Techniques to increase silage stability and starch availability and the effects of heat stress abatement systems on reducing heat load in dairy cattle

by

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B.S., University of Wisconsin-River Falls, 2010
M.S., Kansas State University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

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Abstract

Four studies were conducted that focused either on silage quality parameters or heat abatement systems to improve cow comfort. Study 1 evaluated the effects of treating whole-plant corn at harvest with a dual-purpose commercial silage inoculant containing *Lactobacillus buchneri* and *Lactococcus lactis* O224 on fermentation and aerobic stability of corn silage through 32 d of ensiling. Inoculating silage to be fed after minimal storage time ($\leq$ 32 d post-harvest) had no effect ($P > 0.05$) on the chemical composition, fermentation variables, aerobic stability or rise in temperature post-harvest. Study 2 was designed to develop a berry processing score (BPS) for sorghum silage as well as evaluate the change in starch digestibility as the level of berry processing increased. A method to evaluate the level of processing in sorghum silage was successfully developed by measuring the percent of starch passing through a 1.7 mm screen. This provides the industry with a standardized method to measure the level of processing in sorghum silage. As BPS increased from 26.28 to 55.05 ± 0.04%, 7-h in situ starch digestibility increased from 50.54 to 82.07 ± 4.94% for unprocessed and heavily processed sorghum silage, respectively ($R^2 = 0.43$). By processing sorghum silage during harvest and measuring the extent of processing, sorghum silage starch digestibility can be enhanced and may serve as a viable alternative to corn silage in the diet of lactating dairy cows in areas of the country where corn silage is a high-risk forage crop due to lack of water. Study 3 evaluated the effects of 2 heat stress abatement systems on barn temperature, micro-environmental temperature, core body temperature (CBT), respiration rate, rear udder temperature, and lying time in lactating dairy cows. The systems evaluated were: direct cooling via feedline soakers and fans, or evaporative cooling via a fan and fog system. The evaporative cooling system was effective ($P = 0.04$) in reducing respiration rates (52.0 vs. 57.9 ± 2.2 breaths per min; $P < 0.01$) and rear udder

temperatures (33.2 vs. 34.5 ± 0.3ºC; $P < 0.01$), and increased daily lying time (11.8 vs. 10.8 ± 0.3 h/d; $P < 0.01$) due to differences in barn THI and airflow. No treatment differences ($P = 0.79$) were detected for CBT, likely due to cooler ambient conditions during the study. Study 4 assessed the effects of the same evaporative and direct cooling systems as in Study 2 but were applied in the holding area prior to afternoon milking, where effects on CBT and micro-environmental temperature in lactating dairy cows were measured in addition to water usage by each system. No significant differences ($P > 0.05$) between direct cooling and evaporative cooling were detected for micro-environmental THI. However, the evaporative cooling system reduced the consumption of water in the holding area while maintaining CBT < 39.0ºC. Future research should be conducted under greater ambient THI to determine if an evaporative cooling system is able to maintain CBT < 39.0ºC, while also comparing CBT and water usage to a soaker system in the holding area.

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Dedication

I dedicate this work to my wife, Jessica Johnson. I am truly grateful for all the sacrifices you have made for me throughout my time here at Kansas State University allowing me to pursue this great achievement. Without your unwavering love and support, I would not be where I am today.

Chapter 1 - Literature Review: Aerobic Stability of Corn Silage and the Effect of Berry Processing on Starch Digestibility of Sorghum Silage

Introduction

The first part of this literature review will focus on the process of silage fermentation and how that process can be affected by the use of silage inoculants. Preservation of silage quality, dry matter (DM), and energy post-harvest requires that plant respiration, proteolysis, aerobic microbial growth, and clostridial activity be limited. Proper ensiling techniques are critical to ensure proper fermentation and maintenance of anaerobic conditions throughout the entire storage period. Many factors affect the rate at which silage becomes unstable at feedout and must be accounted for to ensure adequate fermentation and aerobic stability through feedout.

The second part of this literature review will focus on the benefits of including sorghum silage in the cropping system as well as some of the drawbacks of feeding sorghum silage to dairy cattle. Sorghum [*Sorghum bicolor* (L.) Moench] has become an increasingly important source of forage for dairy producers in regions of the U.S. that routinely experience conditions of drought or insufficient irrigation water during the growing season. These regions tend to be located throughout the southern Great Plains, with Kansas, Texas, and Nebraska being the leading sorghum producers (Undersander et al., 1990). These agricultural states rely heavily on the Ogallala aquifer as the main water source and due to recent dry conditions, such as the 2012 drought, and an increase in ground water usage for irrigation, livestock, and industrial demands, this water source is diminishing at an unsustainable rate (Lazarus et al., 2014). Therefore, water conservation strategies and the use of more drought tolerant crops, such as sorghum, should help to decrease water usage in these areas. However, one of the concerns that many dairy producers and nutritionists have in feeding forage sorghum to dairy cattle is the reduction in starch and fiber digestibility compared to corn silage, and therefore, reduced energy availability for the dairy cow.

The Ensiling Process

The ensiling process is a method utilized to preserve high quality forages for a period of time prior to being fed to livestock. Three main factors must occur for silage to be of high quality and include the following: 1) rapidly excluding air from the silage mass; 2) rapid production of lactic acid leading to a reduction in silage pH; and 3) preventing the infiltration of air into the silage mass during storage (Kung, 2010). Meanwhile, there are four phases that make up the entire ensiling process that must be discussed and include: 1) aerobic, 2) fermentation, 3) stable, and 4) feedout phase (Weinberg and Muck, 1996). Each phase is different and must be managed appropriately in order to maintain silage quality from harvest through feedout.

Aerobic Phase

Under optimum conditions, this phase typically only lasts from a few hours to 2 to 3 days after the silage has been placed into the storage structure. Two important plant enzyme activities occur during the aerobic phase: respiration and proteolysis (Bolsen et al.,1996). Respiration is the complete breakdown of plant sugars to carbon dioxide (CO_2), water, and heat in the presence of oxygen (Bolsen et al., 1985; Muck, 1988). This process is unavoidable and results in a loss of DM and sugars. However, DM loss during this phase should not exceed 7% (Zimmer, 1980) and should be much lower than 7% with proper moisture and adequate pack density (Rotz and Muck, 1994; Robinson et al., 2015). Respiration eliminates oxygen from the silo, creating an anaerobic environment. However, if respiration becomes excessive due to filling the silo too slowly or improperly packing and sealing the silo, excessive loss of DM may occur and the DM lost is typically the most fermentable carbohydrates (McDonald, 1981) resulting in lower energy value of the silage and reduced substrate available for lactic acid fermentation. An estimate of the loss

of net energy from this initial aerobic activity is 1 to 2%, and this loss is essentially inevitable (Woolford, 1984). As a result, the onset of pH decline will be delayed allowing plant microbial activity to continue (Ruxton and McDonald, 1974; Ohyama et al., 1975). Excessive respiration may also lead to unnecessary heat production resulting in Maillard or browning reactions, reducing digestibility of the silage (Bolsen et al.,1996).

Proteolysis also occurs during this phase where plant proteases break down proteins primarily into amino acids and ammonia (McDonald et al., 1991). Research, predominantly in legumes, has shown that plant proteolytic activity is greatest with pH values around 6.0 and activity declines linearly as pH is reduced from 6.0 to 4.0 (Brady, 1961; Finley et al., 1980; McKersie, 1985). However, protease activity at pH 4.0 was still 15 to 35% of that at pH 6.0 indicating that while proteolytic activity is severely reduced at lower pH, some proteolytic activity still occurs.

Fermentation Phase

This phase begins once anaerobic conditions have been reached and continues for 7 to 21 days post-ensiling depending on ensiling conditions and the type of crop harvested. Lactic acid bacteria (LAB) become the predominant microflora present and utilize water-soluble carbohydrates (WSC) to produce primarily lactic acid causing a decrease in silage pH (Muck, 1988; Bolsen et al., 1996). Other types of bacteria are also present during this phase, which compete with LAB for WSC and include enterobacteria, clostridial spores, and yeasts and molds that can all have a negative impact on silage quality (Bolsen et al., 1996). Fortunately, the activity of these bacteria is inhibited at low pH. Due to the production of lactic acid, the pH will decrease to < 4.0 in corn silage if proper fermentation occurs.

Lactic acid bacteria can be divided into two categories; homofermentative LAB and heterofermentative LAB (McDonald et al., 1991). Homofermentative LAB produce only lactic acid by fermenting glucose and other 6-carbon sugars. Heterofermentative LAB produce lactic acid as well but also produce acetic acid, ethanol, and CO_2 (McDonald et al., 1991). A common heterofermentative LAB is *Lactobacillus buchneri*, which will be discussed later.

Stable Phase

During this phase, little biological activity occurs as long as the silo was properly packed and sealed to maintain an anaerobic environment with low pH. If air (oxygen) is able to penetrate the silage mass due to a hole or crack in the storage structure, aerobic microorganisms will become active once again (microbial respiration) leading to an increase in yeast and mold populations, loss of silage DM, and heating (Bolsen et al., 1996). This results in reduced silage quality leading to reduced livestock performance and increased incidence of disease.

Feedout Phase

This phase begins when the silo is opened for feeding allowing oxygen to penetrate into the silage face. Spoilage will eventually occur if the silage is not fed in a timely fashion. Aerobic stability of silage can be measured upon feedout and is a measure of the ability of silage to resist a rise in temperature. Woolford (1990) stated that "the single most important factor which influences the efficiency with which forage crops are conserved as silage is the degree of anaerobiosis achieved in the completed silo" post-harvest. By utilizing proper ensiling practices, one can be assured that an adequate and efficient fermentation will occur and maintain silage quality through feedout.

Factors Impacting Aerobic Stability

It has long been known that the presence of oxygen produces negative effects on silage (Woolford, 1990) and allows various aerobic spoilage microorganisms to become active and multiply, causing aerobic deterioration (Woolford, 1990). Aerobic stability is a term used to define the length of time that silage remains stable after being exposed to oxygen. Multiple measurements for aerobic stability have been used and include: 1) number of hours until temperature of the silage increases 3°C above ambient temperature, 2) number of hours until silage reaches peak temperature, and 3) maximal temperature rise above ambient.

When initially exposed to air, deterioration of silage occurs due to the degradation of preserving organic acids (predominantly lactic acid and some acetic acid) by yeasts primarily (Bolsen et al.,1996; Oude Elferink et al., 2000). The largest losses in DM and nutrients can be seen during the feedout phase (or storage phase if air is able to penetrate the silage mass) due to aerobic organisms (primarily yeasts initially) consuming sugars, fermentation products (lactic and acetic acid), and other soluble nutrients. These soluble nutrients are then converted to CO_2 and water, producing heat in the presence of oxygen through the process of respiration (Muck et al., 1988; McDonald et al., 1991; Bolsen et al., 1996). Once this process has occurred, pH of the silage mass will begin to rise because of decreased levels of lactic acid present providing a favorable environment for mold growth during feedout. With an elevated pH, other microorganisms, such as *Enterobacteriaceae*, *Bacillus* spp. and molds (*Aspergillus, Fusarium, and Pencillium*) (McDonald et al., 1991; Bolsen et al., 1996; Oude Elferink et al., 2000), will become active and lead to further heating and deterioration of the silage mass. Aerobic spoilage will occur in all silages that are opened and exposed to air but the rate at which this occurs depends on multiple factors.

Bolsen et al. (1996) proposed four factors that regulate the amount of heating that will occur including: 1) numbers of aerobic microorganisms in the silage, 2) time exposed to oxygen prior to feeding, 3) silage fermentation characteristics, and 4) ambient temperature. In other words, silage management practices dictate the rate at which silage at the face will deteriorate upon opening the silo for feedout. Some of these management practices include; harvesting at the proper moisture content, rapid filling rate, adequate pack density, immediate covering post-harvest to limit oxygen exposure, and appropriate feedout rates to stay ahead of any aerobic deterioration. If failure occurs in any one of these areas, the silage mass is likely to have greater DM losses and heating leading to reduced silage quality. Woolford (1984) established that DM losses are ~1.5 to 3.0% per day for each 8 to 12°C rise in the silage temperature above ambient.

Proper Harvest and Silo Management

In order to make high quality silage, there are 3 main processes that must occur for proper fermentation to take place. First, air must be removed from the silage mass as quickly as possible post-harvest. Second, a rapid production of lactic acid to reduce silage pH is necessary to eliminate growth of harmful microorganisms, and thirdly, efforts must be taken to prevent air penetration into the silage mass during storage and feedout (Kung, 2010).

The importance of proper moisture content at harvest cannot be overemphasized. Typically, corn silage should be harvested when the DM content is between 30 and 40% with the optimum DM being 32 to 35%. Overly dry forage is more porous and will not pack adequately allowing oxygen to penetrate deeper into the silage mass upon feedout reducing aerobic stability. Dry forage stored as silage is more susceptible to heating (Maillard reaction) and aerobic losses from either plant respiration or aerobic microbial activity (Pitt, 1986).

When filling a silo, in addition to proper moisture, even distribution of forage in the storage structure and proper particle length can ensure that proper packing and adequate pack density will occur, which will enhance aerobic stability due to reduced ability of air to penetrate the silage mass. Bunkers and piles should be filled using the progressive wedge technique where forage is packed into thin, 15 to 20 cm layers. It has been recommended that optimal pack density for bunkers and piles is 224 to 256 kg of DM/m^3 (Ruppel et al., 1995) with more recent evidence showing that this number should be closer to 320 kg of DM/m^3.

After filling the silo, silage should be covered immediately with plastic and weighted down with tires, which should be touching. The recent use of oxygen barrier plastics has been useful in limiting oxygen entry into the silage mass (Borreani et al., 2007).

Silage removal rate during feedout should be adequate to minimize aerobic spoilage. The common recommendation is to feed at least 15 to 30.5 cm off the face each day depending on the time of year. During summer, daily feeding rates should be at least 30.5 cm as aerobic stability is reduced during greater ambient temperatures.

By following these common management recommendations and maintaining anaerobiosis in the silage mass through feedout, silage quality and feeding characteristics of the silage put into storage should be very similar to the crop initially harvested with minimal DM and energy losses occurring during the process.

Silage Inoculants

It is well known that the fermentation process of silage can be enhanced by utilizing silage inoculants (McDonald et al., 1991; Kung et al., 2003; Filya et al., 2007). Microbial inoculants are the dominant silage additive type used in most parts of the world today (Muck, 2012). Silage inoculants are divided into two categories based on how they ferment glucose, a

common plant sugar. Historically, inoculants were based on homofermentative LAB to improve fermentation and increase DM and energy recovery of the silage. The common issue with these inoculants, however, is that they do little to inhibit the growth of yeasts as they tend to produce high levels of lactic acid, which has poor antifungal properties (Weinberg and Muck, 1996). Therefore, silage of this type would be more prone to heating and aerobic stability issues. More recently, a newer class of silage inoculants known as heterofermenters has come to the market to overcome some of the shortfalls of homofermenters.

Many of the inoculants used today contain a combination of homofermentative and heterofermentative LAB to overcome the limitations of using either inoculant alone. This is beneficial as the homofermentative LAB are fast and efficient producers of lactic acid, which decrease the silage pH to minimize the loss of WSC and protein degradation (Weinberg and Muck, 1996; Driehuis et al., 1996). The presence of heterofermentative LAB help during feedout and enhance aerobic stability of silage by reducing the growth and survival of yeasts through the conversion of lactic acid to acetic acid (Driehuis et al., 1996; Kung et al., 1999).

Homofermentative Silage Inoculants

Rapid fermentation of sugars to lactic acid and minimal respiration and proteolysis are necessary for the production of high quality silage (Nadeau et al., 2000). Homofermentative LAB are well known for their high production of lactic acid leading to a rapid drop in silage pH post-ensiling, which increases DM and energy recovery of the silage and suppresses the growth of clostridia and other undesired anaerobic organisms in silage (Oude Elferink et al., 2001). Compared to heterofermenters, homofermenters are more energy efficient where each molecule of glucose produces two molecules of lactic acid (Queiroz et al., 2012). The best of these inoculants has shown to not only enhance fermentation but to also result in improved animal

performance in some studies (Muck, 1993; Weinberg and Muck, 1996). However, these results have not been consistent among all studies (Wohlt, 1989; Kung et al., 1993). A downfall of homofermenters is that they generally have a negative or no impact on aerobic stability of silages during feedout because lactic acid has poor antifungal properties (Kung et al., 1991; Sanderson, 1993). As a result, a heterofermentative inoculant may be beneficial.

Heterofermentative Silage Inoculants

A newer class of silage inoculants entered the market in the late 1990's known as heterofermenters. This type of inoculant can be divided into two types: facultative and obligate heterofermenters. Facultative heterofermenters produce mainly lactic acid (85%) from hexose sugars (i.e. glucose), but cannot degrade pentose sugars, whereas obligate heterofermenters degrade both hexose and pentose sugars into lactic acid, acetic acid, ethanol, and CO_2 (Hammes et al., 1992; Schleifer and Ludwig, 1995).

A disadvantage of heterofermenters is that they are less energy efficient compared to homofermenters due to the production of CO_2. However, this disadvantage is offset by the major advantage of heterofermentative type inoculants ability to enhance aerobic stability (Kung and Ranjit, 2001; Kleinschmit et al., 2005) of silage during feedout due to its ability to convert lactic acid to acetic acid, which has good antifungal properties to help minimize aerobic deterioration. One very common type of obligate heterofermenter is *Lactobacillus buchneri.*

Lactobacillus buchneri

Inoculation of silage with *L. buchneri* was first suggested to improve aerobic stability by Muck (1996). *L. buchneri* did not become approved by the FDA, however, until 2001 (Muck, 2004) and entered the U.S. silage market in 2002 (Mari et al., 2009). Since that time, many studies have been conducted showing its effect on increasing acetic acid from lactic acid and

reducing the number of yeasts and molds present, thus improving aerobic stability upon feedout (Kleinschmit and Kung, 2006). These bacterial strains continue to grow slowly even after the active fermentation phase has ended, producing acetic acid from sugars or lactic acid (Muck, 2012). In a meta-analysis conducted by Kleinschmit and Kung (2006), inoculation with *L. buchneri* decreased the concentrations of lactic acid and increased the concentrations of acetic acid in corn silage. In this same meta-analysis, at a higher level of inoculation (> 100,000 cfu/g vs. < 100,000 cfu/g), greater levels of acetic acid were seen with the greater application rates (Kleinschmit and Kung, 2006). A 10 and 100-fold decrease in yeast levels were seen in silages inoculated at lower and higher levels, respectively. Associated with these lower yeast numbers, aerobic stability was improved significantly for silages treated at higher inoculation levels (> 100,000 cfu/g) compared with uninoculated silage (503 vs. 25 h), while silage inoculated at < 100,000 cfu/g remained aerobically stable for just 35 h (Kleinschmit and Kung, 2006). As a result of the increase in acetic acid, a decrease in the ratio of lactic:acetic acid will occur. The common lactic:acetic acid recommendation is $\geq$ 3:1 (Kung and Stokes, 2001). However, if an inoculant containing *L. buchneri* is used, this ratio is likely to be less than 3:1.

A detailed pathway showing the anaerobic degradation of lactic acid to acetic acid by *L. buchneri* can be found in Figure 1.1. The primary end products from anaerobic lactic acid degradation via *L. buchneri* are acetic acid, 1,2-propanediol, and ethanol (Oude Elferink et al., 2001). Concentrations of 1,2-propanediol as high as 2 to 4% of DM have been reported (Driehuis et al., 2001; Nishino et al., 2002, 2003a,b). The ability to convert lactic acid to acetic acid is strongly influenced by pH. The conversion of lactic acid to acetic acid by *L. buchneri* occurred more readily at a low (< 4) than high (> 5) pH (Oude Elferink et al., 2001). When this

conversion occurs, inhibition of yeasts and molds will result as acetic acid is a better inhibitor than lactic acid (Moon, 1983).

Harmful Bacteria in Silage

Undesirable bacteria are also present in the silage mass and can reduce aerobic stability of silage. Undesirable organisms that can cause anaerobic spoilage include clostridia and enterobacteria, while undesirable organisms that can cause aerobic spoilage include yeasts, bacilli, listeria, and molds. Not only can these spoilage organisms have an impact on aerobic stability and silage quality, but may also have a detrimental effect on the animal causing reduced performance and increased disease incidence.

Yeasts

Under anaerobic conditions yeasts ferment sugars to ethanol and CO_2 (Schlegel, 1987; McDonald et al., 1991). As a result, this ethanol production causes a decrease in the amount of sugar available for lactic acid fermentation. Under aerobic conditions yeasts degrade lactic acid to CO_2 and H_2O causing a rise in silage pH allowing for the growth of many spoilage organisms (McDonald et al., 1991). Lactate-assimilating yeasts are usually the initial cause of aerobic deterioration in silage (Pahlow et al., 2003). This yeast activity produces a favorable environment for mold growth during the feedout stage. The presence of oxygen enhances the survival and growth of yeasts during storage (Donald et al., 1995), while high levels of acetic acid reduce yeast survival during storage (Driehuis and Van Wikselaar 1996; Oude Elferink et al., 1999).

Clostridial Bacteria

Clostridia, if present, are a major concern and are the principle anaerobic microorganism detrimental to silage quality (Muck, 1988). There are two main groups of clostridia bacteria:

saccharolytic and proteolytic. Saccharolytic clostridia ferment carbohydrates and organic acids to butyric acid, CO_2 and hydrogen, whereas proteolytic clostridia ferment amino acids to CO_2, ammonia, and amines (Muck 1988). The production of butyric acid and ammonia by clostridia bacteria is a major concern as they have been linked with reduced feed intake and milk production and increased disease incidence in ruminant animals (Neumark, 1967; Wilkins et al., 1971; Conrad et al., 1977). The conversion of lactic acid to butyric acid results in an increase in silage pH allowing other harmful bacteria to grow.

Generally, LAB produce sufficient lactic acid and lower pH quickly enough to keep clostridia from growing. However, a quick pH drop is more difficult to achieve when forage is harvested at a low (< 30%) DM content. Therefore, proper DM at harvest is very important in minimizing clostridial fermentation. If clostridial silage is fed to dairy cattle, increased incidence of ketosis may result as butyric acid present in the silage can be converted to a ketone body, β-hydroxybutyrate in the liver. In addition, cows fed clostridial silage are likely to have reduced feed intake, further increasing the incidence of ketosis.

Sorghum Silage for Dairy Cattle

Forage sorghum is often planted either in total or partial replacement for corn silage as the latter can be a high-risk forage crop under certain climatic conditions such as drought and high ambient temperatures. Forage sorghum is a warm-season annual used for silage production and commonly fed to dairy cattle in many regions of the U.S. When compared to corn, sorghum has a more efficient use of water using approximately 30 to 50% less water (McCorkle et al., 2007; Mahanna, 2015). In addition, sorghum has a greater ability to extract water from deeper soil layers (Farré and Faci, 2006). This combination makes sorghum more heat and drought tolerant when compared to corn. This is especially important in areas where irrigation is limited

and where elevated temperatures combined with drought are commonly seen. Miron et al. (2007) studied the water use efficiency of conventional and brown midrib (BMR) forage sorghum silage compared to corn silage. Conventional and BMR forage sorghum varieties showed improved water use efficiencies of 51 and 18%, respectively, compared with corn silage. Likewise, Farré and Faci (2006) found greater water use efficiency, biomass production, and yield for sorghum compared with corn under minimal irrigation. These data show that planting sorghum for silage should help in reducing water use on-farm.

Currently, there are five major types of sorghum grown: grain sorghum, forage sorghum, sudangrass, sorghum-sudangrass, and sorghum-almun. For this literature review, the focus will be on forage sorghum silage. Different varieties of forage sorghum are available and include: conventional, BMR, photosensitive (PS), and brachytic dwarf varieties. Brown midrib varieties in both corn and sorghum are well known for their reduced lignin content and therefore, increased digestibility of the whole plant. Dry matter digestibility is typically greater for corn silage compared with conventional sorghum silage (Grant et al., 1995). However, when BMR sorghum silage is fed, due to its reduced lignin content, digestibility levels approach those commonly seen in conventional corn silage (Grant et al., 1995). Photosensitive varieties remain in the vegetative stage until day length is less than approximately 12 h. These varieties produce high yields but low digestibility and retain whole plant moisture making them difficult to ensile and get a proper fermentation. The brachytic dwarf variety results in a plant height of about 1.37 to 1.8 m, which is roughly half the height compared to conventional varieties, which stand at ~2.74 to 3.6 m (Bernard and Tao, 2015; Jordan, 2015). However, yield for the brachytic dwarf varieties is similar to conventional forage sorghum, as the brachytic dwarf forage sorghum has a

shorter internode distance and produces the same number and size of leaves compared to conventional forage sorghum (Jordan, 2015).

Chemical Composition of Sorghum Silage

Chemical composition data of corn and sorghum silage can be found in Table 1.1. In general, forage sorghum contains greater concentrations of protein, fiber, lignin, sugar, and ash but lower levels of starch when compared to corn silage. Neutral detergent fiber digestibility (NDFD) is greater for corn silage when compared with conventional sorghum silage. However, when BMR sorghum silage is compared to conventional corn silage, NDFD is similar due to reduced lignin levels and altered chemical composition of the lignin in these hybrids (Bucholtz et al., 1980; Cherney et al., 1991; Vogel and Jung, 2001). It is recommended to harvest sorghum silage during the early to late dough stage of maturity to optimize fiber and starch digestibility (Bernard, 2015). If harvest occurs prior to this, levels of starch will be lower, reducing the energy level of the whole plant and excessive plant moisture is likely, which could lead to the potential of an undesirable fermentation. However, if the plant matures beyond the late dough stage, the starch will become less digestible to rumen microbes due to increased binding of starch in the protein matrix.

Sorghum silage generally contains more stem and less leaf, head, and ear, which results in forage with greater fiber concentrations compared with corn silage (Contreras-Govea et al., 2010). Forage sorghum varieties with the BMR gene, however, produce forage that has reduced lignin concentrations and greater NDFD (Contreras-Govea et al., 2010). Schmid et al. (1976) compared the nutritive value of corn and forage sorghum silage. Eleven corn hybrids and 14 sorghum silage hybrids were compared. On average, the proportion of leaves and ears were greater and the proportion of stalk was lower for corn silage compared with sorghum silage

resulting in greater acid detergent fiber levels for sorghum silage as lignin tends to be more heavily located in the stems of the plant.

Sorghum Silage Effects on Milk Yield

Previous research comparing the response of milk production to feeding either corn silage or sorghum silage as the primary dietary forage has generally shown advantages for corn over sorghum. However, some studies have shown that particular hybrids of forage sorghum, particularly BMR, can approach the performance levels commonly seen when feeding corn silage.

In a study done by Grant et al. (1995), lactating dairy cows were fed diets containing 65% forage provided by either normal or BMR forage sorghum, alfalfa silage, or corn silage. Dry matter intake (DMI) was lowest (20.4 vs. 19.6 kg/d) for cows receiving either the normal sorghum or alfalfa silage based diets, respectively, and greatest for cows receiving the BMR sorghum or corn silage diets (25.3 and 23.1 kg/d, respectively). Milk yield was lower for cows fed the normal sorghum silage compared with the other three treatment diets. No differences were found for milk yield between BMR sorghum, alfalfa, or corn silage. The authors concluded that the results seen in this study were due to the large differences in lignin concentrations between normal sorghum silage (10.3%) and BMR sorghum, alfalfa, and corn silage (7.5, 8.0, and 6.3%, respectively; Grant et al., 1995). Even though cows fed BMR sorghum silage consumed 2.2 kg/d more DM compared with cows fed corn silage, this failed to improve milk yield in cows fed BMR sorghum silage. This indicates that cows fed BMR sorghum were less efficient at converting feed into milk and is likely an indicator of reduced digestibility for the BMR sorghum silage. Also, corn silage fed in this study contained 55.4% NDF, which is high when compared to the 2001 NRC values for normal corn silage of 45.0% NDF (NRC, 2001) and

40.9% NDF from samples submitted to Cumberland Valley Analytical Laboratory from January 1, 2013 through July 1, 2015 (Table 1.1). If a more typical corn silage would have been fed, it is likely that the results seen in this study would have been different, with cows fed corn silage being superior for DMI and milk yield.

Aydin et al. (1999) conducted a similar study consisting of two experiments comparing the levels of DMI and milk yield between normal and BMR sorghum, alfalfa, and corn silage based diets. In the first experiment, NDF content of diets containing normal and BMR sorghum silage was greater (39.7 and 40.3% of DM, respectively) than diets containing alfalfa or corn silage (29.1 and 34.3% of DM respectively). Dry matter intake among diets was not different, while yield of milk, fat, and protein was greatest for cows fed corn silage, intermediate for cows fed either BMR sorghum or alfalfa silage, and lowest for cows fed normal sorghum silage. A second experiment was conducted where all diets contained similar NDF (% of DM) levels. Diets were based on a blend of alfalfa silage fed at 17.5% of DM, and either normal or BMR sorghum silage, or corn silage. Milk yield was greatest for cows fed the BMR sorghum when compared to normal sorghum but not different than corn silage. Dry matter intake did not differ between treatments (Aydin et al., 1999).

To study the effects of two different BMR sorghum genotypes, Oliver et al. (2004) compared diets containing either BMR sorghum-6 or BMR sorghum-18 to diets comprising of either normal sorghum silage or corn silage. All diets were balanced for similar levels of CP, NDF, and starch concentrations. While DMI was not different between any of the four treatment groups, milk yield was lowest for diets containing normal sorghum silage when compared to BMR-6 sorghum and corn silage, but not different than BMR-18 sorghum silage. More recently, forage sorghums containing the BMR-6 gene have been accepted by producers because of their

ability to produce similar DM yields to conventional sorghum silage and their lower lodging potential.

While some studies have shown reduced DMI between diets containing conventional sorghum silage or corn silage (Grant et al., 1995) other studies show no differences (Aydin et al., 1999; Oliver et al., 2004; Colombini et al., 2012). Differences often exist, however, when looking at milk yield or 4% fat-corrected milk yield (FCM) (Grant et al., 1995; Aydin et al., 1999; Oliver et al., 2004), while another study found a reduction in milk yield for cows fed conventional forage sorghum but no difference in 4% FCM yield when compared to corn silage (Colombini et al., 2012). When BMR sorghum silage was fed, however, milk yield was similar to diets containing corn silage (Lusk et al., 1984; Grant et al., 1995; Oliver et al., 2004). These effects are likely due to the reduced lignin levels in the BMR sorghum varieties leading to increased digestibility and therefore, greater energy available to the cow.

Physical Structure of Sorghum Berries

To better understand the chemical factors that could potentially influence the digestibility of sorghum berries in the dairy cow when compared to other silage or grain sources, it is important to understand the anatomy of the sorghum berry. A sorghum berry is made up of three main morphological parts: the pericarp or outercovering, the endosperm, which contains most of the starch, and the germ, or embryo (Rooney et al., 1981). Although there is some variation between hybrid types, for medium-sized sorghum berries, the pericarp, germ, and endosperm make up 6, 10, and 84% of the berry dry weight, respectively (Rooney et al., 1981).

The pericarp is the outermost layer of the berry and its primary role is protection for both the endosperm and embryo within the berry. For this reason, the pericarp is relatively resistant to ruminal bacterial attachment (Huntington, 1997) and therefore, poorly digested in the rumen. If

this outer layer becomes damaged either via mastication by the cow or mechanical processing, ruminal bacteria will gain access to nutrients, primarily starch, within the berry that would have otherwise been inaccessible.

The biological function of the endosperm is to serve as the primary source of nutrients for the embryo until photosynthesis begins after seed emergence (Mohr and Schopfer, 1995; Buchanan et al., 2000). The sorghum endosperm contains primarily starch and protein. In addition, however, the endosperm also contains the following storage proteins which are hydrophobic in nature: albumins, globulins, glutelins, and prolamins (kafarin protein in sorghum) (Wong et al., 2009). The endosperm can be broken into four distinct layers: the aleurone, peripheral, vitreous, and floury layers. The aleurone layer is made up of a single layer of cells located directly below the pericarp and contains large amounts of minerals, water soluble vitamins, enzymes, and oils (Rooney at al., 1981).

The peripheral endosperm layer lies directly beneath the aleurone layer and consists of cells which contain small starch granules. This layer can be anywhere from 2 to 6 endosperm cells thick (Rooney et al., 1981). Starch granules within this layer are embedded in a dense protein matrix, which when combined make up what is called the starch-protein matrix that will be discussed later. This region is very dense, hard, and resistant to digestion (Rooney and Phlugfelder, 1986). Much of the starch in the peripheral endosperm is bound in the protein matrix and is unavailable for digestion unless the berry is processed in some way to disrupt the matrix causing release of the starch granules. When this occurs, bacteria can attach to these starch granules and begin to hydrolyze the starch to volatile fatty acids in the rumen, which serve as the primary energy source for the cow.

The vitreous endosperm is located beneath the peripheral endosperm and is also embedded within the starch-protein matrix (Rooney et al., 1981; Shull et al., 1990). A continuous interface is formed between protein and starch limiting digestion of the starch granules located here. Just like the peripheral endosperm, starch located in this endosperm region is indigestible to the cow unless the protein matrix is broken in some way. If this occurs, rumen bacteria will again gain access to the starch granules.

The floury endosperm is the inner most region of the endosperm and contains loosely packed starch granules with little or no starch-protein matrix present (Rooney et al., 1981). The little protein that is present is readily solubilized, allowing greater access of enzymes to starch granules (Hoffman and Shaver, 2010). Generally, more and larger starch granules are found in this layer (Rooney and Pflugfelder, 1986). Because of the reduced protein matrix, the starch present is much more digestible than in previously mentioned layers. However, the cow must first gain access to starch granules within the floury endosperm to be able to hydrolyze that starch and use as an energy source. By physically processing the sorghum berries, starch digestibility should be improved and provide the cow with greater energy compared to unprocessed sorghum.

The embryo, or germ, of the sorghum berry serves in the transport of moisture, microorganisms, and solubilized endosperm components (Rooney at al., 1981). The embryo has also been shown to play a major role in water uptake and mold susceptibility of the berry (Glueck and Rooney, 1980).

What is Starch?

Starch is a glucan composed of two polymers, amylose and amylopectin (Rooney and Pflugfelder, 1986; Kotarski et al., 1992). Amylose is a linear polymer linked together by α-1,4

bonds and typically comprises 20 to 30% of starch in normal cereal grain (Rooney and Pflugfelder, 1986; Kotarski et al., 1992). Amylopectin is a larger, branched polymer with α-1,4 bonds and α-1,6 branch points every 20 to 25 glucose units (Rooney and Pflugfelder, 1986). Amylopectin typically makes up 70 to 80% of starch in most cereal starches (Rooney and Pflugfelder, 1986).

