E	High input	39 ± 3; 35 – 44	9943.6 ± 254.1; 9637.0 – 10371.4	Holstein	Tiestall	Bucket milking system

-- Herd enrolled < 1 year in DHI testing

Table 2- Pasture quality measures, including dry matter, crude protein, neutral detergent fiber, and acid detergent fiber- on low input (LI) and high input (HI) systems across season

	LI			HI	
	Spring	Summer	Fall	Spring	Fall
Dry matter (%)	39.7	29.9	--	20.3	--
Crude protein	16.1	16.2	19.3	18.7	21.5
NDF	48.8	54.5	46.9	50.1	44.9
ADF	32.6	35.7	29.1	33.7	28.9

-- Pasture quality measures not determined in the fall

Table 3- Percentage of focal cows on low input (LI) and high input (HI) farms by season (spring, summer, fall) across stage of lactation, parity, milk yield, locomotion score, body condition score, and somatic cell score.

	LI			HI	
	Spring	Summer	Fall	Spring	Fall
Stage of lactation					
Early	27.1	6.0	28.3	0	33.1
Mid	33.3	36.7	29.4	56.6	4.5
Late	39.6	57.3	42.4	43.4	62.4
Parity					
1	7.1	11.9	15.2	0	7.3
2	30.0	32.4	48.2	24.1	44.1
3	23.2	18.2	13.5	27.6	22.9
≥ 4	39.7	37.5	23.2	48.3	25.7
Milk yield category					
Low	48.7	54.9	62.6	44.6	66.1
High	51.4	45.1	37.4	55.4	33.9
Locomotion					
1	97.9	87.8	90.8	96.6	100
2	2.1	8.8	7.3	3.45	0
3	0	3.4	1.9	0	0
Body condition score					
< 2	5.6	1.7	0	0	0
2.0 – 2.75	63.6	77.8	94.1	79.3	68.4
3.0 – 3.75	30.8	20.5	5.9	20.7	31.6
≥ 4	0	0	0	0	0
Somatic cell score					
< 4	89.1	79.9	78.8	82.8	87.3
≥ 4	10.9	20.1	21.2	17.2	12.7

Table 4- Changes in behavior on low input farms between fixed effects of parity and stage of lactation.

	Parity					Stage of lactation			
	1	2	3	≥ 4	*P*-value	Early	Mid	Late	*P*-value
Steps (n/d)	3828.3 ± 158.3	3914.6 ± 100.7	3964.9 ± 143.7	4174.2 ± 132.6	0.35	4261.3 ± 91.4^{a}	3950.2 ± 78.8^{b}	3700.0 ± 78.2^{c}	< 0.01
Lying time (h/d)	8.3 ± 0.2	8.3 ± 0.1	8.2 ± 0.2	7.9 ± 0.2	0.39	7.5 ± 0.1^{a}	8.3 ± 0.1^{b}	8.7 ± 0.1^{c}	< 0.01
Lying bout duration (min/bout per d)	84.6 ± 5.8^{*}	89.3 ± 3.5^{*}	99.1 ± $5.1^{*\dagger}$	101.6 ± $4.3^{\dagger}$	0.04	82.7 ± 3.5^{a}	99.4 ± 2.9^{b}	98.9 ± 2.8^{b}	< 0.01
Lying bouts (n/d)	8.9 ± 0.5^{a*}	7.9 ± $0.3^{ab*\dagger}$	7.0 ± $0.5^{b\dagger}$	7.2 ± $0.4^{ab\dagger}$	0.03	7.9^{a}	7.2^{b}	8.2^{a}	< 0.01

$^{a, b, c}$ Means with different superscripts varied within a row and fixed variable after Tukey adjustment (*P* < 0.05).

$^{*, \dagger}$ Means with different superscript symbols varied within a row and fixed variable prior to Tukey adjustment only (*P* < 0.05).

Table 5- Changes in behavior on high input farms between fixed effects of parity and stage of lactation.

