

**Effect of Varying Rumen Degradable and Undegradable Protein on Milk
Production and Nitrogen Efficiency in Lactating Dairy Cows under Summer
Conditions**

**A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

**Jeffrey D. Kaufman
December 2016**

DEDICATION

I dedicate my work to my beautiful wife, Jordan Kaufman and my beloved furry companions,

Abraham and Sampson.

ACKNOWLEDGEMENTS

A thank you of infinite magnitude goes to my wife, Jordan Kaufman, for providing paramount support and encouragement throughout my endeavors and tribulations. Without her, I could never have imagined or dreamed of accomplishing my milestones. Love you Jordan! I would like to thank my family for their continual support. To my parents, Michael and Jinger Kaufman, I have the greatest respect and admiration for raising me and encouraging me to strive for the best and follow my dreams. Thank you for always teaching me that hard work and determination is contagious and can open as many doors as needed. I hope one day I can follow in your shoes and be as remarkable as you.

To my advisor, Dr. Agustín Ríos for pushing me to succeed, having confidence in my abilities, providing employment, offering advice, bestowing your friendship, and enhancing my knowledge in science and life. Everything was a lesson with you and I respect your teaching methods. I entered into this program ignorant of dairy nutrition and have developed exponentially into a relevant scientist in the field. Your knowledge of dairy cow nutrition is endless and vast, and you inspire me to achieve similar knowledge. I am grateful and lucky to have worked with such a great scientist and person. Being your student might have been stressful at times, but I am stronger, smarter, and significant as a scientist and I respect you for that.

To the members of my committee, Drs. Peter Krawczel, Gina Pighetti, J. Travis Mulliniks, and Arnold Saxton thanks for the expertise, opinions, and help you have provided me along this adventure. I would like to thank Dr. Arnold Saxton for his wizardry and vast

knowledge of statistics and providing guidance for my statistics minor. You never stopped impressing me and allowing me to pick your brain, and I respect you for that.

All the help I gained throughout my program have not been in vain. I could have not asked for a better group of individuals to assist and provide expertise toward my project. I am very grateful and fortunate for the group of friends and family I gained while at the University of Tennessee. Special thanks to the UTK Animal Science graduate students and my office mates (Ronique Beckford, Kaysie Jennings, and Kimberly Kassube) for the continued support and magnitude of laughter throughout the years. The Animal Science team is dedicated, driven, kind, and encouraging people that never stopped believing in my work and accomplishments. I convey my utmost gratitude for these people and every aspect they had in helping me complete this thesis. Finally, thanks to everybody for their support, help, encouragement, and friendship throughout my time at UTK and Knoxville.

ABSTRACT

The objective is to determine the effect of reducing nitrogen input through feeding low rumen degradable protein (RDP) and rumen undegradable protein (RUP) proportions on milk production, nitrogen efficiency and metabolism in heat-stressed cows. Forty-eight mid-lactating, Holstein cows were assigned to treatments using a randomized block design in a 2x2 factorial arrangement of treatments (n = 12/treatment). Treatments included two levels of RDP (10 and 8%) and two levels of RUP (8 and 6%). From d 1 to 21, a common diet (10% RDP-8% RUP) was fed to cows followed with their respective treatment diets fed from d 22 to 42 of the study. Cows were housed in a freestall barn and exposed to the prevailing temperature and humidity of July and August with no supplemental cooling. Milk samples were collected and analyzed, and plasma was harvested for analysis of metabolites from d 42. Treatment differences were tested using the MIXED procedure of SAS and reported as least square means \pm [plus or minus] standard error of the mean. Rectal temperatures increased from a.m. to p.m., indicating cows were experiencing heat stress. The 10% RDP treatment decreased vaginal temperatures compared with 8% RDP in the 8% RUP (39.0 vs. $39.4 \pm 0.14^{\circ}\text{C}$), but remained unchanged in the 6% RUP treatment (39.4 vs. $39.3 \pm 0.14^{\circ}\text{C}$). The 8% RDP treatment increased energy-corrected milk (ECM) compared with 10% RDP in the 6% RUP treatment (31.7 vs. 29.4 ± 0.76 kg/d), but reduced ECM in the 8% RUP treatment (32.5 vs. 33.0 ± 0.76 kg/d). The 8% RDP treatment improved nitrogen utilization efficiency compared with 10% RDP (35.1 vs. $31.6 \pm 0.76\%$). The 6% RUP treatment improved nitrogen utilization efficiency compared with 8% RUP (35.1 vs. $31.6 \pm 0.76\%$). The 8% RDP treatment increased glucose concentrations compared with the 10% RDP treatment (3.13 vs. 2.98 ± 0.07 mmol/L). The 8% RDP treatment decreased insulin

concentrations compared with the 10% RDP treatment (15.8 vs. 20.9 ± 1.55 $\mu\text{U/mL}$). Therefore, diets with low RDP and RUP may increase nitrogen utilization efficiency and metabolism without reducing milk production in heat-stressed dairy cows.

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CHAPTER I: LITERATURE REVIEW

Introduction

The conversion of nitrogen from feedstuffs into milk nitrogen is referred as milk nitrogen efficiency and is relatively low at less than 30% (Kohn et al., 2005; Huhtanen and Hristov, 2009). Improvements in nitrogen efficiency is required for the dairy industry to reduce the expulsion of dietary nitrogen into the environment. Greater than 70% of nitrogen intake is excreted as feces and urine (Wilkerson et al., 1997; Rius et al., 2010). Nitrogen in excreta may be harmful to the environment, whereby nitrogen runoff from soil can deteriorate rivers and lakes, ammonia can volatilize in the atmosphere deteriorating air quality, and nitrates can leach into groundwater causing harmful drinking water (Power and Schepers, 1989).

The intensive production of milk in the dairy industry has been under public scrutiny to minimize nitrogen excretion and improve environmental stewardship. Current feeding practices have been set by industry recommendations from NRC (2001) for CP levels in the diet of mid-lactating dairy cows ranging from 16.0 to 17.0% CP of DM with RDP and RUP proportions ranging from 10.0 to 11.0% of DM for RDP and 6.0 to 7.0% of DM for RUP. The NRC (2001) estimates these recommendations to provide adequate nitrogen to the rumen microbes and toward the whole body of the cow. Crude protein fractions of RDP and NPN provide necessary nitrogen sources for rumen microbes, whereas RUP provides nitrogen towards body tissues. However, recent research suggests that feeding reduced dietary CP, RDP, and RUP below recommendations improves milk nitrogen efficiency (Broderick, 2003; Rius et al., 2010).

However, high environmental temperatures and humidity, resulting in heat stress, further limit efficient nitrogen utilization by increasing urinary nitrogen excretion (Kamiya et al., 2005). Heat stress is an environmental burden that impairs productivity and reduces revenue for dairy

producers. In high-producing dairy cows, heat stress reduces DMI, milk production, and milk protein synthesis and increases break down of muscle, which reduces nitrogen efficiency (Schneider et al., 1988; West, 1998). As a result, heat stress can result in an annual \$1.2 billion economic cost to the U.S. dairy industry (Key et al., 2014). Losses are exacerbated due to an increase in urinary excretion of nitrogen during heat stress, and a major contributor towards the influential cost of the diet is the CP content.

Unfortunately, the current recommendations of CP are not adjusted during periods of heat stress in dairy cattle, which over predicts requirements and limits the ability to properly optimize nitrogen utilization leading to improvements in milk synthesis. Huber et al. (1994) have indicated the necessity to increase the protein density of diets in heat-stressed dairy cows to improve milk production. However, reports indicate that feeding low CP diets increases milk production in lactating cows exposed to heat stress (Higginbotham et al., 1989a; Arieli et al., 2004). Thus, reducing RDP and RUP below industry recommendations may allow for improved nitrogen efficiency and milk production in dairy cows experiencing heat stress.

Protein and Nitrogen Metabolism

Ruminal Protein Degradation

Dietary protein supplied to the rumen has different outcomes according to digestibility of the protein and passage time within the rumen. Factors affecting digestibility are due to the chemical structuring of proteins (i.e. determinant on the quantity and types of peptide bonds present), the microbial population, and interrelationships with energy nutrients (e.g.

carbohydrates). Factors affecting passage time of dietary protein are determined mostly by the amount of feed intake and the physical form of feedstuffs (Satter and Roffler, 1975; Bach et al., 2005). Evaluation of these factors contribute toward an effective dietary protein management strategy.

The initial process of protein degradation in the rumen is microbial action on feed particles. Approximately 50% of the rumen microbes contain proteolytic enzymes to breakdown undigested feed protein (Prins et al., 1983). The degradation of proteins by the microbial population results in AA and peptides (Bach et al., 2005) and are incorporated into microbial CP. Microbial CP is a direct supply of AA and nitrogen after being absorbed post-ruminally in the intestines.