Starch-Protein Matrix

The starch-protein matrix refers to the combination of starch, prolamins and other proteins (albumins, globulins, and glutelins) in the endosperm and has been defined as "a physiochemical impediment to starch digestion in ruminants" (Owens et al., 1986). This matrix is responsible for binding starch granules together and the degree of binding determines the digestibility of the starch (Kotarski et al., 1992). In sorghum, hydrophobic kafarin proteins are the primary prolamin proteins in the starch-protein matrix, and comprise 70 to 80% of the protein in whole grain sorghum (Wong et al., 2009). Kafarin proteins can be broken into 1 of 4 subclasses (α, β, γ, and δ). The α-kafarins make up 80 to 84% of the total fraction in vitreous endosperms and 66 to 71% in floury endosperms, while γ-kafarin makes up about 7 to 8% in vitreous endosperms and 10 to 13% in floury endosperms (Watterson et al., 1993). Kafarins tend to be more hydrophobic on average when compared to other prolamin proteins such as zein in corn. As prolamin-kafarin proteins enlarge with advancing maturity β- and γ- kafarins form a cross-linked network and α- and δ-kafarins penetrate this network forming a hydrophobic starch-protein matrix (Buchanan et al., 2000). These cross-links are more pronounced in sorghum than corn, which helps to explain the lower digestibility of starch granules in sorghum (Rooney and Pflugfelder, 1986). When this cross-linking of kafarin proteins becomes excessive, vitreous endosperm will result. Previous research in corn has shown that varieties with greater percent

vitreous endosperm resulted in decreased in vitro and in situ starch digestibility (Philippeau et al., 2000; Correa et al., 2002; Ngonyamo-Majee et al., 2008). Vitreousness increases with increasing maturity at harvest (Phillipeau and Michalet-Doreau, 1997) so differences among hybrids are greatest when harvested at a more mature state. For sorghum to be a viable energy source, the protein matrix within the endosperm must be disrupted or starch granules will be unavailable to rumen microbes for digestion.

Importance of Kernel Processing

While forage sorghum silage is not typically processed, kernel processing via on-board kernel processors have been used extensively in the harvest of corn silage in an effort to reduce kernel particle size leading to increased total tract starch digestibility for the dairy cow. Ferreira and Mertens (2005) established a method to determine the degree of kernel processing, or breakage, in whole plant corn harvested as silage. This was accomplished by shaking undried corn silage samples through a series of screens with differing apertures ranging from 19 mm on the top to 1.18 mm on the bottom in addition to a pan. This method has been adapted for use in forage testing laboratories and measures the proportion of starch passing through a 4.75 mm screen using a Ro-Tap machine. The development of a kernel processing score has given the industry a standard by which to measure the degree of kernel processing in corn silage. General recommendations are to have $\geq$ 70% of the starch pass through the 4.75 mm screen (Rock River Laboratory, Watertown, WI). Reducing particle size of the kernel fraction disrupts the starch-protein matrix found in grains of various grain crops (corn, sorghum, wheat, etc.). This increases the surface area of the starch allowing rumen microbes to better access and more completely digest the starch portion prior to passing out of the rumen.

Kernel processing of corn silage harvested with a roll gap setting of 1 to 2 mm has been shown to increase starch digestibility (Rojas-Bourrillon et al., 1987; Johnson et al., 1996; Bal et al., 2000) and reduce particle size by 15 to 30% (Schurig and Rodel, 1993; Roberge et al., 1998). Greater total tract starch digestibility (96.1 vs. 90.7%) was observed in growing steers fed processed corn silage compared with unprocessed corn silage (Rojas-Bourrillon et al., 1987), while greater ruminal in situ starch degradation was seen for processed versus unprocessed corn silage (Bal et al., 1998). Increased milk production when fed processed corn silage has been reported (Johnson et al., 1996; Bal et al., 2000) and this increase is due in large part to the increased digestibility of processed corn silage evidenced by lower fecal starch levels (Johnson et al., 1996).

Sorghum Starch Digestibility

Ruminal starch fermentation rates vary and are influenced by grain type, type of processing, diet, and ruminant species (Owens et al., 1986; Theurer, 1986). When comparing the composition and kernel structure of corn and sorghum, the two are actually very similar (Rooney at al., 1980). Starch granules are very similar in size, shape, and composition but major differences are seen in the type and distribution of proteins surrounding the starch granules within the endosperm between corn and sorghum (Rooney and Pflugfelder, 1986). In general, sorghum contains a higher proportion of peripheral endosperm (Rooney and Sullins, 1973; Rooney and Miller, 1982), which as mentioned earlier, is the endosperm region that is extremely dense, hard and resistant to digestion (Rooney and Pflugfelder, 1986). A primary purpose of the peripheral endosperm is to protect the starch located within this layer and therefore, is resistant to digestion unless the starch-protein matrix is broken in some way. This protein matrix adheres

starch and protein more tightly in sorghum than in corn and is the main reason for lower digestibility often seen with sorghum (Rooney and Pflugfelder, 1986).

Fortunately, the digestibility of the starch contained in sorghum can be improved by certain processing methods including: steam-flaking, rolling, grinding, and early harvest prior to complete development of the starch-protein matrix. If one or more of these methods are employed, the nutritional value of sorghum can be improved to a similar level as corn (Rooney and Pflugfelder, 1986).

Ward et al. (1965) recognized the importance of sorghum silage and the digestibility of starch retained within and its impact on animal performance. In this study, control sorghum silages were harvested and ensiled in a conventional manner, while the treatment sorghum silage had the berry heads removed from the silage, passed through a hammermill, and then added back to the original silage. Mean starch digestibility for the ground head silage was greater compared to the control silage due to the level of processing (Ward et al., 1965). The processing method was effective at disrupting the protein matrix and therefore, rumen bacteria were able to access the starch granules contained within the sorghum berries.

Numerous researchers have attempted to enhance the digestibility of starch in sorghum silage to increase animal performance by either processing the berries before or after harvest. Oliveira et al. (1993) found increased starch digestibility in dairy cows fed steam flaked sorghum grain compared to rolled sorghum grain. Steers fed processed sorghum silage had greater average daily gains ranging from 8 to 29% and greater feed conversions of ~19% compared with steers fed unprocessed sorghum silage (Fox et al., 1970; Pund, 1970). In contrast, other studies have shown no improvement in performance of growing cattle fed processed grain sorghum silages (Brethour and Duitsman, 1971; Smith et al., 1984). Differences among these studies are

likely due to differences in maturity at harvest and the degree of processing applied to the sorghum silage. Smith et al. (1985, 1986) suggested that the performance of growing cattle to processed grain sorghum silage was influenced by stage of maturity at harvest.

Conclusions

The efficiency of corn silage fermentation and aerobic stability of corn silage is impacted by multiple factors that affect the quality of the final product produced. Ensuring proper management from harvest through feedout will increase the likelihood of producing high-quality silage with high energy value to be fed to livestock. The use of silage inoculants can help in the production of high quality silage through the production of lactic acid or by improving aerobic stability during feedout through the effects of *L. bucherni*, or both if a dual-purpose silage inoculant is used.

Sorghum is well known for its increased drought tolerance compared to corn as it requires 30 to 50% less water making it an attractive forage source to producers in areas of the country that routinely experience drought-like conditions. A major drawback of sorghum, however, is the reduced starch digestibility compared to corn due to differences in prolamin proteins between the two crops. Starch digestibility in sorghum can be improved by processing the sorghum berries and disrupting the starch-protein matrix. Increased starch digestibility should then lead to improved animal performance as a result of increased energy availability in the processed silage.

Figures and Tables

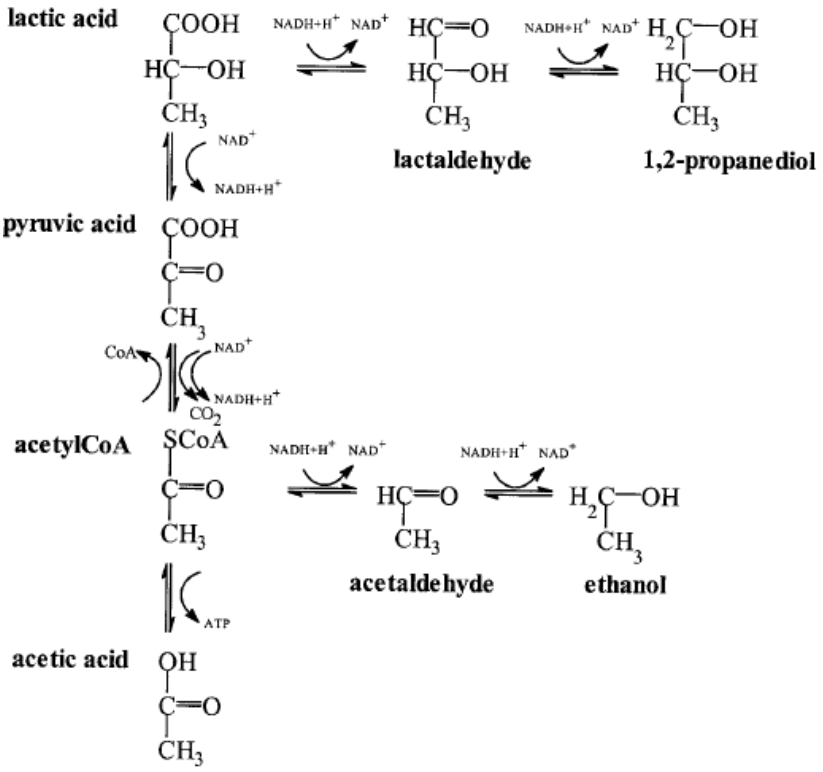

Figure 1.1 Proposed pathway for anaerobic degradation of lactic acid by *Lactobacillus buchneri* into equimolar amounts of 1,2-propanediol and acetic acid and trace amounts of ethanol (Oude Elferink et al., 2001).

Table 1.1 Chemical composition (mean ± standard deviation) of corn, conventional forage sorghum, or BMR forage sorghum

	Corn Silage[1]	Forage Sorghum[1]	
Item, %	Conventional	Conventional	BMR
n =	8,640	1,498	132
DM	35.2 ± 4.9	32.8 ± 5.3	34.0 ± 6.5
CP	8.1 ± 1.1	9.8 ± 2.4	10.6 ± 3.2
ADF	25.3 ± 3.3	34.4 ± 4.59	34.3 ± 4.5
NDF	40.9 ± 5.0	53.0 ± 6.8	54.2 ± 7.2
NDFD, 30 h	56.5 ± 4.4	48.7 ± 7.0	54.0 ± 8.3
Lignin	3.2 ± 0.6	5.0 ± 0.9	4.6 ± 0.9
Sugar	1.3 ± 0.8	4.2 ± 2.3	5.3 ± 3.0
Starch	32.1 ± 6.5	11.7 ± 8.0	10.3 ± 8.8
Fat	3.2 ± 0.3	2.7 ± 0.4	2.9 ± 0.4
Ash	4.1 ± 1.6	9.1 ± 3.5	8.9 ± 3.1
Ca	0.25 ± 0.20	0.51 ± 0.35	0.44 ± 0.13
P	0.23 ± 0.04	0.23 ± 0.06	0.26 ± 0.07
Mg	0.16 ± 0.05	0.33 ± 0.11	0.32 ± 0.09
K	1.14 ± 0.28	2.02 ± 0.76	2.23 ± 0.84

[1]Analysis of silage samples submitted to Cumberland Valley Analytical Laboratory from January 1, 2013 through July 1, 2015.

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Chapter 2 - Literature Review: The Effects of Heat Stress on the Dairy Cow

Introduction

Heat stress greatly affects the dairy industry every year and it has been estimated that annual losses in the U.S. due to heat stress alone total $897 million (St. Pierre et al., 2003) even when current, economically feasible heat abatement systems are used. This number is only likely to increase in the future as a result of increasing production levels in dairy cattle leading to greater body heat production, and also a shift trending towards locating dairy cattle in hotter climates. Advances in management by using cow cooling systems and nutritional strategies have helped to lessen some of the losses incurred due to heat stress but significant economic losses still occur. Lactating dairy cattle tend to be most affected by heat stress; however, it is important that producers not forget about dry cows, heifers, and young calves as these groups also suffer from heat stress, leading to decreased growth development, health, and reproductive performance.

The alleviation of heat stress in dairy cattle by use of water evaporation and forced-air movement has long been a topic of research leading to the common recommendation of cooling dairy cattle using a combination of both water and air movement. Seath and Miller (1948) were one of the first researchers to investigate the use of water evaporation and air movement over cows and its impact on the cow. However, this method of cooling would not be applied to the dairy industry until many years later, primarily because the technology to do so was not readily available at the time. More recently, water availability and efficiency of use has become a major concern in the dairy industry leading to the need to discover new, more efficient methods to cool cows using less water. In addition, the rise in energy costs in summer from the use of ventilation systems in an effort to sustain adequate airflow over cows has led some producers to question the efficacy of their cooling systems. Typically, cow cooling systems are operated based on the

temperature of the environment. Temperature humidity index (THI) is an index used to measure heat stress in dairy cows and uses both environmental temperature and relative humidity. Other important considerations, however, not included in the THI calculation, include air velocity, wetness of the hair coat, and radiation, all of which can impact the actual THI experienced by the cow.

To understand why and how heat stress affects dairy cattle, it is important to first understand the biology of the cow and what is happening physiologically to cause the decrease in performance that we see annually throughout the summer months.

Etiology of Heat Stress

The biological mechanism by which heat stress causes decreased milk yield and fertility is partly explained by reduced feed intake (West, 2002, 2003). However, it also includes altered endocrine status, a reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier et al., 2005), which results in decreased nutrients and energy available for production. It has been estimated that energy requirements during heat stress are increased by 25 to 30% (NRC, 1989; Fox and Tylutki, 1998), primarily due to increased energy expenditure on panting, sweating, production of heat shock proteins (Tomanek, 2010), and increased Na+/K+ ATPase activity (Gaffin and Hubbard, 1996).

Due to the reduction in feed intake during heat stress, a majority of dairy cows enter into negative energy balance (NEBAL) (Baumgard et al., 2008). While this is similar to the NEBAL observed in early lactation, differences in nutrient partitioning are apparent between early lactation and heat stressed cows (Baumgard et al., 2008).

In early lactation, dry matter intake (DMI) is insufficient to support peak milk yield, causing cows to enter into NEBAL as they simply cannot consume enough energy to meet their

requirements for maintenance and milk production (Drackley, 1999). Therefore, these cows begin to mobilize adipose tissue in order to meet their energy requirements. Changes in both carbohydrate and lipid metabolism ensure movement of dietary and tissue derived nutrients to the mammary gland (Bauman and Currie, 1980). Many of these changes are regulated by endogenous somatotropin, which naturally increases during periods of NEBAL (Bauman and Currie, 1980). Somatotropin is known to promote non-esterified fatty acid (NEFA) export from adipose tissue by heightening the response of adipose tissue to β-adrenergic signals and by blocking lipogenesis and glucose utilization (Bauman and Vernon, 1993). In an early lactation cow not under heat stress, circulating insulin (an anti-lipolytic signal) levels are reduced because of reduced insulin sensitivity (Baumgard et al., 2008), which allows for mobilization of adipose tissue resulting in increased NEFA concentration (Bauman and Currie, 1980; Rhoads et al., 2004). These elevated NEFA serve as a significant energy source for the cow and spares glucose to be used by the mammary gland to support milk production (Bell, 1995) by decreasing glucose uptake by skeletal muscle (Randle, 1998). Early lactation hypoglycemia heightens catecholamine's effect on adipose tissue lipolysis (Galaster et al., 1981) as part of the normal stress response.

Similarly, heat stressed dairy cows also enter into NEBAL due to a reduction in DMI, but unlike the early lactation cow, heat stress induced NEBAL does not result in elevated plasma NEFA as insulin levels remain elevated. Chronically heat stressed dairy cows tend to have reduced somatotropin levels (Mohammed and Johnson, 1985; Li et al., 2006). The greater levels of insulin sensitivity are likely a mechanism by which cattle decrease metabolic heat production, as oxidizing glucose is more efficient (Baldwin, 1980). Elevated insulin blocks the breakdown of adipose, making the heat stressed cow metabolically inflexible and unable to rely on adipose

tissue to meet its energy needs. Therefore, glucose is used as an energy source at a greater rate in heat stressed animals in an attempt to generate less metabolic heat. This results in less glucose being available for the mammary gland to produce lactose, leading to decreased milk production. Recent studies have shown that heat stressed animals secrete ~200 to 400 g/d less milk lactose compared with pair-fed, thermal-neutral controls (Rhoads et al., 2009; Wheelock et al., 2010) due to increased glucose uptake by extramammary tissues. In a recent study, two groups (heat stressed or cooled) of early lactation cows were pair fed. Non-esterified fatty acid levels were similar for each group prepartum but the heat stressed group showed a significant reduction in NEFA levels postpartum, indicating impaired adipose tissue mobilization (Lamp et al., 2015).

Rhoads et al. (2009), studied the percent decline in milk production that could be attributed to decreased DMI and what percent was due to other factors. Two groups (heat-stressed and cooled) were pair-fed. With similar DMI, cows exposed to heat stress had a milk yield of 21.5 kg/d, while the pair-fed cows under thermoneutral conditions produced 29.0 kg/d. Thus, factors other than reduced DMI are responsible for 64% of the milk loss during heat stress. These authors hypothesized that because heat stressed dairy cows do not mobilize adipose tissue, glucose-sparing mechanisms that normally prevent severe reductions in milk yield during periods of inadequate feed intake are not present (Rhoads et al., 2009).

In addition to reduced feed intake, the thermoregulation process leads to increased blood flow to the skin bringing heat up from the body core and dissipating that heat via evaporation from the skin and respiratory tract. Thus, blood flow to internal organs (i.e. splanchnic tissues) is reduced by up to 50% (McGuire et al., 1989; Hall et al., 2001) and therefore, there is less movement of water and nutrients via the portal system that would have been used to support milk production (Finch, 1986). Enterocytes within the small intestine are extremely sensitive to

oxygen and nutrient restriction (Rollwagen et al., 2006). With reduced blood flow to the small intestine during heat stress, conformational changes and reduced intestinal barrier function result from intestinal hypoxia (Hall et al., 2001; Lambert, 2009). As will be discussed later, heat stress can also lead to ruminal acidosis (Mishra et al., 1970; Kadzere et al., 2002), which further compromises the integrity of the intestinal barrier (Plaizier et al., 2008). Due to tight junction dysfunction between enterocytes, increased paracellular movement of lipopolysaccharide (LPS) from the lumen into blood occurs, causing an inflammatory response (Bouchama and Knochel, 2002; Mani et al., 2012). By infusing LPS into the mammary gland, insulin levels increased, indicating that LPS contributes to elevated insulin levels in heat stressed cows (Waldron et al., 2006). Greater levels of insulin from LPS further reduce the mobilization of adipose tissue as an energy source in the heat stressed cow leading to increased glucose uptake by insulin-dependent tissues (adipose and muscle), leaving less glucose available for the mammary gland (non-insulin dependent).

Temperature Humidity Index

Heat stress is caused by a combination of environmental factors including the following: air temperature, relative humidity (RH), solar radiation, air movement, and precipitation. Many different indices have been developed which combine these different environmental factors to measure the level of heat stress, but their use has generally been limited due to poor data availability (Bohmanova et al., 2007). Most heat stress studies conducted today focus mainly on two of the environmental factors: air temperature and RH. Relative humidity refers to the actual moisture content in ambient air relative to that of saturated air (Thompson, 1998) and has an impact on the rate of evaporative heat loss through the skin and lungs. The amount of moisture in the air becomes increasingly more important as air temperature increases. Temperature

humidity index is a single value representing the combined effects of temperature and RH. Temperature humidity index was originally developed by Thom (1958) and was later adapted for use in cattle by Berry et al. (1964). Traditionally, a THI of 72 was thought to be the point at which milk production declined and other effects of heat stress began in dairy cows. This was discovered, however, using cows producing just 15.5 kg/d of milk. More recently, Zimbelman et al. (2009), re-evaluated the THI threshold and hypothesized that the higher producing dairy cows today are more susceptible to heat stress and that the effects of heat stress start well before a THI of 72. It was concluded that a new THI threshold for lactating dairy cows producing greater than 35 kg/d should be 68 (Zimbelman et al., 2009). As milk production of dairy cattle continues to increase, cattle become more sensitive to heat stress conditions and have a reduced temperature threshold at which milk loss begins to occur (Berman, 2005). This is due in part to the fact that as production levels rise, metabolic heat production is increased, causing the animal to become more heat stressed. Heat production from cows producing 18.5 and 31.6 kg/d of milk was 27.3 and 48.5% greater than non-lactating cows (Purwanto et al., 1990). In addition, as milk production increased from 35 to 45 kg/d, the threshold temperature for heat stress was reduced by 5°C (Berman, 2005).

Temperature humidity index is used today in the dairy industry to estimate the cooling requirements of dairy cattle in order to improve management strategies to alleviate the effects of heat stress. It is beneficial to use THI as it incorporates both temperature and RH into one value.

Consequences of Heat Stress

Heat stress can affect the cow to varying degrees depending on stage of lactation. Typically, heat stress affects high producing, lactating dairy cows first as they produce the greatest amount of metabolic heat as a result of their greater DMI and milk yield (Coppock et al.,

1982). For this reason, producers commonly focus on this group first when cooling dairy cattle. Recent research, however, has shown that if cows are not cooled during the dry period, heat stress can cause major production losses in the subsequent lactation and negatively affect the young, growing calf as well (Tao et al., 2011; Tao et al., 2012a).

Heat Stress during Lactation

Lactating dairy cows exposed to high ambient temperatures, RH, radiant energy from the sun, or their combination respond with reduced milk yield. Heat stress during lactation can have major and long-lasting effects on DMI, milk yield and composition, and reproduction. Milk yield and DMI were shown to decrease by 1.8 kg and 1.4 kg, respectively, for each 0.55°C increase in core body temperature (CBT) (Johnson et al., 1963; Umphrey et al., 2001). In another study by Zimbelman et al. (2009), milk production decreased by 2.2 kg/d for every 24 h spent above 68 THI or when minimum THI exceeded 65.

Seasonal patterns in milk yield and composition are evident in cattle. The month of parturition is known to have a large impact on milk yield and composition in the following lactation. Milk protein yield is directly affected by temperature where, as ambient temperature increases, protein levels typically decrease (Collier et al., 2012). One reason for this may be due to the production of heat shock proteins in response to heat stress by mammary epithelial cells, which would reduce milk protein synthesis (Collier et al., 2008). Cows that calved in December produced the highest levels of milk and milk protein, while those calving in June produced the lowest, 92.8% of the maximum (Barash et al., 2001). In the same study, it was found that average milk production declined by 0.38 kg/°C and average protein production was reduced by 0.01 kg/°C (Barash et al., 2001). Along with a reduction in milk yield and milk protein, milk fat also typically decreases in the summer months. McDowell et al. (1976) found that milk fat,

solids-not-fat, and milk protein percentage decreased 39.7, 18.9, and 16.9%, respectively, when air temperature increased from 18 to 30°C.

It would be expected that feed efficiency would decrease in cows exposed to heat stress. The increase in maintenance associated with heat stress causes nutrients to be shifted away from production processes due to mechanisms put in place by the animal in trying to maintain normal CBT. Therefore, anything that can be done to keep the cow cooler will result in greater feed efficiency and increased production levels.

The major challenge in high producing dairy cows under heat stress is to dissipate heat produced by metabolic processes. Cows that are housed in hot climates produce additional heat relative to cool climates because of the greater physical activity (i.e. panting) necessary to increase cooling in hot conditions. Improvements in genetics and management will continue to increase feed intake and milk yield in dairy cows leading to even greater metabolic heat production.

Heat Stress during the Dry Period

Although lactating cows are often thought of as the most important group to cool, dry cows also suffer from heat stress if not adequately cooled. Tao et al. (2012b) found that cows under heat stress during the dry period tend to have increased circulating insulin levels and decreased plasma glucose concentration during the postpartum period. Also, cooled cows had increased circulating NEFA in early lactation compared with non-cooled cows. These data indicate that when cows are cooled during the dry period, the mobilization of adipose tissue postpartum is enhanced to support greater milk yield due to decreased circulating insulin levels. The decreased glucose levels in the cooled cows postpartum likely indicates that because of

greater milk production, more glucose was being used to produce larger volumes of milk lactose leaving less glucose in the blood compared with non-cooled cows.

In a study done by Tao et al. (2011), heat stress conditions tended to decrease mammary epithelial cell proliferation rate leading to an increase in production in the subsequent lactation for cooled cows compared to heat stressed cows (33.9 vs. 28.9 kg/d, respectively). In addition, heat stressed cows consumed less feed during the dry period when compared to cooled cows. By using heat abatement strategies during the dry period, we can greatly enhance milk production in the following lactation due to increased mammary epithelial cell proliferation rate, leading to increased mammary secretory cells.

Heat stress is also known to affect immune function and increase the incidence of metabolic disease of dairy cows. The immune system consists of the non-specific innate immune function, which is the first line of defense to pathogens, and the specific adaptive immune function, which generates memory of pathogen exposure. Both types of immune function can be affected by thermal stress. Cows cooled during the dry period showed greater neutrophil function as measured by oxidative burst at 2 and 20 d post-partum compared to heat stressed cows (do Amaral et al., 2009; 2010; 2011). Since neutrophils are the first line of defense against disease, this indicates that cooled cows would be able to better fight off an infection and maintain their immune system, particularly during the transition period. In addition, when cows were injected with ovalbumin, cooled cows responded with superior production of IgG during the dry period, indicating impaired humoral immunity in heat stressed cows during late gestation (do Amaral et al., 2011). When cows were induced with *Streptococcus uberis* at 5 d postpartum, cooled cows had greater numbers of white blood cells

and neutrophils before and during the challenge (Thompson et al., 2014). This also shows that cows cooled during the dry period have an improved immune system in early lactation.

Decreased immune function in heat stressed dairy cows, particularly during the transition period, increases the risk of metabolic disease postpartum. Thompson et al. (2012) found that cows exposed to heat stress during the dry period had greater incidence of postpartum disorders including mastitis, retained fetal membranes, and respiratory problems. With decreased white blood cell and neutrophil function in heat stressed dairy cows, an increase in postpartum disorders is not unexpected.

Cooling cows during the entire dry period cannot be overemphasized. When nonlactating dairy cows were cooled during the close-up period (final 3 weeks prior to parturition) only, milk production improved 1.4 kg/d through 60 DIM compared with non-cooled cows (Urdaz et al., 2006). When cows received cooling throughout the entire dry period, however, milk production increased 2.5 to 5 kg/d over non-cooled cows (Tao et al., 2011, 2012b; Thompson et al., 2012). Therefore, any time and money spent on cooling cows during the entire dry period will be well worth the time and effort, and will be money well spent.

Effects of Heat Stress on the Calf

Heat stress not only affects older animals but the young growing calf as well. Depending on the stage of gestation, nutrition can have a large effect on fetal growth and immune function of the neonate. Malnutrition, as seen during heat stress due to a decrease in DMI, as well as during late gestation, has been linked to inferior birth weights of calves, increased incidence of dystocia, and greater mortality and morbidity rates (Wu et al., 2006).

Calves born to heat stressed dams often have reduced birth weights (Collier et al., 1982; Tao et al., 2012a). Several factors may contribute to impaired fetal growth during late gestation

under heat stress. One such factor is that heat stressed cows typically have a shorter gestation length, accounting for ~40% of the reduction in birth weights observed from heat stressed dams (Monteiro et al., 2014). Another factor leading to impaired fetal growth is decreased uterine (Oakes et al., 1976; Dreiling et al., 1991; Reynolds et al., 2006) and mammary blood flow (Lough et al., 1990). This reduction in blood flow leads to decreased transport of oxygen and nutrients from the dam to the calf, resulting in impaired fetal growth (Monteiro et al., 2014). The last two months of gestation are critical to bovine fetal development and accounts for ~60% of body weight gain prior to birth (Bauman and Currie, 1980). According to Muller et al. (1975), the fetus of a Holstein dairy cow has an average daily gain of 0.5 kg in the final week of gestation. Another factor that may lead to decreased birth weight is the fact that DMI of the dam is reduced when exposed to heat stress, leaving fewer nutrients for the fetus to grow. Placental hormones (placental lactogen and pregnancy-associated glycoprotein) delivered to the placenta are also reduced because of decreased uterine blood flow during heat stress (Collier et al., 1982; Bell et al., 1989; Thompson et al., 2013), which also affects the growing fetus.

Calves born to heat stressed or cooled cows weighed 36.5 kg and 42.5 kg, respectively (Tao et al., 2012a). While no differences were seen in colostrum IgG content, calves born to heat stressed dams were less efficient in absorbing IgG from the colostrum and had lower serum IgG concentrations for the first 28 d of life than calves born to cooled cows (Tao et al., 2012a). In contrast, Nardone et al., (1997) found that colostrum from heat stressed dams contained lower levels of IgG. These data indicate a reduction in passive transfer of immunity in calves exposed to heat stress in-utero. These results confirm that calf body weight and immunity can be impacted significantly by heat stress during the final weeks of gestation.

Heat Stress and Lameness

Every year with the arrival of late summer or early fall (typically 60 to 90 days post-heat stress), producers see a seasonal increase in lameness. Factors associated with the increase in lameness are heat stress, cow comfort and housing (increased standing times), and nutrition. Mean lying time decreased from 10.9 to 7.9 h/d from the coolest to the hottest part of the day and time spent standing in the alley increased from 2.6 to 4.5 h/d from the coolest to the hottest part of the day (Cook et al., 2007). Other studies have shown similar decreases in lying time during heat stress (Overton et al., 2002; Legrand et al., 2011). Ideally, high producing dairy cows should by lying down for a minimum of 12 h/d (Cook et al., 2007). Oftentimes, non-infectious lesions are greatest following summer heat stress due to changes in daily time budgets and physiological adaptations, both of which result in greater risk for ruminal acidosis and the subsequent production of inferior claw horn (DeFrain et al., 2013). Claw horn lesions, such as sole ulcers, are believed to develop from increased pedal bone mobility due to changes in the corium at calving (Lischer et al., 2002). Factors that lead to an increase in standing time such as heat stress may intensify these changes by further compromising the structure of the claw. Time spent standing when the cow should be lying down may stress the bond between the third phalanx and the claw horn capsule, a bond that is already weakened around calving (Tarlton et al., 2002). Extra care should be taken during the summer months not to overcrowd pens as this will lead to an increase in stocking density, which in turn leads to an increase in standing times. This will cause increased pressure on the foot, leading to lameness. Also, with sprinklers often being used throughout the summer, the cow environment is often wetter, which can also be detrimental to hoof health.

Heat Stress Effects on Reproduction and Fertility

Heat stress is a major contributing factor to low fertility in dairy cattle. Heat stress has been shown to alter the following: duration of estrus, estrus intensity, conception rate, uterine function, endocrine status, follicular growth and development, luteolytic mechanisms, early embryonic development, and fetal growth (Jordan, 2003). One of the reasons why reduced conception rates are seen during summer heat stress is a result of the intensity of estrus being reduced, leading to fewer cows being found in estrus and inseminated at the proper time. In summer months, dairy cows had just half the number of mounts per estrus compared to those in winter months (Thatcher and Collier, 1986). Wilson et al. (1998) found that serum estradiol concentrations were reduced in cows under heat stress from day 11 to 21 of the estrous cycle, further explaining why fewer cows are found in estrus throughout the summer months. Also, the incidence of anestrus and silent ovulation are increased in summer (Her et al., 1988; Al-Katanani et al., 2002). When exposed to heat stress, the uterine environment of the cow becomes compromised due to reduced blood flow to the uterus and an increase in uterine temperature, leading to loss of the embryo (Roman-Ponce et al., 1978). The combination of these factors will lead to reduced pregnancy rates during the summer months.

Heat stress also delays follicle selection and lengthens the follicular wave, and thus has potential adverse effects on oocyte quality. Wolfenson et al. (1995) found that preovulatory follicles from heat stressed cows emerged two to four days earlier and may result in ovulation of older follicles, resulting in reduced fertility. Summer heat stress reduces the degree of dominance of the dominant follicle and more medium-sized subordinate follicles survive (Jordan, 2003). This can lead to a situation where more than one dominant follicle develops, which could explain the increase in twinning that is commonly seen in cows conceiving during

the summer months. Heat stress leads to an increase in the number of small (2 to 5 mm) follicles during day 11 to 15 of the estrous cycle, which in turn leads to a decrease in function of the dominant follicle (Trout et al., 1998). In a study done by Al-Katanani et al. (2002), effect of season and exposure to heat stress on oocyte development was studied. The authors found that the number of embryos that developed to the blastocyst stage on day 8 after insemination was decreased during the warm season (April to September) versus cool season (October to March).

Temperature increases of just 0.5°C above normal body temperature have been shown to lead to reduced pregnancy rates in dairy cattle (Gwazdauskas et al., 1973; Wolfenson et al., 1988; Thatcher et al., 2010). In an effort to study the effects of CBT on reproduction in lactating dairy cattle, cows were cooled 7 times per day for 30 minutes by sprinklers and forced ventilation. Another group of cows were housed under conditions without fans and sprinklers. The wetting cycles for the cooled group consisted of 30 seconds of wetting followed by 4.5 minutes of forced ventilation with two hours between cooling periods. CBT for cooled cows was maintained below 39.0°C throughout the duration of the study while CBT of non-cooled cows was greater than 39.0°C for most of the day. Conception rate at first insemination was greater (59%) in cooled cows versus non-cooled cows (17%). Pregnancy rates were also measured at days 90, 120, and 150 post-breeding. Cooled cows had greater pregnancy rates at all 3 time-periods (44, 59, and 73 vs. 14, 31, and 31, respectively; Wolfenson et al., 1988). Thus, according to these data, by using cooling methods to maintain CBT below 39.0°C, the impact of heat stress on reproduction throughout the summer months can be reduced.

More recent data, however, shows that reproduction and fertility may be affected below 39.0°C rectal temperature. Recipient cows in an embryo transfer study showed decreased probability of pregnancy once average daily rectal temperatures exceeded 38.0°C and continued

to decrease linearly as rectal temperature increased (Vasconcelos et al., 2011). Rectal temperatures in this study were taken between 0600 and 1000 h which is when we would expect cows to have the lowest rectal temperatures. This explains why reproductive efficiency began to decrease at 38.0°C, which is below the normal body temperature of a cow (38.5°C).

Heat stress can greatly affect the growing embryo, and the greatest susceptibility of the embryo to heat stress is immediately after the onset of estrus and during the early post-breeding period. Embryos become more resistant to the effects of heat stress as development progresses. Ealy et al. (1993) looked at how the developing embryo responded to heat stress on day 1, 3, 5, or 7 of development. Embryos were most susceptible to heat stress on day 1, but once the embryo reached day 3 of development, it became more resistant, but not completely resistant to the effects of heat stress. Therefore, by keeping the cow cooler throughout the breeding period through the use of heat abatement systems to maintain lower rectal temperatures, embryo survival should increase. Another strategy producers could consider using during summer heat stress is embryo transfer in place of timed artificial insemination. Embryo transfer may increase the number of pregnancies generated during the summer, but producers should consider the economic aspect of this strategy. Implanting embryos that developed under thermoneutral conditions into heat stressed cows 7 days post-estrus can bypass the critical time period (days 1 to 7) when embryos are most sensitive to heat stress (Putney et al., 1989).

Heat stress often induces NEBAL in the dairy cow due to a reduction in DMI. As a result, fewer nutrients are supplied to the reproductive system for ovarian function and embryo growth. With reduced DMI during heat stress, cows experience reduced levels of insulin and glucose in blood. Insulin is required for the development of follicles and has beneficial effects on oocyte quality. In addition, glucose is the primary fuel for the ovary and embryo (De Renis et

al., 2003). With decreased levels of insulin and glucose, fertility will be reduced. The lactating dairy cow is going to first direct nutrients to growth, maintenance, and lactation before supplying the reproductive organ.