	Parity					Stage of lactation			
	1	2	3	≥ 4	*P*-value	Early	Mid	Late	*P*-value
Steps (n/d)	1572.3 ± 363.1	2055.9 ± 119.9	2180.2 ± 126.8	2027.8 ± 127.5	0.40	1857.5 ± 153.0	2018.3 ± 136.3	2001.4 ± 122.7	0.68
Lying time (h/d)	10.7 ± 1.1	11.1 ± 0.3	11.4 ± 0.4	10.7 ± 0.4	0.61	9.8 ± 0.4[a]	11.5 ± 0.4[b]	11.6 ± 0.4[b]	< 0.01
Lying bout duration (min/bout per d)	155.8 ± 21.7[a]	88.8 ± 7.2[b]	83.5 ± 7.6[b]	82.9 ± 7.6[b]	0.01	78.0 ± 9.2[a]	116.1 ± 8.2[b]	114.2 ± 7.4[b]	< 0.01
Lying bouts (n/d)	9.0 ± 2.1	11.7 ± 0.6	13.0 ± 0.7	11.0 ± 0.7	0.05	10.6 ± 0.7	11.6 ± 0.7	11.4 ± 0.7	0.39

[a, b, c] Means with different superscripts varied within a row and fixed variable after Tukey adjustment ($P < 0.05$).

Table 6- Farm production measures obtained from Dairy Herd Information records for 2017 (mean ± SD; range) and management details

Farm	No. of DHI test dates in 2017	Herd Size	Rolling herd average (kg)	Mean Herd SCS	Dominant Breed	Housing; Milking System
A	9	36 ± 4; 30 – 41	--	2.1 ± 0.4; 1.6 – 2.9	Crossbred	Compost bedded pack; parallel parlor
B	8	77 ± 6; 69 – 85	3643.4 ± 67.5; 3583.8 – 3782.1	2.8 ± 0.3; 2.4 – 3.3	Jersey	Compost bedded pack; herringbone swing parlor
C	7	40 ± 8; 26 – 50	7938.4 ± 154.8; 7709.7 – 8135.6	2.8 ± 0.1; 2.5 – 2.9	Holstein	Tiestall; bucket milking system
D	8	39 ± 3; 35 – 44	9943.6 ± 254.1; 9637.0 – 10371.4	2.7 ± 0.6; 1.9 – 3.6	Holstein	Tiestall; bucket milking system
E	10	56 ± 6; 46 – 63	6460.4 ± 180.5; 6155.2 – 6665.5	2.7 ± 0.6; 1.6 – 3.7	Holstein	Concrete based pen; herringbone parlor

-- Herd enrolled in DHI testing < 1 year, making annual rolling average unavailable

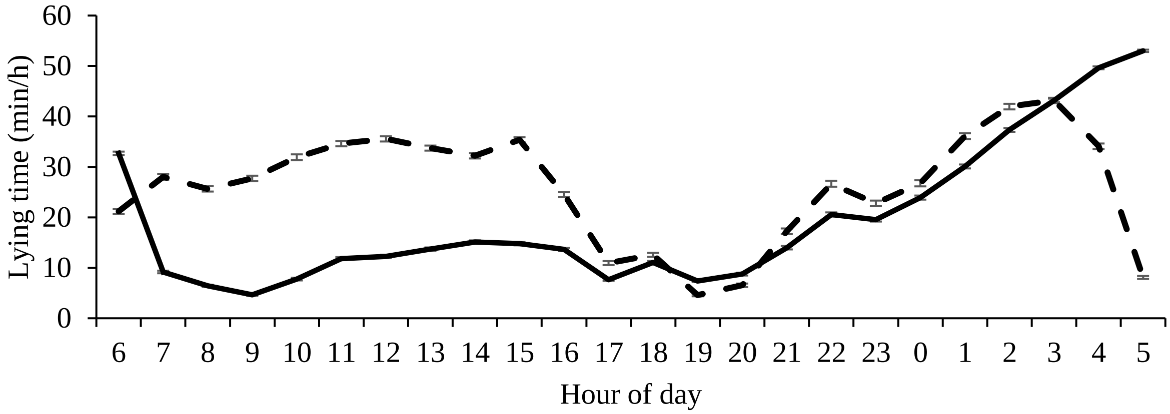

Figure 1- Mean lying time on low input (solid line) and high input farms (dashed line) with bars representing SE