Microbial use of protein in the rumen varies depending on solubility. Highly soluble proteins degrade rapidly in the rumen, and less soluble proteins avoid degradation by rumen microbes. Globular proteins are found in most feedstuffs (i.e. mostly plant sources) and are low in molecular weights with some containing multiple disulfide bonds (Van Soest, 1994; NRC, 2001). The chemical bonds within and between protein chains are a large determinant of degradability from rumen microbes (Bach et al., 2005). Disulfide bonds reduce the degradability of proteins in the rumen because enzymes capable of breaking them down are rarely present in rumen microbes. Feedstuffs with the most insoluble protein (i.e. fibrous proteins) have been found to be forages, soy hulls, dried distillers grains, fish meal, and meat and bone meal (Blethen et al., 1990; NRC, 2001). Therefore, varying degradability of dietary protein is used to provide nitrogen for rumen microbes and for the animal.

Crude Protein Fractions

Crude protein is fractioned into RDP and RUP, which are defined as absorbable true protein, where RDP consists of NPN and true protein nitrogen (NRC, 2001). Total mixed rations fed to lactating dairy cows contain multiple feedstuffs that vary in their ruminal degradation and passage of CP, digestibility, and absorption of peptides and AA. Typical dairy TMR forage to concentrate ratio can range from 50 to 60% for forages and 40 to 50% for concentrates in order to meet requirements for both the microbial population and the animal (NRC, 2001). Feedstuff selection for the fractions of RDP and RUP of a dairy TMR requires careful consideration to achieve desired productive goals.

Non-protein nitrogen is instantaneously soluble and available for rumen microbial utilization. Examples of NPN sources are AA, urea, biuret, and ammonium bicarbonate. Some NPN can substitute protein nitrogen sources in the diet, lower the cost of the dietary nitrogen, and increase nutrient space in the diet by providing a higher concentration of nitrogen. Urea, at 287% CP, is the primary source of NPN in dairy cow rations (NRC, 2001). Urea is normally fed at around 1.5% of the dairy cow ration for microbial need of ammonia (NRC, 2001). Rumen microbes convert some NPN along with other fragments of RDP into microbial CP.

The second fraction of CP is the true protein portion of RDP, which is fermented by ruminal bacteria to produce microbial CP. Rumen degradable protein is degraded in the rumen by bacteria into ammonia and free AA, then is converted into microbial CP. The microbial CP flows in the liquid and solid phase of digesta to be digested and absorbed as AA and peptides, providing 50 to 80% of the absorbable true protein (Bach et al., 2005). Therefore, the microbial CP leaving the rumen to the small intestine is a function of the availability and efficiency of RDP

utilization from rumen microbes. Unfortunately with current recommendations, the excess supply of RDP over the cow's requirements promotes catabolism of AA and ammonia production, which in turn reduces the efficiency of turning RDP into microbial CP (NRC, 2001).

The third fraction of CP is the other true protein proportion, RUP, which avoids degradation by rumen microbes and travels directly to the abomasum and small intestine for direct use by the animal. The absorption of RUP can vary depending on form and the indigestible portion which will be excreted in feces. Rumen undegradable protein is mostly fibrous protein and non-bovine sourced animal protein (i.e. blood meal, fish meal, feather meal, etc.). Additionally, plant sourced protein can be chemically manipulated (e.g. protected soybean meal) to become less degradable in the rumen similar to animal protein sources. Within the small intestine, RUP is digested and approximately 80% is absorbed as AA along with the microbial CP to be utilized by tissues (Satter and Roffler, 1975; Owens et al., 2014). Rumen undegradable protein is important for providing a higher quality AA profile (i.e. supply of EAA to meet AA requirements) to high-producing dairy cows compared to microbial CP (Bach et al., 2000; NRC, 2001). Therefore, supplementing high-quality RUP sources in TMR may improve the absorption and utilization of AA in tissues and provide greater amounts of EAA (Chen et al., 1993; Erasmus et al., 1994).

Metabolizable Protein

Metabolizable protein refers to the true utilizable protein and AA after absorption into the intestine of the cow (NRC, 2001; Owens et al., 2014). Metabolizable protein is the product of microbial CP, RUP, and the less abundant endogenous CP [i.e. derivation of enzymes and

epithelial cell sloughing (Lapierre and Lobley, 2001; Wang et al., 2007)]. Studies indicate that 80% of microbial CP is true protein, and that true protein from microbial CP and RUP is 80% digestible in the small intestine (NRC, 2001; Owens et al., 2014). Most of the MP is used to sustain synthesis of milk in high-producing dairy cows. The efficiency of MP is projected to be constant at 0.67 for lactation (NRC, 1989), and Rius et al. (2010) determined that for every gram of milk protein yield, 1.5 g of MP is required. Thus, manipulation of RUP determines, in part, the quantity and quality (i.e. EAA profile) of the MP supplied and proteins and AA available to the cow.

Nitrogen Recycling

Nitrogen in the form of urea is either recycled as part of endogenous CP (i.e. via saliva and blood) or excreted via urine. Recycling of nitrogen is the movement of nitrogen from the blood and saliva back to the rumen (Calsamiglia et al., 2010), and research suggests that approximately 15 to 40% more of the total nitrogen intake can potentially be recycled (Lapierre and Lobley, 2001). Storm et al. (2013) reported that the urea recycled through saliva is proportional to 63% of urea present in the blood. Every absorbed nitrogen containing compound passes through the liver (i.e. major site of nitrogen metabolism) via the portal vein, and ammonia is the major nitrogenous substrate utilized in the liver to synthesize urea. Recycling urea nitrogen will decrease the requirements for feeding RUP and reduce the amount of NPN output in milk and total nitrogen present in urine. With less urea nitrogen being excreted, more microbial CP will be synthesized resulting in highly digestible protein and AA supply for milk

protein synthesis. Overall, reducing RDP can increase nitrogen recycling and reduce urinary nitrogen excretion, whereby milk production can be sustained and possibly improved.

Effects of Intense Environmental Temperatures

Evaluation of Heat Stress

Summer climate conditions in the U.S. can deliver a harsh environment for high-producing dairy cows with warm to hot environmental temperatures [avg. 31°C (NOAA, 2010)]. A 2006 event of extreme summer climate in California reported deaths of more than 30,000 dairy cows (Baumgard and Rhoads, 2012). Thus, accurately identifying heat stress is required to successfully manage high-producing cows during warm climates. The magnitude of heat stress is commonly measured as temperature-humidity index [**THI**; eq. 1 (Dikmen and Hansen, 2009)]. Temperature-humidity index represents the combined linear effect of environmental temperature (**TC**; °C) and relative humidity (**RH**; %) towards thermal load experienced by livestock. Therefore, correctly assessing temperature and humidity for THI during warm climates is an easy tool to assess heat stress in dairy cows.

$$\text{THI} = (1.8 \cdot \text{TC} + 32) - [(0.55 - 0.0055 \cdot \text{RH}) \cdot (1.8 \cdot \text{TC} - 26)] \quad (\text{eq. 1})$$

Temperature-humidity index assesses the severity and intensity of heat stress and demonstrates the ability to predict heat stress, but concern over THI levels and identifying accurate body temperatures in cows has been questioned (Dikmen and Hansen, 2009). Previous

reports indicate that lactating cows undergo heat stress at a threshold THI level of 72 (Armstrong, 1994). However, new reports have indicated significant decreases in milk yield at a threshold THI level of 68 for high-producing cows (Cook et al., 2007). The reduced threshold for high-producing cows results from their increases in intake and production contributing to greater metabolic heat (Purwanto et al., 1990; West et al., 2003). Hammami et al. (2013) stated that the assessment also has the following limitations: 1) represents empirical information and not measured, 2) classifies all animals being effected similarly by environmental effects, and 3) lacks the inclusion of confounding effects (e.g. solar radiation and physiological animal differences). Unfortunately, the THI method of predicting heat stress experienced by cattle is not consistent for all, but currently presents the easiest, cheapest, and most labor-free method to assess heat stress.

Core Body Temperature and Respiration Rate

In addition to evaluating heat stress using THI, the more reliable and accurate but more difficult method is by direct assessment of core body temperature (Collier et al., 2006). Various methods and technologies have been utilized to continuously observe core body temperature, including rumen temperature boluses (Bewley et al., 2008; Ipema et al., 2008), implanted udder thermistors (Bitman et al., 1984; Lefcourt and Adams, 1996), and abdominal transmitters (Brown-Brandl et al., 2005). Since these methods can be expensive or invasive to integrate, the measurement of core body temperature is most commonly assessed through rectal temperatures and is consider the best indicator of physiologic response to heat stress (Silanikove, 2000). Measurement of rectal temperature takes into account correction factors of radiation, conduction,

convection, evaporation, and metabolic heat (Finch, 1986); however, rectal temperatures more labor-intensive and are only a snapshot in time toward the effects of warm climates. Rectal temperature information will need to be assessed multiple times throughout the day to accurately monitor heat stress in the cows.