Heat stress and its effects on reproduction can extend into the fall months as well as it takes approximately 40 to 50 days for antral follicles to develop into large dominant follicles and ovulate (Wilson et al., 1998). If heat stress occurs during the time of follicular development, both the follicle and oocyte become damaged, resulting in a less fertile oocyte, thus reducing fertility.

As milk production per cow continues to increase each year across the U.S., new and improved ways to better manage heat stress are going to be necessary as milk production and fertility are inversely related. High producing dairy cattle have greater metabolic heat production due to increased DMI and milk production levels which leads to greater rectal temperatures thus, affecting embryonic survival and development.

Heat Stress Abatement Strategies

Dairy cows are well known for their significant heat production due to ruminal fermentation and metabolic processes. All this heat must be exchanged with the environment or body temperature will increase. There are 4 principle routes by which cattle may exchange heat with the environment. Three of these methods fall under the category of sensible routes of heat loss and include conduction, convection, and radiation. All three of these mechanisms work on a temperature gradient (Kadzere et al., 2002; West, 2003; Collier et al., 2006). The fourth method that a dairy cow can use to remove excess body heat in summer is known as evaporation and works on a vapor pressure gradient. This is the primary method of heat loss at greater ambient temperatures experienced in summer (Kadzere et al., 2002; West, 2003; Collier et al., 2006). If

the cow fails to dissipate more heat than what is taken in from the surrounding environment, body temperature will increase.

Cooling the Cow

Supplemental airflow provided in summer increases the relative rates of convective heat transfer. Convection is the movement of heat from the cow's body to the surrounding environment by the movement of air past the cow's body. When cool air comes in contact with a warm body, a layer of air surrounding the body surface is heated and this heated air then rises, moving away from the body and carrying heat with it, hence cooling the cow (Kadzere et al., 2002; Collier and Gebremedhin, 2015). At low ambient temperature, air velocity impacts heat loss via convection where, as air velocity increases, the effects of convective cooling increase, making the animal feel cooler (Kadzere et al., 2002; Collier and Gebremedhin, 2015). If there is a significant difference between air temperature and the cow's body surface, convection will provide considerable cooling. However, as air temperature rises, the efficiency of heat loss via convection decreases and once air temperature exceeds body surface temperature, heat flow will reverse resulting in elevated cow body temperature (Collier et al., 2006). Therefore, convective cooling becomes less effective at greater ambient temperatures and another form of cooling (evaporation of water from the skin) should be employed to effectively cool the cow.

Increasing air velocity between 0.5 and 3.0 m/s over a cow's body surface would reduce the boundary layer insulation and therefore, be expected to increase the convective heat loss from the cow (Berman, 2004). Using dairy cows not supplemented with soakers at an air temperature of 30°C, increasing air velocity from 0.2 to 0.9 m/s (0.5 to 2.0 mph) resulted in only a slight increase in convective heat loss (-7 to 64 watts/m^2). However, when air velocity was increased to 2.2 m/s (5 mph) convective heat loss increased to 227 watts/m^2 (Hillman et al.,

2001). When the cow was soaked at either 40 min or 20 min intervals, convective heat loss was reduced with increased frequency of soaking as greater evaporative heat loss occurred. Meanwhile, other researchers have found minimal gains in convective heat loss when air flow rates were increased in cows exposed to various levels of soaking at 30°C (Gebremedhin and Wu, 2001, 2002). Therefore, current convective cooling systems are unable to sufficiently reduce the heat load placed on the cow at high ambient temperatures.

Researchers at the University of Missouri did extensive work in defining the different mechanisms of heat loss in dairy cattle (Kibler and Brody, 1949, 1950, 1952). A major finding from this research was the accounting of heat loss mechanisms in dairy cattle over a range of environmental temperatures. The authors concluded that when ambient temperatures exceeded 21°C, heat loss from the cow occurs primarily via moisture evaporation from the skin and lungs. More recently, applying water onto cows and allowing that water to evaporate has been shown to enhance evaporative cooling allowing the cow to feel cooler (Hillman and Gebremedhin, 1999; Stowell, 2000; and Hillman et al., 2001). In contrast to convective heat loss, evaporative, or latent heat loss, works on a vapor pressure gradient and is dependent on the RH of the environment surrounding the cow.

A hair coat that has been wetted to the skin is known to enhance the flow of moisture and heat away from the animal (Chastain and Turner, 1994), assuming adequate air velocity is present. With an air velocity over the body surface of 2.2 m/s (~5.0 mph), total heat loss increased by 45% from 476 to 689 watts/m^2 for no soaking and 40 min interval soaking, respectively. When the soaking interval was reduced to 20 min, total heat loss increased by 90 and 31% when compared to no soaking and 40 min interval soaking, respectively (Hillman et al., 2001). Of the total heat loss (sum of convective, radiant, and evaporative heat loss), evaporative

heat loss accounted for 50, 82, and 96% of total heat loss at 30°C for no soaking, soaking at 40 min intervals, and soaking at 20 min intervals, respectively. Meanwhile, convective heat loss accounted for just 48, 19, and 5% of total heat loss for no soaking or soaking at either 40 min or 20 min intervals, respectively (Hillman et al., 2001). Others have found similar results showing that evaporative cooling is enhanced with increased soaking of the hair coat as well as increased air velocity (Gebremedhin and Wu, 2001, 2002). These researchers evaluated the effect of soaking the body surface area to 25, 50 or 75% and found that as wetness of the total body surface increased, evaporative heat loss also increased, whereas convective heat loss was relatively unchanged. Therefore, while wetting the cow results in reduced convective heat loss, the overall heat loss is greater when the animals body surface is wetted (typically via sprinklers or soakers) to the skin due to increased evaporative heat loss (Gebremedhin and Wu, 2001, 2002; Hillman et al., 2001), and this evaporative heat loss is enhanced by increased air velocity.

Several studies have been performed that show cooling cows using a combination of supplemental airflow and wetting of the body surface results in reduced cow body temperature (Flamenbaum et al., 1986; Igono et al., 1987; Turner et al., 1992; Brouk et al., 2003). Wetting of the body surface alone has also been shown to reduce core body temperature in dairy cattle (Igono et al., 1985; Brouk et al., 2003), although not as effective as cooling using a combination of airflow and water. Cooling with supplemental airflow alone has shown to have minimal benefits in cooling cows in summer (Lin et al., 1998; Brouk et al., 2003) due to a reduced temperature gradient between the cow's body surface and the surrounding environment, therefore reducing the efficiency of convective heat loss.

Factors other than air velocity and wetting of the hair coat that also impact evaporative heat loss include ambient temperature, RH, and thermal and solar radiation (Collier and

Gebremedhin, 2015). As ambient temperature increases, cows become more reliant on evaporative cooling from the skin surface compared to sensible routes of heat loss (Gebremedhin and Wu, 2001, 2002; Collier and Gebremedhin, 2015). At an air temperature of 10 to 20°C, evaporative heat loss accounted for just 20 to 30% of the total heat loss, but when air temperatures exceeded 30°C, evaporative heat loss accounted for ~85% of total heat loss (Maia et al., 2005). Meanwhile, heat loss via evaporation becomes much less efficient as RH of the surrounding environment increases due to a reduced moisture gradient between the skin and the surrounding environment. Gebremedhin and Wu (2001) found that as RH increased from 20 to 80%, evaporative heat loss decreased by ~45%.

Nutrition

Although ventilation and cooling systems will have a greater impact on minimizing production and feed intake losses due to heat stress, nutrition is another way by which we can alleviate some of the heat stress put on dairy cattle during the hot summer months. Water is the most important nutrient for dairy cattle. Without water, DMI and milk production will decrease as milk is 87% water. Cows acclimated to 21.1°C and then exposed to 32.2°C ambient temperature for 2 weeks increased water consumption 110% and water losses from the respiratory tract and skin surface increased 55 and 177%, respectively, at the greater temperature (McDowell and Weldy, 1960). These changes lead to increased water intake. Adequate water supply must be available at all times under hot conditions. Studies in climate chambers suggested that water needs under heat stress are 1.2 to 2-fold greater than that required of cows housed under thermoneutral conditions (Beede, 1993). Water should be placed in close proximity to the cows and in the shade. Cows will often choose to continue lying down in shade versus standing up to get a drink of water if they need to walk through the sun to get there.

Having water available as the cow exits the parlor is also very important. Beede (2006) found that cows drank as much as 50 to 60% of their total daily water intake within 1-hour post-milking. The author recommends providing 61 cm of linear trough space per cow when exiting the parlor. Inside the pen, a minimum of 2 water sources should be available and cows should never have to walk more than 15 meters to access water. A common recommendation is to provide 7.6 cm of linear trough space per cow in each pen (Beede, 2006).

Heat stress has long been known to adversely affect rumen health. Cows suffering from heat stress begin to pant in order to dissipate heat. This increased respiratory rate results in more carbon dioxide (CO_2) being exhaled, reducing blood concentrations of carbonic acid to bicarbonate (HCO_3^-) necessary to maintain blood pH, resulting in respiratory alkalosis (Benjamin, 1981). In order to be an effective blood pH buffering system, the body needs to maintain a 20:1 HCO_3^- to CO_2 ratio (Baumgard et al., 2008). Due to the hyperventilation induced decrease in blood CO_2, the kidney secretes HCO_3^- into the urine to maintain this ratio (Benjamin, 1981). This reduces the amount of HCO_3^- that can be used (via saliva) to buffer and maintain a healthy rumen pH (Baumgard et al., 2008). In addition, panting cows drool more, which reduces the amount of saliva containing HCO_3^- that normally would end up in the rumen. The reductions in saliva HCO_3^- content and the decreased amount of saliva entering the rumen make the heat stressed cow much more susceptible to subclinical and acute ruminal acidosis (Baumgard et al., 2008).

One common practice in the dairy industry in an attempt to minimize metabolic heat production in dairy cows is to feed during the cooler parts of the day. This would mean feeding early in the morning before it gets hot and again later in the evening after temperatures have cooled down. A cow's peak heat production occurs 3 to 4 hours after eating (Staples, 2007).

Feeding early in the morning to allow for peak heat production to occur prior to the hottest part of the day can alleviate some of the heat stress put on the cow. By feeding a second time in the evening, fresh feed is delivered, stimulating the cow to come to the feed bunk to eat after consuming very little feed throughout the day. In two University of Florida studies, lactating dairy cows having greater rectal temperatures averaging 41.0°C consumed 79% of their total daily DMI during the cooler part of the day (1600 to 0800) compared to cows with a rectal temperature of 39.3°C, which consumed 59% of feed during the cooler part of the day (Schneider et al., 1984; Mallonee et al., 1985).

It is common to alter diets fed to lactating dairy cows during the summer months in an effort to reduce the effects of heat stress. During summer, it is common to increase the energy density of the diet to account for the expected decline in DMI. This is usually done by feeding extra concentrates and reducing forage levels. In doing this, however, rumen pH will decline, leading to increased risk for rumen acidosis in a cow that already is at high risk due to less HCO_3^- entering the rumen. In addition, it is common for nutritionists to increase the crude protein value of the ration in an attempt to account for the drop in DMI. If there is excess protein in the diet, however, energy must be used to convert the excess protein to urea in the liver which is then excreted in the urine. This process then leads to excess heat production in the animal (West, 1997). All these factors lead to an unhealthy rumen, which is why we see increased laminitis and milk fat depression during the summer months.

Another common and preferred way to increase energy density of the diet is to increase the amount of fat in the diet. Fat contains 2.5 times the energy level of concentrates and are utilized with a greater efficiency for milk production. Fats also have a decreased heat increment compared to starchy and fibrous feeds. Total heat loss decreased by 4.9 and 7.0% when cows

were fed whole cottonseed at 15% of dietary DM or whole cottonseed plus 0.54 kg/d of calcium salts of palm oil distillate, respectively (Holter et al., 1992). Fat supplementation effects on milk production have not been very consistent. With the use of fats in high fiber diets fed during heat stress, one may be able to maintain milk production and reduce the risk of rumen acidosis.

Mineral levels are another area of nutrition that needs to be checked during times of heat stress. When a cow is heat stressed, minerals are lost due to increased sweating. Potassium is the primary electrolyte lost in sweat of cattle. Along with the loss of potassium in sweat, the drop in DMI during heat stress results in less potassium intake through the diet. For this reason, it is common to increase potassium concentrations. It is recommended that dietary potassium levels be increased to 1.5% of diet DM during the summer (Staples, 2007). In a study done by Schneider et al. (1984) potassium concentration of the diet were increased from 1.0 to 1.5% using KCl, which resulted in an increase in milk production from 39.7 to 40.8 kg/d.

Sodium is another mineral that should be fed at high levels during the summer. The heat stressed cow excretes more sodium in the urine. Just like potassium, with a decrease in DMI during heat stress, sodium intake is reduced. It is recommended that sodium concentrations be increased to 0.45 to 0.60% of dietary DM (Staples, 2007). When sodium concentration of the diet was increased from 0.67 to 0.96% using $NaHCO_3$, DMI increased (39.9 vs. 42.8 kg/d) and milk production also increased (39.5 vs. 40.8 kg/d) (Schneider et al., 1984).

Trace mineral nutrition is key area that should be considered to aid cows coping with heat stress. Any type of stress alters the efficiency of the immune system, making the cow more susceptible to infectious disease. Trace minerals that play a key role in immune function, oxidative metabolism, and energy metabolism in ruminants include zinc, copper, manganese, selenium, chromium, cobalt, and iron (Overton and Yasui, 2014). If any of these minerals are

lacking in the diet, immune function may be compromised, leading to increased incidence of disease, particularly during the transition period. When these trace minerals are lacking, inadequate amounts of anti-oxidant enzymes are synthesized, leading to potential tissue damage (Overton and Yasui, 2014). Common stressors such as heat stress lead to the accumulation of free radicals. If the anti-oxidants that prevent accumulation of free radicals are not present, damage may occur (Andrieu, 2008).

Conclusions

Heat stress in the U.S. dairy industry leads to significant economic losses every year. Much research has been carried out looking at the causes for reduced milk production and it goes beyond just the typical drop in DMI often seen in the summer. The consequences of heat stress on the dairy cow can occur during lactation and the dry period. Also, calves born from cows exposed to heat stress conditions during the dry period have decreased levels of passive immunity and compromised immune function as well as decreased birth weights. Due to many factors such as increased standing time and stress in the summer, an increase in lameness is seen that often carries into the fall months of the year. Also, reproduction and fertility take a major hit in the summer months. Throughout the years, different heat abatement strategies have been implemented. This can occur by cooling the cow directly or cooling the environment in which the cow is housed. Finally, nutritional programs can be implemented during the summer that will decrease or limit metabolic heat production in high producing dairy cows. Water, however, must not be overlooked as a decrease in water intake will surely lead to a decrease in milk production.

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Chapter 3 - Evaluation of SiloSolve® FC Treated Silage on Fermentation and Aerobic Stability in Ag Bags

Abstract

The effect of treating whole-plant corn at the time of harvest with a dual-purpose commercial silage inoculant containing *Lactobacillus buchneri* LB1819 DSM22501 and *Lactococcus lactis* O224 DSM11037 on the fermentation and aerobic stability of corn silage through 32 d of ensiling was investigated. Corn silage was either left untreated and used as the control (C) or treated with a silage inoculant (T; SiloSolve® FC, Chr. Hansen Animal Health and Nutrition, Milwaukee, WI) at a rate of 2.5×10^5 cfu/g. Three silage bags were used with 2 replications per treatment within each bag. Samples were collected from each section of each bag on d 0, 2, 4, 8, 16, and 32 after ensiling. Samples were analyzed for chemical composition, fermentation variables, and aerobic stability. Inoculation had no effect on the chemical composition of the silage or on any fermentation variables measured other than the mold count (log_{10} cfu/g), where treated silage had reduced mold counts compared to C (0.10 and 0.64 ± 0.10, respectively). Silage pH were not different between treatments with means of 4.04 and 4.05 ± 0.03 for C and T silages, respectively. Silage pH decreased from 5.57 and 5.68 ± 0.11 on d 0 of ensiling to 3.61 and 3.58 ± 0.03 on d 32 of ensiling for C and T, respectively. Lactic acid and acetic acid levels did not differ at any of the time points measured resulting in a similar lactic acid:acetic acid ratio. Yeast counts (log_{10} cfu/g) were not different between treatments with means of 6.07 and 6.12 ± 0.23 for C and T silages, respectively. Aerobic stability was numerically greater for T on each day of sampling post-harvest but failed to reach a treatment effect with means of 74.7 and 95.2 ± 15.5 h for C and T, respectively. Rise in temperature of the silage mass post-harvest was similar for C and T and followed a pattern seen with properly

fermented corn silage. In this study, inoculating silage to be fed after minimal storage time (≤ 32 d post-harvest) had no effect on the chemical composition, fermentation variables other than the mold count, aerobic stability or rise in temperature post-harvest.

Key words: corn silage, aerobic stability, *Lactobacillus buchneri*

Introduction

All silage exposed to oxygen will eventually deteriorate as a result of aerobic microbial activity (Jonsson, 1989). The use of silage inoculants to ensure an efficient fermentation of sugar to lactic acid has become widely used and accepted today in an effort to minimize respiration and proteolysis by plant enzymes (Nadeau et al., 2000) as well as aerobic deterioration. Improving the quality and extent of fermentation can be accomplished by applying lactic acid based additives (McDonald et al., 1991; Kung et al., 2003; Filya et al., 2007). In addition to limiting respiration and proteolysis, lactic acid based inoculants also manipulate the fermentation process and inhibit the activity of clostridia bacteria and other aerobic microorganisms such as yeast and mold (Kung et al., 2003; Rooke and Hatfield, 2003). As a result, the aerobic stability of silage is often enhanced when using specific silage inoculants whose main role is to initiate a rapid front-end fermentation after harvest and prevent heating of the silage mass upon feedout and therefore, prolonging bunk life. This can be accomplished by using a reputable silage inoculant that contains both homofermentative and heterofermentative lactic acid bacteria (LAB).

These dual-purpose silage inoculants are commonly used today and were developed to overcome the limitations of silage inoculants containing either type of bacteria alone. Positive effects on the aerobic stability of corn silage have been reported when using these dual-purpose inoculants in a laboratory setting (Weinberg et al., 2002; Huisden et al., 2009; Reich and Kung,

2010). However, few studies have been conducted under field conditions looking into the effect of *Lactobacillus buchneri* on the aerobic stability of corn silage. Inoculation with *L. buchneri* at ensiling is commonly recommended to minimize aerobic deterioration at the farm level (Mari et al., 2009). Studies performed under field-conditions, however, have indicated that the effect of *L. buchneri* is often less than expected when compared to laboratory-scale studies (Mari et al., 2009; Kristensen et al., 2010; Tabacco et al., 2011).

Lactococcus lactis 0224 has also been shown to be beneficial due to its oxygen scavenging ability. The combination of these two specific LAB strains (*L. buchneri* and *L. lactis*) helps to ensure the rapid removal of oxygen post-ensiling, thus enhancing the fermentation process and minimizing the loss of water soluble carbohydrates and also helps minimize aerobic spoilage during feedout through the inhibition of yeasts and mold growth, particularly when silage has been stored for short periods of time and has not yet fully fermented.

The objective of this study was to compare the effect of a dual-purpose commercial silage inoculant containing specific LAB strains to an untreated control and its effect on silage fermentation, chemical analysis, and aerobic stability of corn silage stored under field-conditions with minimal storage time ($\leq$ 32 d). We hypothesized that inoculation would improve the fermentation and aerobic stability of corn silage resulting in higher quality silage at feedout.

Materials and Methods

Whole plant corn (Pioneer P1602AMX, Pioneer Hi-Bred International, Des Moines, Iowa) was grown at the Kansas State University Dairy Unit (Manhattan, KS) and later harvested using a self-propelled John Deere forage harvester equipped with a mechanical processor. Chopped corn silage was stored in 3 silage bags (60.96 to 76.2 m long and 3.05 m in diameter) and were prepared in the following manner: initially, 4 loads (~15 ton per load) were chopped

without a silage inoculant applied resulting in ~60 ton of untreated silage. Then, an inoculant (SiloSolve® FC, Chr. Hansen Animal Health and Nutrition, Milwaukee, WI) containing *Lactobacillus buchneri* LB1819 DSM22501 and *Lactococcus lactis* O224 DSM11037 was applied at a rate of 0.20 L/ton of fresh forage resulting in an application rate of 2.5×10^5 cfu/g at the forage harvester for the next 8 loads of silage (~120 ton of silage). The inoculant was then turned off for the next 4 loads (~60 ton) and then turned back on again for the remainder of the bag. This same process was carried out for all 3 silage bags. This resulted in 2 sections of control silage (**C**) and 2 sections of inoculated silage (**T**) in each of the 3 bags. Sections of control and inoculated silage were identified by markings painted on the bags during the filling process.

Aerobic Stability Measurement

Silage samples were obtained from each section (at least 0.9 m from the mark indicating a shift from control to inoculated silage to ensure all silage was untreated or treated, respectively) of the bag on day 0, 2, 4, 8, 16, and 32 after ensiling. Samples removed during collection were then mixed completely and separated into a 2-kg sample for aerobic stability measurement. The aerobic stability sample was placed on ice and transferred to the lab and placed in a refrigerator until all samples reached a similar temperature of approximately 2 to 4°C below room temperature. Samples were then loosely placed into styrofoam buckets (Minno Therm NPLS-X88-12, Plastilite Corporation, Omaha, Nebraska) and aluminum foil was placed over the top of each bucket to avoid contamination and drying out of the silage, while still allowing oxygen to infiltrate the silage sample. A temperature data logger (HOBO U12-008 equipped with TMC20-HD temperature probes, Onset Computer Corporation, Pocasset, MA) was placed in the geometric center of the forage mass for each bucket and recorded temperature continuously

every 15 min until the silage sample exceeded 3°C above room temperature at which point the data logger was stopped. An additional HOBO U12-008 temperature logger was placed within the room to track room temperature (~24°C) continuously at 15 min intervals. Aerobic stability was defined as the number of hours silage remained stable before a 3°C rise in temperature above room temperature.

Sampling and Chemical Analysis

In addition, a ~500 g sample for chemical, fermentation, and yeast and mold count analysis was collected, immediately placed on ice, and shipped within 24 h to Rock River Laboratory (Watertown, WI) for analysis. Chemical analysis was performed using Near Infrared Reflectance spectroscopy (NIR), while fermentation and yeast and mold count analyses were performed using wet chemistry techniques. Finally, 2 more ~500 g samples were collected and immediately placed into a freezer set at -15.5°C for additional analysis if the decision was made to do so in the future. In total, ~3.5 to 4.0 kg of silage was removed per sampling site for each day of the study.

At the time of ensiling, a temperature data logger (HOBO U12-015, Onset Computer Corporation, Pocasset, MA) was inserted ~1.5 m into each section of each bag and continuously recorded temperature of the silage mass every 15 min until 21 d post-harvest. The loggers were then removed and downloaded using the HOBOware software package (Onset Computer Corporation, Pocasset, MA). Two additional data logging devices (HOBO U23-001, Onset Computer Corporation, Pocasset, MA) were placed near the silage storage area to track ambient temperature and relative humidity levels every 15 min throughout the 21-d study period.

Logger Calibration

The 2 data loggers used to track silage temperature (HOBO U12-008 and HOBO U12-015) were calibrated to ensure similar temperature responses between all devices. This was done by placing all devices at room temperature for 10 min while each device recorded room temperature every 30 sec. Following this calibration test, another test was performed where all devices were placed in a 38.0°C water bath and temperature readings were taken every 30 sec for 10 min. Average temperature and standard deviations were compared between each of the data loggers to ensure similar temperature responses.

Statistical Analysis

This study was run as a generalized randomized complete block design where bag was used as the blocking factor and individual silage samples collected from each section of each silage bag served as the experimental unit. Each treatment was replicated twice within each block. Statistical analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The model statement included the fixed effects of treatment and day as well as their interaction. Bag and the interaction of bag and treatment were included as random effects and day was used as a repeated measure using the covariance structure giving the lowest Bayesian information criterion (BIC) value for each variable. Contrasts of least squares means were used to test significant differences among treatments. All microbial data (yeast and mold) were transformed to $\log_{10}$ and are presented on a wet weight basis. Chemical data are presented on a DM basis. Silage aerobic stability data were measured until a 3°C rise in temperature was observed. Confidence intervals are reported at 95% and statistical significance between treatments was declared at $P < 0.05$ and a tendency at $0.05 \leq P \leq 0.10$.

Results and Discussion

The chemical composition and fermentation analysis data of chopped corn silage by day of sampling after harvest are presented in Tables 3.1 and 3.2, respectively. The DM content of the silage showed little variation from d 0 through d 32 and ranged from 29.3 to 31.4 ± 1.7%. The concentrations of CP, ADF, and aNDFom were not affected ($P > 0.05$) by inoculation at any time point measured. Previous studies have also reported that inoculation with *L. buchneri* did not affect these chemical composition components (Kleinschmit et al., 2005; Kleinschmit and Kung, 2006a). Likewise, concentrations of starch, sugar, and NFC were not affected by inoculation at any time point after harvest.

Silage pH was not different ($P = 0.79$) between C and T at any time points measured but, as expected, there was a day effect as silage pH gradually decreased with time of storage ($P < 0.01$). The concentrations of lactic acid ranged from 0.0 ± 0.5% of the DM on d 0 to > 6.0 ± 0.5% of the DM on d 32. However, no treatment effect ($P > 0.05$) was observed during any of the time periods. Acetic acid followed a similar pattern as lactic acid but no significant treatment effects were observed ($P > 0.05$). As a result, the lactic acid:acetic acid ratio also did not differ at any of the time points measured in this study. The lactic acid:acetic acid ratio had a mean of 3.5:1 and 3.7:1 ± 0.2 over the entire study period for C and T, respectively. Field recommendations have suggested a desirable lactic acid:acetic acid ratio of greater than 3:1, indicating a more dominant homolactic fermentation (Kung and Stokes, 2001; Kleinschmit and Kung, 2006a). Although not seen in the current study, silage treated with an *L. buchneri* containing silage inoculant typically have a reduced lactic acid:acetic acid ratio of < 3:1 (Kleinschmit and Kung, 2006a). In a meta-analysis conducted by Kleinschmit and Kung (2006b), inoculation with > 1×10^5 cfu/g of *L. buchneri* decreased the concentrations of lactic

acid and increased the concentrations of acetic acid in corn silage resulting in silage with greater pH. These changes are attributed to *L. buchneri* converting lactic acid to acetic acid, ethanol and 1,2 propanediol under anaerobic conditions (Oude-Elferink et al., 2001). Because of the minimal storage time used in the current study (≤ 32 d), it is possible that insufficient time was allowed for these processes to occur. Kleinschmit and Kung (2006a) reported that a major increase in acetic acid was observed as early as 56 d for silage treated with *L. buchneri* at a rate of 1×10^5 cfu/g. This, however, is still beyond the time period measured in the current study. In contrast, Filya (2003) observed increased acetic acid levels in silages inoculated with *L. buchneri* within 2 d of ensiling in low DM corn and sorghum silages (23.5 and 22.2% DM, respectively), while Driehuis et al. (1999) observed that it took 20 d to observe this effect in corn silage. However, in the Filya (2003) study, the inoculant was applied at a rate of 1×10^6 cfu/g, which is 4× greater than the application rate used in the current study likely explaining the observed increase in acetic acid levels on d 2 of ensiling. More recently, Queiroz et al. (2012) found that acetic acid concentrations did not differ between treated and untreated silage until 7 d of ensiling. However, application rates were 5×10^5 cfu/g, exceeding the application rate used in the current study by 2×. While not shown in Table 3.2, both untreated and treated silages had undetectable concentrations of 1,2-propanediol throughout the study period. This is in agreement with Kleinschmit and Kung (2006a) where 1,2-propanediol concentrations were not detectable until 42 d post-ensiling in inoculated silage.

Other possible explanations for the lack of treatment effects seen may be due to either inconsistency in application of the inoculant at the silage harvester or the lower rates of inoculation often used at the farm level, which have been reported to be less effective (Kleinschmit and Kung, 2006b). Proper application rates are critical to derive value from

inoculants. If inoculation rates are less than recommended, these inoculants will likely have little impact on silage quality and fermentation (Muck, 1989). Also, the biology of ensiling is very complex making it virtually impossible to guarantee an ensiling response to inoculants under all conditions.

Ammonia-nitrogen (NH_3-N) concentrations were not different ($P = 0.57$) between treatments but increased with advancing storage time. This is in agreement with Filya (2003) who reported increasing amounts of NH_3-N through 90 d of ensiling and Kleinschmit and Kung (2006a) who reported steady increases in NH_3-N through 361 d of ensiling. No differences ($P = 0.63$) in ethanol concentrations were detected between treatments. We would have expected to see lower concentrations of ethanol along with a reduction in the numbers of yeasts present in silages inoculated with *L. buchneri*. However, this was not the case as yeast concentrations were not different between treatment groups at any time point measured post-harvest. These results are in agreement with Kleinschmit and Kung (2006a) where no differences in the number of yeasts detected were found between inoculated and untreated silage until 42 d of ensiling, which is beyond the time period measured in the current study. If the sampling period would have been extended, we would have likely seen reduced numbers of yeast present in the treated silage due to the conversion of lactic acid to acetic acid by the *L. buchneri* bacteria present in the treated silage. As previously reported, acetic acid is an inhibitor of yeast growth upon aerobic exposure at feedout (Kleinschmit and Kung, 2006b). There was, however, a treatment effect ($P = 0.02$) for mold count where silage treated with the inoculant showed reduced mold counts when compared to the untreated silage. A difference was found ($P < 0.05$) on d 0 (day of harvest), while no differences were found after d 0. It is not clear why there was such a drastic difference

in mold count on d 0, but because no differences were found during subsequent sampling days, the significance of the treatment effect is questioned.

Upon exposure to oxygen, silage temperature begins to increase rapidly and reaches a maximum in less than 24 h because of lactate-assimilating yeasts rapid growth potential in the presence of oxygen (Lowes et al., 2000; Tabacco et al., 2009). Aerobic stability in this study was defined as the number of hours that silage is exposed to oxygen prior to a 3°C rise in temperature above ambient temperature. An increase in temperature is an easy indicator of the extent and intensity of aerobic deterioration because the oxidation process is accompanied by the evolution of heat (Honig and Woolford, 1980). Upon initial oxygen exposure, treated silage had numerically greater hours to aerobic deterioration at each time point measured, but no significant differences ($P = 0.37$) between treatments were found for aerobic stability hours. Over the entire study period, untreated silage remained stable for 74.7 ± 15.5 h, while treated silage remained stable for 95.2 ± 15.5 h, equating to a difference of 20.5 h between untreated and treated silages. While both treatments appeared to remain stable beyond the time period that silage is thought to typically be exposed to oxygen on the farm during feedout (12 to 24 h), it has been reported that oxygen may penetrate up to 0.9 m (3 ft) into the silage face prior to feeding depending on pack density (Holmes, 2013). If silage is fed at a rate of ~15 cm/d (6 in/d), silage from both treatments would begin to deteriorate prior to being fed. However, if silage is fed at the recommended rate of ~30 cm/d (1 ft/d), treated silage would remain stable through feedout, while the untreated silage may still begin to deteriorate prior to being fed given the aerobic stability hours for each treatment in the study. Time to aerobic deterioration for the untreated silage ranged from 7.3 ± 1.9 h on d 0 to 106.5 ± 31.9 h on d 16. Time to aerobic deterioration for the treated silage ranged from 8.7 ± 1.9 h on d 0 to 137.3 ± 23.5 h on d 8.

Lactobacillus buchneri is an obligate heterofermentative LAB that produces high levels of acetic acid in silage through the anaerobic degradation of lactic acid. Laboratory results using this microorganism have shown promising results with regard to aerobic stability (Kung and Ranjit, 2001; Filya et al., 2002; Ranjit et al., 2002). Previous work performed in laboratory settings have shown enhanced aerobic stability when silage was treated with an inoculant containing *L. buchneri* alone or in combination with a homofermentative LAB (Driehuis et al., 2001; Filya, 2003). Studies performed in the field, however, have shown conflicting results. Field studies have often shown reduced aerobic stability when compared to studies performed in a laboratory setting (Mari et al., 2009; Kristensen et al., 2010; Tabacco et al., 2011). The current study is in agreement with this as aerobic stability reached a maximum of 137 h on d 8 for the treated silage. The lack of an overall treatment effect for aerobic stability in this study is not surprising as no difference was found in the number of yeasts present in each silage treatment.

Temperature of the silage mass was also measured for the first 21 d after ensiling (Figure 3.1). Both the untreated and treated silages followed similar patterns and a pattern that would be expected for properly fermented corn silage (Kung, 2008). No differences were found between treated and untreated silages ($P = 0.74$). The control silage had a temperature of 34.52 ± 2.78°C on d 0 and reached a maximum temperature of 39.42 ± 2.78°C on d 4 after harvest resulting in an increase of 4.90°C. The treated silage had a temperature of 34.05 ± 2.80°C on d 0 and reached a maximum of 39.33 ± 2.80°C on d 5 after harvest resulting in an increase of 5.28°C. Silage will increase in temperature as part of the normal fermentation process that occurs within the first week after harvest. Properly ensiled corn silage will increase in temperature by 8 to 11°C (15 to 20°F) after harvest (Bolsen et al., 1996) due to residual oxygen present, but should not exceed these levels (Kung, 2008), and then gradually decline to levels near ambient temperature. Values

in this study were less than that recommended indicating adequate packing ensuring minimal oxygen entry into the silage mass helping to ensure a proper fermentation.

Conclusions

Treating whole-plant corn with a dual-purpose silage inoculant containing *Lactobacillus buchneri* LB1819 DSM22501 and *Lactococcus lactis* O224 DSM11037 applied at a rate of 2.5 × 10^5 cfu/g failed to improve the aerobic stability of corn silage stored for a short duration ($\leq$ 32 d) under field conditions. No differences were found between treatments for any chemical composition measures or fermentation variables with the exception of mold counts where treated silage contained lower levels of mold. While we would have expected to see greater differences between treatments, it is likely that because of the short storage time used, the silage inoculant had insufficient time to fully exert its effects. Other possible explanations for the lack of treatment effects may be due to either inconsistency in application of the inoculant at the silage harvester or the lower rates of inoculation often used at the farm level, which have been reported to be less effective. This study shows that it may be beneficial to inoculate silage at a greater concentration than what is currently recommended to see the most benefits. Future research looking into the effect of this same silage inoculant on the aerobic stability and fermentation variables after silage has been stored for a longer duration as well as inoculating at greater concentrations would be beneficial. Overall, this silage inoculant, when applied at a rate of 2.5 × 10^5 cfu/g, appears to offer little advantages over untreated silage if silage is to be stored for only a short duration of < 1 mo prior to feedout.