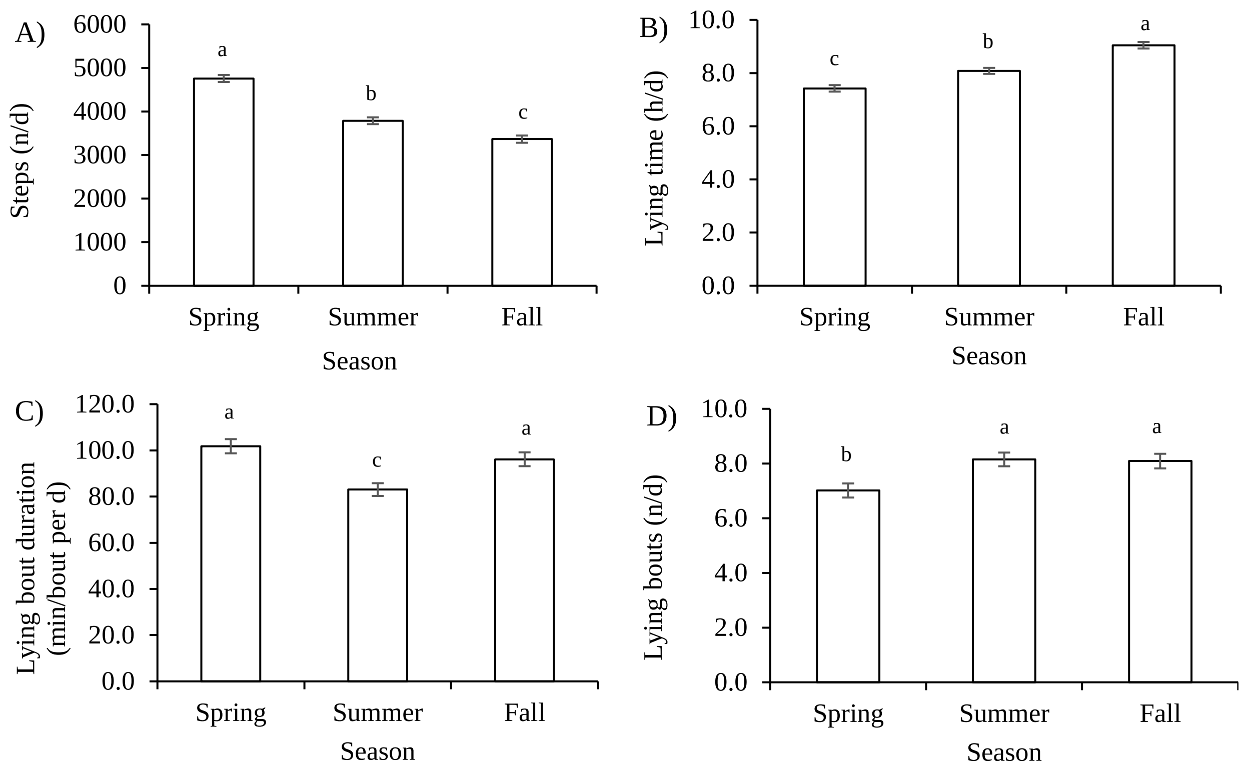

Figure 2- Lying behavior on low input farms across season (spring, summer, fall). Error bars represent SE and differing letters represent $P < 0.05$.