A more informative method over rectal temperatures for core body temperature assessment have been investigated using intravaginal temperature loggers inserted into blank controlled internal drug release (**CIDR**) devices (Dikmen et al., 2008; Burdick et al., 2012) to measure vaginal temperatures. Vaginal measurement is convenient and informative due to a constant, cyclical 24 h/d temperature assessment as cows move freely throughout their environment (Collier et al., 2006). Dikmen et al. (2008) reported increased vaginal temperatures at peak environmental temperatures of 39°C at 1400 h, with a THI average of 88. Vickers et al. (2010) stated there is a moderate to strong relationship between rectal temperatures and vaginal temperatures depending on stage of lactation: in 24 h postpartum cows ($r = 0.81$) and peak lactation cows (DIM = 98; $r = 0.46$). However, Burdick et al. (2012) reported stronger correlations ($r = 0.92$) between the two assessments of core body temperature when cows experienced ambient temperatures of approximately 31°C. Therefore, vaginal temperatures can be an innovative method to assess body temperature patterns and physiologically adaptations of dairy cows during warm climates.

Another method of core body temperature assessment during heat stress is udder, cutaneous, and milk temperatures, which are often measured using infrared thermography guns. Udder temperatures were evaluated in dairy cows and were found to be less variable compared to other external parts (i.e. rump, shoulder, etc.) of the animal and have a linear relationship with increasing THI (Zahner et al., 2004). West et al. (1999) measured milk temperature using a

thermocoupler attached to the milking system. They report a strong correlation ($r = 0.78$) with rectal temperatures claiming the values are more consistent and superior to rectal temperatures. Rhoads et al. (2009b) measured body temperature using a 5 cm² shaved patch of hair from the shoulder of the cow. They report cutaneous surface and rectal temperatures similarly increased in heat-stressed cows compared with thermoneutral cows. Notably, external cutaneous and udder temperatures reportedly are lower than internal rectal temperature values. All other core body temperature assessments are excellent and quick physiological indicators of heat stress in dairy cows that can be assessed by producers, but rectal temperature still remains the gold standard towards evaluation of physiological effect of heat stress.

Respiration rates (breaths/min) are also used to determine if and how severe cows are experiencing heat stress. Measurement of respiration rates represents panting patterns during heat stress. Panting is the increased frequency of respirations and loss of CO₂ by pulmonary ventilation with a decrease in tidal volume (West, 2003). Panting allows increases in ventilation of the upper respiratory tract in order to dissipate internal heat via evaporation (Silanikove, 2000). Multiple studies looking into effects of heat stress on dairy cows report respiration rate increases up to two fold during peak heat stress from approximately 40 to 80 breaths/min (Higginbotham et al., 1989b; Rhoads et al., 2009a). West et al. (1999) reported a difference of 53 breaths/min for cows experiencing a peak THI of 83.7 (109 breaths/min) compared with cows experiencing thermoneutral conditions (56 breaths/min). Assessing ventilation rate along with core body temperature can be an effective and simple analysis of heat stress (Baumgard and Rhoads, 2012). Proper evaluation and measurement of core body temperature and ventilation rates can allow for accurate assessment of heat stress in dairy cows and allow for proper management adjustments to combat negative effects.

Adaptations to Heat Stress

Adaptations to heat stress causes physiological and metabolic changes in livestock. Kibler and Brody (1953) established that with increasing ambient temperature and low to high humidity, the physiological adaptations towards suppressing thermal load in dairy cows shifts from non-evaporative methods (i.e. convection, conduction, and radiation) to evaporative methods (i.e. sweating and panting). Sweating and panting can compromise animal health [e.g. respiratory alkalosis, ketosis, and rumen acidosis (Collier et al., 1982)]. In the southeastern United States the opportunity of sweating as a means of cooling is compromised from high relative humidity, which makes the cow rely on panting. Panting increases release of CO₂ via ventilation, which can cause respiratory alkalosis and elevation in blood pH (West, 2003). Increased blood pH can cause urinary excretion of bicarbonate, consequently resulting in reduced rumen buffering capabilities and occurrence of metabolic acidosis (Constanzo, 2014). Adaptations from panting and the inability to effectively release heat can overall impact the total production resulting from the cow.

Cows in heat stress shift from productive functionality to survival instincts by dissipating heat and maintaining homeostasis via every avenue possible. Multiple physiological changes occur in not only acid-base chemistry, but also in digestive function, endocrine function, and partitioning of nutrients during warm climates (West, 2003). Some of these effects result from reduced DMI, but also from the adaptations to stress. The temperature sensitive receptors from the skin signals for neural response to the hypothalamus to allow for adaptive processes (Christison and Johnson, 1972; Spiers et al., 2004). The change in digestive function alters rumen fermentation of dietary ingredients by manipulating the molar propionate to acetate ratio

(Kelley et al., 1967) and limiting the supply of energy and protein to the animal (McGuire et al., 1989). The cow results in altering the partitioning of nutrients away from secondary tissues (e.g. mammary gland for lactation) and utilizes energy and protein towards maintaining homeostasis (Baumgard and Rhoads, 2013).

Acute and chronic exposure to heat stress each has its own effect through physiological adaptations. There is a strong relationship with environment and energetics of animals (Wheelock et al., 2010). Increases in maintenance costs and animal energy expenditure are present when animals go from mild to severe heat stress (Baumgard and Rhoads, 2013). However, adaptations toward heat stress influences these responses. Lactating cows experiencing acute intense environmental heat have increased energy metabolic rates, but during chronic heat stress these rates decreased (Bianca, 1965). Lactating cows experiencing acute heat stress forego elevated catecholamine (i.e. epinephrine, norepinephrine, and dopamine) and glucocorticoid (i.e. cortisol) concentrations that potentiates metabolic energy toward poor homeorhetic adaptations to favor homeostasis (Thompson et al., 1963; Collier et al., 1982). Chronic exposure to heat lowers circulating concentrations of growth hormone, thyroxine, and glucocorticoids, which are hormones directly related to regulating metabolic rate; therefore, lowering metabolic rate to lower total body heat production (Collier et al., 1982). Nonetheless, both acute and chronic effects cause inhibition of fatty acid mobilization, stimulation of glucose uptake by muscle and adipose tissues, and catabolism of AA from tissues. Hence, developing a comfortable environment and appropriate diets for high-producing dairy cows during heat stress is paramount towards controlling metabolic rates for proper milk production.

Energy Expenditure and Balance

The NRC (1989) states that at 35°C maintenance energy expenditures increase by 7 to 25% compared with thermoneutral conditions due to the adaptive changes during heat stress. When compared with cows that are not lactating, high-producing cows yield 48% more heat (Purwanto et al., 1990). The energy used towards releasing heat and maintaining a homeostatic condition limits energy used to produce milk (West, 2003). Production of internal heat during thermoneutral conditions is normally dissipated through the non-evaporative methods of physiological cooling to maintain body temperature homeostasis. However, when dairy cows experience thermal load, releasing heat is managed through evaporative methods (i.e. panting) as mentioned earlier, which is highly energy dependent. The production of internal heat and the energy required to release the heat increases the energy maintenance requirements.

According to Moore et al. (2005), heat-stressed cows exhibit a negative energy balance. Heat stress prevents cows from consuming adequate amounts of nutrients to supply enough energy for maintenance and production needs, resulting in negative energy balance (Baumgard and Rhoads, 2009). Therefore, negative energy balance reportedly alters the endocrine status, reduces rumination and nutrient absorption, and increases maintenance requirements (Rhoads et al., 2009a; Soriani et al., 2013). Wheelock et al. (2010) demonstrated that cows experiencing a constant cyclical THI range of 72 to 82 went from a positive energy balance (3.95 Mcal/d) during THI of 64 to a negative energy balance (-2.97 Mcal/d). As a result, those researchers reported in two separate studies that cows experiencing heat stress lose approximately 45 to 50 kg of BW (Rhoads et al., 2009a; Wheelock et al., 2010). The overall consequences from

negative energy balance result in lack of energy and nutrients essential for high milk production, along with life threatening decreases in BW (Moore et al., 2005).

Dry Matter Intake

Dairy cows are expected to reduce DMI ranging from 22 to 55% in mid-lactation cows during heat stress to correct for the energy losses due to evaporative heat loss (NRC, 1981). Johnson et al. (1962) reported a linear reduction of 0.23 kg/d of DMI when THI exceeded 70. A recent study reported a decline in DMI of 7.45 kg/d and a range of 29 to 37% decrease for animals experiencing a peak THI of 82 compared with 64 during thermoneutral conditions (Rhoads et al., 2009a; Shwartz et al., 2009). The reductions in DMI have been suggested to be an adaptation in order to prevent metabolic heat production from rumen fermentation (Fuquay, 1981). Metabolic heat production further intensifies the heat already being placed on the animal from the environment (West et al., 2003). Consequently, reductions in DMI prevent adequate supply of nutrients to meet maintenance and production requirements in heat-stressed lactating cows.