Figures and Tables

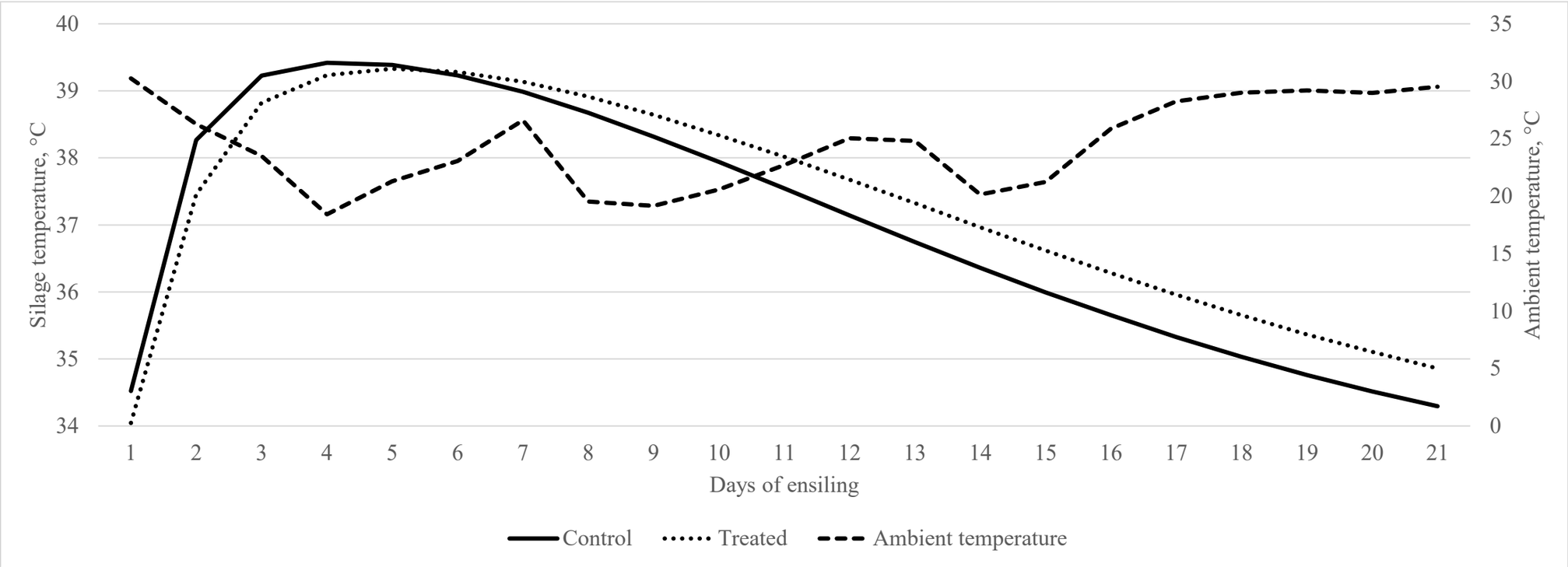

Figure 3.1 Temperature of the silage mass for untreated (Control) and treated (SiloSolve® FC; Chr. Hansen Animal Health and Nutrition, Milwaukee, WI) silage by days of ensiling. Treatment: $P = 0.74$.

Table 3.1 Chemical composition (DM basis) of corn silage samples by day of ensiling

	Treatment[1]															
	0 d		2 d		4 d		8 d		16 d		32 d			*P*-value		
Item, %	C	T	C	T	C	T	C	T	C	T	C	T	SE	Trt	Day	Trt × Day
DM	31.2	31.4	30.9	30.9	31.1	31.1	29.6	29.3	30.4	31.0	30.1	30.1	1.7	0.90	0.05	0.83
CP	8.07	8.10	7.83	7.86	7.86	7.91	7.77	7.95	7.88	7.58	8.18	8.07	0.29	0.79	0.25	0.55
aNDFom	40.43	41.32	41.51	42.16	40.20	40.68	40.90	42.59	40.57	41.62	40.16	40.28	1.65	0.24	0.01	0.39
ADF	24.35	24.73	25.12	25.50	24.48	24.80	24.98	25.98	25.08	25.07	25.24	25.36	1.00	0.40	0.40	0.67
Lignin	4.29	4.21	3.88	3.82	3.69	3.62	4.06	4.05	3.84	3.82	3.79	3.74	0.17	0.70	0.01	1.00
Ash	5.58	5.47	5.71	5.65	5.05	5.49	5.12	5.07	4.67	4.78	4.71	4.58	0.50	0.84	< 0.01	0.90
Starch	27.96	27.40	25.97	24.87	26.89	27.28	26.31	23.51	24.85	25.72	24.41	24.15	2.16	0.61	0.06	0.01
Sugar	2.60	2.78	2.20	2.08	1.93	1.79	1.68	1.84	2.44	2.57	1.90	2.33	0.33	0.67	< 0.01	0.94
NFC	43.47	42.73	42.27	41.83	44.10	42.89	43.48	42.02	44.10	43.41	44.21	44.59	2.02	0.35	< 0.01	0.40

[1]C = untreated corn silage; T = corn silage treated with SiloSolve® FC (Chr. Hansen Animal Health and Nutrition, Milwaukee, WI).

Table 3.2 Effect of applying a silage inoculant on fermentation indices and aerobic stability of corn silage ensiled for a short duration

	Treatment[1]															
	0 d		2 d		4 d		8 d		16 d		32 d			*P*-value		
Item	C	T	C	T	C	T	C	T	C	T	C	T	SE	Trt	Day	Trt × Day
pH	5.57	5.68	3.92	3.90	3.80	3.81	3.77	3.72	3.58	3.63	3.61	3.58	0.11	0.79	< 0.01	0.50
Lactic acid, % of DM	0.00	0.00	4.53	4.74	4.64	4.73	5.34	6.21	6.33	6.18	6.92	6.31	0.50	0.88	< 0.01	0.17
Acetic acid, % of DM	0.00	0.00	1.36	1.38	1.30	1.32	1.28	1.35	1.29	1.19	1.44	1.25	0.10	0.32	< 0.01	0.78
Lactic:Acetic acid ratio	0.0	0.0	3.3	3.4	3.6	3.6	4.2	4.6	5.1	5.3	5.0	5.1	0.3	0.51	< 0.01	0.84
Propionic acid, % of DM	0.00	0.00	0.09	0.10	0.00	0.00	0.00	0.00	0.05	0.05	0.13	0.08	0.05	0.72	0.01	0.97
Butyric acid, % of DM	0.00	0.00	0.00	0.05	0.08[a]	0.00[b]	0.00	0.04	0.00	0.00	0.00	0.00	0.02	0.89	0.23	0.06
Ethanol, % of DM	0.00	0.00	0.43	0.43	0.58	0.41	0.64	0.54	0.52	0.58	0.56	0.55	0.07	0.63	< 0.01	0.26
NH_3-N, % of CP	0.020	0.020	0.038	0.039	0.050	0.049	0.057	0.058	0.062	0.057	0.079	0.065	0.006	0.57	< 0.01	0.69
Yeasts, $\log_{10}$ cfu/g	6.70	6.51	5.97	6.18	6.41	6.12	6.19	5.89	6.16	6.43	5.00	5.60	0.50	0.81	0.02	0.38
Molds, $\log_{10}$ cfu/g	2.31[a]	0.58[b]	0.00	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.43	0.02	0.02	0.32
Aerobic stability, h	7.3	8.7	76.6	93.0	100.5	106.3	101.5	137.3	106.5	128.2	55.9	97.5	31.9	0.37	< 0.01	0.74

[1]C = untreated corn silage; T = corn silage treated with SiloSolve® FC (Chr. Hansen Animal Health and Nutrition, Milwaukee, WI).

[a,b]Means in rows within day of ensiling with unlike superscripts differ ($P < 0.05$).

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Chapter 4 - Development of a Berry Processing Score for Sorghum Silage and Processing Effects on Sorghum Silage Starch Digestibility

Abstract

Two studies were conducted in an effort to develop a berry processing score (BPS) for sorghum silage, similar to the kernel processing score currently used for corn silage. In both studies, sorghum silage samples were collected from commercial farms in Kansas and randomly assigned to 1 of 4 processing levels differing in roll gap spacing: unprocessed (UNP), 1.5 (1.5P), 1.0 (1.0P), or 0.5 (0.5P) mm. Samples were processed through a roller mill in the Grain Science & Industry grain processing laboratory at Kansas State University. After drying, individual sorghum silage samples were placed into a Ro-Tap particle separation machine (W. S. Tylor, Mentor, OH) fitted with screens containing square apertures of 9.50, 6.70, 4.75, 4.00, 3.35, 2.80, 2.36, 1.70, 1.18, and 0.6 mm (in addition to a pan) for 10 min. Whole samples, as well as separated fibrous and whole berry portions were sent to Rock River Laboratories (Watertown, WI) and analyzed for percent starch retained on each screen. In Study 1, as the roll gap spacing was reduced, mean particle size (MPS) was also reduced (2.16, 2.15, 2.07, and 2.00 ± 0.05 mm for UNP, 1.5P, 1.0P, and 0.5P, respectively). Whole berries per g of sample weight were reduced from 10.0 to 0.3 ± 1.2 as the roll gap spacing was reduced, indicating successful processing of the samples. Percent starch retained on the 2.36 mm screen was reduced, while the percent starch passing through the 1.7 mm screen increased as the level of processing increased. The percent starch of the whole berry sample retained on the 2.8 mm screen was 49.31 and 5.26 ± 6.07%, while percent starch retained on the 1.7 mm screen was 4.2 and 48.04 ± 6.07% for UNP and 0.5P, respectively. Thus, from these data, we determined that the appropriate screen to use in determining a BPS for sorghum silage is the 1.7 mm screen. In Study 2, data for BPS

showed that as the level of processing increased, BPS also increased from 26.28 to 55.05 ± 0.04% for UNP and 0.5P, respectively. This resulted in greater 7-h in situ starch digestibility for 0.5P compared to UNP (82.07 vs. 50.54 ± 4.94%, respectively). The relationship between BPS and fecal starch was low ($R^2 = 0.09$), primarily due to the small contribution of sorghum silage to overall dietary starch. By processing sorghum silage during harvest and measuring the extent of processing, sorghum silage starch digestibility can be greatly enhanced and may serve as a viable alternative to corn silage in the diet of lactating dairy cows in areas of the country where corn silage is a high-risk forage crop due to lack of water.

Key words: sorghum silage, processing, starch digestion

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] has become an increasingly important forage crop for dairy producers, particularly in the Midwestern and plains regions of the U.S. that routinely experience conditions of drought or insufficient water. Forage sorghum is often planted either in total or partial replacement of corn silage as the latter can be a high-risk forage crop under certain climatic conditions such as excessively dry and high ambient temperatures. Forage sorghum is a warm-season annual used for silage production and commonly fed to dairy cattle in many regions of the U.S. When compared to corn silage, sorghum silage is more water efficient, using approximately 30 to 50% less water (McCorkle et al., 2007; Mahanna, 2015) making sorghum more heat and drought tolerant. This is especially important in areas where irrigation is limited and where elevated temperatures combined with drought are commonly observed. Miron et al. (2007) reported that conventional and brown midrib forage sorghum varieties showed improved water use efficiencies of 51 and 18%, respectively, when compared to corn silage. Likewise, Farré and Faci (2006) found greater water use efficiency, biomass

production, and yield for sorghum compared to corn silage under minimal irrigation. These data show that planting sorghum for silage should help in reducing water use on the farm.

An often-cited downfall of harvesting sorghum for silage is the reduced starch digestibility and milk yield often observed when replacing corn silage in the diet (Grant et al., 1995; Aydin et al., 1999; Oliver et al., 2004). In general, the sorghum berry contains a higher proportion of peripheral endosperm (Rooney and Sullins, 1973; Rooney and Miller, 1982), which is extremely dense, hard and resistant to digestion (Rooney and Pflugfelder, 1986) in comparison to corn. A primary purpose of the peripheral endosperm is to protect the starch located within and therefore, is resistant to digestion unless the starch-protein matrix is broken in some way. This protein matrix adheres starch and protein more tightly in sorghum than in corn and is the main reason for lower digestibility and milk yield often seen with sorghum (Rooney and Pflugfelder, 1986).

Processing of corn silage through rollers during harvest often increases starch digestibility (Rojas-Bourrillon et al., 1987; Bal et al., 2000; Weiss and Wyatt, 2000; Andrae et al., 2001; Schwab et al., 2002). While forage sorghum is not typically processed at harvest, kernel processing via on-board kernel processors have been used extensively in the harvest of corn silage in an effort to reduce kernel particle size leading to increased total tract starch digestibility in the dairy cow. Ferreira and Mertens (2005) established a method to determine the degree of kernel processing, or breakage, in whole plant corn harvested as silage. However, no such method has been developed for sorghum silage.

Therefore, the objectives of the current study were: 1) to determine the mean particle size (MPS), percent material retained on each screen of the Ro-Tap machine, and starch distribution after applying different levels of processing; 2) to develop a berry processing score (BPS) for

sorghum silage, similar to the kernel processing score used with corn silage; 3) to evaluate 7-h in-situ starch digestibility of sorghum silage when exposed to different levels of processing; and 4) to determine the current BPS of sorghum silage samples collected from commercial dairy farms and the relationship between BPS and fecal starch.

Materials and Methods

Study 1

In an effort to determine MPS as well as develop a system for determining a BPS for sorghum silage, sorghum silage samples (Croplan BMR 108, Croplan Genetics, St. Paul, MN) were collected from 3 commercial dairy farms in Kansas. Samples were collected using the quartering technique (Rock River Laboratories, Watertown, WI) and then brought to the Grain Science & Industry grain processing laboratory at Kansas State University for processing. Eight samples were collected from each dairy resulting in a total of 24 samples. Upon returning to the lab, samples were either left unprocessed (**UNP**) and used as the control, or run through a 9 × 6 roller mill (Ross Machine & Mill Supply, Inc., Oklahoma City, OK) using a roll gap spacing of either 1.5 (**1.5P**), 1.0 (**1.0P**), or 0.5 (**0.5P**) mm. Preliminary data collected showed that a roll gap spacing of > 1.5 mm did not enhance processing of the sorghum berries when compared to unprocessed sorghum silage. From each dairy, two samples were left unprocessed and two samples were processed at one of the aforementioned roll gaps. Samples were then dried in a forced-air oven at 55°C for 72 h to ensure complete removal of moisture resulting in samples weighing ~100 g on a DM basis. Following DM determination, samples were separated using a Ro-Tap 3-dimensional separator (W. S. Tylor, Mentor, OH) fitted with screens containing square apertures of 9.50, 6.70, 4.75, 4.00, 3.35, 2.80, 2.36, 1.70, 1.18, and 0.6 mm (in addition to a pan). This screen combination was chosen based on preliminary data collected where different screen

sizes and combinations were tried. Ultimately, the screen combination chosen resulted in the most even distribution of the sorghum silage samples across all screens. Samples were placed into the Ro-Tap machine for 10 min to determine MPS and the percent material retained on each screen by weight was calculated. Mean particle size (minimal cross-sectional dimension) was determined by calculating the weight of residue retained on each screen and determining the geometric mean particle size as described by ANSI (1993), except the square instead of the diagonal dimension of apertures were used.

Whole sorghum berries retained on the 4.00, 3.35, 2.80, 2.36, and 1.70 mm screens were hand-separated from the remaining sample, counted, and weighed. Preliminary research showed that all whole sorghum berries were retained below the 4.75 mm screen and above the 1.70 mm screen. Once separated, whole sorghum berry samples and the remaining fibrous samples (with whole sorghum berries removed) were sent to Rock River Laboratories (Watertown, WI) for DM, starch, and fiber (aNDF) determination using the wet chemistry technique. Material retained on the 9.50, 6.70, and 4.75 mm screens were combined into a single sample prior to analysis since no whole berries were retained on these screens.

Study 2

Study 2 aimed to evaluate the impact of processing on in situ starch digestibility of sorghum silage. Twelve, ~500 g sorghum silage samples were collected from 6 commercial farms in Kansas resulting in a total of 72 samples. The 12 samples from each farm were split into 1 of 4 treatments as in Study 1: unprocessed (UNP), 1.5 mm processed (1.5P), 1.0 mm processed (1.0P), and 0.5 mm processed (0.5P). This resulted in 3 samples for each treatment from each farm in the study. Two of the 3 samples were dried and placed through the Ro-Tap 3-dimensional separator, while the third sample was analyzed for starch digestibility using the in-

situ procedure outlined below. Unless otherwise noted, sample collection and laboratory procedures followed similarly as those described in Study 1. Following separation of the sorghum silage samples, samples were divided into material retained above and below the 1.7 mm screen. Samples were then analyzed for starch content at Rock River Laboratories (Watertown, WI) to determine the percent of total starch passing through the 1.7 mm screen to determine the BPS for each sample. A BPS was calculated as follows:

BPS = (Starch passing through 1.7 mm screen (g) / Total sample starch (g)) × 100

In addition, sorghum silage samples and a representative fecal sample were collected from 17 dairy farms feeding ≥ 2.27 kg of DM/d per cow from sorghum silage. Sorghum and fecal samples were frozen immediately and later dried in a forced-air oven at 55ºC for 72 h to ensure complete removal of moisture. Sorghum samples were then placed into the Ro-Tap 3-dimensional separator to divide samples into material passing through or retained above the 1.7 mm screen as previously described. Sorghum and fecal samples were sent to Rock River Laboratories (Watertown, WI) for starch analysis.

In Situ Procedures

All in situ analysis was conducted at Rock River Laboratories (Watertown, WI). Upon receiving the sorghum silage samples, all samples were placed in a 60ºC forced-air oven and dried overnight. Once dried, samples were ground to pass through a 6-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Dried and ground samples were then weighed into pre-weighed and labeled Ankom R510 bags (5 × 10 cm, 50 µm pore size; Ankom Technology, Macedon, NY). Bags were filled to ~3.0 g resulting in a 30 mg/cm^2 sample to surface area ratio. Four bags were weighed per sample with 1 bag serving as the 0 h digestion time point by soaking for 20 min in warm (~39.0ºC) water. The other 3 samples were soaked prior to rumen

incubation. Samples were incubated in single bags in each of 3 cows, yielding 3 replicates. All in situ bags (including two standards within each cow) were placed within a mesh laundry bag containing a 200-g weight to keep samples positioned in the ventral rumen of the cow. Three lactating, Holstein dairy cows consuming a diet consisting of corn silage, alfalfa haylage, and concentrate were used for the in situ ruminal incubations. After 7-h of rumen incubation, samples were removed and immediately placed in ice water to stop the fermentation process. Samples were then rinsed within a small, portable washing machine with two, 5 min cycles or until the rinse water was completely clear. Washed bags were then dried in a forced-air oven at 60ºC for 24 h and weighed to determine DM disappearance. Digested bags were cut open and residues were then composited for nutrient analysis. Ruminal disappearance was then calculated as follows:

(((Sample nutrient amount (g) – residue nutrient amount (g)) / sample nutrient amount (g)) × 100)

Statistical Analysis

Study 1 and Study 2 were analyzed as a generalized randomized complete block design where farm was used as the blocking factor and individual silage samples collected from each farm served as the experimental unit. Each treatment was replicated twice within each block. Statistical analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The statistical model for sorghum silage retained on each screen, percent of starch retained in the whole sample and whole sorghum berry sample included the fixed effects of screen and the interaction between screen and roll gap spacing, while the random effects included farm and the interaction between farm and roll gap spacing. The statistical models for MPS, BPS, 7-h in situ starch digestibility, and chemical composition included the

fixed effects of roll gap spacing, while farm and the interaction of farm and roll gap spacing were included as random effects in the model. Degrees of freedom were approximated using the method of Kenward-Roger (ddfm = kr). The ESTIMATE statement was used to analyze for differences between unprocessed and processed samples, as well as to check for linear and quadratic effects. Means were determined using the least squares means statement and included the PDIFF option. The REG procedure in SAS was used to generate linear regression models to determine the relationship between BPS and MPS, BPS and 7-h in situ starch digestibility, and BPS and fecal starch. Confidence intervals are reported at 95% and statistical significance between treatments was declared at $P < 0.05$ and a tendency at $0.05 \leq P \leq 0.10$.

Results and Discussion

Study 1

As shown in Figure 4.1, as the roll gap spacing was reduced, there was a tendency ($P = 0.09$) for MPS to also decrease (2.16, 2.15, 2.07, and 2.00 ± 0.05 mm for UNP, 1.5P, 1.0P, and 0.5P, respectively). This is in agreement with data evaluating the effect of kernel processing corn silage on MPS (Schurig and Rodel, 1993; Roberge et al., 1998; Bal et al., 2000). However, MPS in the current study was reduced just 7.4% comparing UNP and 0.5P samples, whereas MPS was reduced 15 to 30% comparing kernel processed to unprocessed corn silage. Evaluating MPS provides a quantifiable measure of how thoroughly materials are chopped and how physically effective that material will be at stimulating rumination in the cow.

After sample separation, material retained on each screen was measured (Figure 4.2). While no differences ($P > 0.05$) were found between treatments for screen size $\geq$ 3.35 mm, there was a significant reduction ($P < 0.05$) in material retained on the 2.8 and 2.36 mm screens as roll gap spacing was reduced. This led to an increase in material retained in the pan for 0.5P (9.04 ±

0.65%) samples compared to UNP and 1.5P (7.23 and 7.32 ± 0.65%, respectively) sorghum silage samples ($P < 0.05$). These data are in agreement with the results seen for MPS.

Whole sorghum berry weight as a percent of total sample weight was analyzed to measure the effectiveness of the different roll gaps chosen for the study (Figure 4.3). Whole sorghum berry weight as a percent of total sample weight was reduced ($P < 0.001$) for processed samples compared to UNP (12.34, 3.53, 1.07, and 0.15 ± 1.4% for UNP, 1.5P, 1.0P, and 0.5P). Whole berries per g of sample weight were reduced ($P < 0.001$) from 10.0 to 0.3 ± 1.2 whole berries per g of sample as the roll gap was reduced from UNP to 0.5P, indicating successful processing of the samples (Figure 4.4). Also, the percent of starch processed was greater ($P < 0.01$) for processed samples compared to the unprocessed sorghum silage samples (Figure 4.5). These data indicate that the different roll gaps chosen for this study were effective at processing the sorghum berries.

Percent starch retained by screen for the whole sorghum silage sample is shown in Figure 4.6. The percent starch retained on the 2.36 mm screen was reduced as the level of processing increased (or as the roll gap was reduced). In contrast, the percent starch passing through the 1.7 mm screen was greater for 0.5P (18.90, 22.31, 29.45, and 36.92 ± 2.93 for UNP, 1.5P, 1.0P, and 0.5P, respectively).

After hand-separating the whole sorghum berries from the sorghum silage sample following passage through the Ro-Tap machine, the percent starch retained on each screen was analyzed to help in determining on which screen whole berries were retained after processing (Figure 4.7). For UNP sorghum silage, 49.31 and 42.22 ± 6.07% of the starch from whole berries was retained on the 2.8 and 2.36 mm screens, respectively. Therefore, greater than 90% of whole sorghum berries in unprocessed sorghum silage were unable to pass through the 2.36

mm screen. For medium processed samples, 50.92 and 47.12 ± 6.07% of starch from whole sorghum berries was retained on the 2.36 mm screen for 1.5P and 1.0P, respectively. Meanwhile, for the 0.5P treatment, just 10.29 ± 6.07% of whole sorghum berry starch was retained on the 2.36 mm screen, while 48.04 ± 6.07% of starch from whole sorghum berries was retained on the 1.7 mm screen, indicating that only the smallest whole sorghum berries remained unprocessed. No whole berries were able to pass through the 1.7 mm screen.

Based on the results described above, we have determined that the appropriate screen to use in determining a BPS for sorghum silage is the 1.7 mm screen. As shown in Figure 4.7, a significant amount of starch from whole sorghum berries was still being retained on the 1.7 mm screen, while no whole sorghum berries were able to pass through the 1.7 mm screen. In order to account for this, we decided to use the 1.7 mm screen in determining a BPS for sorghum silage samples, where a BPS for any sorghum silage sample can be calculated by measuring the percent of starch passing through the 1.7 mm screen of the Ro-Tap machine.

Study 2

In Study 2, we looked to build off the results of Study 1 to determine a BPS for samples collected from commercial farms and then processed through the roller mill as mentioned previously. We were also interested in determining the impact of increased BPS on 7-h in situ starch digestibility to give an indication of the impact of increasing BPS on starch digestibility in the dairy cow.

The chemical compositions of the sorghum silage treatments are presented in Table 4.1. The DM content, starch, and aNDF levels were similar between all treatments in the current study. Other researchers have also reported no effect of processing on chemical composition of silages (Rojas-Bourrillon et al., 1987; Andrae et al., 2001; Zobell et al., 2002). In contrast, CP

decreased with increased level of processing ($P = 0.04$). Dry matter disappearance was positively correlated with the level of processing where, as the level of processing increased, DM disappearance also increased ($P = 0.04$).

Given the reduced starch digestibility and milk yield often observed when replacing corn silage in the diet with sorghum silage (Grant et al., 1995; Aydin et al., 1999; Oliver et al., 2004), methods to enhance starch digestibility of sorghum silage are important if sorghum silage is to be a viable alternative to corn silage when fed to lactating dairy cattle in the future. Improving sorghum genetics via plant breeding, harvesting at the correct stage of maturity, storing silage for a longer period of time, thereby allowing silage bacteria to breakdown the protein matrix and therefore, increasing starch digestibility (Hoffman et al., 2011), and processing of the sorghum berries are a few methods by which starch digestibility may be improved. For this study, we focused on the latter. In general, sorghum contains a higher proportion of peripheral endosperm (Rooney and Sullins, 1973; Rooney and Miller, 1982), which is extremely dense, hard and resistant to digestion (Rooney and Pflugfelder, 1986), when compared to corn. A primary purpose of the peripheral endosperm is to protect the starch located within and therefore, is resistant to digestion unless the starch-protein matrix is broken in some way.

The starch-protein matrix refers to the combination of starch, prolamins and other proteins (albumins, globulins, and glutelins) in the endosperm and has been defined as "a physiochemical impediment to starch digestion in ruminants" (Owens et al., 1986). This matrix is responsible for binding starch granules together and the degree of binding determines the digestibility of the starch (Kotarski et al., 1992). In sorghum, hydrophobic kafarin proteins are the primary prolamin proteins in the starch-protein matrix, and comprise 70 to 80% of the protein in whole grain sorghum (Wong et al., 2009). Kafarins tend to be more hydrophobic on average

when compared to other prolamin proteins such as zein in corn. The cross-links present in the protein matrix of sorghum are more pronounced than in corn, which helps to explain the often-cited lower digestibility of starch granules in sorghum compared to corn (Rooney and Pflugfelder, 1986).

In determining a BPS for each sorghum silage sample, based on the results from Study 1 and as previously mentioned, we decided to measure BPS as the percent of starch passing through the 1.7 mm screen. The method used to develop a system to determine a BPS for sorghum silage samples was adopted from Ferreira and Mertens (2005), where they developed a method to evaluate kernel processing of corn silage samples as the percent of starch passing through a 4.75 mm screen. Due to the much smaller size of sorghum berries compared to corn kernels, it was necessary to use a screen with a smaller square aperture size.

As shown in Figure 4.8, BPS increased as the level of processing increased (26.28, 34.64, 40.30, and 55.05 ± 0.04% for UNP, 1.5P, 1.0P, and 0.5P, respectively; $P < 0.001$). The unprocessed sample represents sorghum silage as it was collected from on-farm silage bunkers and indicates that processing of the sorghum berries could be greatly improved in the field. While BPS was improved when processed at either 1.5 or 1.0 mm compared to UNP, berry particle size could be reduced even further when processed at a roll gap spacing of 0.5 mm to enhance starch digestibility. Applying this to a forage harvester in the field will no doubt be more difficult when considering the amount of silage material that must pass through the processing unit.

In the current data set of sorghum silage samples, there was an inverse relationship between BPS and MPS, although there was considerable variation in BPS at a given MPS

(Figure 4.9) resulting in a low correlation ($R^2 = 0.20$) between BPS and MPS. These results are similar to those observed by Ferreira and Mertens (2005).

As a result of the increased BPS, 7-h in situ starch digestibility also increased as the level of processing increased ($P < 0.01$). Seven-h in situ starch digestibility was lowest for UNP (50.54 ± 4.94%), intermediate for 1.5P and 0.5P (66.76 and 68.95 ± 4.94%, respectively) and greatest for 0.5P (82.07 ± 4.94%). These results indicate that processing was effective at disrupting the protein matrix, allowing rumen microbes to attach and degrade the starch particles. These data are in agreement with Huntington (1997), who concluded that processing increases the rate of starch digestion with the effects being greater for grains with more vitreous endosperm, such as sorghum. While little work has been conducted with sorghum silage, similar results have been found when processing corn silage where processing during harvest reduced kernel particle size and increased total-tract starch digestibility (Bal et al., 2000; Johnson et al., 2002; Cooke and Bernard, 2005). Improving starch digestibility via processing not only will affect milk production, but also ruminal pH and fiber digestibility (Firkins et al., 2001), and the type, amount and, absorption of fuels (i.e. acetate, propionate, lactate, glucose) available to the cow (Allen, 2000).

Based on the results for BPS and its relationship with greater 7-h in situ starch digestibility (Figure 4.11; $R^2 = 0.43$), we are recommending that in order for sorghum silage samples to be considered adequately processed, ≥ 50% of the starch should pass through the 1.7 mm screen. As shown in Figure 4.10, 7-h in situ starch digestibility can be greatly enhanced to > 80% when processed at 0.5 mm. A common goal to achieve for 7-h in situ starch digestibility is ~85% (Rock River Laboratories, Watertown, WI). Therefore, the current data shows that starch digestibility of sorghum can become quite digestible and similar to starch digestibility in corn

when adequately processed. If < 30% of the starch is able to pass through the 1.7 mm screen, samples should be considered poorly processed and will have poor digestibility (~50%) in the rumen of the dairy cow (Figure 4.10). This confirms producers and nutritionists concerns of the reduction in starch digestibility observed when replacing a portion of the corn silage in the diet with sorghum silage. When BPS is between 30 and 50%, samples should be considered intermediately processed. Seven-h in situ starch digestibility was greater for 1.5P and 1.0P compared to UNP, but still lower than 0.5P. Therefore, sorghum silage should be harvested using a sorghum silage processor with the roll gap spacing set as tight as possible, which just happens to be ~0.5 mm.

Commercial forage testing laboratories routinely offer fecal starch analysis as a diagnostic tool to analyze starch digestibility in lactating dairy cows. Fecal starch was shown to account for 94% of the variation in total tract starch digestibility (Fredin et al., 2014) and therefore, could be used as an on-farm tool to measure digestibility of the total diet. However, there are some challenges when using fecal starch analysis on-farm. Often multiple starch containing feeds are included in the diet. Therefore, the feed responsible for greater than normal fecal starch levels may not always be apparent. Also, between cow fecal starch variation can be high due to differences in passage rates.

Mean BPS for sorghum silage samples collected from 17 commercial dairy farms was 45.77 ± 18.18% with a maximum of 63.13% and a minimum of 18.88%. Fecal starch concentrations (DM basis) had a mean of 2.90 ± 1.72% with a maximum of 6.72% and a minimum of 0.54%. Data from the current study showed a low correlation ($R^2 = 0.09$; $P = 0.25$) between BPS and fecal starch concentrations (Figure 4.12). This could be due to the fact that all diets on all farms included other starch containing feeds and the percent of dietary starch coming

from sorghum silage was low. Farms selected for the present study were feeding ~2.27 kg of sorghum silage in the diet (DM basis). This made up only 6.05 ± 3.58% of the total dietary starch, with a minimum of 2.23% and a maximum of 13.11%. Therefore, other feeds in the diet (corn silage or ground corn primarily) contributed more to the differences in fecal starch than did sorghum silage. The actual percent of total dietary starch that a single feed must contribute to significantly affect fecal starch values is unknown, but likely exceeds 20%. Previous research evaluating the effect of kernel processing on total tract starch digestibility of diets containing corn silage suggested that corn silage contributed ~27% to ~67% of total dietary starch (Bal et al., 2000; Weiss and Wyatt, 2000; Schwab et al., 2002). In these studies, corn silage would have a greater effect on total tract starch digestibility and fecal starch concentrations than sorghum silage starch contribution of the farms in the current study. In addition, due to difficulties in finding dairy farms feeding $\geq$ 2.27 kg/d per cow, there was a large variation in milk production (31.8 to 45.5 kg/d), dry matter intake (20.5 to 27.3 kg/d), and days in milk (80 to 250) between herds. This could also explain the low correlation between BPS and fecal starch due to differences in passage rates depending on stage of lactation and milk production.

Conclusions

From these data, we were able to develop a method to calculate a BPS for sorghum silage samples measured as the percent of starch passing through a 1.7 mm screen. The development of a BPS for sorghum silage will give the industry a standard by which to measure the degree of processing in sorghum silage. Our data also showed that 7-h in situ starch digestibility was increased as BPS increased. Therefore, by increasing the level of processing in sorghum silage, we may be able to enhance starch digestibility sufficiently, allowing sorghum silage to replace at least a portion of corn silage in the diet without the commonly seen decrease in starch

digestibility, and therefore milk production. This may be especially important in areas of the country that are at increased risk of drought-like conditions and may have limited access to water during the growing season. When evaluating BPS from sorghum silage samples collected from commercial farms, the relationship between BPS and fecal starch was low due to the small inclusion rate of sorghum silage in these diets.