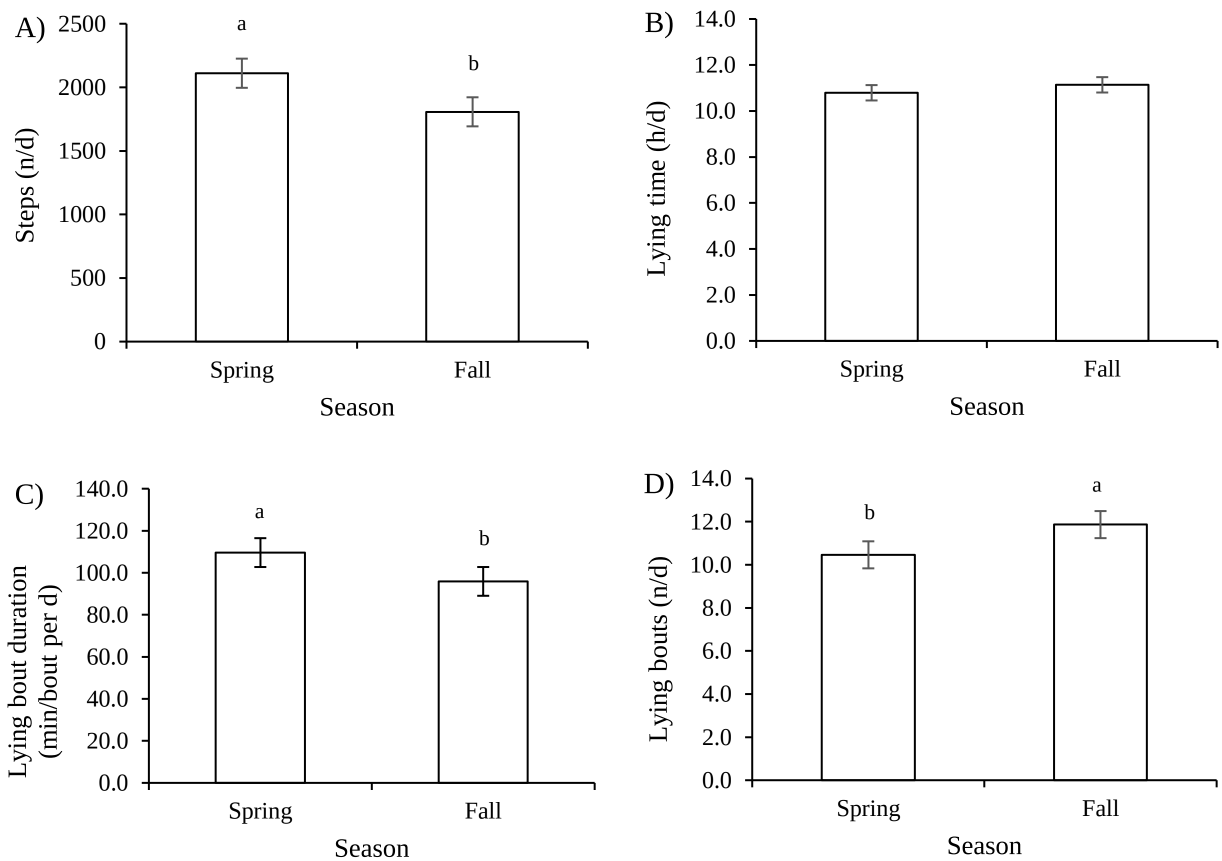

Figure 3- Lying behavior on high input farms across seasons (spring, fall). Error bars represent SE and differing letters represent *P* < 0.05.

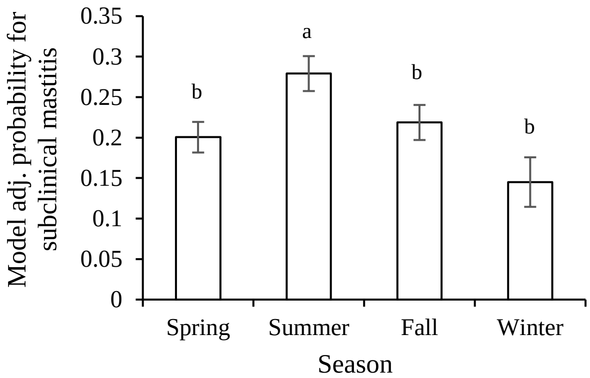

Figure 4- The model adjusted probability for subclinical mastitis by season of the year. Error bars represent SE and differing letters represent *P* < 0.05.

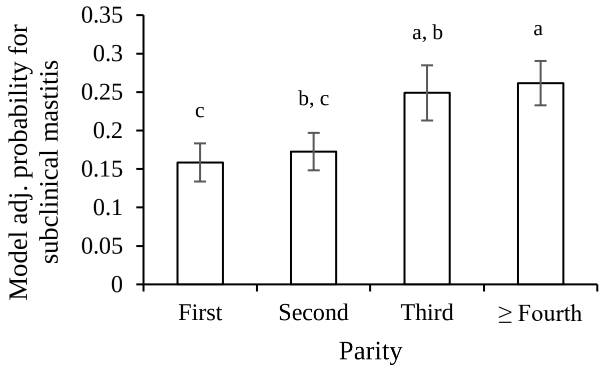

Figure 5- The model adjusted probability for subclinical mastitis by parity. Error bars represent SE and differing letters represent $P < 0.05$.