Feeding behavior and intake is altered during periods of intense environmental climates. Bernabucci et al. (2010) stated that thermoneutral cows consume 12 to 15 meals/d, but following heat stress their feeding frequency reduces to 3 to 5 larger meals/d. Larger meals contributes to greater acid production in the gut and resulting rumen acidosis. However, recent research has discovered the inhibitory neurotransmitter that regulates satiety and body temperature in the hypothalamus [i.e. γ -aminobutyric acid (**GABA**)] may stifle DMI reductions during heat stress (Wang et al., 2013; Cheng et al., 2014). Dry matter intake is partially improved consequently

from reduced core body temperature present in the hyperthermic animal (Cheng et al., 2014). High concentrations of supplemental rumen-protected GABA increased DMI by 4.3% in early lactation dairy cows compared with lower GABA supplementation (Wang et al., 2013). Additionally, the inhibition of the satiety center in the central neural network allows for increased intake and possibly improved feeding bouts. The reduction in DMI possibly lowers metabolic heat production, which suppresses the rise in core body temperature during heat stress.

Milk Production and Composition

Heat stress causes a 30 to 50% reduction in milk yield (Rhoads et al., 2009a; Wheelock et al., 2010), but also affects milk quality by reducing milk lactose, fat, and protein production (Bernabucci and Calamari, 1998; Calamari and Mariani, 1998). The reduction of milk quality is another contribution to the annual revenue losses experienced by producers due to heat stress. With proper nutritional management during warm climates, the quality of milk production can potentially be improved through dietary RDP and RUP manipulation. Understanding how these milk components are individually affected by dietary and metabolic factors would provide data to develop better nutritional management tools and improve production for producers.

Normal lactose percentages present in lactating cows in thermoneutral conditions are scarcely variable, remaining constant at 4.85% (NRC, 2001). However, research has demonstrated approximately a 3.0% reduction in milk lactose during warm climates. Milk lactose percent was reduced during heat stress (4.64%) compared with thermoneutral conditions (4.75%) in lactating cows (Rhoads et al., 2009a). A similar study by Wheelock et al. (2010) showed comparable results where milk lactose decreased in heat-stressed dairy cows (4.71%)

compared with thermoneutral cows (4.90%). Lactose synthesis is highly dependent on glucose utilization in the Golgi apparatus of mammary epithelial cells (Anderson et al., 1985). Resulting milk lactose concentrations osmotically regulate the synthesis of milk (Kronfeld, 1982; Zhao, 2014). Therefore, reductions in milk lactose agree with reported 30 to 50% losses in milk yield from cows in heat stress conditions compared with thermoneutral conditions (Rhoads et al., 2009a; Wheelock et al., 2010). Milk lactose concentrations are important in determining and possibly sustaining milk yield in heat-stressed cows.

Volatile fatty acids, specifically acetate and butyrate, are utilized to produce milk fat content typically at 3.0 to 4.0% of milk composition (Oldham, 1984; NRC, 2001). During high environmental temperatures of 37.7°C, acetate production decreased the total VFA production by 50% influencing lowered milk fat concentrations greatest compared with butyrate (Weldy et al., 1964; Kelley et al., 1967). However, a more recent study has shown a 15% increase of 0.66% in milk fat concentrations for cows experiencing heat stress (4.04%) compared with thermoneutral conditions [3.38% (Wheelock et al., 2010)]. Arieli et al. (2004) reported similar milk fat concentrations and yields for heat-stressed lactating cows consuming a 15.1 and 16.7% CP diet. The milk yield for this study was also similar between the two different dietary protein treatments, which might explain the lack of difference between the treatments. Consequently, the production of milk fat may be increased during warm climates based on the dietary TMR formulation.

Propionate, an additional primary VFA produced from carbohydrate metabolism, provides the substrate necessary for AA synthesis. The AA are absorbed and utilized for milk protein synthesis in the mammary gland. Kelley et al. (1967) reported a 30% decrease in propionate when cows experienced 37.7°C compared with 18.2°C; therefore, altering energy

utilization away from milk protein synthesis. Bernabucci et al. (2002) reported a deleterious effect of heat stress in the synthesis of caseins and a decrease in milk protein percentage and milk protein yield. Comparatively, Rhoads et al. (2009b) reported a reduction of 0.1% in milk protein concentration for heat-stressed cows when compared with thermoneutral cows fed reduced intake. Therefore, alterations of carbohydrate metabolism and reductions of protein synthesis are part of the changes in the post-absorptive tissues that account for the losses from heat stress in lactating dairy cows.

Management Strategies for Heat Stress

Several management strategies can be employed to relieve the effects of heat stress in lactating dairy cows (Beede and Collier, 1986): 1) physical modification to environmental mitigation factors (West, 2003; Baumgard and Rhoads, 2012); 2) genetic selection and improvement for heat-tolerant cows (Collier et al., 1981; Finch, 1985); and 3) nutritional management strategies. Incorporating the first approach can be an effective strategy toward alleviating heat stress. Providing artificial or natural shade and increasing ventilation through housing structures can be a cost-effective and immediate approach in controlling and enhancing productivity during warm climates. New technology in physical protective techniques have resulted in improved intake and productivity, however, these systems are often not properly employed or not used at the farm level.

The utilization of selecting for genetic improvements toward heat tolerance is scarcely advantageous. The genetic selection for high-producing dairy cows and heat tolerance is counterproductive physiologically (Beede and Collier, 1986). Cows producing larger amounts of

milk consume larger amounts of feed, resulting in greater metabolic heat production, which is further exacerbated by intense summer conditions (West, 2003). There is lack of research stating selection for more genetically adaptable dairy cows benefit against heat stress by maintaining productivity. Introducing heat-tolerant dairy cows that produce large amounts of milk would be desirable, but does not seem physiologically plausible. However, the third strategy through nutritional management can potentially be advantageous and cost-effective for heat-stressed lactating cows by providing innovative ways to reduce metabolic heat and altering the partitioning of nutrients toward lactation.

Heat Increment of Feedstuff

Heat increment consists of heat of fermentation and nutrient metabolic heat (Maynard et al., 1979). Various feedstuffs have different increments of heat due to differences in digestibility and metabolism. Heat increment from feed in high-producing lactating cows can contribute up to 67% of the total internal heat produced (Chandler, 1994). The protein proportion of the diet contributes a high increment of heat from the feed (Fuquay, 1981), mostly due to the great energy cost of urea production. Therefore, manipulating feed sources (specifically reduction of protein) for heat-stressed dairy cows to reduce the increment of heat can possibly help improve the reductions present in DMI.

Feeding fat sources has been known to reduce the heat of fermentation due to its low metabolic heat production. Huber et al. (1994) indicated that fat sources increase milk yield for dairy cows in summer conditions. However, diets fed with 5.5% of fat or more are at risk of reducing fiber digestion and DMI (Bauman et al., 2008; Shwartz et al., 2009). Addressing

consumption of fat is important in controlling metabolic heat, however limitations on the amount supplied in the diet prevent supplemental fat from being used solely to lower heat of fermentation. Comparatively, the supplementation of appropriate fiber and carbohydrates provides rumen microbes ample nutrients for adequate energy supply.

Dietary Energy Value

The energy value of the diet in lactating dairy cows is important to provide ME needed for maintenance and lactation performance (Cadorniga and Satter, 1993). The majority of ME available to dairy cows comes from VFA produced from ruminal fermentation (Annison and Armstrong, 1970). Volatile fatty acids, previously mentioned, are short chain fatty acids as two to four carbon compounds primarily referred to as acetate, propionate, and butyrate, respectively. Volatile fatty acids provide a large majority of the energy required by the ruminant animal contributing towards milk protein, fat, lactose, and yield in the lactating dairy cow.

Heat stress reduces the apparent amount of VFA production in the rumen (Weldy et al., 1964; Beede and Collier, 1986). In the ruminant, the major end product of rumen fermentation of fiber and starches that is utilized by the animal are VFA. Previous research shows that due to reduced DMI during heat stress, VFA concentrations (i.e. specifically acetate and propionate) are lowered by approximately 10.5% (Moody et al., 1967; McDowell et al., 1969). An additional contribution to lowered VFA concentrations may be from behavioral sorting and selection of nutrients by the animal during heat stress. High increment of forages contributes to selective forage reduction and causes continual consumption of concentrates (Anderson et al., 1985). The acetate to propionate ratio would be reduced in response to the selective behavior, which also

contributes to rumen pH reductions. Heat-stressed dairy cows adapt their eating patterns to minimize heat increment from rumen fermentation.

Recent research has suggested that fiber content of the diet does not contribute to the reductions in DMI. Cummins (1992) reported that dairy cows experiencing heat stress had similar DMI compared with thermoneutral cows that were fed higher or lower fiber diets. Another study concurred that with increased dietary NDF ranging from 30.2 to 42.0% of DM, DMI was reduced linearly; however, there were no DMI differences in thermoneutral conditions compared with heat stress conditions (West et al., 1999). The fiber content then may not provide as high a heat increment as does the non-fibrous carbohydrates present in the diet. Thus, the studies suggest total energy intake is the main contributor to reductions in DMI and not necessarily the fiber content of the diet.