Figures and Tables

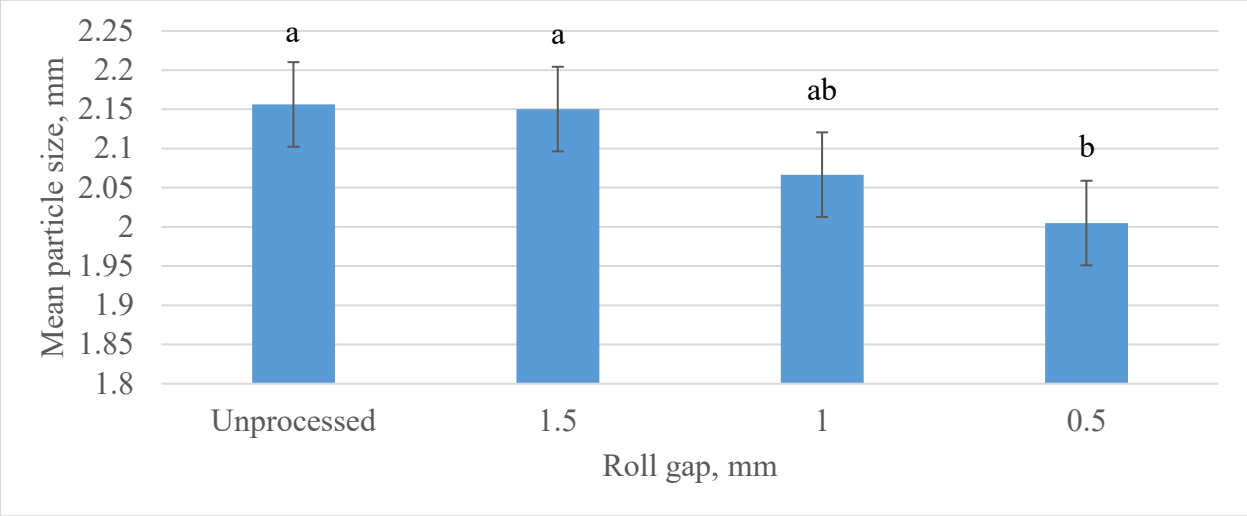

Figure 4.1 Least squares means for mean particle size at each roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm).
Treatment effect: $P = 0.09$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P = 0.11$
Linear: $P = 0.04$
Quadratic: $P = 0.82$
[a,b]Means differ ($P < 0.05$)

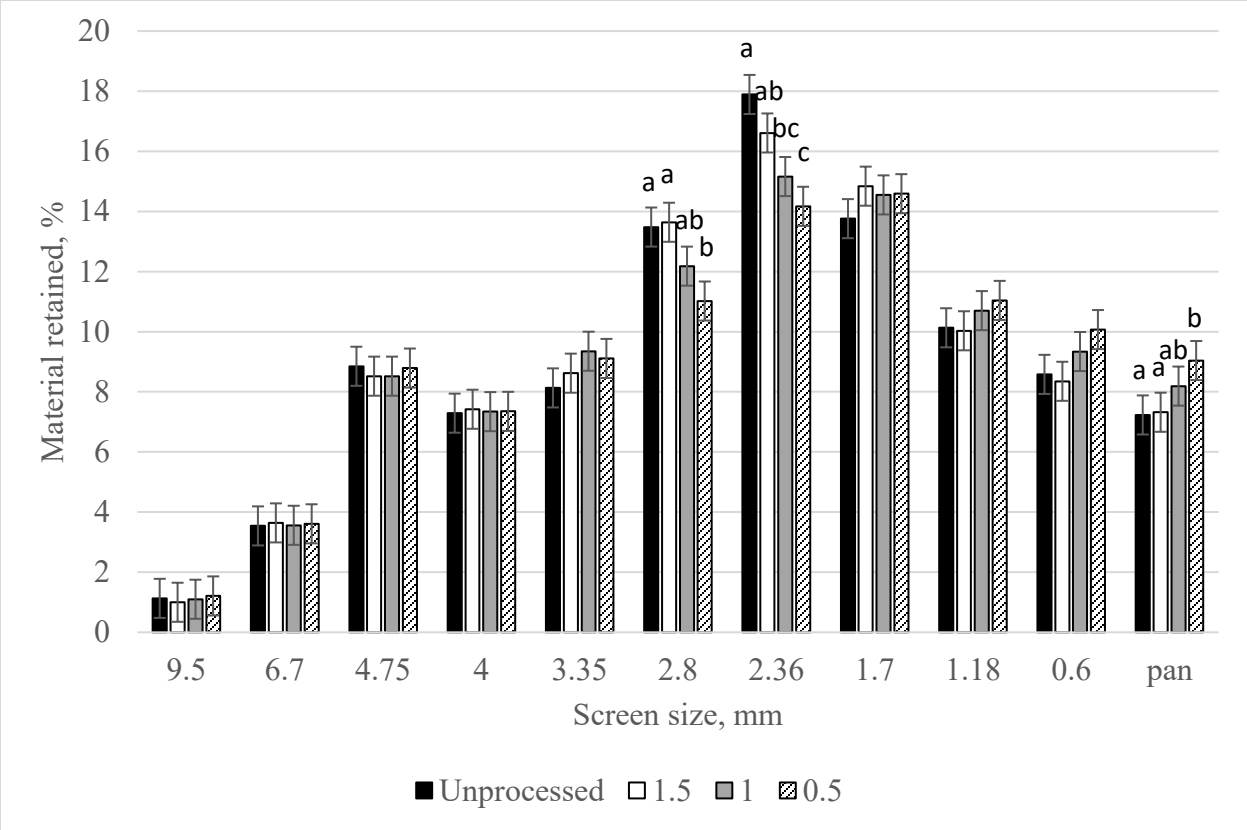

Figure 4.2 Percent material retained on each screen at each roll gap spacing (unprocessed, 1.5, 1.0 or 0.5 mm) after complete separation using a Ro-Tap particle separator (W. S. Tylor, Mentor, OH).
Screen: $P < 0.001$
Rollgap × screen: $P = 0.11$
[a,b,c]Means within screen size differ ($P < 0.05$)

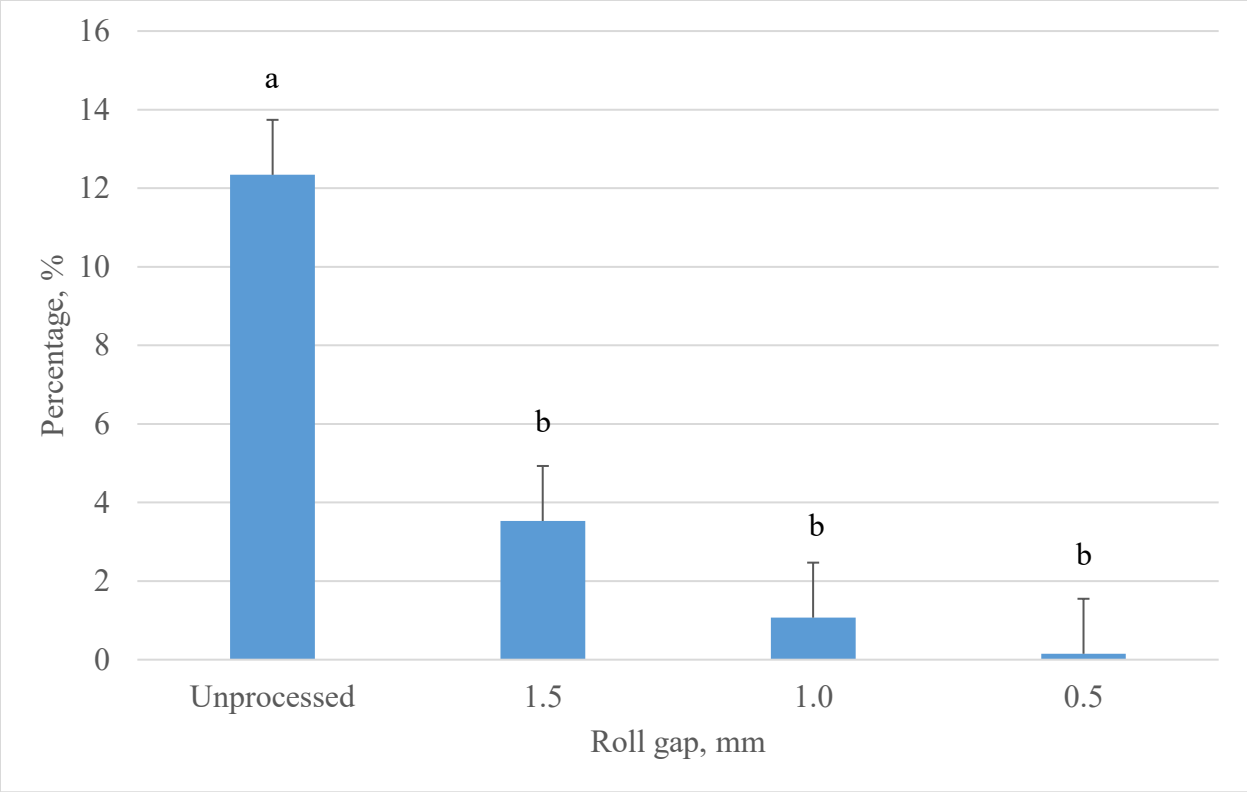

Figure 4.3 Whole sorghum berry weight as a percent of total sample weight at each roll gap spacing (unprocessed, 1.5, 1.0 or 0.5 mm) after complete separation using a Ro-Tap particle separator (W. S. Tylor, Mentor, OH).
Treatment effect: $P < 0.01$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P < 0.001$
Linear: $P = 0.09$
Quadratic: $P = 0.61$
[a,b]Means differ ($P < 0.05$)

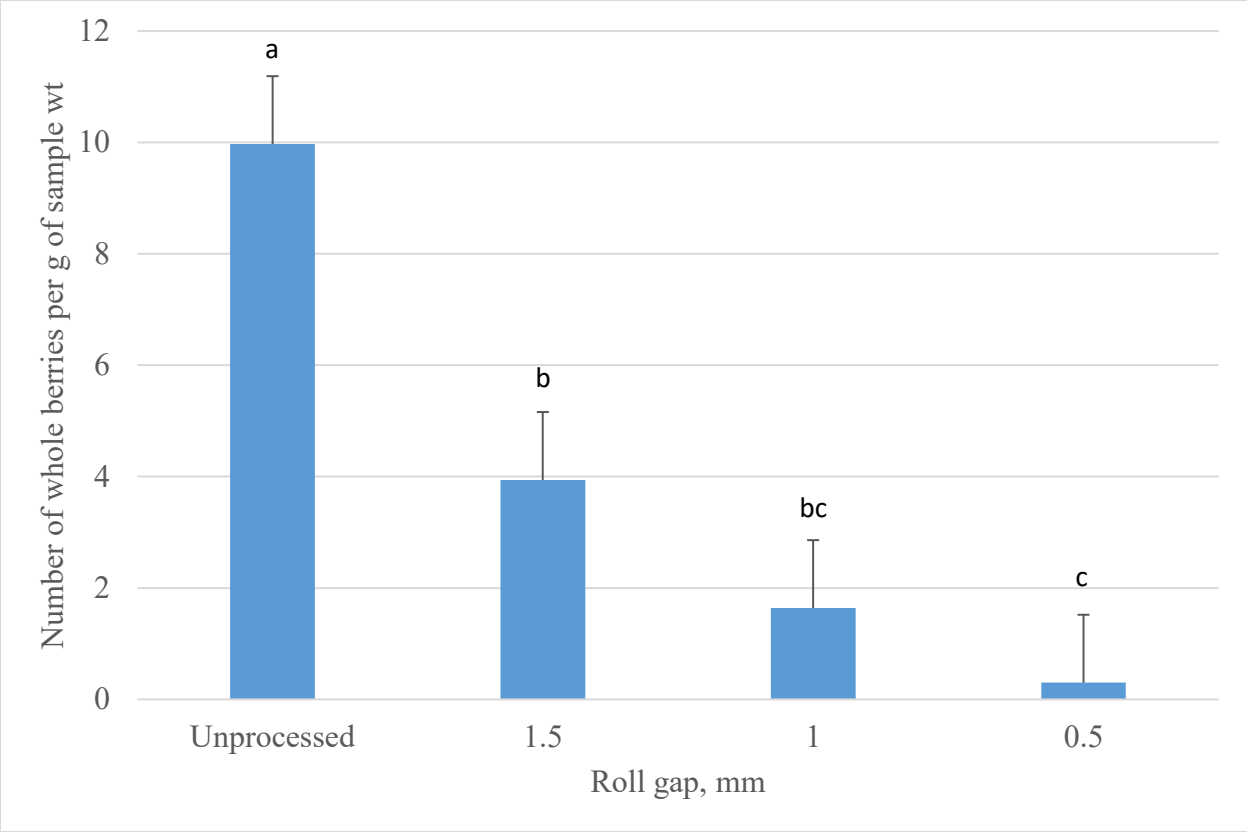

Figure 4.4 Whole berries per gram of sample for each roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm).
Treatment effect: $P < 0.001$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P < 0.001$
Linear: $P = 0.02$
Quadratic: $P = 0.65$
[a,b,c]Means differ ($P < 0.05$)

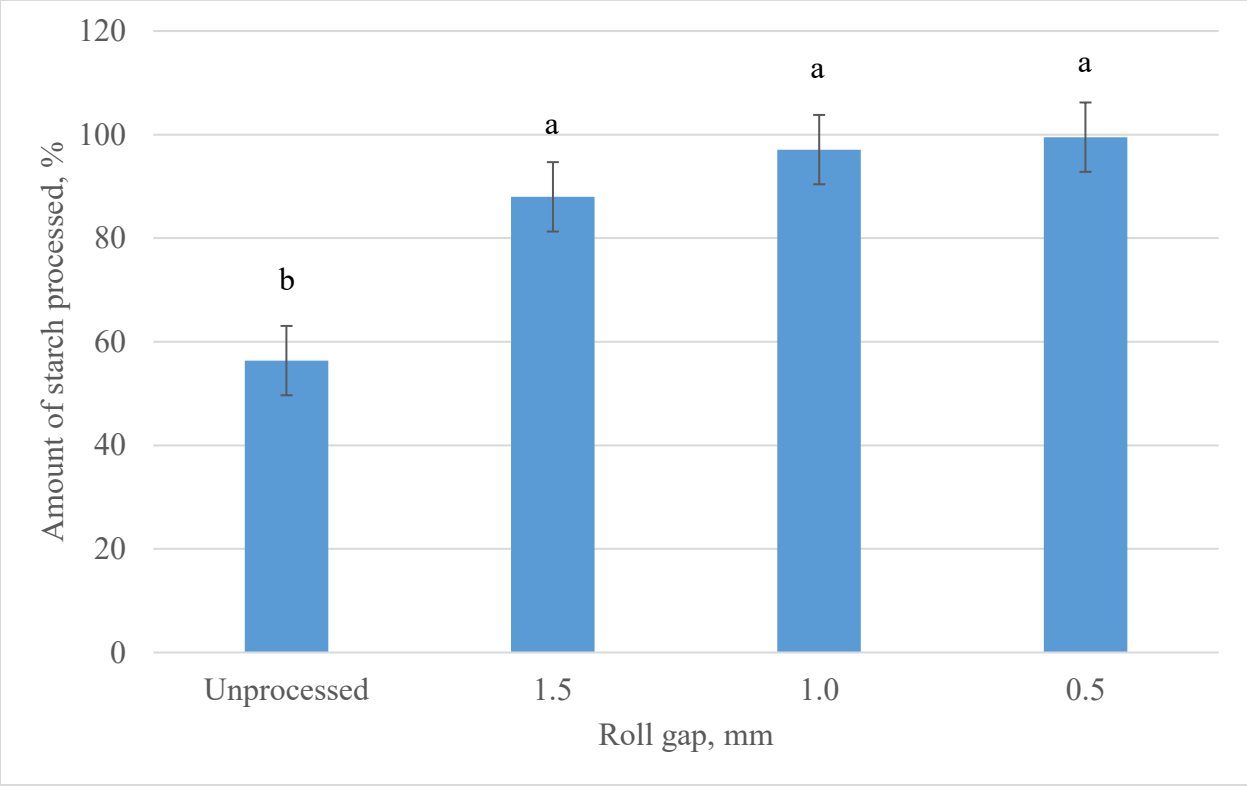

Figure 4.5 Percent of whole sample starch processed at each roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm). Measured as the percent of starch contained in the processed sorghum berries divided by the percent of starch contained in the whole, unprocessed sorghum berries.
Treatment effect: $P < 0.01$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P < 0.01$
Linear: $P = 0.22$
Quadratic: $P = 0.67$
[a,b]Means differ ($P < 0.05$)

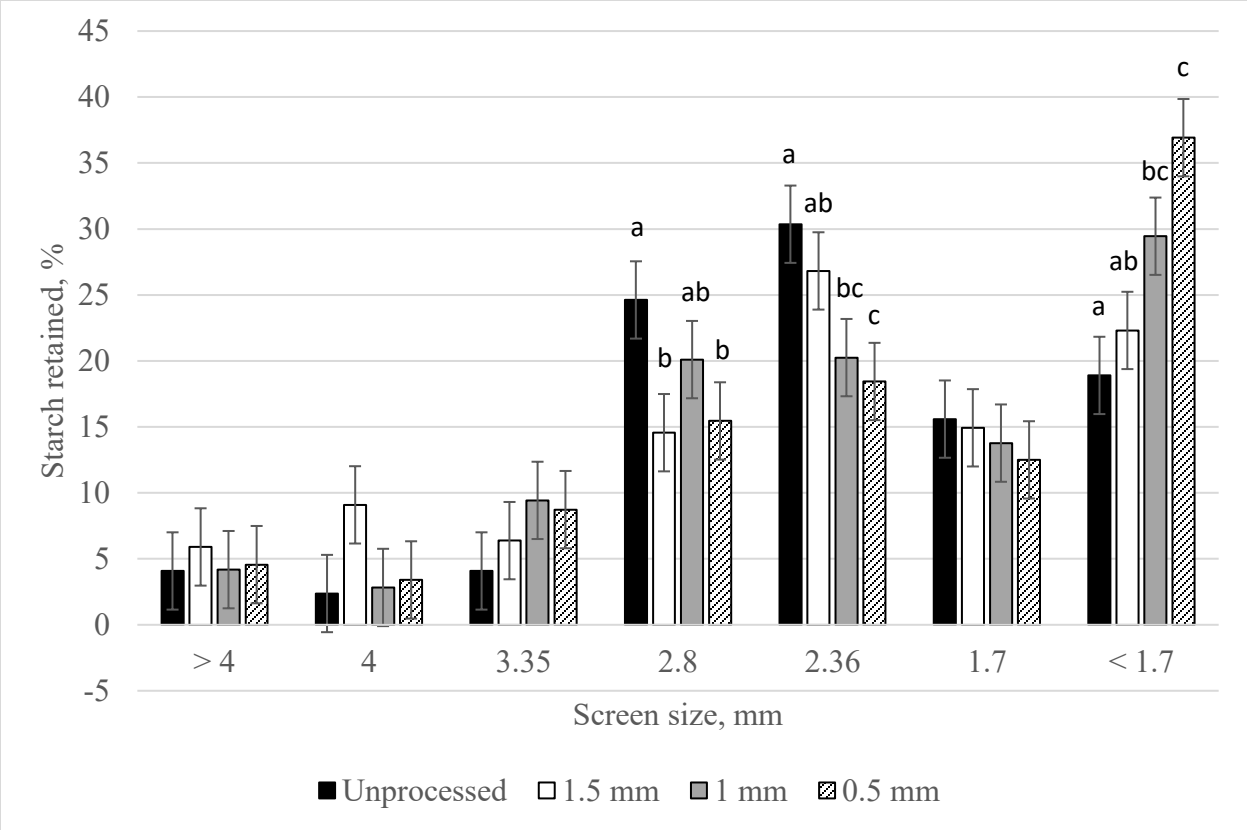

Figure 4.6 Percent starch retained by screen for each roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm) of whole sorghum sample after complete separation using a Ro-Tap particle separator (W. S. Tylor, Mentor, OH**)**.
Screen: $P < 0.001$
Rollgap × screen: $P < 0.01$
[a,b,c]Means within screen size differ ($P < 0.05$)

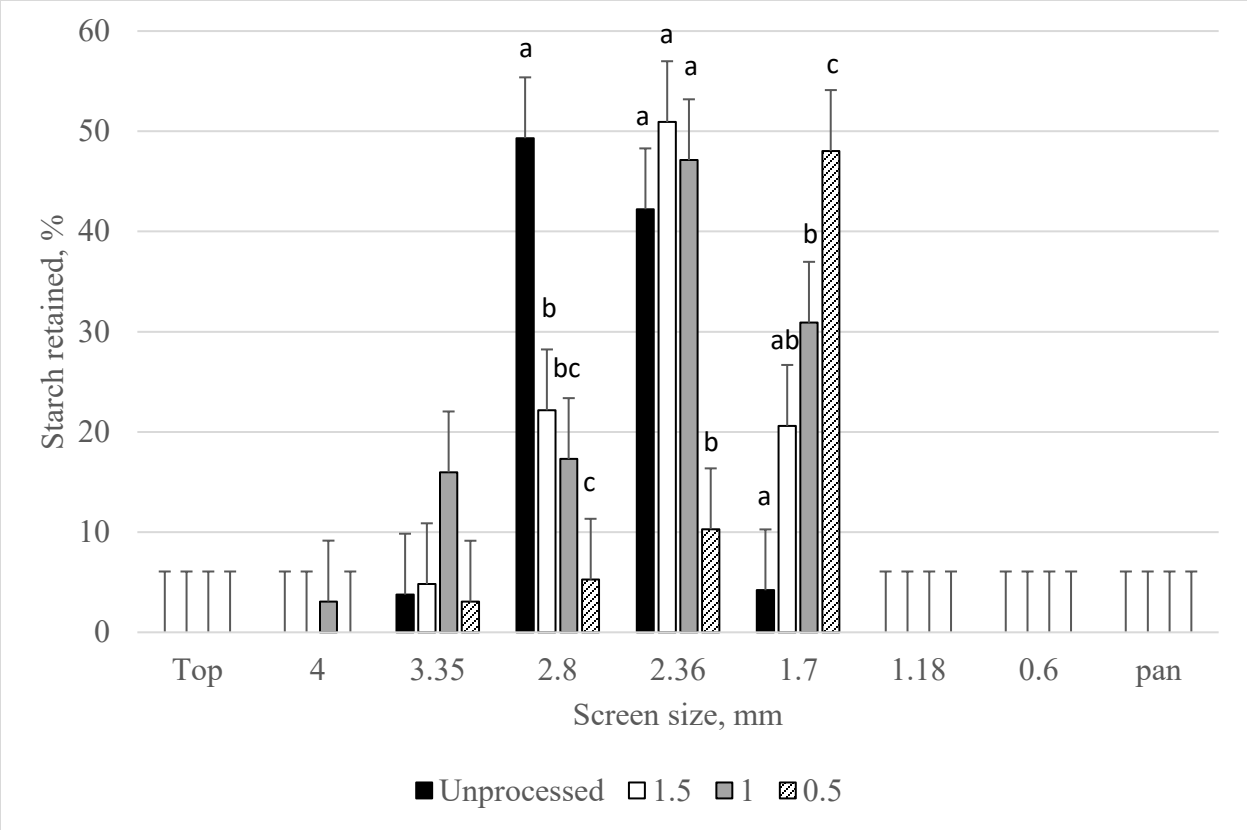

Figure 4.7 Percent starch retained by screen for each roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm) of whole berry portion of sorghum sample.
Screen: $P < 0.001$
Rollgap × screen: $P < 0.0001$
[a,b,c]Means within screen size differ ($P < 0.05$)

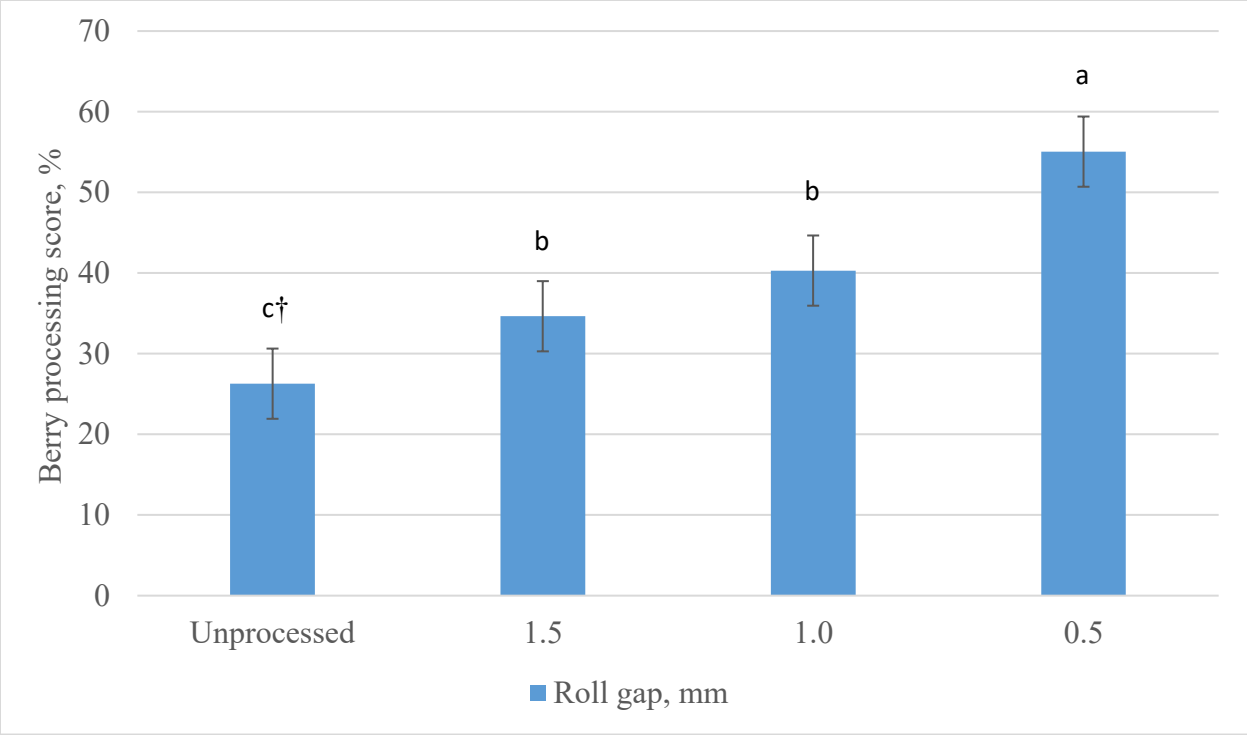

Figure 4.8 Berry processing score (BPS) by roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm) measured as a percent of total starch passing through the 1.7 mm screen.
Treatment effect: $P < 0.001$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P < 0.001$
Linear: $P < 0.001$
Quadratic: $P = 0.29$
[a,b,c]Means differ ($P < 0.05$)
[†]$P = 0.10$ vs. 1.5 mm

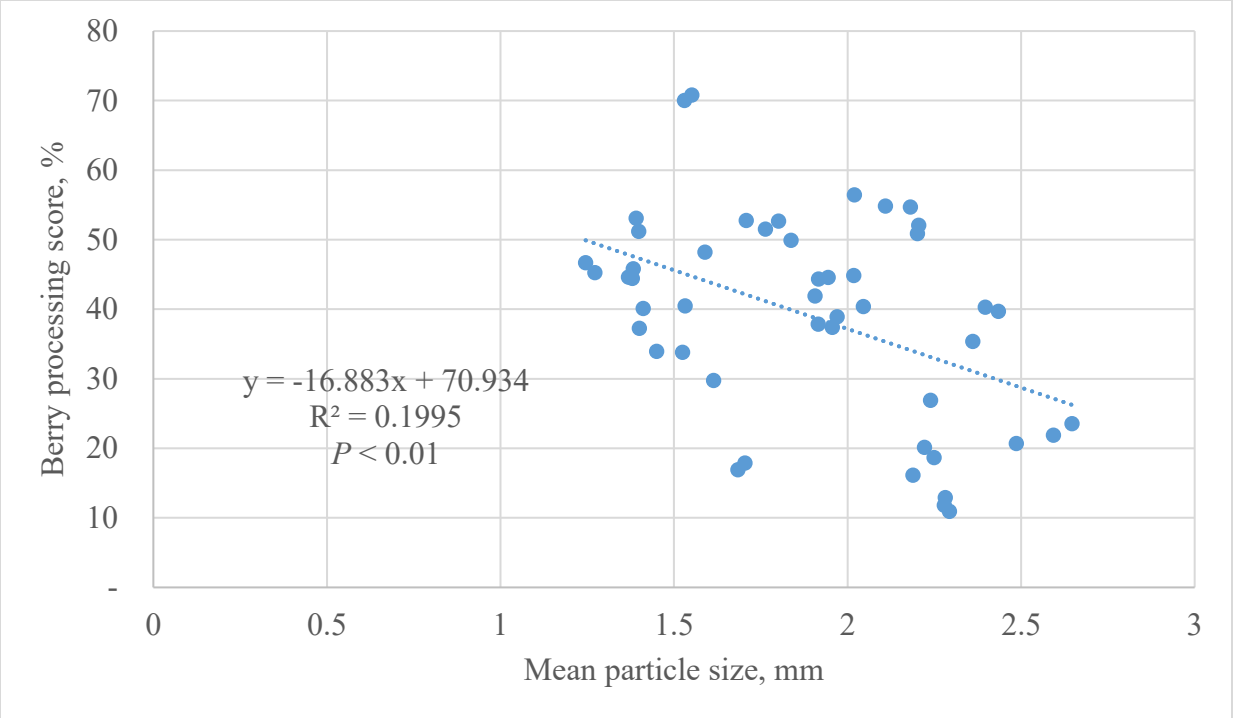

Figure 4.9 Relationship between berry processing score (BPS) and mean particle size (MPS). Berry processing score was defined as the percent of starch passing through the 1.7 mm screen.

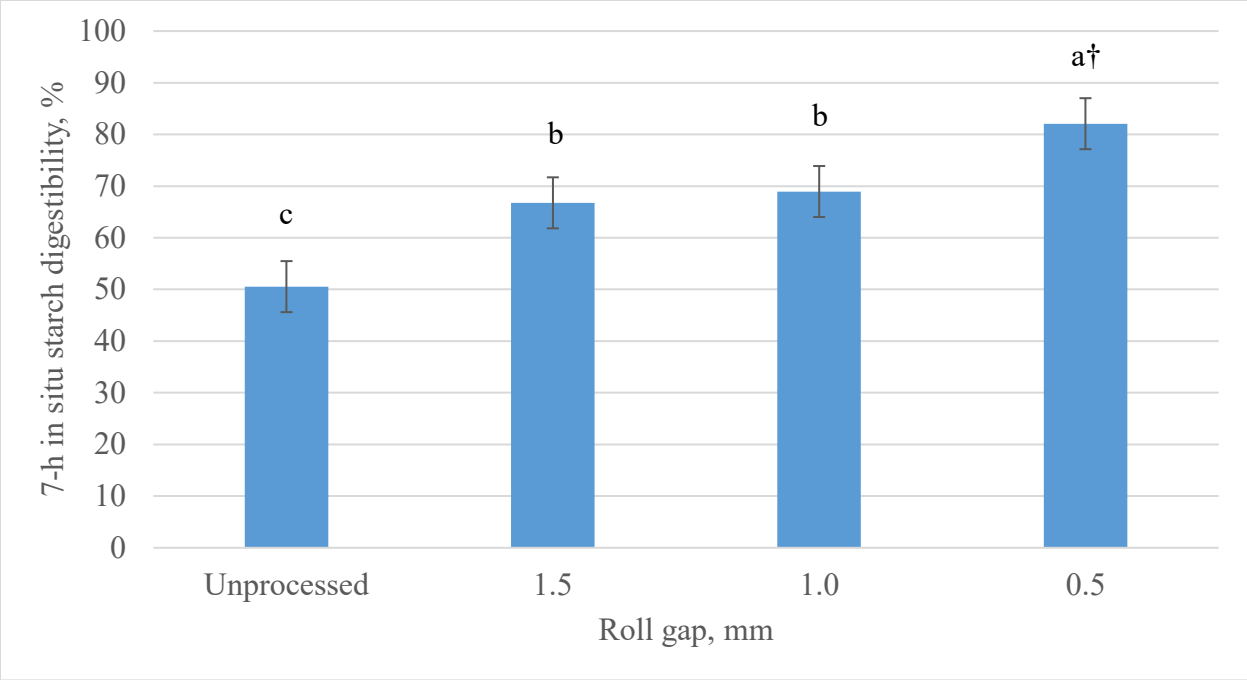

Figure 4.10 Least squares means for 7-h in situ starch digestibility by roll gap spacing (unprocessed, 1.5, 1.0, or 0.5 mm).
Treatment effect: $P < 0.01$
Unprocessed vs. processed (1.5, 1.0, and 0.5 mm): $P < 0.01$
Linear: $P = 0.04$
Quadratic: $P = 0.37$
[a,b,c]Means differ ($P < 0.05$)
[†]$P = 0.07$ vs. 1.0 mm

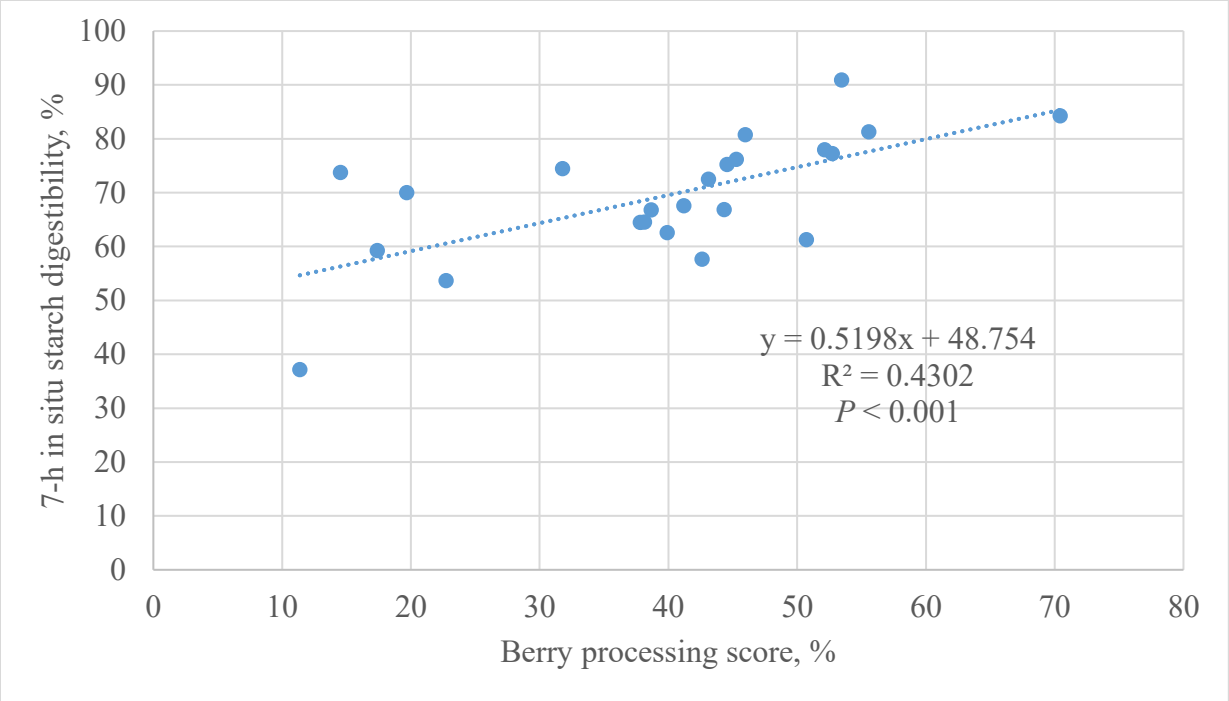

Figure 4.11 Relationship between 7-h in situ starch digestibility and berry processing score (BPS). Berry processing score was defined as the percent of starch passing through the 1.7 mm screen.

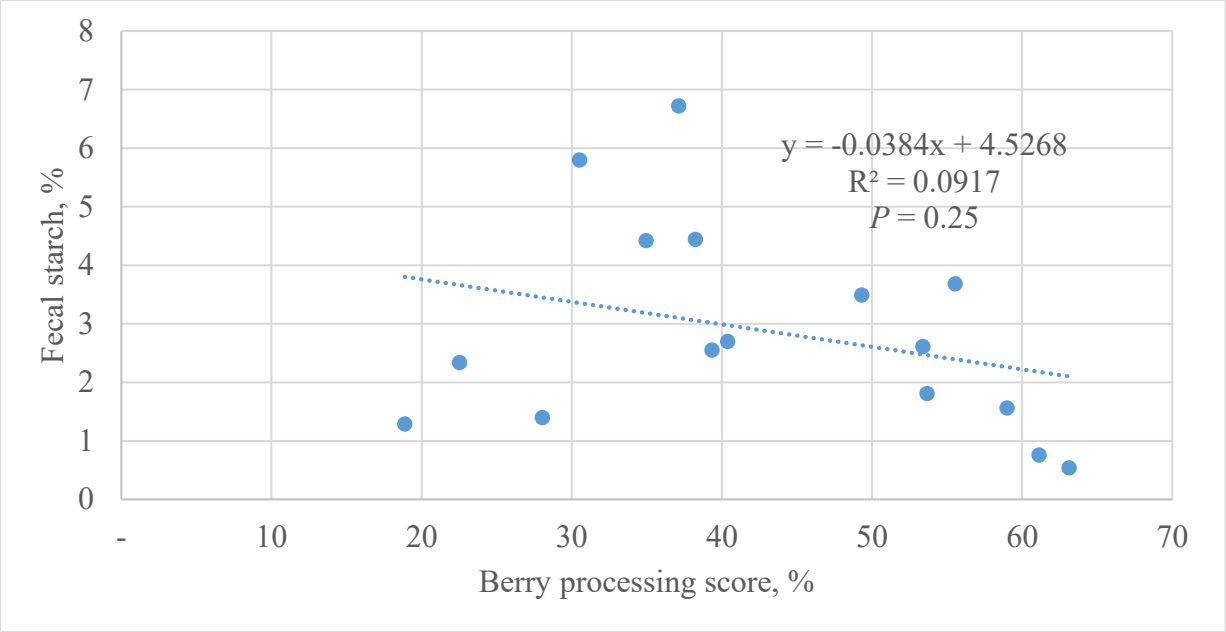

Figure 4.12 Relationship between fecal starch and berry processing score (BPS). Berry processing score was defined as the percent of starch passing through the 1.7 mm screen.