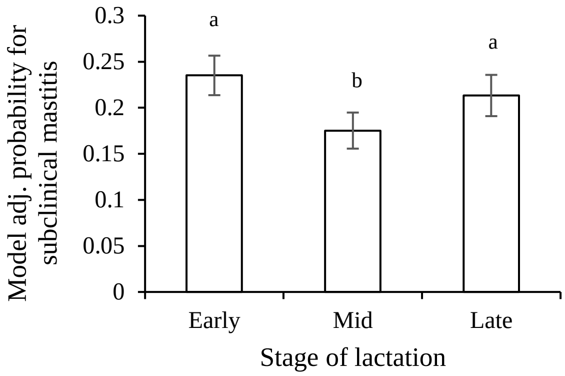

Figure 6- Model adjusted probability for subclinical mastitis by stage of lactation. Error bars represent SE and differing letters represent $P < 0.05$.

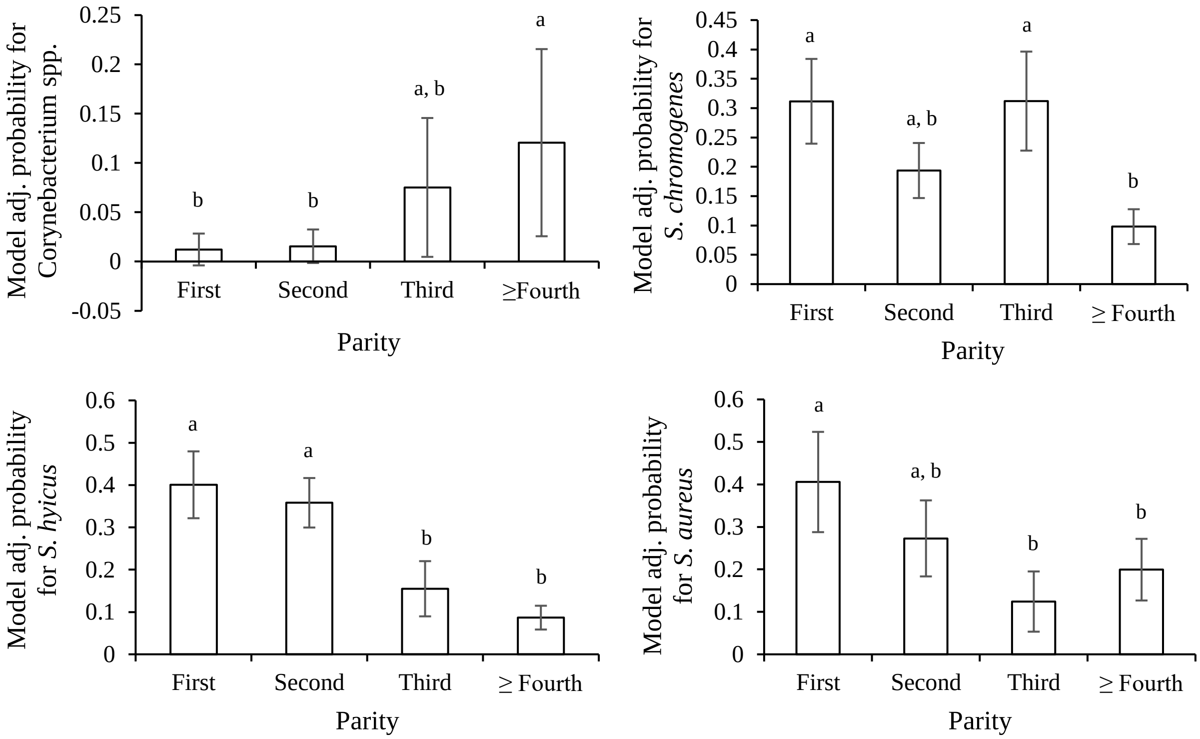

Figure 7- Model adjusted probability for mastitis-causing organisms across parities. Error bars represent SE and differing letters represent $P < 0.05$.

VITA

Victoria L. Couture was born on May 12, 1995 in Richmond, KY. Victoria graduated from Madison County High School in 2013 and continued her education at the Eastern Kentucky University, receiving a Bachelor’s of Science degree in Animal Studies in 2016 while working at Stateland Dairy. In January 2017, Victoria began to study for her Master’s of Science in animal science at the University of Tennessee. While at UT, Victoria travelled across the state, presenting at Master Dairy Modules and aiding with milk quality research. In addition, she has presented her research at national and international conferences and submitted a manuscript for peer-review publication.