Crude Protein Utilization

Previous research suggested that CP should be provided in greater amounts to heat-stressed cows in order to adjust for reduced milk production from lowered DMI. Reports indicate that dairy cows under heat stress fail to properly utilize the greater amount of dietary CP efficiently because it is greater than their requirement, and they decrease their metabolic ability to recycle nitrogen (Higginbotham et al., 1989b; West, 1998). Lack of nitrogen being recycled results in AA being catabolized by the liver to provide an amine group to produce urea. Concentrations of BUN indicate catabolism of proteins and AA from tissues and ammonia production in the rumen (Shwartz et al., 2009; Wheelock et al., 2010). Heat-stressed cows increased BUN levels by 71%, with an immediate acute increase of 47%, compared with cows in

thermoneutral conditions and fed the same plane of nutrition (Shwartz et al., 2009; Wheelock et al., 2010). Higginbotham et al. (1989a) reported reductions in BUN levels when heat-stressed cows were fed diets with reduced CP and protein degradability. A major factor into that reduction was providing less RDP in the diet limiting over-production and conversion of ammonia and catabolism of AA being used to produce urea in the liver. The reduction of CP and RDP can improve nitrogen efficiency and possibly increase milk production in heat-stressed dairy cows.

Previous work has recommended providing less RUP in the diets of lactating dairy cows experiencing heat stress compared with thermoneutral cows (Huber et al., 1994). The lower RUP concentration of 6.4% RUP in the diet presented greater uptake and utilization of AA (Higginbotham et al., 1989b). Blood urea nitrogen levels were 30% lower in the cows fed the 6.4% RUP diet compared with 7.7% RUP of DM. Similarly, Higginbotham et al. (1989a) reported a 24% reduction in BUN concentrations when heat-stressed cows were provided 6.5 vs. 5.4% RUP of DM. Conversely, Arieli et al. (2004) showed no differences among BUN concentrations in diets containing 16.7 and 15.1% CP with the same proportions of RUP in the diets. One possible explanation towards their finding is lack of large differences in RUP amounts (6.0 vs. 5.4% RUP of DM) of the diets compared with that of the previous studies. Lowered BUN indicates a greater efficiency towards AA utilization during milk protein synthesis for cows consuming lower RUP levels during heat stress.

Common use for efficient nitrogen utilization toward specific tissues, such as muscle and mammary, can be assessed through BUN, blood creatinine, 3-methyl-histidine, and MUN. Dairy cows experiencing heat stress or inadequate nutrient intake increase BUN, creatinine, 3-methyl-histidine, and MUN levels (i.e. deaminate skeletal muscle AA for energy) due to the inefficiency

of nitrogen utilization to help prevent significant reductions in milk production (Schneider et al., 1988; Bell, 1995; Wheelock et al., 2010; Lamp et al., 2015). The deamination of skeletal muscle AA results in the carbon skeletons being used for intermediates in the TCA cycle and gluconeogenesis, and the amino groups are excreted via urinary urea nitrogen (Anderson et al., 1985). Lamp et al. (2015) reported a 58% increase in plasma 3-methyl-histidine in heat-stressed lactating cows, demonstrating an increase in muscle proteolysis. Schneider et al. (1988) similarly reported increased plasma creatinine concentrations in heat-stressed cows (1.20 mg/dL) compared with thermoneutral conditions (1.06 mg/dL). Assessment of these metabolic factors may allow future research to gain important information on how nutritional management can improve nitrogen efficiency during heat stress.

Milk Urea Nitrogen

Milk urea nitrogen, which is directly proportional to BUN (Oltner and Wiktorsson, 1983; Kauffman and St-Pierre, 2001), is the measurement of the amount of urea nitrogen present in the milk coming from the mammary gland of the cow. Urea nitrogen rapidly dissipates throughout multiple body fluids, including milk; therefore, the recycling of urea nitrogen in the liver to tissues can also be determined through MUN (Broderick and Clayton, 1997). Both BUN and MUN have a direct correlation to the amount of urea nitrogen being excreted through urine by the cow (Ciszuk and Gebregziabher, 1994; Kauffman and St-Pierre, 2001). Additionally, Hof et al. (1997) concluded that MUN concentrations can be used to represent the spare nitrogen not utilized by microbial synthesis in the rumen. Therefore, MUN can be a useful, non-invasive tool

towards calculating and predicting utilization of nitrogen throughout the body of the animal and its efficiency [see following equations adapted from Wattiaux and Karg (2004)].

$$\text{Urine nitrogen output (g/d)} = 0.0283 * \text{MUN (mg/dL)} * \text{BW (kg)}; \quad (\text{eq. 2})$$

$$\text{Fecal nitrogen output (g/d)} = \text{nitrogen intake (g/d)} - \text{predicted urine nitrogen output (g/d)} - \text{milk nitrogen (g/d)}; \quad (\text{eq. 3})$$

$$\text{Nitrogen efficiency (\%)} = 100 * \text{milk nitrogen (g/d)} / \text{nitrogen intake (g/d)} \quad (\text{eq. 4})$$

Dairy cows experiencing intense summer climates are less efficient in recycling and utilizing nitrogen (Kamiya et al., 2005; Wheelock et al., 2010). Milk urea nitrogen concentrations for lactating cows in thermoneutral conditions range from 8.0 to 12.0 mg/dL (Kauffman and St-Pierre, 2001; Kohn et al., 2002; Broderick and Reynal, 2009). Research looking into the effects of heat stress on MUN is minimal, but improvement in nitrogen efficiency has been demonstrated. Arieli et al. (2004) reported an 8.1% reduction in MUN concentrations in heat-stressed dairy cows fed a 15.1% CP (14.8 mg/dL) versus a 16.7% CP diet (16.1 mg/dL; both containing a constant 38.5% RUP of %CP). Hence, reduced protein diets for heat-stressed cows increases the efficiency and recycling of nitrogen throughout the body of the animal and it is not being wasted as urea nitrogen in bodily fluids such as milk and urine. With measurement of blood metabolites, we may be able to make assumptions on how the animal utilizes that nitrogen and not exclusively how efficient they are at using the nitrogen.

Effect of Heat Stress on Energy Metabolism

Non-Esterified Fatty Acid Metabolism

Thermoneutral cows lose substantial amounts of BW (> 50 kg) from mobilization of stored triglycerides due to reduced DMI in order to supply required energy, whereas heat-stressed cows lack the ability and may only lose weight based on reduced gut fill (Rhoads et al., 2009a). Rhoads et al. (2009a) reported cows had circulating NEFA levels of 305 $\mu\text{Eq/L}$ compared with 128 $\mu\text{Eq/L}$ in heat-stressed lactating dairy cows; the difference is a 138% greater NEFA level response in the thermoneutral cows that are feed restricted. The same authors reported similar results with plasma NEFA concentrations increasing by 63% in thermoneutral cows in negative energy balance compared with heat-stressed cows (Wheelock et al., 2010). Additionally, the NEFA levels for heat-stressed cows did not differ from thermoneutral cows on a normal plane of nutrition. Heat-stressed cows result in using other sources for energy, which negatively affects the partitioning of nutrients. The manipulation of dietary protein for cows has been explored as an opportunity to limit the use of AA for energy and change the partitioning of nutrients. Arieli et al. (2004) reported plasma NEFA concentrations remained stable when heat-stressed lactating cows were fed 15.1% CP diets (136 $\mu\text{Eq/L}$) when compared with 16.7% CP diets (133 $\mu\text{Eq/L}$). Results indicate heat stress directly effects the expenditure of energy in lactating dairy cows, and feeding lower CP diets has no effect on improving mobilization of adipose tissue.

Ketone Metabolism

Typical blood BHBA thresholds for signifying ketotic conditions in dairy cows range from 0.97 to 1.20 mmol/L for clinical and subclinical ketosis (Ospina et al., 2010; Oetzel, 2015). Dale and Brody (1954) proposed that heat-stressed lactating cows undergo metabolic ketosis to satisfy energy requirements by mobilizing fatty acids or NEFA that often result in incomplete oxidation producing the ketone BHBA. However, lactating cows experiencing heat stress have displayed the lack of NEFA-derived ketone, BHBA, utilization for energy requirements compared with thermoneutral conditions (do Amaral et al., 2009; Lamp et al., 2015). Cows in thermoneutral negative energy balance are found to use NEFA and BHBA in order to spare glucose for the synthesis of milk (Baumgard and Rhoads, 2013). Similarly, BHBA is utilized in the mammary gland for milk fat synthesis through de novo synthesis to free fatty acids (Anderson et al., 1985). Dale and Brody (1954) conducted two experiments for heat-stressed cows and thermoneutral cows with a lowered plane of nutrition. They found that blood ketone concentrations failed to increase during heat stress, but increased in thermoneutral cows. Comparatively, do Amaral et al. (2009) reported a 40% increase in BHBA in thermoneutral cows compared with heat-stressed cows, and Lamp et al. (2015) similarly reported a lack of BHBA oxidation in heat-stressed cows. The lack of BHBA utilization follows that of NEFA for heat-stressed lactating cows, and demonstrates that heat-stressed lactating cows have post-absorptive changes negatively impacting productivity and energy metabolism.