Table 4.1 Chemical composition and DM disappearance of sorghum silage processed at different roll gap spacing

Item	Roll gap spacing, mm				SE	*P*-value
	Unprocessed	1.5 mm	1.0 mm	0.5 mm		
DM, %	32.6	32.2	32.0	31.5	1.4	0.14
CP, % of DM	8.9[a]	8.8[a]	8.7[ab]	8.4[b]	0.5	0.04
Starch, % of DM	19.3	18.5	17.2	17.4	4.6	0.55
aNDF, % of DM	47.6	47.9	48.2	48.1	2.7	0.95
DM Disappearance, %	28.6[a]	29.9[ab]	33.4[bc]	34.4[c]	1.5	0.04

[a,b,c]Means within a row with differing superscripts differ ($P \leq 0.05$).

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Chapter 5 - The Effects of an Evaporative Cooling System on Reducing Heat Load in Lactating Dairy Cows

Abstract

A study was conducted to evaluate the effect of two cooling systems on barn temperature, collar temperature, core body temperature (CBT), respiration rate, rear udder temperature, and lying time in lactating Holstein dairy cows. The study design was a switchback design where cows were moved between barns for 6 d, therefore exposing treatment groups to each barn for a total of 3 d. Twenty lactating Holstein dairy cows were randomly assigned to 1 of 2 treatment groups with 10 cows per treatment group: CONV, which refers to the time period when cows were housed in a conventional, open-sidewall freestall barn that utilized feedline soakers and fans located over the feedline and stalls as its principal source of cow cooling, and TUNNEL, which refers to the time period when cows were housed in a tunnel-ventilated freestall barn utilizing an evaporative cooling system. The cooling system in the tunnel-ventilated barn (TUNNEL) was effective at reducing barn and collar temperature and THI, while RH was increased in comparison to the conventional, open-sidewall barn (CONV). Most of the differences found were during the afternoon h. Lower THI in the cow environment for TUNNEL failed to result in treatment differences for CBT, however, with CONV and TUNNEL having similar CBT of 38.6 ± 0.04ºC. TUNNEL cows had reduced respiration rates compared to CONV (52.0 vs. 57.9 ± 2.2, respectively) and this difference was greater during the afternoon h (1600 h) with average respiration rates of 55.4 and 63.0 ± 2.6 breaths per min for TUNNEL and CONV, respectively. Similar results were found for rear udder temperatures where TUNNEL cows had reduced rear udder temperatures overall (33.2 vs. 34.5 ± 0.3ºC) and during the

afternoon period (34.0 vs. 34.9 ± 0.4ºC) compared to CONV. Cows housed in the TUNNEL barn had increased lying time by 1 h/d compared to CONV (11.8 vs. 10.8 ± 0.3 h/d). CONV cows tended to have a greater number of lying bouts/d (11.8 vs. 10.8 ± 0.6 bouts/d for CONV and TUNNEL, respectively), while TUNNEL had a greater duration of lying bout (57.5 vs. 69.3 ± 3.3 min/bout for CONV and TUNNEL, respectively). Overall, the evaporative cooling system was effective in reducing barn THI leading to reduced respiration rates and rear udder temperature and increased daily lying time. No treatment differences were detected for CBT, however, likely a result of the cooler ambient conditions under which the study took place.
Key words: heat stress, evaporative cooling, core body temperature, lying behavior

Introduction

Heat stress greatly affects dairy cattle behavior and physiological processes (Collier et al., 2006) every year throughout the U.S. Not only does heat stress reduce milk production but also greatly decreases efficiencies for growth and reproduction and leads to animal welfare issues such as lameness. It has been estimated that heat stress costs the U.S. dairy industry $897 million annually (St-Pierre et al., 2003). Total U.S. dairy cow numbers in 2003 was ~9,082,000 (NASS, 2003). Therefore, annual heat stress related losses were $99 per cow per year, or $0.27 per cow per day. Given the greater ambient temperatures and genetic selection for greater milk production, annual heat stress losses today likely exceed $100 per cow. Even though progress has been made in limiting the negative effects of heat stress on dairy cattle, we continue to see the negative effects of heat stress in reduced feed intake, milk production and reproduction, and increased susceptibility to disease in today's high producing dairy cows.

Core body temperature (CBT) and total daily lying time are very important in the production and profitability of dairy cattle. Maintaining a normal CBT is critical for lactating

dairy cows to sustain production and reproduction throughout the summer months. Milk production has been shown to decline when rectal temperature exceeds 39.0°C for more than 16 h (Igono and Johnson, 1990). In addition, reproductive efficiency and fertility have been shown to decrease when CBT exceeds 39.0°C (Gwazdauskas et al., 1973; Wolfenson et al., 1988; Thatcher et al., 2010). Meanwhile, mean daily lying time decreased from 10.9 to 7.9 h/d from the coolest to the hottest part of the day (Cook et al., 2007) and others have also found similar reductions in lying time in heat stressed dairy cows (Overton et al., 2002; Legrand et al., 2011). Ideally, high producing dairy cows should by lying down for a minimum of 12 h/d (Cook et al., 2007). Grant (2007) proposed that each additional hour of lying time results in an increase of 0.91 to 1.59 kg/d of milk. In addition, when cows do not have adequate lying times, animal welfare issues and lameness may be a concern (Fregonesi and Leaver, 2001). Therefore, cooling systems that are able to reduce CBT and increase daily lying times in summer are necessary and could greatly increase profitability of the dairy herd.

It has been reported that 94% of U.S. dairies use some form of heat abatement (USDA, 2010). In order to reduce the heat load placed on the dairy cow in summer, 1 of 2 methods are currently employed to reduce the negative impacts of heat stress on dairy cattle: environmental modification (i.e. evaporative cooling) or utilizing methods to enhance heat dissipation from the skin of cattle (i.e. soaking). Currently, feedline soakers with fans located over the feedline and bedding area is the most common cow cooling method used on dairy farms (USDA, 2010). However, there has been much concern from producers about excessive energy and water usage from these types of systems, particularly because these systems continue to run whether cows are present within the pen or not. Therefore, the development of heat abatement systems that maximize the efficiency of water and energy use on the dairy is paramount.

Evaporative cooling systems equipped with a fogging system have been used to decrease air temperature around the cow and increase heat exchange between the cow and the environment (Berman, 2006). The fog cools the air as it moves through the facility aided by movement of air provided from strategically placed fans throughout the barn. Fan placement and spacing is of utmost importance in order to achieve adequate effective cooling velocity over the cows. The objective for this study was to evaluate the use of high velocity fans equipped with a fogging system and measure its effects on barn and collar temperature humidity index (THI), respiration rates, rear udder skin temperature, CBT, and lying times in lactating Holstein dairy cows.

Materials and Methods

This study was conducted in August 2016 on a commercial dairy in Nebraska that contained a tunnel-ventilated freestall barn and an open-sidewall, conventional freestall barn. The tunnel-ventilated barn contained ECV72 fans (CYC723230460, 1.83 m ECV72 with deflectors, 230/460V, 3 HP) provided by VES Environmental Solutions (Chippewa Falls, WI) equipped with a fogging system, as the main source of cooling. Fans were located over the freestalls with a spacing between fans of 18.3 m. The fog system would cycle on and off throughout the late morning and afternoon hours, determined by the temperature and relative humidity (RH) levels within the facility. Each ECV72 fan within the facility was rated to move air at a rate of ~1,700 m^3/min. The fog system ran at ~492,148 kg/m^2 of pressure resulting in a water droplet size of 10 to 17 microns with a flow rate of ~0.136 L/min per nozzle with 15 nozzles per fan. The conventional freestall barn had 122 cm basket fans (1 HP; 566 m^3/min) located over the stalls, 91.5 cm basket fans (1 HP; 325 m^3/min) located over the feedbunk, and

included a feedline soaker system that turned on and off intermittently, determined by ambient temperature. Spacing between fans located over the feedbunk and freestalls was 9 m.

Pen dimensions for each pen used in the study were 129.8 m in length and 12.6 m in width for the tunnel-ventilated barn, and 128 m in length and 12.8 m in width for the conventional barn. Total number of cows in each pen was 200 and 205 for the tunnel-ventilated and conventional freestall barn, respectively, resulting in similar stocking density based on feedbunk space (each pen utilized 61 cm headlocks) and number of stalls. Freestall dimensions were also similar between barns. Both barns used sand as the source of bedding. Throughout the trial, cows were milked 3 times per day and a total mixed ration (TMR) was fed at least twice daily. The TMR was formulated to meet or exceed the predicted nutrient requirements (NRC, 2001) for energy, protein, vitamins and minerals. The Institutional Animal Care and Use Committee at Kansas State University approved all experimental procedures and all measures were taken to avoid unnecessary discomfort to animals throughout the study.

Experimental Design and Treatments

Twenty lactating Holstein dairy cows were randomly assigned to 1 of 2 treatment groups. Group 1 was made up of 10 cows that averaged 166 ± 34 DIM and 40 ± 3 days carried calf. Group 2 consisted of 10 cows averaging 155 ± 9 DIM and 40 ± 3 days carried calf. This study utilized a switchback design where both groups of cows were moved between barns every 24 h for 6 consecutive days, therefore exposing both groups to each barn environment for a total of 3 d. **TUNNEL** consists of the time periods when cows in Group 1 or Group 2 were located in the tunnel-ventilated freestall barn while **CONV** refers to the time periods when cows in Group 1 or Group 2 were located in the conventional freestall barn.

Throughout the study, ambient temperature and RH were measured at 1-min intervals with 2 weather stations located throughout the farm. Weather stations were composed of a sensor (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) and a solar radiation and moisture shield (M-RSA; Onset Computer Corporation, Pocasset, MA). Within each barn, 3 weather stations (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) were placed throughout the pen to track pen temperature and RH at 1-min intervals.

Each cow in the study was fitted with a neck collar that contained a sensor (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) to track temperature and RH of the micro-environment as the cow moved throughout the facilities. Each cow also received an intravaginal stainless-steel temperature logger (HOBO U12, Onset Computer Corporation, Pocasset, MA) attached to a blank controlled internal drug-releasing device (CIDR; Pfizer Animal Health, New York, NY) that recorded vaginal temperature at 1-min intervals. Before the start of the study, each vaginal probe was validated in a water bath with a certified thermometer to ensure similar temperature responses. In addition, each cow was fitted with an electronic data logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) that was attached to the medial side of the right, hind leg using vet wrap. The acceleration data logger was placed in a position such that the x-axis was parallel to the ground, the y-axis was perpendicular to the ground pointing upward, and the z-axis was parallel to the ground pointing away from the sagittal plane. The loggers recorded the *g*-force on the x, y, and z-axes at 1-min intervals throughout the study. All recording devices were pre-programmed to begin recording at 1200 h on d 1 of the study. Each of the data loggers were removed from the cows at the end of the study and downloaded using Onset HOBOware software (Onset Computer Corporation, Pocasset, MA), which converted the *g*-force readings into degrees of tilt. These data were

exported into Microsoft Excel (Microsoft Corporation, Redmond, WA), and the degree of vertical tilt (y-axis) was used to determine the position of the animal, such that readings < 60° indicated the cow standing and readings ≥ 60° indicated the cow lying down (Ito et al., 2009). Standing and lying bouts of < 2 min were ignored because these readings were likely associated with leg movements at the time of recording (Endres and Barberg, 2007). Total daily lying time (min/d), frequency of lying bouts (n/d), and average lying bout duration (min/bout) were calculated for each cow. The average bout duration was calculated by dividing the daily lying time by the number of bouts for that day for each cow. All data loggers were programmed and managed by a single computer, allowing for synchronization of time.

Individual cow measurements for respiration rate and rear udder temperature were taken daily in the morning (0900 h) and afternoon (1600 h). Respiration rate was measured by counting the number of flank movements for 30 sec and then multiplying by 2. Body surface temperature was taken using an infrared thermometer gun (Raytek Raynger MX; Model: 4KM98).

Statistical Analysis

Mean hourly THI data was calculated using the formula THI = (9/5 x T_{db} + 32) – [0.55 – (0.55 x RH/100)] x [(9/5 x T_{db} + 32) – 58], where T_{db} is dry-bulb temperature (°C; Zimbelman et al., 2009). Vaginal temperatures were used to determine mean 24-h CBT and mean hourly CBT. In addition, CBT data were used to determine the duration of time (h/d) cows maintained a CBT above or below various temperatures each day including: < 38.6ºC, ≥ 38.6ºC but < 39.0ºC, and ≥ 39.0ºC. Lying behavior data were summarized by analyzing the angles recorded by the leg data loggers and total lying time was calculated for each cow. Lying behavior was also broken into 3

different time periods around milking times to evaluate lying behavior during different periods of the day (0400 to 1000 h, 1200 to 1800 h, and 2000 to 0200 h).

Data for ambient and barn conditions, collar data, and CBT were averaged by hour prior to analysis and assessed in a switchback design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Model effects included treatment, hour, and the interaction of treatment and hour. Treatment and the interaction of treatment and time of day was the model effect used for respiration rate and rear udder skin temperature, while treatment was the model effect for lying time data. Individual cows were considered to be the experimental unit and was included as the random effect in addition to day. The repeated measure was utilized using the covariance structure giving the lowest Bayesian information criterion (BIC) value for each variable. Means were determined using the least squares means statement. Confidence intervals are reported at 95% and statistical significance between treatments was declared at $P < 0.05$ and a tendency at $0.05 \leq P \leq 0.10$.

Results

Average ambient temperature during the study was 22.3 ± 3.4ºC, and average RH was 78.1 ± 14.2% resulting in an average THI of 70.1 ± 4.6 throughout the study (Figure 5.1; Table 5.1). Average minimum and maximum temperatures throughout the study were 18.1 ± 1.8ºC and 26.2 ± 2.6ºC, respectively, while minimum and maximum RH were 58.5 ± 7.0 and 94.7 ± 3.0, respectively. This resulted in a minimum and maximum THI throughout the study of 64.3 ± 3.2 and 74.3 ± 3.9, respectively.

Barn Environment

Barn temperature was cooler for TUNNEL compared to CONV ($P = 0.02$) with the main difference being detected during the afternoon hours (1300 to 1800 h; Figure 5.2). As expected,

RH was greater for TUNNEL compared to CONV ($P < 0.01$) as a result of the evaporative cooling system used (Figure 5.3). Temperature humidity index was reduced for TUNNEL compared to CONV ($P = 0.04$) with the primary difference being detected during the afternoon hours (Figure 5.4). The differences observed between barns were expected and indicate more effective cooling for TUNNEL as shown by the reduced THI levels. Due to the fogging system being present in the tunnel barn, RH levels were greater as a result of water evaporation.

Collar Data

Collar temperature was cooler for TUNNEL compared to CONV ($P < 0.01$) with the main difference being detected during the early morning (0400 to 0700 h) and afternoon hours (1400 to 1900 h; Figure 5.5). As expected, RH was greater for TUNNEL compared to CONV ($P < 0.01$; Figure 5.6). Temperature humidity index was reduced for TUNNEL compared to CONV ($P < 0.01$) with the primary differences being detected during the afternoon hours (Figure 5.7).

Vaginal Temperature (CBT)

Core body temperature did not differ ($P = 0.79$) between treatment groups (Figure 5.8) with an average CBT of 38.6 ± 0.04ºC and 38.6 ± 0.04ºC for CONV and TUNNEL, respectively. When looking at CBT by zone (Table 5.2), no differences were found between treatment groups. While there were numerical differences between treatments, CONV and TUNNEL cows spent similar time (h/d) within each CBT zone (< 38.6, ≥ 38.6, and ≥ 39.0ºC) resulting in a lack of treatment effect ($P > 0.05$).

Respiration Rates and Rear Udder Skin Temperature

Respiration rates were reduced in TUNNEL cows compared to CONV cows (Tables 5.3 and 5.4). CONV had an average daily respiration rate of 57.9 ± 2.2 breaths per min (BPM), while TUNNEL had an average respiration rate of 52.0 ± 2.2 BPM ($P < 0.01$). When broken

into the morning (0900 h) and afternoon (1600 h) time periods, respiration rates were reduced for TUNNEL cows in the morning (48.6 vs. 52.9 ± 2.0 BPM; $P = 0.03$) and afternoon (55.4 vs. 63.0 ± 2.6 BPM; $P < 0.01$) periods compared to CONV.

Rear udder temperature (Tables 5.3 and 5.4) averaged 34.5 and 33.2 ± 0.3ºC for CONV and TUNNEL, respectively ($P < 0.01$). When broken into the morning (0900 h) and afternoon (1600 h) time periods, udder temperature was reduced for TUNNEL cows both in the morning (32.5 vs. 34.1 ± 0.2ºC; $P < 0.01$) and afternoon (34.0 vs. 34.9 ± 0.4ºC; $P < 0.01$) compared to CONV.

Lying Time

Average daily lying time data can be found in Tables 5.3 and 5.5. CONV cows had reduced lying time by 1 h/d compared to TUNNEL cows (10.8 vs. 11.8 ± 0.3 h/d; $P < 0.01$). When data were divided into 3 different time periods between milkings, TUNNEL cows spent a greater ($P < 0.01$) percentage of time within each period lying down. TUNNEL cows spent > 50% of their time lying down in each time period, while CONV cows spent > 50% of their time lying down only during the first-time period (0400 to 1000 h). CONV cows averaged 11.8 ± 0.6 bouts/day, which tended to be greater than TUNNEL cows that had a mean of 10.8 ± 0.6 bouts/day ($P = 0.08$). When broken into the different time periods, no differences were detected between treatment groups for any of the 3 time periods ($P = 0.32$). Lying bout duration was greater ($P < 0.01$) for TUNNEL compared to CONV and averaged 69.3 and 57.5 ± 3.3 min/bout for TUNNEL and CONV, respectively. When broken into the 3 individual time periods there was a tendency ($P = 0.08$) for TUNNEL to have a greater lying bout duration. No differences ($P > 0.05$) were detected for lying bout duration during the 0400 to 1000 h or the 2000 to 0200 h time period. During the 1200 to 1800 h time period, however, there was a significant treatment

effect ($P < 0.05$) where TUNNEL cows had greater lying bout duration with a mean of 90.1 ± 7.2 min/bout compared to CONV with a mean of 61.8 ± 7.2 min/bout.

Discussion

Ambient conditions throughout the study were much cooler than anticipated but still adequate to achieve heat stress conditions for lactating dairy cattle. We expect that had this study been conducted under greater ambient temperatures, greater differences between treatment groups would have been detected.

Much research in heat stressed dairy cattle has been conducted where heat stressed cows have reduced milk yield as a result of reduced dry matter intake (DMI) and a shift in post-absorptive metabolism and nutrient partitioning (Rhoads et al., 2009; Wheelock et al., 2010). Heat stress is also known for its negative effect on reproductive performance in dairy cattle by decreasing the intensity and duration of estrus (Younas et al., 1993; Hansen and Arechiga, 1999) and reducing estradiol concentrations in blood (Wilson et al., 1998). This results in reduced conception rates (Morton et al., 2007) and compromises early embryonic development (Roman-Ponce et al., 1977; Hansen et al., 2001). As a result of genetic selection for milk yield and increased management of high producing dairy cows, more efficient cooling systems must be employed to offset the greater heat production by high producing dairy cows.

As shown by the barn and collar temperature, RH, and THI data, the evaporative cooling system utilized in the tunnel-ventilated freestall barn (TUNNEL) was effective at reducing air temperature and THI during the afternoon hours while RH levels increased during this same time period. By applying a sensor to the neck collar of each cow, we were able to track temperature and RH levels of the micro-environment the cow was exposed to as she moved throughout the facility. We thought this would be a better indicator than relying on sensors placed within the

barn as the neck collar sensor would give us a better indicator of the micro-environmental conditions the cow was exposed to within the barn throughout the day. Due to the proximity of the sensor to the cow's body, collar temperature sensor readings were greater than barn and outside ambient sensors due to heat given off from the cow's body surface.

Over the years, many different types of cooling systems have been developed in an effort to enhance heat loss in dairy cattle. Evaporative cooling systems have been shown to effectively cool the environment where cows are housed (Ryan et al., 1992; Ortiz et al., 2010). This creates a greater temperature gradient between cow and environment by cooling the air allowing the cow to dissipate more body heat.

The ECV72 fans used in the present experiment were high velocity fans that incorporated a fogging system. The fog evaporates as it moves through the air cooling the environment around the fan and fog. Hinds (1999) studied the effects of 3 different water droplet sizes (20, 30, and 100 micron) and the amount of time required for evaporation. A water droplet of 20 microns required 254 sec to fall 10 feet, while a 100-micron water droplet required just 10 sec to drop the same distance. Therefore, as water droplet size increases, evaporation time also increases. Hinds (1999) also studied the effects of differing RH levels on water droplet evaporation times. It was found that a 20-micron water droplet evaporates in just 1 sec at 50% RH, while it took 20 sec to evaporate when RH increased to 70%. Therefore, water droplet size and RH levels are important considerations when choosing an effective cooling system. Due to evaporation of the fog, RH levels will increase, which decreases the vapor pressure gradient between the cow and environment leading to less efficient evaporative heat loss. Thus, the benefit of decreasing air temperature must be greater than the effect of increasing RH levels or greater heat stress will occur. If the air velocity used with fogging systems is not adequate, small

water droplets will begin to accumulate on the cows' surface hair and act as an insulating barrier preventing dissipation of body heat (Hahn, 1985).

Even though the barn temperature and THI were reduced slightly for TUNNEL cows during the afternoon hours, this failed to result in treatment differences for CBT throughout the day as well as for CBT by zone. This is likely a result of the lower ambient temperatures experienced during the study leading to a lack of treatment effect for CBT. Core body temperature is used as the primary indicator of the severity of heat stress experienced by the animal. An increase in CBT is a physiological response of dairy cows resulting from an imbalance of heat production and heat dissipation. Had this study taken place during greater ambient temperatures, we would expect to have seen significant treatment differences for CBT. Both treatments were effective at preventing a rise in CBT under the conditions of the current study.

Research shows that a CBT of 39.0°C is a very critical temperature for lactating dairy cows. Milk production has been shown to decline when rectal temperatures exceed 39.0°C for more than 16 h (Igono and Johnson, 1990). Much research has been conducted studying the impact of elevated CBT on reproductive efficiency and it was found that conception rate and fertility decreased once CBT exceeded 39.0°C (Gwazdauskas et al., 1973; Wolfenson et al., 1988). More recent data, however, showed that reproduction and fertility may be affected below 39.0°C CBT. Recipient cows in an embryo transfer study showed decreased probability of pregnancy once rectal temperatures (taken between 0600 h and 1000 h) exceeded 38.0°C and continued to decrease linearly as rectal temperature increased (Vasconcelos et al., 2011). Cows in the current study spent minimal time above a CBT of 39.0ºC due to the lower ambient conditions under which the study was conducted.

Although CBT between treatments were similar, treatment differences for respiration rate and rear udder temperature were apparent. Historically, 60 BPM has been considered the threshold at which cows are considered to be heat stressed. Both TUNNEL and CONV cows maintained average daily respiration rates below the 60 BPM threshold as a result of the cooler ambient temperatures during the study. However, during the afternoon hours, CONV cows had respiration rates that exceeded 60 BPM, while TUNNEL maintained respiration rates below 60 BPM indicating that the evaporative cooling system for TUNNEL cows was more effective at reducing heat stress when looking at respiration rates alone. Likewise, rear udder temperatures were reduced for TUNNEL cows compared to CONV cows throughout both the morning and afternoon periods. Both treatment groups, however, maintained udder temperatures below 35.0ºC, which is considered the threshold at which milk yield loss begins (Collier et al., 2006). The low udder temperatures signify that cows in this study were able to adequately dissipate body surface heat brought up from internal organs via peripheral vasodilation and central vasoconstriction of blood vessels (Farooq et al., 2010). The reduction in udder temperature seen during the study for TUNNEL cows is in agreement with Berman (2006) and indicates that heat flow from internal organs to the body surface was less than the amount of heat removed via water evaporation. The forced ventilation from the evaporative cooling and fogging system was effective at cooling the air around the cow, thus decreasing udder temperature due either to decreased blood flow to the skin or increased removal of heat from the body surface. If skin surface temperature remains below 35.0°C the cow is able to dissipate heat via all 4 routes of heat exchange (conduction, convection, radiation, and evaporation) (Collier et al., 2006) and is more likely to maintain euthermia.

One of the primary goals of heat abatement should be to encourage dairy cows to lie down. This will benefit the cow with reduced incidence of lameness from reduced standing time during heat stress (DeFrain et al., 2013) as well as greater milk production (Grant, 2007). Even though ambient conditions during the study were mild resulting in similar CBT between treatment groups, the evaporative cooling system used in the current study was able to increase daily lying time in cows housed in the tunnel-ventilated freestall barn (TUNNEL) compared to the conventional freestall barn (CONV), a result of increased evaporative cooling and airspeed over the resting area, thus encouraging cows to lie down. Cows will often increase total daily standing time when heat stressed in an attempt to dissipate body heat due to greater body surface area being exposed allowing increased heat loss via convection and evaporation. Cook et al. (2007) found that mean daily lying time decreased from 10.9 to 7.9 h/d from the coolest to the hottest part of the day. Other studies have shown similar decreases in lying time during heat stress (Overton et al., 2002; Legrand et al., 2011). This study indicates that TUNNEL cows had increased cow comfort and more efficient cow cooling as shown by the increased lying time in TUNNEL cows, but still similar CBT between treatment groups. TUNNEL cows were able to lie down and still maintain euthermia even though less body surface area was exposed to allow for maximum convective and evaporative heat loss. Conversely, CONV cows stood for 1 h/d more in order to maintain euthermia via increased body surface area available to dispose of extra body heat. Ideally, high producing dairy cows should by lying down for a minimum of 12 h/d (Cook et al., 2007). Benefits of increased lying time are that mammary blood flow is enhanced by ~25% when the animal is resting (Rulquin and Caudal, 1992; Delamaire and Guinard-Flament, 2006), resulting in increased flow of nutrients to the mammary gland. Grant (2007) proposed that each additional hour of resting time results in an increase of 0.91 to 1.59 kg/d of

milk. As a result, cooling within the pen should be focused primarily over the resting area, as this is where cows should spend 50% of their day, as well as over the feedbunk as cows typically spend up to 20% of their day eating. Therefore, by focusing cow cooling over the living space (resting area and feedbunk) where cows spend ~70% of the day, we can maximize cow cooling efficiency. In the current study, using the data by Grant (2007), we could expect that TUNNEL cows would produce 0.91 to 1.59 kg/d more milk compared to CONV cows when looking at lying time data only. Thus, if we can encourage cows to lie down more when heat stressed by increasing cooling over the resting area, one could expect increased levels of production along with reduced incidence of lameness, which commonly increases during summer months.

When lying time data were broken into 3 different time periods throughout the day, TUNNEL cows spent a greater percentage of time lying down within each period compared to CONV. When looking at lying bout duration, an interesting observation was found where lying bout duration was greatest during the hottest part of the day (1200 to 1800 h) for TUNNEL cows, while CONV cows had their lowest lying bout duration during the afternoon period. This would indicate that the evaporative cooling system (ECV72 fans) was effective at keeping cows cool during the hottest part of the day, allowing cows to continue lying for a longer duration and therefore, resulting in increased total daily lying times. This shows the importance of focusing heat stress abatement systems over the resting area in order to encourage cows to lie down during heat stress.

Conclusions

The results of the current study show that the evaporative cooling system used in the tunnel-ventilated freestall barn was effective at reducing barn and collar temperature and THI, while increasing RH levels when compared to the cooling system utilized in the open-sidewall

conventional freestall barn. This resulted in reduced respiration rates and rear udder temperatures for cows housed in the tunnel-ventilated freestall barn, while CBT did not differ between treatment groups. Interestingly, lying bout duration was maximized during the afternoon period (1200 to 1800 h) for TUNNEL cows indicating effective cooling by the evaporative cooling system utilized in the current study. This led to increased daily lying time by 1 h/d for TUNNEL cows. This study shows the importance of cooling cows over the resting area to encourage cows to lie down during heat stress.

Figures and Tables

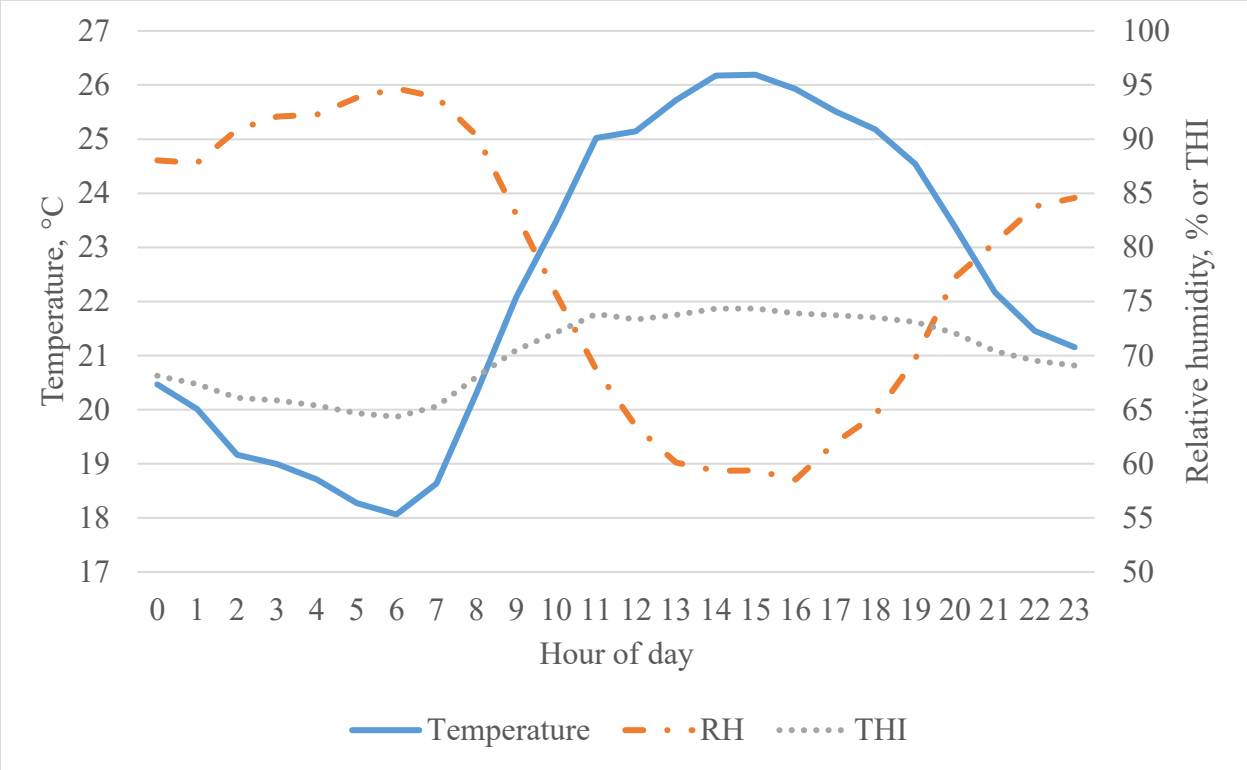

Figure 5.1 Average ambient temperature, relative humidity (RH), and temperature humidity index (THI) by hour.

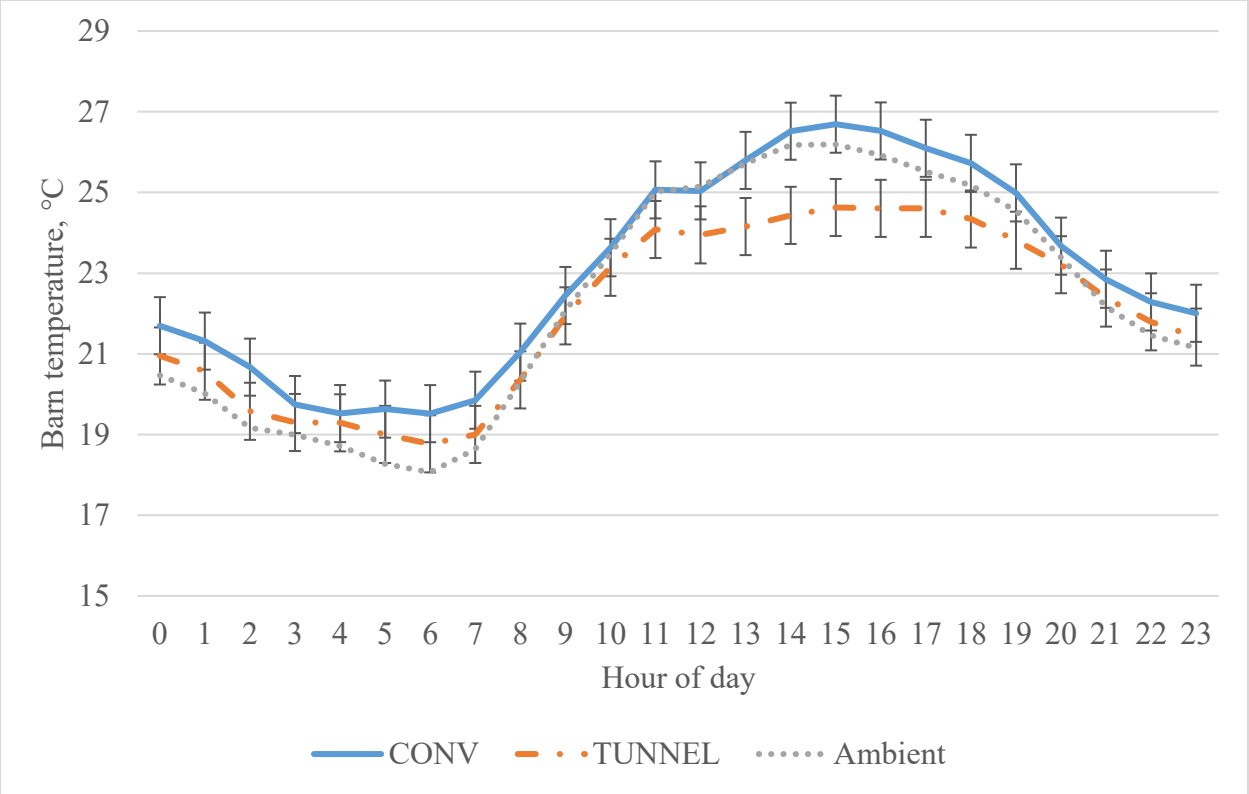

Figure 5.2 Effect of cooling treatment (CONV vs. TUNNEL) on barn temperature by h of d. Ambient temperature data is also shown for comparison.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P = 0.02$) and treatment × hour ($P = 0.89$).

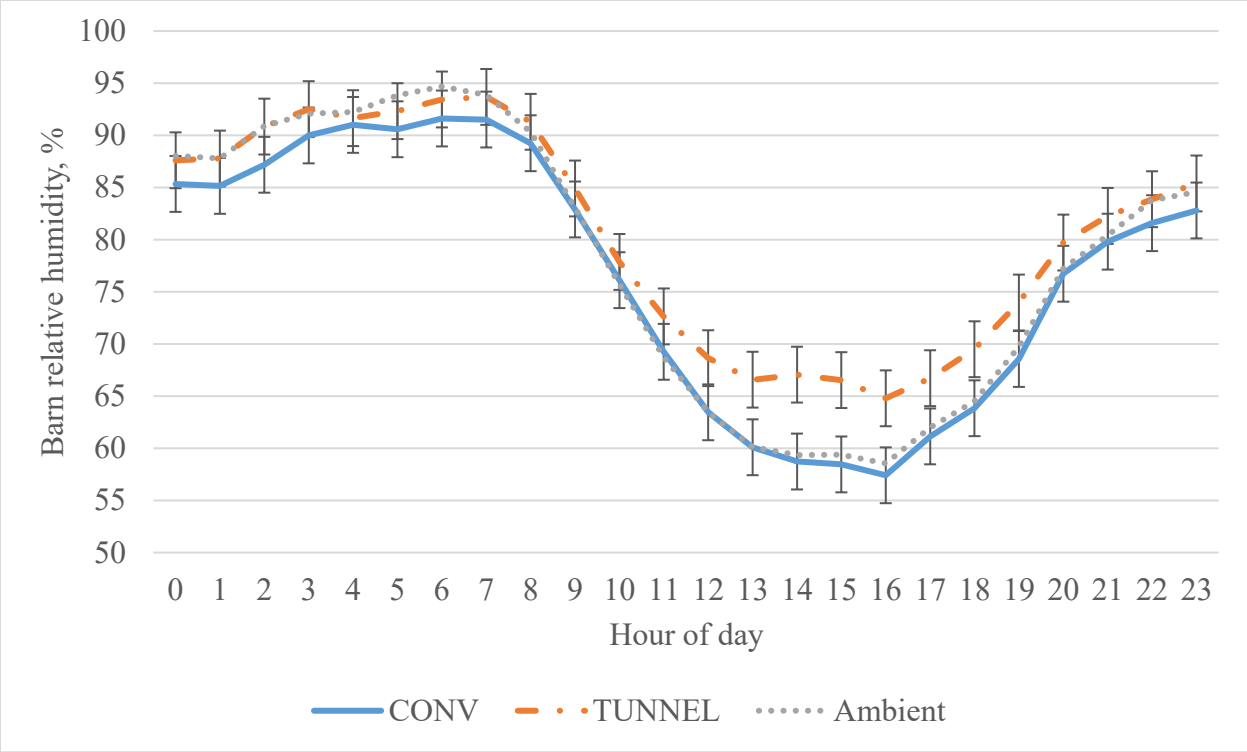

Figure 5.3 Effect of cooling treatment (CONV vs. TUNNEL) on barn relative humidity (RH) by h of d. Ambient RH data is also shown for comparison.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P < 0.01$) and treatment × hour ($P = 0.87$).

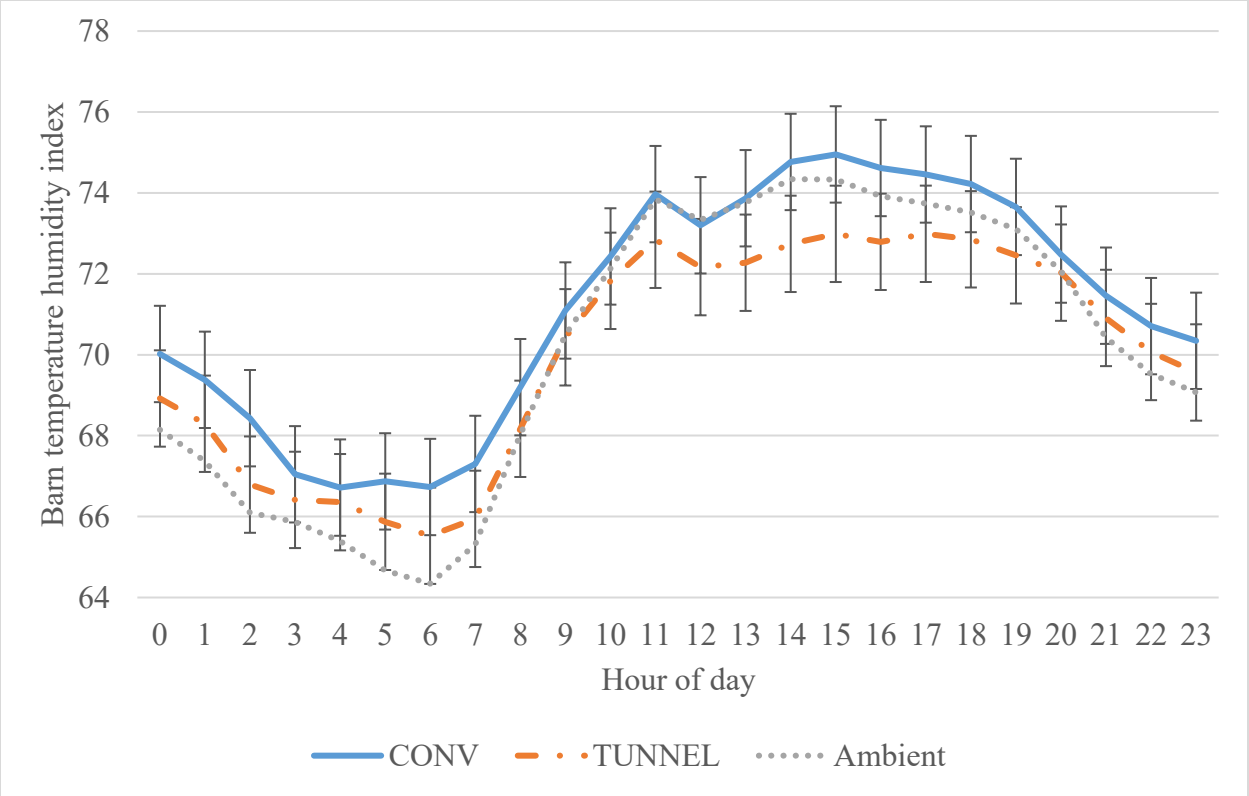

Figure 5.4 Effect of cooling treatment (CONV vs. TUNNEL) on barn temperature humidity index (THI) by h of d. Ambient THI data is also shown for comparison.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P = 0.04$) and treatment × hour ($P = 0.99$).

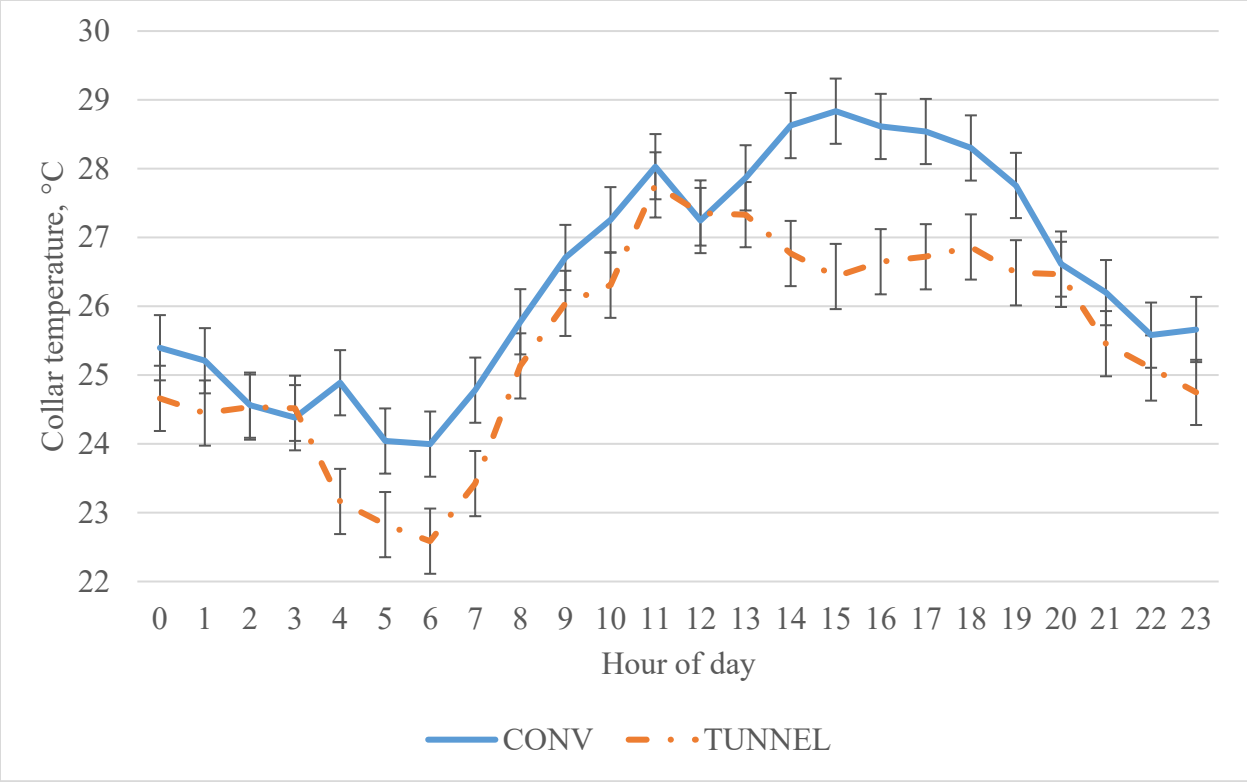

Figure 5.5 Effect of cooling treatment (CONV vs. TUNNEL) on collar temperature by h of d. CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P < 0.01$) and treatment × hour ($P < 0.01$).

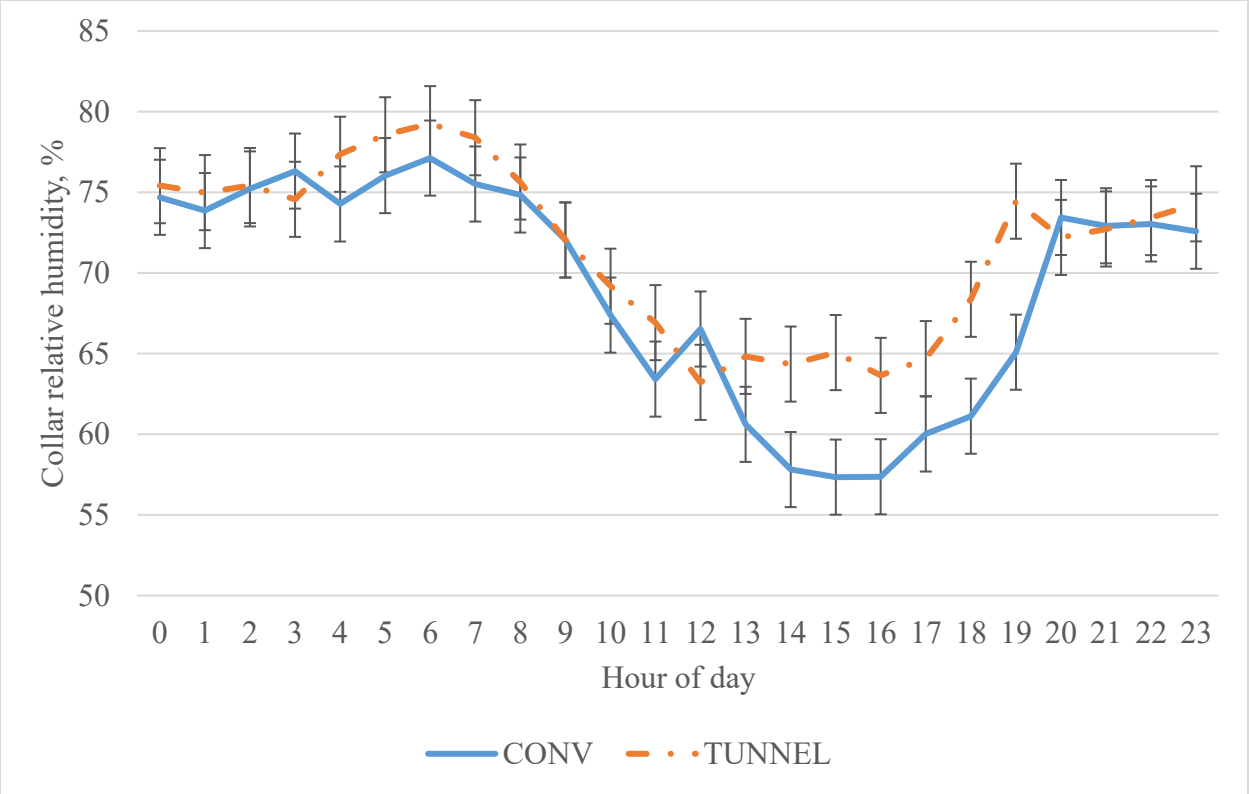

Figure 5.6 Effect of cooling treatment (CONV vs. TUNNEL) on collar relative humidity by h of d.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P < 0.01$) and treatment × hour ($P < 0.01$).

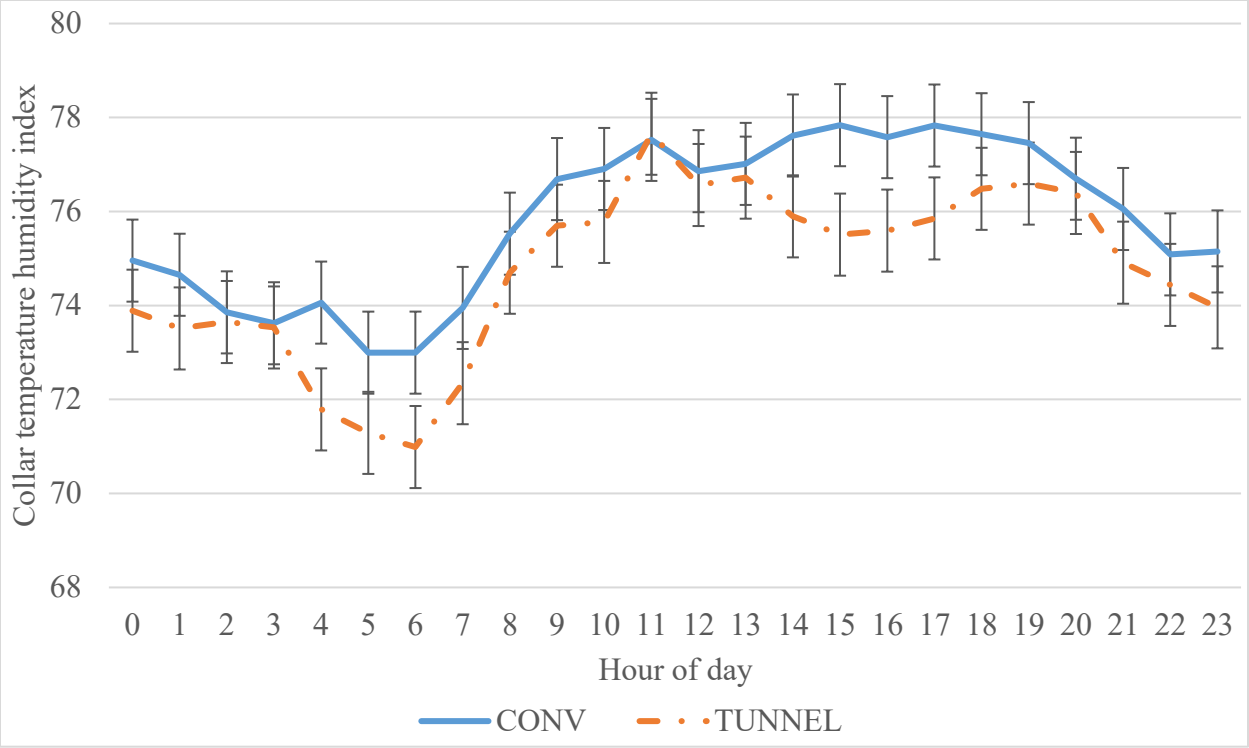

Figure 5.7 Effect of cooling treatment (CONV vs. TUNNEL) on collar temperature humidity index by h of d.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P < 0.01$) and treatment × hour ($P < 0.01$).

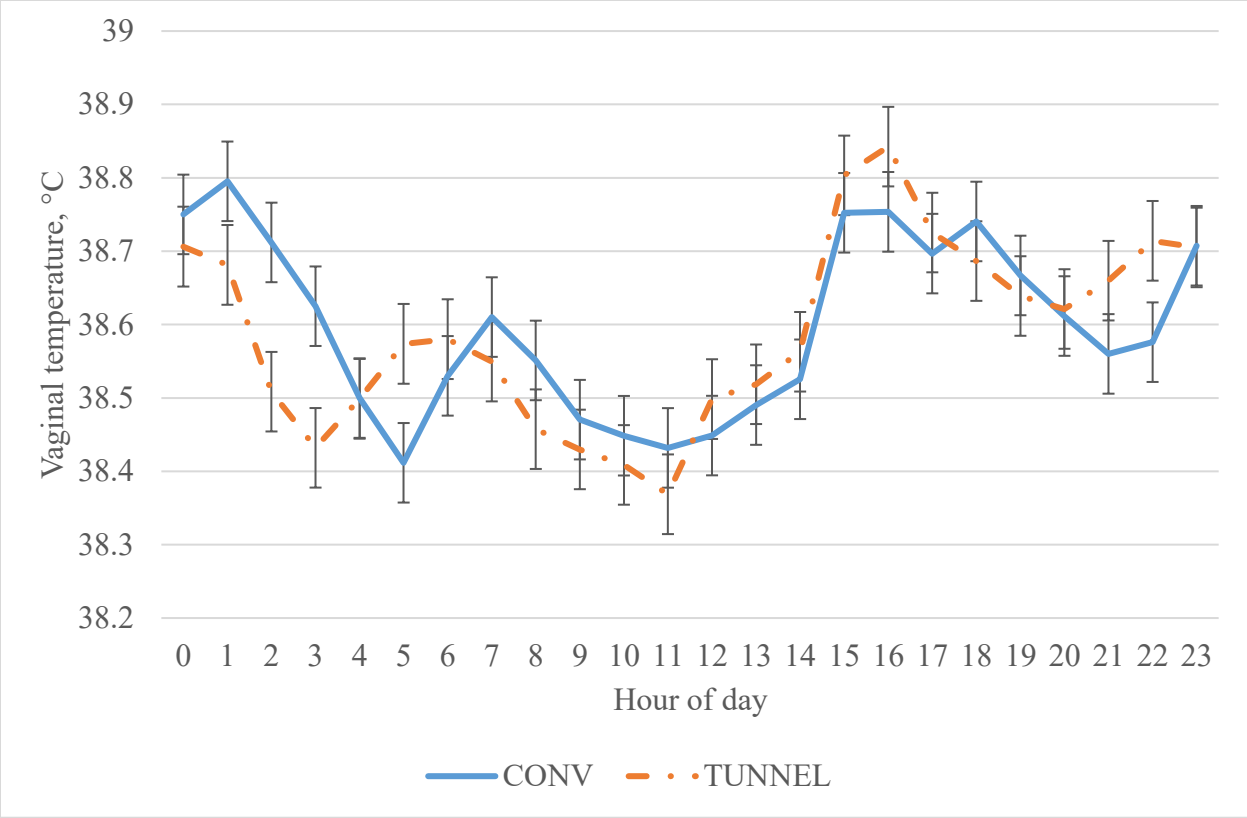

Figure 5.8 Effect of cooling treatment (CONV vs. TUNNEL) on vaginal temperature (CBT) by h of d.
CONV = conventional open-sidewall freestall barn with feedline soakers and fans located over the feedline and freestalls. TUNNEL = Tunnel-ventilated freestall barn equipped with an evaporative cooling system over the freestalls. Treatment ($P = 0.79$) and treatment × hour ($P < 0.01$).

Figure 5.9 Photo showing the evaporative cooling system (ECV72 fans and fog) over the resting area in the tunnel-ventilated freestall barn.

Figure 5.10 Photo showing the cooling system (feedline soakers with fans over the feedline) in the open-sidewall, conventional freestall barn. Fans were also located over the resting area.

Table 5.1 Environmental conditions throughout the study

Item	Minimum	SD	Maximum	SD	Mean	SD
Temperature, ºC	18.1	1.8	26.2	2.6	22.3	3.4
Relative humidity, %	58.5	7.0	94.7	3.0	78.1	14.2
THI[1]	64.3	3.2	74.3	3.9	70.1	4.6

[1]Temperature humidity index. THI = (9/5 x T_{db} + 32) – [0.55 – (0.55 x RH/100)] x [(9/5 x T_{db} + 32) – 58], where T_{db} is dry bulb temperature (ºF) and RH is relative humidity, presented as a decimal.

Table 5.2 Effect of cooling treatment on time (h/d) spent within each core body temperature (CBT) zone for each treatment throughout the study

	Treatment[1]			
CBT[2], ºC	CONV	TUNNEL	SE	*P*-value
< 38.6	13.4	14.2	1.08	0.12
≥ 38.6	7.9	7.3	0.59	0.22
≥ 39.0	2.7	2.5	0.59	0.60

[1]CONV refers to cows housed in the open-sidewall conventional freestall barn, while TUNNEL refers to cows housed in the tunnel-ventilated freestall barn.
[2]CBT was broken into 3 zones: h/d with CBT < 38.6°C; h/d with CBT ≥ 38.6°C but < 39.0°C; h/d with CBT ≥ 39.0°C.

Table 5.3 Effect of cooling treatment on respiration rate, udder temperature, and lying time data for each treatment throughout the study

	Treatment[1]			
Item	CONV	TUNNEL	SE	*P*-value
Respiration rate, BPM[2]	57.9	52.0	2.2	< 0.01
Udder temperature, ºC	34.5	33.2	0.3	< 0.01
Lying time, h/d	10.8	11.8	0.3	< 0.01
Lying bouts, n/d	11.8	10.8	0.6	0.08
Lying bout duration, min	57.5	69.3	3.3	< 0.01

[1]CONV refers to cows housed in the open-sidewall conventional freestall barn, while TUNNEL refers to cows housed in the tunnel-ventilated freestall barn.
[2]Breaths per minute.

Table 5.4 Effect of cooling treatment on respiration rate and udder skin temperature during the morning and afternoon observation periods for each treatment throughout the study

	Treatment[1]			*P*-value		
Item	CONV	TUNNEL	SE	Trt	Time	Trt × Time
Respiration rate, BPM[2]						
0900 h	52.9	48.6	2.0	0.03	< 0.01	0.32
1600 h	63.0	55.4	2.6	0.004	< 0.01	0.32
Udder temperature, ºC						
0900 h	34.1	32.5	0.2	< 0.01	< 0.01	0.10
1600 h	34.9	34.0	0.4	< 0.01	< 0.01	0.10

[1]CONV refers to cows housed in the open-sidewall conventional freestall barn, while TUNNEL refers to cows housed in the tunnel-ventilated freestall barn.
[2]Breaths per minute.

Table 5.5 Effect of cooling treatment on the percent of time spent lying down within 3 time periods throughout the day

	Treatment[1]			*P*-value		
Item	CONV	TUNNEL	SE	Trt	Time	Trt × Time
Lying time, %						
0400 – 1000 h	51.6[a]	58.5[b]	0.03			
1200 – 1800 h	42.7[a]	54.4[b]	0.03	< 0.01	< 0.01	0.36
2000 – 0200 h	49.2[a]	57.7[b]	0.03			
Lying bouts, n/time period						
0400 – 1000 h	2.9	3.1	0.21			
1200 – 1800 h	2.7	2.6	0.21	0.32	0.05	0.21
2000 – 0200 h	2.8	3.1	0.21			
Lying bout duration, min						
0400 – 1000 h	76.9	80.4	7.22			
1200 – 1800 h	61.8[a]	90.1[b]	7.22	0.08	0.88	0.02
2000 – 0200 h	76.0	77.7	7.22			

[1]CONV refers to cows housed in the open-sidewall conventional freestall barn, while TUNNEL refers to cows housed in the tunnel-ventilated freestall barn.
[a,b]Means within a row with differing superscripts differ ($P \leq 0.05$).

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Chapter 6 - The Effects of Different Cooling Systems in the Holding Area on Temperature Humidity Index and Core Body Temperature of Lactating Dairy Cows

Abstract

A study was conducted on a commercial dairy farm to evaluate the effects of evaporative and direct (soaking) cooling systems applied in the holding area on core body temperature (CBT) in lactating Holstein dairy cows. A second objective of the study was to determine total water use by each system. The study design was a 3 × 3 Latin square with 3 groups of cows receiving each of 3 treatments over 3 time periods. Thirty-six lactating Holstein dairy cows were randomly assigned to 1 of 3 treatment groups with 12 cows per treatment group: FS where the fog was shut off and only the fans and soaker lines were in operation; FF where the soaker lines were shut off and only the fans and fog were in operation; and FS+FF where fans, fog, and the soaker lines were all operating. Holding area temperature, relative humidity (RH), and temperature humidity index (THI) were measured using temperature and RH loggers attached to a neck collar on each cow in the study. Temperature of the holding area was reduced for FS+FF compared to FF and FS (27.82, 28.03, and 27.82 ± 0.69ºC, respectively). Relative humidity, however, was increased for FS+FF compared to FF and FS (75.28, 70.84, and 72.10 ± 1.15, respectively). This resulted in a lower THI for FS+FF compared to FF and FS (77.80, 78.62, and 78.39 ± 1.29, respectively). Core body temperature was greater for FF compared to FS and FS+FF, but all treatments were able to maintain a CBT < 39.0ºC throughout the study period. Time to lie down post-milking as well as the duration of the first lying bout were not different. Water usage was lowest for the evaporative cooling system (FF), which used 20.86 ± 0.25

L/min, compared to the direct cooling system (FS), which used 30.96 ± 0.26 L/min. When comparing cow cooling systems, evaluating the systems effectiveness in minimizing a rise in CBT as well as its overall water usage is important. Under the conditions of the current study, the use of the FF system was able to reduce the consumption of water in the holding area while still preventing a major rise in CBT. Future research should be conducted under greater ambient conditions to determine if an evaporative cooling system is able to maintain a CBT < 39.0ºC and compare CBT and water usage to a soaker system in the holding area.

Key words: heat stress, holding pen, evaporative cooling

Introduction

It has been estimated that heat stress costs the U.S. dairy industry ~$900 million annually (St-Pierre et al., 2003). While measures can be taken to help dairy cattle cope with heat stress and minimize the economic losses during summer, significant economic losses still occur each year throughout the U.S. Holding areas have been identified as one of the highest risk areas for heat stress on dairy farms today (Collier et al., 2006). It is common for dairy cattle to remain in the holding area for up to 60 min or more prior to each milking. Therefore, cows may be in the holding area for 3 h or more per day, making this area critical for cow cooling to enhance cow comfort and minimize a rise in core body temperature (CBT). Cows are often crowded together in the holding area making CBT rise at a greater rate compared to other barn locations.

It has been reported that 94% of U.S. dairies use some form of heat abatement (USDA, 2010). In order to reduce the heat load placed on the dairy cow in summer, 1 of 2 methods are primarily utilized in the holding area to reduce the negative impacts of heat stress: environmental modification (i.e. evaporative cooling), or more commonly, utilizing direct cooling methods to enhance heat dissipation from the skin of cattle (i.e. soaking). Both types of systems use fans

located over the cows to bring fresh air into the holding area environment and blow that air down over the cow increasing convective and evaporative heat loss. Water and air movement together are most effective in promoting heat dissipation from the dairy cow, especially in the holding area. Cows that had access to cooling in the holding area (fans and sprinklers) had reduced CBT and greater milk yield compared to cows not cooled in the holding area (Wiersma and Armstrong, 1983; Collier et al., 2006).

Water availability has become a large concern in recent years for many areas of the country where dairy farms are prevalent. Therefore, research of cooling systems that minimize water usage while still cooling cows effectively is important. Evaporative cooling systems (i.e. fogging systems) have the advantage of reduced water consumption compared to direct cooling systems (i.e. soakers), but research comparing the use of each type of system in the holding area is lacking. Therefore, the objective of the current study was to determine the type of holding area cooling system most effective in preventing a major rise in CBT during and after exiting the holding area and to determine the overall water usage from each system.

Materials and Methods

This study was conducted on a commercial dairy in Nebraska during August 2016. All cows were housed in a tunnel-ventilated freestall barn, which contained ECV72 fans (CYC723230460, 1.83 m ECV72 with deflectors, 230/460V, 3 HP) and a fogging system provided by VES Environmental Solutions (Chippewa Falls, WI), as the main source of cooling. Throughout the trial, cows were milked 3 times per day and a total mixed ration (TMR) was fed at least twice daily. The TMR was formulated to meet or exceed the predicted nutrient requirements (NRC, 2001) for energy, protein, vitamins and minerals. The Institutional Animal Care and Use Committee at Kansas State University approved all experimental procedures and

all measures were taken to avoid unnecessary discomfort to animals throughout the study. Treatments were applied in the holding area prior to the afternoon milking. The holding area contained 2 different types of cooling systems: 1) fans and soakers; and 2) fans coupled with a fogging system. The fans (BLT503230460V, 1.27 m BLAST fan, 230/460V, 1.5 HP) and fogging system were provided by VES Environmental Solutions (Chippewa Falls, WI).

Experimental Design and Treatments

Thirty-six lactating Holstein dairy cows were randomly assigned to treatment groups in a replicated, 3 × 3 Latin square design consisting of 3 periods. Group 1 was made up of 12 cows that averaged 40.04 ± 5.15 kg/d of milk and 46 ± 3 DIM in their first lactation. Group 2 consisted of 12 cows averaging 53.59 ± 5.36 kg/d of milk and 45 ± 3 DIM in their 2nd or greater lactation and Group 3 consisted of 12 cows averaging 53.15 ± 5.40 kg/d of milk and 44 ± 2 DIM, also in their 2nd or greater lactation. During each replicate of the Latin square, each group of 12 cows was exposed to each of 3 treatments. Treatments applied in the holding area during the afternoon milking included: **FS**, where the fog was shut off and only the fans and soaker lines were in operation; **FF**, where the soaker lines were shut off and only the fans and fog were turned on; and **FS+FF**, where fans, fog, and the soaker lines were all operating. Treatments were turned on as cows entered the holding area and continued until all cows had entered the milking parlor. The fog system operated on cycles of 4-min ON:1-min OFF, while the soaker system operated with a controller (C-440S, Edstrom Industries Inc., Waterford, WI) that cycled between 4 quadrants. The fog system operated at a pressure of ~92,148 kg/m^2 resulting in a water droplet size of 10 to 17 microns with a flow rate of ~0.136 L/min per nozzle with 7 nozzles per fan over the holding area and 12 nozzles per fan on 4 intake fans. A water meter was

installed at the beginning of the study to measure water usage by each cooling system in the holding area.

Throughout the study, ambient temperature and RH were measured at 1-min intervals with two weather stations located throughout the farm. Weather stations were composed of a sensor (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) and a solar radiation and moisture shield (M-RSA; Onset Computer Corporation, Pocasset, MA).

Each cow in the study was fitted with a neck collar that contained a sensor (HOBO Pro V2, Onset Computer Corporation, Pocasset, MA) to track temperature and RH of the micro-environment as the cows moved throughout the facilities. Each cow also received an intravaginal stainless-steel temperature logger (HOBO U12, Onset Computer Corporation, Pocasset, MA) attached to a blank controlled internal drug-releasing device (CIDR; Pfizer Animal Health, New York, NY) that recorded vaginal temperature at 1-min intervals. Prior to starting the study, each vaginal probe was validated in a water bath with a certified thermometer to ensure similar temperature responses. In addition, each cow was fitted with an electronic data logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA) that was attached to the medial side of the right, hind leg by using vet wrap. The acceleration data logger was placed in a position such that the x-axis was parallel to the ground, the y-axis was perpendicular to the ground pointing upward, and the z-axis was parallel to the ground pointing away from the sagittal plane. The loggers recorded the *g*-force on the x, y, and z-axes at 1-min intervals throughout the duration of the study. All recording devices were pre-programmed to begin recording at 1200 h on d 1 of the study. Each of the data loggers were removed from the cows at the end of the study and downloaded using Onset HOBOware software (Onset Computer Corporation, Pocasset, MA), which converted the *g*-force readings

into degrees of tilt. These data were exported into Microsoft Excel (Microsoft Corporation, Redmond, WA), and the degree of vertical tilt (y-axis) was used to determine the position of the animal, such that readings < 60° indicated the cow standing and readings ≥ 60° indicated the cow lying down (Ito et al., 2009). Standing and lying bouts of < 2 min were ignored because these readings were likely associated with leg movements at the time of recording (Endres and Barberg, 2007). All data loggers were programmed and managed by a single computer, allowing for synchronization of time.

Statistical Analysis

Mean hourly temperature humidity index (THI) data was calculated using the formula THI = (9/5 x T_{db} + 32) – [0.55 – (0.55 x RH/100)] x [(9/5 x T_{db} + 32) – 58], where T_{db} is dry-bulb temperature (°C; Zimbelman et al., 2009). Vaginal temperatures were used to determine mean CBT around the time of milking when cows were exposed to treatments. Time to lie down post-milking was summarized by analyzing the angles recorded by the leg data loggers. Milking time data was collected for each cow at each milking and from this, we could calculate the exact milking time and the time from milking to first lie down post-milking for each cow in the study.

Data for ambient conditions were averaged by hour of day, while CBT and collar data were averaged by min around milking time prior to analysis and assessed in a Latin square design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The fixed effects model included treatment, time, and their interaction for CBT and collar data, while treatment was the model effect used for lying time data. Random effects included cow and day and the experimental unit was assumed to be each individual cow. Degrees of freedom were approximated by the method of Kenward-Roger (ddfm = kr). The repeated statement for CBT and collar data included time with the subject being the interaction of cow and day nested within

treatment, while the repeated statement for lying time data included day with the subject being cow nested within treatment. For all variables measured, the covariance structure resulting in the lowest Bayesian information criterion (BIC) was used. The ESTIMATE statement was used to test for significant treatment differences at each time point around milking. If the first data collection (-60 min) prior to milking time was different between treatments, that variable was included in the model as a covariate. Interaction effects were partitioned using the SLICE option and means were determined using the least squares means statement and included the PDIFF option. Confidence intervals are reported at 95% and statistical significance between treatments was declared at $P < 0.05$ and a tendency at $0.05 \leq P \leq 0.10$.

Results

Layout of the holding area cooling treatments are shown in Figure 6.1. Environmental conditions throughout the duration of the study are shown in Figure 6.2.

All treatments had similar collar temperature (-60 min) prior to entering the holding area (Figure 6.3). Collar temperature was reduced ($P < 0.01$) around the time of milking for FS+FF compared to FF and FS (28.03, 27.82, and 27.05 ± 0.69ºC for FF, FS, and FS+FF, respectively). Collar temperature tended ($P = 0.07$) to be greater for FF compared to FS with significant treatment differences occurring as cows approached the milking parlor (-10 to 20 min around milking time). There was also a significant treatment × time interaction ($P < 0.01$) where FF had reduced collar temperature -40 min before milking, but greater collar temperature from -10 to 20 min around milking compared to FS.

Due to differences at time point -60 around milking, -60 min was used as a covariate in the analysis (Figure 6.4). Collar RH, as expected, was greater ($P < 0.01$) for FS+FF compared to FF and FS (70.84, 72.10, and 75.28 ± 1.15 for FF, FS, and FS+FF, respectively). Overall RH for

FF was reduced ($P = 0.02$) compared to FF. There was a significant treatment × time interaction ($P < 0.01$) where FF had greater collar RH levels at -40 min prior to milking, but reduced collar RH from -20 to 20 min around milking compared to FS.