The reduction of CP fractions may potentially shift post-absorptive metabolism toward utilization of ketones for energy requirements in heat-stressed lactating cows. Arieli et al. (2004) compared the effect of two dietary protein treatments in heat-stressed lactating cows [15.1% CP

(9.7% RDP and 5.4% RUP) and 16.7% CP (10.7% RDP and 6.0% RUP) of DM]. The authors reported that treatments had the same concentrations of BHBA (0.94 and 0.94 mmol/L), but overall BHBA concentrations were increased during heat stress with lower dietary CP compared to industry recommended amounts. Since the same study reported lack of a NEFA response in heat-stressed cows, the increased concentrations of BHBA reported may be inept to make a full conclusion on ketone metabolism in lowered CP diets. The study did not report any changes between milk fat concentrations between low and high CP diets; therefore, a possible assumption is that due to alteration in VFA production in the rumen less BHBA is being metabolized and sent to mammary tissue.

Glucose Metabolism

Reduced glucose levels in circulating blood pools indicates that glucose becomes a favored source of energy for heat-stressed lactating cows (Wheelock et al., 2010). Studies suggest the reasoning behind increased glucose energy use is the increased efficiency of glucose oxidation compared with NEFA to minimize metabolic heat production (Baldwin et al., 1980). Shwartz et al. (2009) reported cows experiencing heat stress had a 12.0% reduction in blood glucose concentrations. Similarly, Wheelock et al. (2010) reported a 8.8% reduction in glucose concentrations compared with cows in thermoneutral conditions. The decrease in glucose concentrations may partially explain the 200 to 400 g/d losses in milk lactose and 24% loss in milk yield during heat stress (Wheelock et al., 2010; Baumgard and Rhoads, 2013). The reduction in glucose could potentially be caused from the acute onset of heat stress reducing

glucocorticoid and catecholamine levels (Baumgard and Rhoads, 2013) or from the reduction in propionate production in the rumen due to reduced DMI (Kelley et al., 1967).

Dietary protein plays a significant role in the utilization of glucose for energy required to breakdown and synthesize proteins and AA. Providing lower proportions of RDP limits energy used to synthesize microbial CP, therefore allowing more glucose to be used towards milk production. Taylor et al. (1991) reported increased milk lactose concentrations in heat-stressed lactating cows fed lowered 8.5% RDP (4.87%) compared with 10.8% RDP (4.75%), suggesting that glucose utilization may possibly be increased for milk synthesis in cows fed lower RDP concentrations. However, Higginbotham et al. (1989a) reported no differences in blood glucose concentrations when heat-stressed lactating cows were fed high (18.5% of DM) and low (16.1% of DM) CP diets with varying degradability (65 and 60% of CP). Comparatively, Arieli et al. (2004) reported similar blood glucose concentrations with no differences among feeding varying amounts of CP. Therefore, glucose concentrations have been found to remain stable when feeding varying amounts of CP, but have the potential to increase towards milk lactose production with different amounts of degradability during heat stress.

Insulin

As DMI decreases for lactating cows experiencing heat stress, the typical dogma would be that insulin levels would decrease. However, heat stress increases blood insulin concentrations in lactating cows. Itoh et al. (1998) reported an increase of 38.6% in insulin concentrations between heat stress (28°C) and thermoneutral cows (18°C). Likewise, Wheelock et al. (2010) reported a 27.3% increase in insulin concentration for lactating cows exposed to a

peak THI of 82.2 or 38.9°C. Heat stress presents a model in which intake is reduced and insulin levels increase. In swine, heat stress increased whole body insulin sensitivity (Fernandez et al., 2015). Similarly in humans, hot water baths and saunas are therapeutically used and subsequently increase insulin sensitivity (McCarty et al., 2009). Research has reported an increased response in insulin from a glucose tolerance test in heat-stressed cows compared with thermoneutral cows (Wheelock et al., 2010). Secretion of insulin may have possibly been increased from the pancreas. High-producing lactating cows may utilize this occurrence as an opportunistic homeorhetic adaptation towards heat stress to limit fatty acid mobilization and increase uptake and utilization of glucose (Randle, 1998) possibly due to the high heat increment from oxidation of NEFA.

Opportunities for Reduced Protein Diets in Warm Climates

Overall the number of dairy cows present in the world is decreasing at a rate of approximately 3% annually (Capper et al., 2009), and the world population is increasing at a rate of 1.5% annually. Milk production will need to improve for the current dairy cow population in order to maintain the supply of milk products per capita. During warm climates, the reduction in milk produced causes a major problem in improvements in milk production. Reducing RDP and RUP may allow for greater synthesis of milk protein, and may also shift metabolic adaptations toward enhancing energy expenditure for milk production instead of releasing body heat. Therefore, the limited amount of dairy cows may then provide sufficient amounts of milk and not be suppressed during warm climates, while improving the environmental stewardship of the dairy industry.

**CHAPTER II: FEEDING LOW RUMEN DEGRADABLE AND
UNDEGRADABLE PROTEIN IMPROVED MILK PRODUCTION,
NITROGEN EFFICIENCY, AND METABOLISM IN LACTATING DAIRY
COWS UNDER SUMMER CONDITIONS**

Introduction

Ruminants compared to other livestock excel in the conversion of low-quality feed products into nutritious food products for human consumption. However, lactating dairy cows inefficiently convert dietary nitrogen into milk nitrogen, defining milk nitrogen efficiency (Bequette et al., 1998; Castillo et al., 2000). Poor conversion of nitrogen results in greater urinary and fecal nitrogen excretion into the environment. Increases in environmental ammonia and nitrate contamination is detrimental to dairy industry sustainability and hazardous to human populations through atmospheric changes and water contamination. Thus, limiting excess nitrogen release into the environment is a pivotal aim for the dairy industry.

Unfortunately, exposure to intense summer climates negatively affects DMI and mechanisms responsible for efficient nitrogen utilization and milk synthesis in dairy cattle (West et al., 2003; Kamiya et al., 2005; Shwartz et al., 2009). Previous research has indicated the necessity for providing protein and energy dense diets in lactating cows during warm climates in order to adjust for DMI reductions that prevent adequate milk synthesis (Hassan and Roussel, 1975; Huber and Chen, 1992). Conversely, other studies have demonstrated that reduced DMI only contributes to approximately 50% of milk synthesis reductions during warm climates (Rhoads et al., 2009b; Shwartz et al., 2009; Wheelock et al., 2010). The same studies also indicate that the additional 50% reduction in milk synthesis is limited by deviations in protein and energy metabolism; therefore, nitrogen efficiency is reduced.

Manipulation of dietary CP fractions, specifically RDP and RUP, can be effective nutritional management techniques to reduce the impact on production and utilization of nitrogen during warm climates in lactating cows. Reduction of dietary RDP to 8.5% from 10.8% of DM

in cows during warm climates had improved milk yield and no reductions in DMI (Zook, 1982; Taylor et al., 1991). Similarly, 76% of research over 12 yr reported feeding higher RUP levels from 50 to 60% of CP negatively impact milk production (Santos et al., 1998). Reductions in dietary RDP and RUP concentrations for thermoneutral cows can promote nitrogen recycling, which reduces nitrogen waste and increases capture of nitrogen (Higginbotham et al., 1989b; Kalscheur et al., 2006; Wang et al., 2007; Rius et al., 2010) compared with concentrations recommended by NRC (2001). Therefore, efficient nitrogen utilization may improve nutrient partitioning toward synthesizing milk and milk protein with reduced dietary RDP and RUP concentrations during warm climates.

In regards to the present study, we hypothesized that altering CP fractions through reduced RDP and RUP will maintain milk production, while improve the nitrogen efficiency and metabolism in lactating dairy cows exposed to warm climates. The objectives of this study were to 1) reduce nitrogen input without compromising milk production, 2) assess the effects of RDP and RUP on milk production, nitrogen efficiency, and energetic and metabolic parameters, and 3) evaluate the accuracy of the NRC (2001) model in predicting production and requirements for cows fed varying RDP and RUP amounts during warm climates.

Materials and Methods

Animals, Housing, and Management

All experimental procedures were pre-approved by the Institutional Animal Care and Use Committee of the University of Tennessee. Thirty multiparous and 18 primiparous Holstein

cows [144 ± 49 DIM] were used from the East Tennessee AgResearch and Education Center (ETREC) dairy herd. Cows were housed in freestalls at the ETREC dairy facility. During the pre-treatment period (d 1 to 21 of the study), cows were housed in ambient temperatures common for East Tennessee with heat abatement previously explained by do Amaral et al. (2009); however, no sprinkler systems were utilized. Environmental temperature ($^{\circ}\text{C}$) and relative humidity (%) were measured at 10 min intervals using HOBO Pro v2 Series probes (Onset Computer Corporation, Bourne, MA). Temperature-humidity index (THI) was assessed at maximum and minimums based on the equation from Dikmen and Hansen (2009). Throughout the treatment period (d 22 to 42 of the study), all cows experienced 10 h of unabated daily summer temperatures. At 2000 to 1000 h, THI ranged from 69 to 76; thereafter, the environment was at a THI range of 74 to 82 between 1000 and 2000 h (Figure 1 in Appendix).

Monitoring and assessing thermal load was accomplished through core body temperature and respiration rate. Rectal temperatures were measured twice daily at 1000 and 1500 h in 4 cows from each treatment group ($n = 16$ cows) using a GLA M700 (GLA Agricultural Electronics, San Luis Obispo, CA; accuracy $\pm 0.1^{\circ}\text{C}$) battery-operated digital read out thermometer. Vaginal temperatures were assessed every 10 min using intravaginal temperature loggers (DS1921G Thermochron iButton Device, Maxim Integrated, San Jose, CA; accuracy $\pm 1.0^{\circ}\text{C}$) inserted into a modified blank controlled internal drug release (CIDR; Elanco) devices that were adapted from Dikmen et al. (2008) and Burdick et al. (2012). Intravaginal temperature loggers were calibrated in vitro prior to using in a 37°C water bath at 10 min intervals to determine if the calibrations were accurate. In vivo vaginal temperatures were measured rotationally in 24 cows during four 5 d periods of the treatment period. Both sets of 24 cows were assessed twice during the treatment period. Between rotations the prepared CIDR were

removed, rinsed with water, cleaned in detergent soap, sanitized in chlorhexidine, and dried.

Respiration rates were measured three times weekly in 5 cows from each treatment group (n = 20 cows) at 1100 h by counting flank movements for 15 s and reported as breaths/min.

Cows were milked twice daily at 0900 and 1900 h, and milk production was automatically recorded at each milking. Body weights and BCS, determined according to the method by Wildman et al. (1982), were recorded once weekly on each cow after 0900 h milking. Daily milking and weekly BW were used to calculate energy balance according to Wheelock et al. (2010) and NRC (2001). Lactating cows experiencing high environmental temperatures typically enter into negative energy balance according to the van't Hoff-Arrhenius equation (Fuquay, 1981; Beede and Collier, 1986). Resulting effects of negative energy balance increases energy maintenance requirements at heights of 25% (NRC, 1989). Therefore, negative energy balance determination was multiplied by 1.25 for all cows during the treatment period.

Experimental Diets

Cows were individually fed a TMR at 10% daily refusals once daily at 0900 h using electronic Calan Broadbent feeding system (American Calan Inc., Northwood, NH) and self-propelled TMR mixer (Data Ranger, American Calan Inc., Northwood, NH). Cows were arranged into 4 treatment groups equalized on DIM, parity, milk production, BCS, and pregnancy status, then randomly assigned to receive 1 of the 4 treatment diets varying in percent RDP and RUP of DM basis. Dietary treatments were formulated with 2 levels of RDP (10% and 8%) and 2 levels of RUP (8% and 6%) in a factorial arrangement of treatments equaling 4 dietary treatments: 1) 10% RDP, 8% RUP; 2) 8% RDP, 8% RUP ; 3) 10% RDP, 6% RUP; and 4)

8% RDP, 6% RUP. The combinations of dietary treatment groups results in dietary CP amounts of 18%, 16%, and 14%. Diets were formulated to meet NRC (2001) recommendations for NE_L, minerals, and vitamins for a mid-lactation dairy cow during warm climates weighing 622 kg (BCS = 3.0), producing 36.5 kg of milk/d containing 4.0% fat and 3.0% protein, and consuming 19.6 kg/d of DM. Final diet composition contained a 50% forage to 50% concentrate ratio for all 4 treatment diets (Table 1). During the pre-treatment period, cows were fed 10% RDP with 8% RUP and then fed their respective experimental dietary treatments during the treatment period. Forage components, concentrate, and orts samples were collected twice weekly and stored at -20°C, then pooled and evaluated by treatment on an equivalent weight basis, and submitted to Dairy One (Ithaca, NY) for chemical analysis (Table 2).

Sample Collection and Analyses

Milk samples were collected from successive milkings on d 18, 19, and 20 of the pre-treatment period and d 39, 40, and 41. Individual samples were analyzed for fat, protein, lactose, SNF, and MUN by infrared analyses (Foss MilkoScan, Eden Prairie, MN; United Lab DHIA, Blacksburg, VA). Milk SCC were analyzed by flow cytometry (Foss Fossomatic FC, Eden Prairie, MN) at United Lab DHIA (Blacksburg, VA). Energy-corrected milk was calculated by using the equation provided by Tyrrell and Reid (1965). Predicted values were calculated (eq. 2-4) for urinary nitrogen, fecal nitrogen, and nitrogen efficiency using the provided equations by Kauffman and St-Pierre (2001) and Wattiaux and Karg (2004).

Blood samples were collected twice via coccygeal venipuncture on the last day of the pre-treatment and treatment period and immediately placed on ice (10 mL; Becton Dickinson

and Co., Franklin Lanes, NJ). Plasma was obtained by centrifugation at 1,500 x g for 20 min at 4°C within 2 h of blood collection. Individual plasma aliquots were stored at -20°C until analyses were conducted for AA, BHBA, glucose, insulin, and NEFA (Garverick et al., 2013; McCarthy et al., 2015). All plasma BHBA, glucose, and NEFA concentrations were measured enzymatically using commercially available kits (Sigma-Aldrich, St. Louis, MO; Wako Diagnostics, Mountain View, CA) through microplate spectrophotometer (BioTek Synergy H1 Multi-Mode Reader, Winooski, VT). For BHBA, glucose, and NEFA the inter- and intra-assay coefficients ranges were $\leq 10\%$. Plasma insulin concentrations were determined by RIA (EMD Millipore's Porcine Insulin RIA) using Wizard² Gamma Counter (Perkin Elmer, Waltham, MA). The daily insulin sample intra-assay coefficients were 1.9%. Plasma free AA were determined using a commercially available kit (Phenomenex EZ:faast, Torrance, CA) and were analyzed using a liquid chromatograph-mass spectrometer (Orbitrap LC-MS, Thermo Scientific).

Statistical Analysis

Nutrient and DM intake, milk yield and composition, BW, BCS, rectal and vaginal temperature, respiration rate, and blood metabolite data were analyzed as a randomized block design with the MIXED model procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC; Table 3).

$$Y_{ijkl} = \mu + D_i + U_j + P_k + A(D*U*P)_{ijkl} + T_m + D*T_{im} + U*T_{jm} + \beta(\chi)_{ijklm} + e_{ijklmn},$$

where Y_{ijklm} = response variable of the i^{th} and j^{th} treatment in the k^{th} parity, l^{th} animal, and m^{th} date, μ = mean, D_i = fixed effect of i^{th} treatment (i = 10% and 8% RDP), U_j = fixed effect of j^{th} treatment (j = 8% and 6% RUP), P_k = random effect of k^{th} parity (k = primiparous and multiparous), $A(D*U*P)_{ijkl}$ = random effect of l^{th} animal in the i^{th} and j^{th} treatment and k^{th} parity, T_m = fixed effect of m^{th} date as repeated measure, $\beta(\chi)_{ijklm}$ = covariate effect of pre-treatment period, and e_{ijklmn} = random error. Non-random time constraints were expected in these dependent variables (exception for milk composites and blood parameters), thus, repeated measures was used for experimental days. The last 7 d of the pre-treatment period were averaged and included as a covariate adjustment in the model for intake and milk production if statistically significant ($P \leq 0.05$). Milk and blood sampling in the pre-treatment period were included as a covariate adjustment for milk and blood components. The last 7 d of the treatment period were used in the statistical analysis of milk production and intake. Unless otherwise stated, significance differences were declared at $P \leq 0.05$ and trend to differ at $P \leq 0.10$. All results are reported as least squares means (\pm SEM).

NRC Model Analysis

Predictive accuracy of the NRC (2001) model was assessed using observed values for production (Rius et al., 2010). Observed least squares means of DMI, milk yield, milk composition, BW, and BCS for each dietary treatment were used as inputs to compare the model predictions. The diet composition was set to actual ingredient values listed in Table 2. The feeding rate for each ingredient was set with the observed DMI for each dietary treatment. Tabular values were used to calculate the nutrient content of each concentrate grain mix and

compared with the observed chemical composition of the concentrate grain mixes in Table 2. An adjustment was warranted in tabular values due to slight deviations from observed values of CP, NDF, and ADF. Since soybean meal, protected soybean meal (SoyPLUS), soybean hulls, and blood and fish meal were the major contributors of CP in the grain mixes, the tabular CP content of the ingredients were adjusted to mimic actual CP contents equal to the observed treatment values. The tabular CP content of protected soybean meal increased from 46.6 to 51.3% DM, soybean hulls increased from 13.8 to 16.8% DM, and blood meal reduced from 95.5 to 86.0% DM. The large 3.0 percentage unit CP adjustment for soybean hull likely reflects formulation errors at the feed mill. The NDF and ADF content of soybean hulls, soybean meal, and protected soybean meal were adjusted in a similar manner to mirror the observed chemical analysis of the concentrate grain mixes. The tabular NDF content of soybean hulls were increased from 60.3 to 63.3% DM, soybean meal was increased from 9.80 to 10.3% DM, and protected soybean meal was reduced from 17.6 to 15.0% DM. The ADF tabular values of soybean hulls were reduced from 44.6 to 40.1% DM, soybean meal increased from 6.20 to 6.80% DM, and protected-soybean meal reduced from 10.6 to 9.5% DM. The adjusted CP, NDF, and ADF values were used alongside the observed forage and other concentrate grain mix ingredients as inputs to the NRC (2001) to generate predicted RDP, RUP, and MP balance and requirements for the cows (Table 4).