All treatments had similar collar THI (-60 min) prior to entering the holding area (Figure 6.5). As a result of the collar temperature and RH data, collar THI was reduced ($P < 0.01$) for FS+FF (78.62, 78.39, and 77.80 ± 1.29 for FF, FS, and FS+FF, respectively. Overall collar THI for FF and FS was not different ($P = 0.10$). There was a significant treatment × time interaction ($P < 0.01$).

All treatments had similar CBT (-60 min) prior to entering the holding area (Figure 6.6). Cows receiving the FF treatment had increased ($P < 0.05$) CBT compared to FS and FS+FF (38.82, 38.75, and 38.75 ± 0.05ºC for FF, FS, and FS+FF, respectively), while CBT between FS and FS+FF was not different ($P = 0.84$). There was a significant treatment × time interaction ($P < 0.01$) where the greatest treatment effects occurred during milking (0 h) and continued up to 60 min post-milking. Core body temperatures at milking (0 h) were 38.86, 38.73, and 38.75 ± 0.05ºC for FF, FS, and FS+FF, respectively.

Time to lie down post-milking was not different ($P = 0.69$) between treatment groups (Figure 6.7). Time to lie down post-milking had a mean of 77.86, 72.30, and 74.95 ± 6.09 min for FF, FS, and FS+FF, respectively. Lying bout duration of the first lying bout post-milking also did not differ ($P = 0.97$; Figure 6.8) between treatment groups with means of 64.39, 65.26, and 65.43 ± 4.86 min for FF, FS, and FS+FF, respectively.

When comparing water usage between the two systems, the soaker system used a mean of 30.96 ± 0.26 L/min compared to 20.86 ± 0.25 L/min for the fogging system. Therefore, when both systems were run simultaneously (FS+FF), water usage averaged ~51.82 ± 0.25 L/min.

Discussion

Holding pen cooling is an essential component of every heat abatement system in use on dairy farms today because of the high animal density, resulting in greater temperature and RH in this area compared to the rest of the barn. Dairy cows may spend as much as 3 h/d in the holding area and if not adequately cooled in the summer months, CBT will increase rapidly leading to milk production and future reproduction losses affecting overall profitability of the dairy farm. Most holding areas rely on natural ventilation with open sidewalls in addition to providing supplemental cooling via circulation fans and a low-pressure soaking system. The current holding area design under study consisted of completely enclosed sidewalls utilizing intake fans to bring fresh air into the holding area and using circulation fans located over the holding area in addition to a soaker system and a fogging system as the source of cow cooling.

Two principle types of cooling systems are available for use in the holding area: direct cooling via soakers, or evaporative cooling via a fogging system or the use of cooling cells. Direct cooling is by far the most common cooling system used in the holding area (USDA, 2010) and cools the animal via wetting cycles that vary based on holding area temperature, and then allows that water to evaporate, carrying heat away from the animal. Wetting of the hair coat with large water droplets penetrates deep down to the skin and is a very effective method of heat loss (Kimmel et al., 1991; Chastain and Turner, 1994), assuming adequate airflow is present. Combining wetting with forced ventilation via circulation fans increases the rate at which water evaporation from the hair coat occurs (Hillman et al., 2001; Gebremedhin and Wu, 2001, 2002), helping to prevent a major rise in CBT. Adequate air velocity prevents moisture from accumulating in the air space around the cow and maintains a humidity gradient between the cow

and the surrounding environment allowing evaporative heat loss to occur. This type of cooling works well under high ambient temperatures and RH levels.

The second type of cooling is evaporative cooling where air surrounding the cow is cooled in an effort to increase the temperature gradient between the cow and the surrounding environment, allowing for more efficient dissipation of body heat. It is important to note that with this type of cooling, we are not wetting the cow directly, but rather, we are trying to cool the air around the cow. With the use of a fogging system, fine water droplets are sprayed into the air and cool the surrounding air as they evaporate leading to a greater temperature gradient between the cow and the surrounding environment. If using evaporative cooling systems, it is important to realize that as air temperature is reduced due to water evaporation, the potential to evaporate moisture from the skin of cattle is also reduced due to greater RH levels. The net effect of evaporative cooling of air must be greater than the loss of cooling from moisture evaporation from the skin of cattle (Collier et al., 2006). One way to overcome some of the increased RH levels is to increase air velocity around the cow. Evaporative cooling systems are most effective under high ambient temperatures combined with low RH, but if enough air velocity is provided, evaporative cooling systems may be used effectively in more humid climates as well. As air velocity is reduced, the convective and evaporative heat loss from the body surface is reduced and increases the impact of the effects of elevated RH on the cow.

Therefore, the goal of the current study was to determine which type of cooling system was most effective under the conditions of the study, which included cooler ambient temperatures combined with elevated RH levels. We chose to use temperature and RH loggers located on the collar of each animal as compared to loggers placed in the holding area, as ambient conditions may not be indicative of the micro-environmental conditions within the

holding area. Cows that received the combination treatment of soaking and fog (FS+FF) had reduced collar temperature, elevated RH, and reduced THI compared to FF and FS. These results were not unexpected due to the greater water usage during FS+FF compared to FF and FS. When comparing FF and FS, FF tended to have greater collar temperature and collar THI beginning at -10 min prior to milking. This helps to explain the rise in CBT for FF at -10 min prior to milking compared to FS.

Due to cooler than expected ambient temperatures throughout the study, CBT remained fairly steady with only a minimal rise when located in the holding area. While CBT was similar between treatments (up to -20 min prior to milking) when located in the holding area, the FF treatment had elevated CBT compared to FS and FS+FF at the time of milking (0 min) and remained elevated up to 1 h post-milking. These data show that once FF cows approached and entered the milking parlor, a rise in CBT occurred, likely a result of not having access to the fogging system within the milking parlor. FF cows had a drier hair coat upon entering the milking parlor compared to FS and FS+FF cows and therefore, likely had reduced evaporative heat loss in the milking parlor from receiving the FF treatment in the holding area. Meanwhile, FS and FS+FF cows were able to rely on evaporative heat loss within the milking parlor due to a wet hair coat from the holding area cooling treatment (soakers). All 3 treatments, however, were able to maintain CBT < 39.0ºC, which has been shown to be a very critical temperature for lactating dairy cows. Once CBT exceeds 39.0ºC, production and reproductive losses begin to occur (Igono and Johnson, 1990; Gwazdauskas et al., 1973; Wolfenson et al., 1988). Therefore, while treatment differences were found, the significance of these differences is questioned as all treatments were able to maintain CBT < 39.0ºC. Had ambient temperatures been greater when

this study was conducted, we would have expected to see even greater differences between treatment groups and likely, CBT exceeding 39.0ºC for one or more of the treatments.

The time to lie down post-milking as well as the duration of the first lying bout post-milking were evaluated to determine if the cooling treatment received in the holding area impacted lying behavior. When cows are heat stressed, standing time increases in an effort to increase body surface area exposed to the environment to maximize heat dissipation (Silanikove, 2000; Berman, 2003; Maia et al., 2005). Allen et al. (2015) found that cows were less likely to lie down with a CBT > 38.8ºC and lying bouts lasted longer when cows had lower CBT. The thought was that if one holding area cooling treatment was superior to the others, these cows would lie down sooner post-milking and have increased duration of the first lying bout as a result of a reduced CBT. While cows receiving the FF treatment maintained a CBT ≥ 38.8ºC out to 80 min post-milking compared to FS and FS+FF, which remained below 38.8ºC for the majority of the time period measured, minimal differences were found between treatments for time from milking to first lie down or lying bout duration, potentially a result of the cooler ambient conditions.

Water availability has become a much bigger issue in recent years in many areas of the country where dairy farms are prevalent. For this reason, research of cooling systems that minimize water usage while still cooling cows effectively is important. As expected, the FF treatment used the least amount of water (20.86 L/min) to cool cows in the holding area. This is in comparison to the soaker treatment (FS), which used 30.96 L/min, on average. This means that the combination treatment (FS+FF) used 51.82 L/min. Assuming cows are exposed to heat stress for 120 d/yr and the cooling system runs for 10 h/d, by using the FF treatment as the cooling source in the holding area, we could save 727,200 L/yr compared to the FS treatment.

The FS+FF treatment would use 1,501,920 and 2,229,120 L/yr more water compared to FF and FS, respectively. While FS and FS+FF did result in reduced CBT compared to FF, all 3 treatments were able to maintain CBT below the critical threshold of 39.0ºC for the duration of the time period measured around milking. Therefore, under the environmental conditions of the current study, the FS+FF treatment is not advised due to its excessive water usage and minimal reduction in CBT compared to FF and FS. When comparing FF and FS, FF has the benefit of reduced water usage in the holding area. While FS resulted in reduced CBT, both cooling systems were able to maintain CBT < 39.0ºC and therefore, would be viable heat stress abatement options in the holding area. Further evaluation should be conducted when cows are exposed to greater ambient temperatures. From this, we could better evaluate whether the reduced water use from an evaporative cooling system (FF) is still able to maintain CBT < 39.0ºC and compare this to CBT from cows cooled directly with a soaker (FS) system.

Conclusions

While the FS+FF treatment applied in the holding area prior to milking was able to reduce the THI of the environment, its use is not advised due to the excessive water consumption as well as the fact that all 3 treatments were able to maintain CBT below the critical threshold of 39.0ºC. There were no treatment differences for time to lie down post-milking or for the duration of the first lying bout. Under the conditions of the current study, the use of the FF system was able to reduce water consumption in the holding area while still preventing a major rise in CBT. Future research should be conducted under greater ambient conditions to determine if an evaporative cooling system is still able to maintain a CBT < 39.0ºC and compare CBT and water usage to a soaker system in the holding area.

Figures and Tables

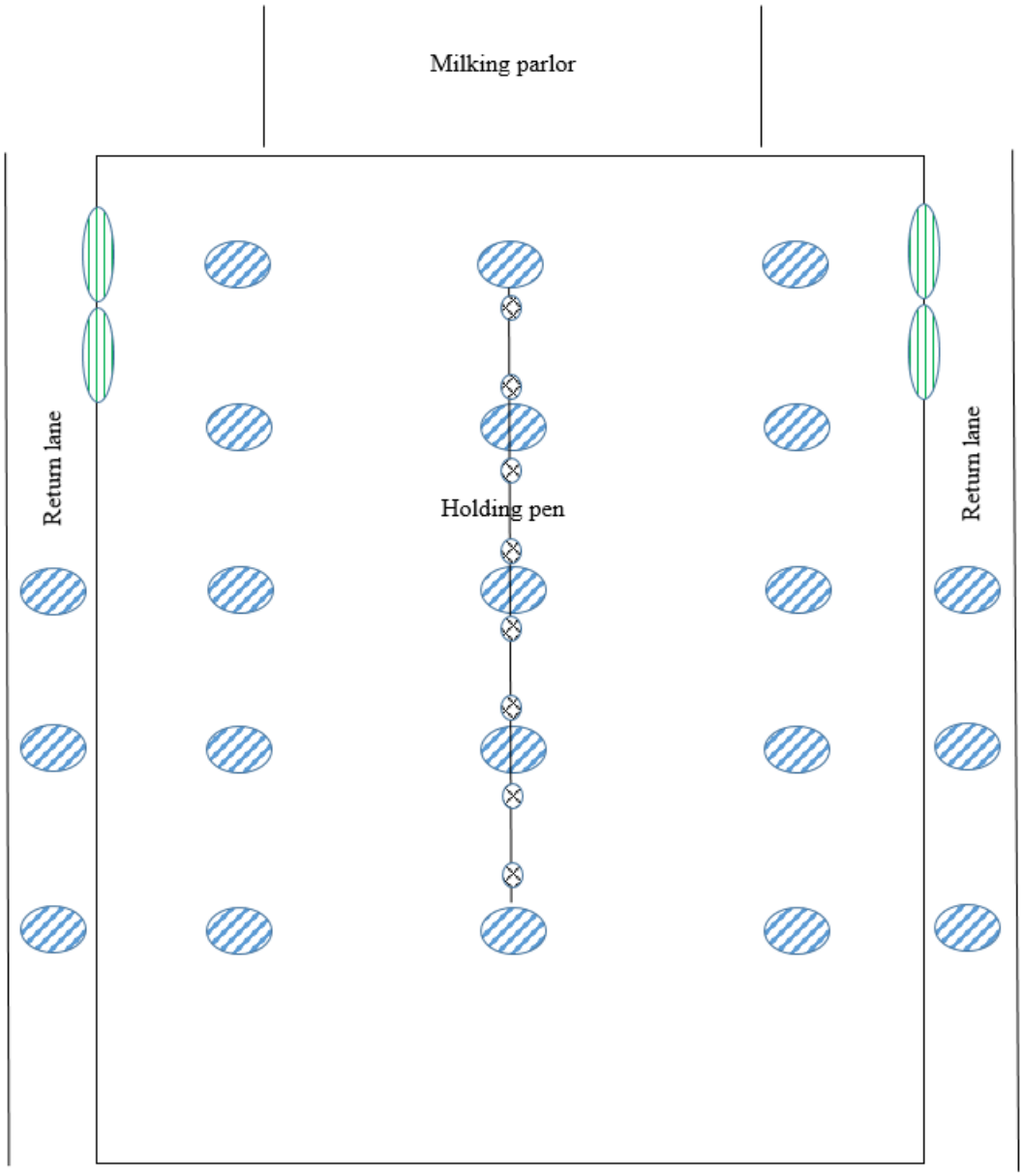

Figure 6.1 Holding area layout showing the location of fans and sprinklers.
- 1.27 m blast fans (VES Environmental Solutions, LLC)
- 1.83 m ECV72 fan (VES Environmental Solutions, LLC)
- Sprinkler heads

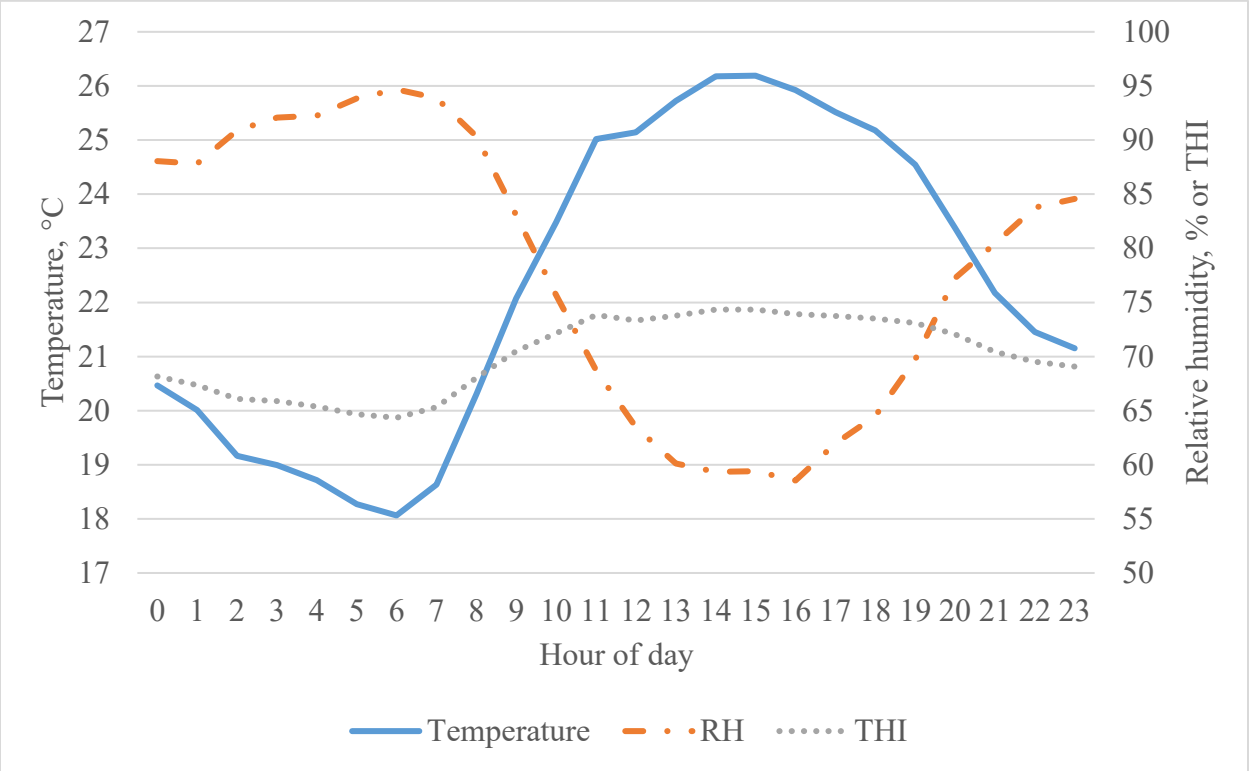

Figure 6.2 Average ambient temperature, relative humidity (RH), and temperature humidity index (THI) by hour throughout the study.

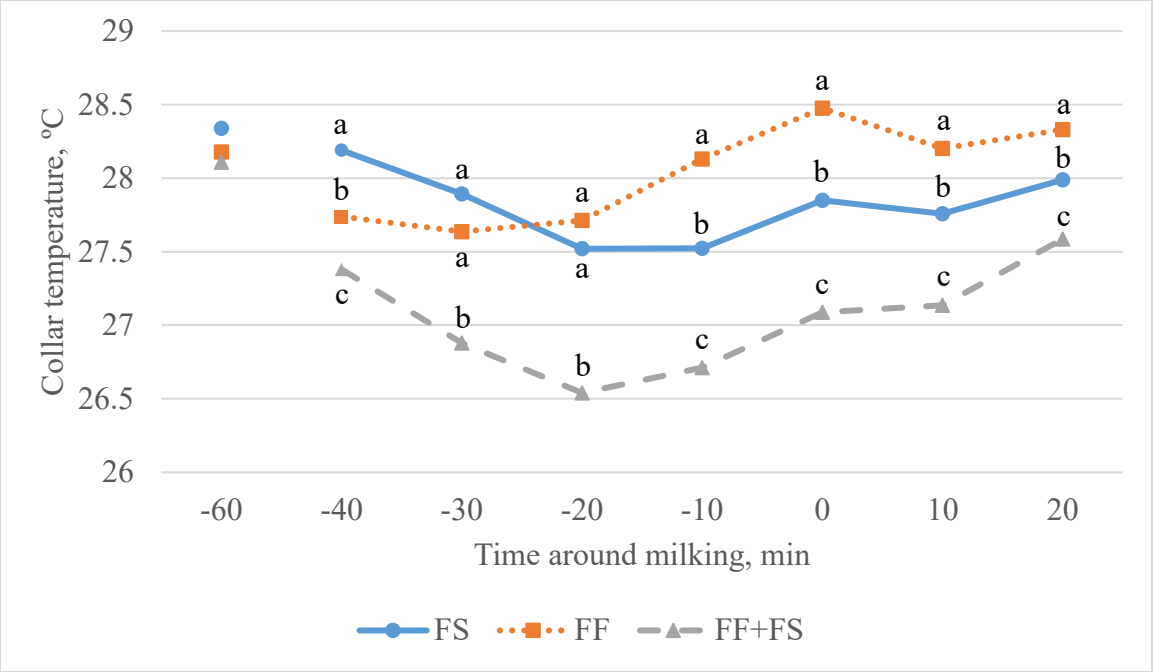

Figure 6.3 Effect of cooling treatment on collar temperature around the time of milking. FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P < 0.01$). Treatment × time ($P < 0.01$). SEM = 0.69.
FS vs. FF: $P = 0.07$
FS vs. FS+FF: $P < 0.01$
FF vs. FS+FF: $P < 0.01$
[a,b,c]Differing letters indicate significant treatment differences ($P < 0.05$) within time around milking

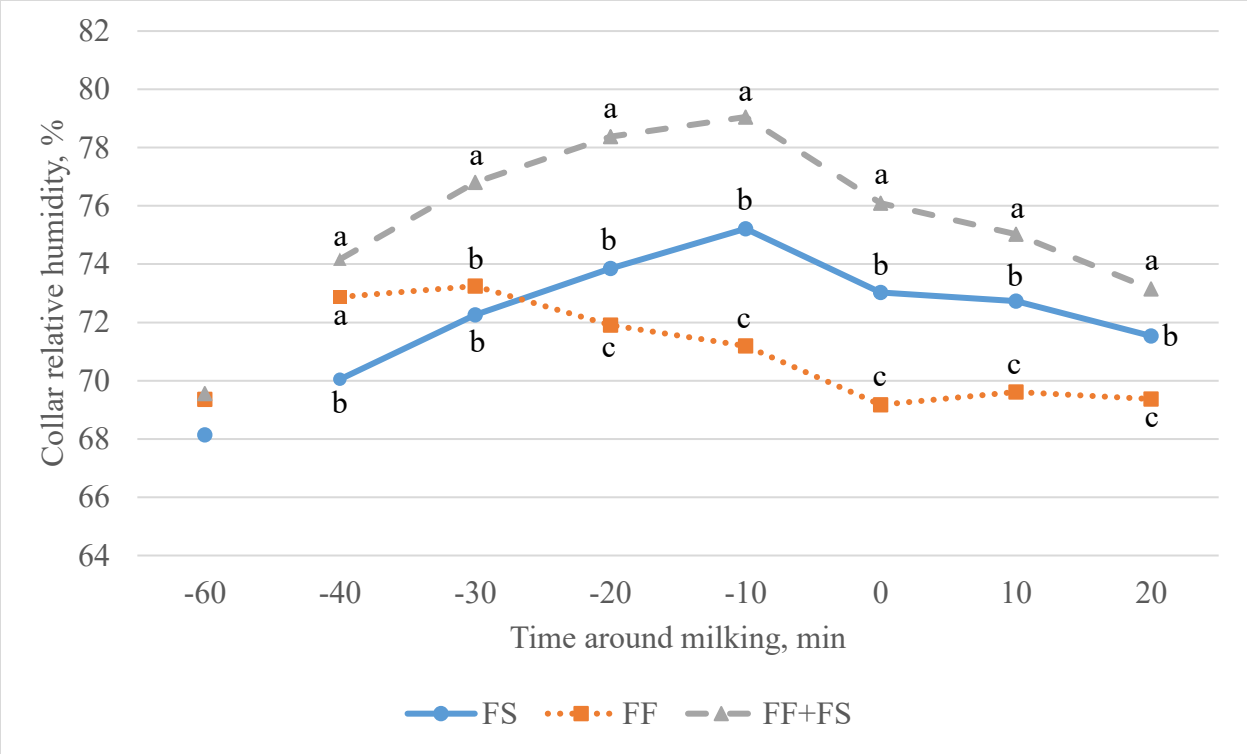

Figure 6.4 Effect of cooling treatment on collar relative humidity around the time of milking. Time point -60 min was included as a covariate because of significant differences.
FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P < 0.01$). Treatment × time ($P < 0.01$). SEM = 1.15.
FS vs. FF: $P = 0.02$
FS vs. FS+FF: $P < 0.01$
FF vs. FS+FF: $P < 0.01$
[a,b,c]Differing letters indicate significant treatment differences ($P < 0.05$) within time around milking

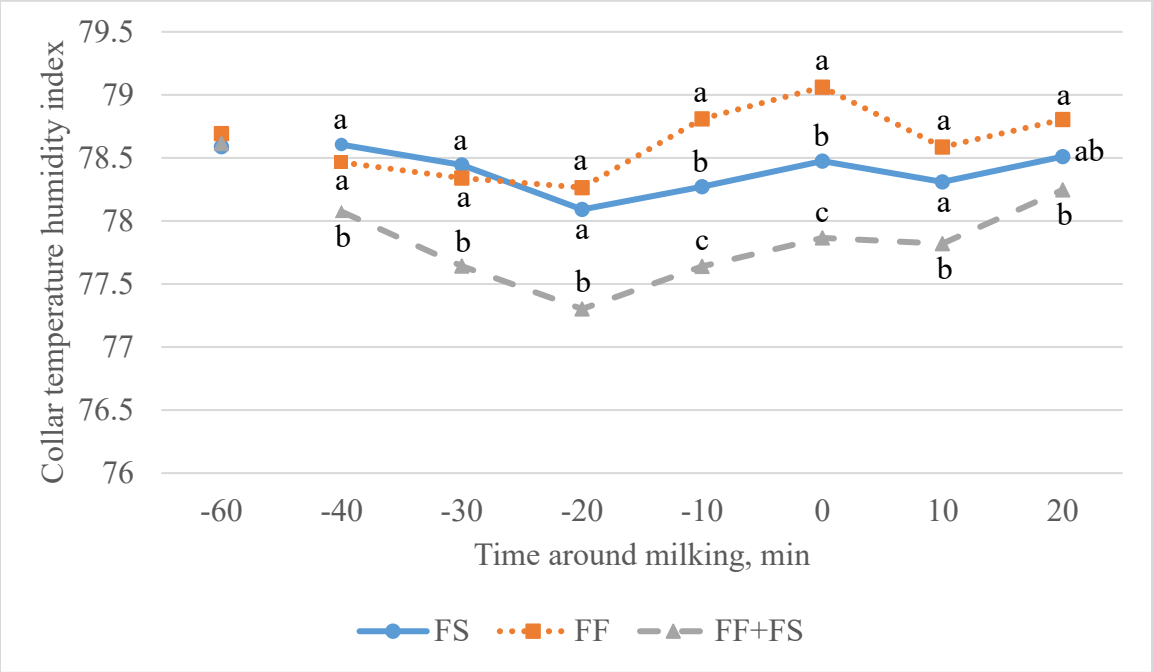

Figure 6.5 Effect of cooling treatment on collar temperature humidity index around the time of milking.
FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P < 0.01$). Treatment × time ($P < 0.01$). SEM = 1.29.
FS vs. FF: $P = 0.10$
FS vs. FS+FF: $P < 0.01$
FF vs. FS+FF: $P < 0.01$
[a,b,c]Differing letters indicate significant treatment differences ($P < 0.05$) within time around milking

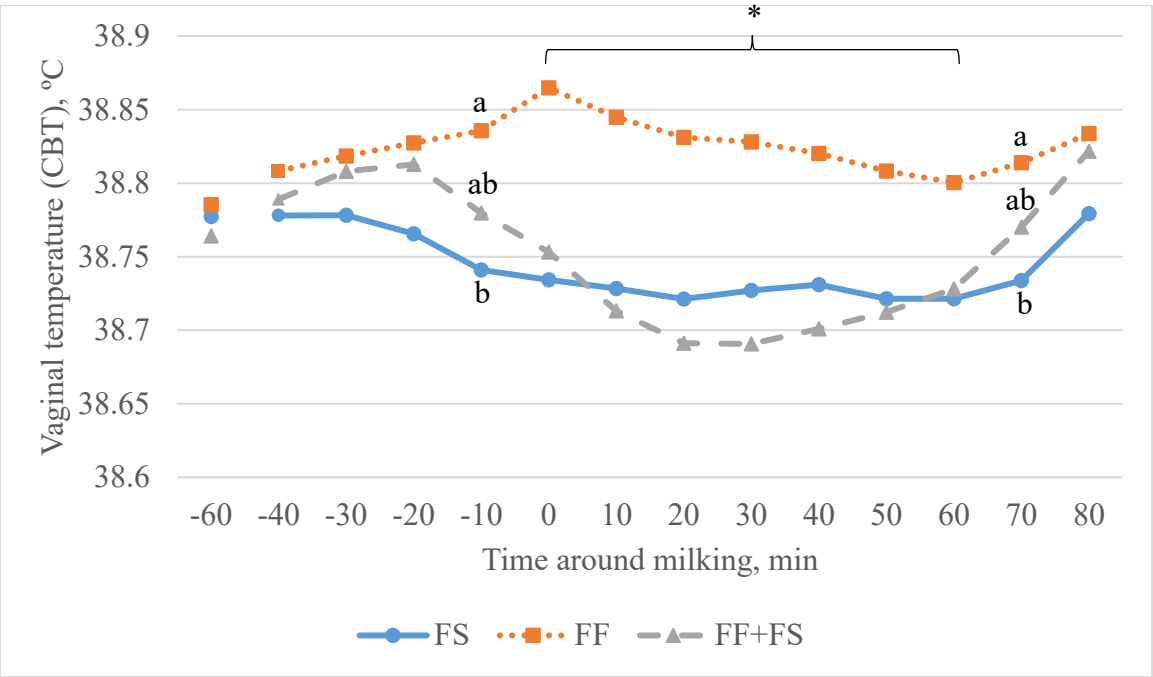

Figure 6.6 Effect of cooling treatment on vaginal temperature (CBT) around the time of milking. FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P = 0.03$). Treatment × time ($P < 0.01$). SEM = 0.05.
FS vs. FF: $P = 0.03$
FS vs. FS+FF: $P = 0.84$
FF vs. FS+FF: $P = 0.01$
*$P < 0.05$ for FF vs. FS and FS+FF
[a,b]Differing letters indicate significant treatment differences ($P < 0.05$) within time around milking

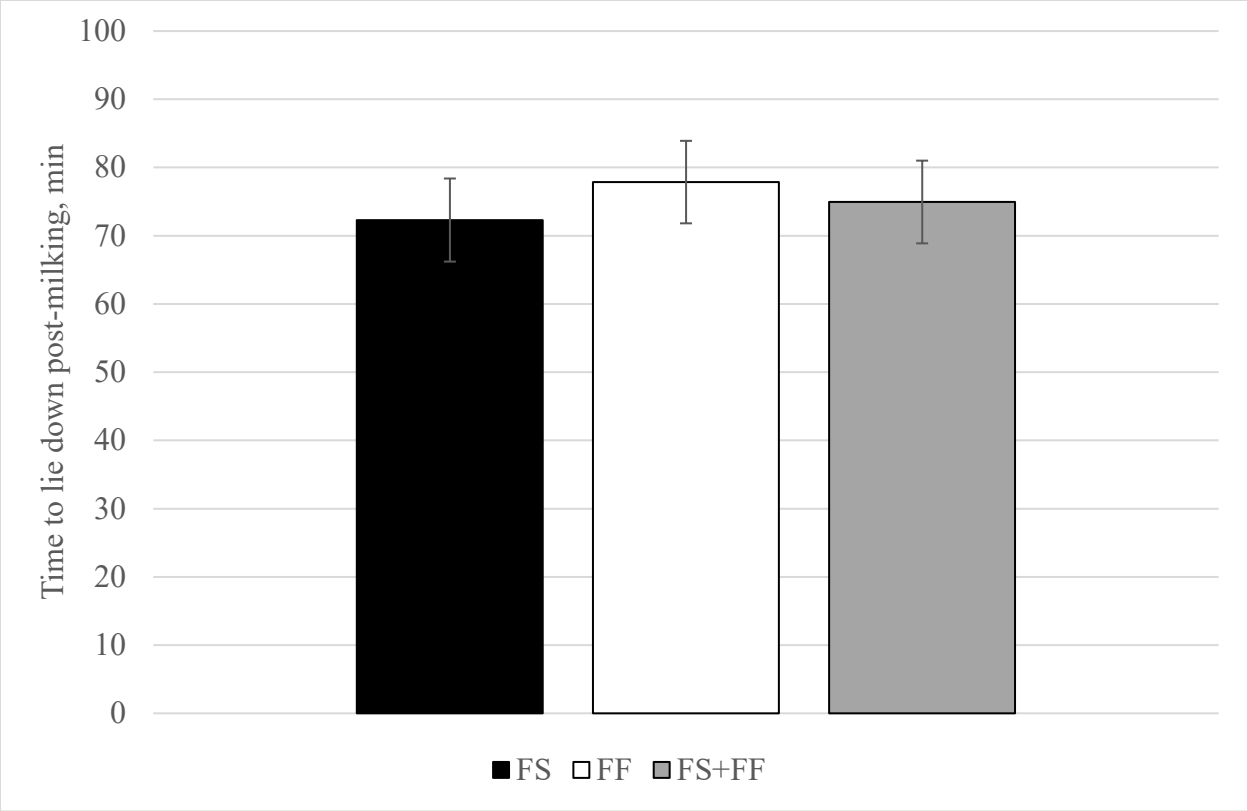

Figure 6.7 Effect of cooling treatment on the time to lie down (min) post-milking.
FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P = 0.69$).

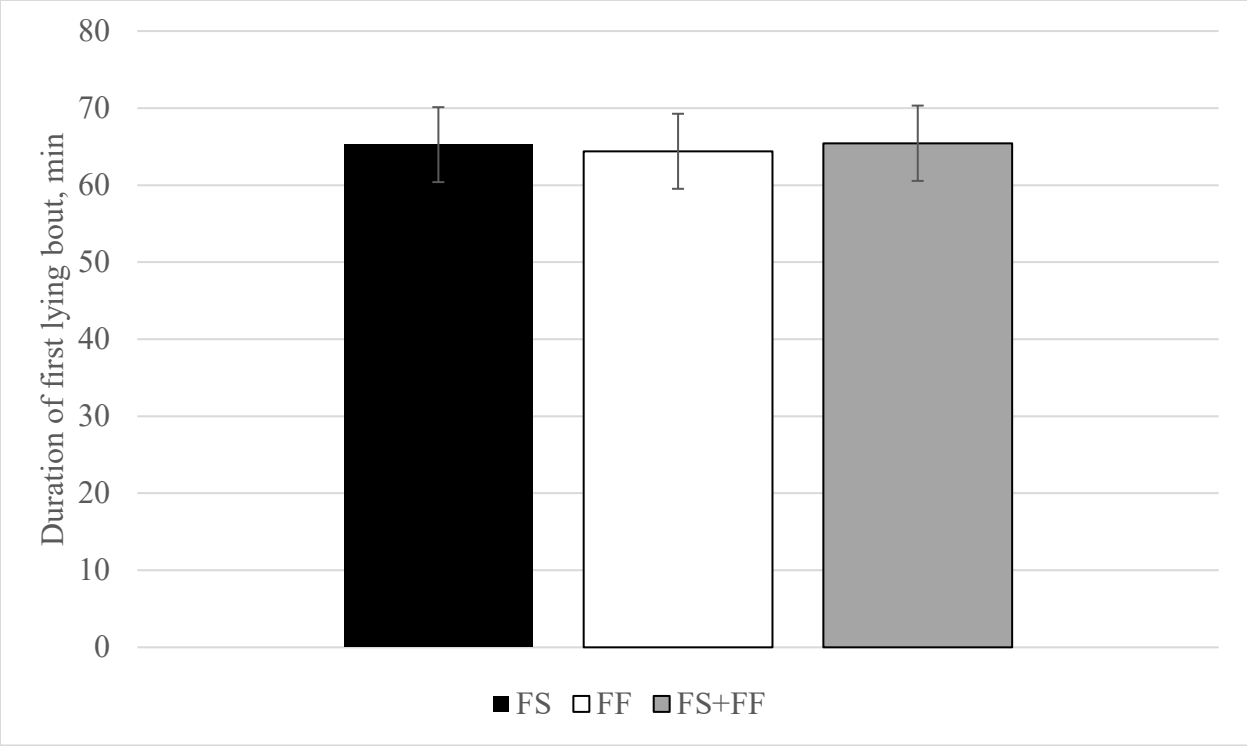

Figure 6.8 Effect of cooling treatment on the duration of the first lying bout (min) post-milking. FS = holding area cooling using fans and sprinklers (direct cooling); FF = holding area cooling using fans and a fogging system (evaporative cooling); FS+FF = holding area cooling using a combination of fans, sprinklers, and the fogging system. Treatment ($P = 0.97$).

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