Results

Animal Performance

Core body temperatures and respiration rates significantly differed ($P < 0.01$) between the a.m. and p.m. measurements during the treatment period (Table 5). The a.m. and p.m. rectal and vaginal temperatures differed by 0.80 and 0.40°C, respectively ($P < 0.01$). Differences between treatments were not significant for rectal temperatures; however, treatment 10% RDP with 8% RUP reported lower vaginal temperatures compared with cows fed other dietary treatments (interaction, $P = 0.04$). Morning and afternoon (1000 and 1500 h) respiration rates differed by 22.6 breaths/min ($P < 0.01$).

The 6% RUP treatment decreased (interaction, $P = 0.03$) DMI by 1.70 kg/d compared with 8% RUP in the 10% RDP treatment, but sustained DMI in the 8% RDP treatment (Table 6). The 8% RDP treatment decreased (interaction, $P = 0.03$) CP intake by 0.29 kg/d compared with 10% RDP in the 6% RUP treatment, but increased CP intake by 0.50 kg/d in the 8% RUP treatment. The 8% RDP treatment increased (interaction, $P = 0.07$) ECM yield by 2.30 kg/d compared with 10% RDP in the 6% RUP treatment, but sustained ECM yield in the 8% RUP treatment. The 10% RDP treatment increased (interaction, $P < 0.01$) milk protein yield by 6.0% compared with 8% RDP in the 8% RUP treatment, but sustained milk protein yield in the 6% RUP treatment. Treatment 8% RDP (6.86 mg/dL) reduced ($P < 0.01$) MUN concentration compared with cows fed 10% RDP (10.2 mg/dL). Likewise, the 6% RUP treatment (7.22 mg/dL) reduced ($P < 0.01$) MUN concentration compared with cows fed 8% RUP (9.82 mg/dL).

Nitrogen Utilization

Treatment 8% RDP (120 g/d) decreased ($P < 0.01$) predicted urinary nitrogen excretion compared with cows fed 10% RDP (178 g/d; Table 7). Similarly, treatment 6% RUP (126 g/d) decreased ($P < 0.01$) predicted urinary nitrogen excretion compared with cows fed 8% RUP (172 g/d). Efficiency of nitrogen utilization improved as CP and nitrogen intake decreased.

Treatment 8% RDP (35.1%) improved ($P < 0.01$) nitrogen efficiency compared with cows fed 10% RDP (31.7%) and likewise for 6% RUP (35.1%) compared with 8% RUP (31.7%).

Blood Metabolites and Amino Acids

Treatment 8% RDP (3.13 mmol/L) increased ($P = 0.04$) plasma glucose concentrations compared with cows fed 10% RDP (2.98 mmol/L; Table 8). Treatment 8% RDP (15.8 μ U/mL) decreased ($P < 0.01$) plasma insulin concentrations compared with cows fed 10% RDP (20.9 μ U/mL). The 10% RDP treatment decreased (interaction, $P = 0.03$) total plasma EAA concentrations (specifically His, Lys, Met, Phe, and Trp) by 270 μ M compared with 8% RDP in the 8% RUP treatment, but reported no EAA concentration differences in the 6% RUP treatment (Table 9). The 8% RDP treatment decreased (interaction, $P < 0.01$) plasma 3-methyl-histidine concentrations by 3.30 μ M compared with 10% RDP in the 6% RUP treatment, but increased 3-methyl-histidine concentrations by 2.65 μ M in the 8% RUP treatment. The 8% RDP increased (interaction, $P < 0.01$) plasma γ -aminobutyric acid (**GABA**) concentrations by 0.031 compared with 10% RDP in the 6% RUP treatment, but decreased GABA concentrations by 0.034 μ M in the 8% RUP treatment.

NRC Model Analysis

Table 4 reports the NRC (2001) predicted supplies and requirements for CP, RDP, and RUP using the observed treatment means. Observed DMI varied for each treatment diet compared with the predicted DMI (19.6 kg/d); therefore, nutrient supply to the cows were variable among treatments. After adjusting for observed DMI, ingredient composition, and milk yield and composition, the NRC (2001) model underpredicted allowable milk yields for cows fed all diets other than with 10% RDP with 8% RUP, which was overpredicted. The predicted responses to RDP on 8% RUP and 6% RUP diets closely resembled the observed responses. The model predicted 3.90 [(10% RDP, 8% RUP) – (8% RDP, 8% RUP), Table 4] and 2.00 kg/d [(10% RDP, 6% RUP) – (8% RDP, 6% RUP)] responses, respectively and observed responses of 2.60 and 3.00 kg/d (Table 6), respectively. The differences present between predicted and observed values fall within the predicting error of the model. However, the responses to RUP on 10% RDP and 8% RDP diets were overpredicted compared with predicted responses of 11.1 [(10% RDP, 8% RUP) – (10% RDP, 6% RUP)] and 9.20 kg/d [(8% RDP, 8% RUP) – (8% RDP, 6% RUP)], respectively and observed responses 6.00 and 0.40 kg/d, respectively.

Discussion

Production Parameters

Health, production, and utilization of nutrients are significantly affected under hyperthermic conditions causing a burden to the dairy industry. Even with new technologies and

advances in heat abatement techniques, summer heat presents problems for high-producing dairy cows (Ravagnolo et al., 2000). Reduction of RDP and RUP can potentially reduce the negative impacts from heat stress on milk production and excretion of nitrogen. Herein, manipulation of RDP and RUP levels is evaluated on intake, milk production, nitrogen efficiency, and metabolism in dairy cows experiencing warm climates.

Dry matter intake was largely unaffected by feeding 8% RDP with 6% RUP compared with higher RDP and RUP levels (Table 6). As expected, CP intake mirrored the supply of CP offered to different treatments. Therefore, cows consuming 10% RDP with 8% RUP had the greatest intake, while cows consuming 8% RDP with 6% RUP had the lowest. In agreement, Arieli et al. (2004) reported similar DMI from a 0.27 kg/d reduction in CP intake, and Taylor et al. (1991) reported similar DMI from a 2.3% reduction of RDP (10.8 to 8.5% RDP of DM). Feeding lower RDP and RUP levels does not reduce DMI; however, the present DMI results may be resulting from a large variability present from inconsistent feeding procedures between people.

Ruminants can consume lower than recommended nitrogen inputs and still sustain milk production (Christensen et al., 1993; Cyriac et al., 2008). In the present study, milk production was largely unaffected by altering RDP and RUP concentrations (Table 6). Production was an average of 31.7 kg/d across all diets, demonstrating that cows fed 8% RDP with 6% RUP at least for a short period can sustain milk production compared with higher RDP and RUP concentrations. Cows fed 10% RDP with 8% RUP diets only output 1.3 kg/d more milk to the 0.96 kg/d more CP consumed when compared with cows fed 8% RDP with 6% RUP. The 1.1 kg/d more consumption of DMI does not greatly produce more milk than feeding less RDP and RUP. We can speculate that the ability for these cows to maintain production could be due to

enhance urea nitrogen recycling and maintenance of microbial CP. Being able to feed a diet with 8% RDP and 6% RUP all year round should be approached with caution since the study was only performed over 7 d. Further research on feeding this concentration ratio of RDP and RUP for a longer period is warranted to make a full determination on improved milk production in heat-stressed cows. Previous research has reported that cows fed a high CP (16.7%) diet compared with a lower CP (15.1%) diet had no differences between milk production for less than 80 d (Arieli et al., 2004). Similarly, Higginbotham et al. (1989b) reported similar to greater milk production when cows were fed diets for less than 50 d with low amounts of CP (16.1% CP) and degradability (10.5 and 9.7% RDP) compared to a high CP and high degradability diet (18.4% CP with 12.0% RDP).

Nitrogen Utilization

Feeding excess RDP and RUP decreases the proportion of nitrogen intake retained in milk resulting in an increase in urinary nitrogen excretion (Kalscheur et al., 2006; Wang et al., 2007). As expected, cows fed lower RDP and RUP had less urinary nitrogen excretion compared with higher RDP and RUP diets. As a result, nitrogen efficiency was greater for cows fed 8% RDP and cows fed 6% RUP (37.6%) by reducing urinary nitrogen output by $\geq 29\%$ when compared with the other dietary treatments. In agreement, previous research has reported increases in nitrogen efficiency when CP decreased from 17.5 to 14.5% of DM basis in the diet for lactating cows (Broderick, 2003; Rius et al., 2010). Arieli et al. (2004) reported a comparable 10.9% improvement in nitrogen efficiency for cows fed 15.1% CP (5.4% RUP) diet compared with a 16.7% CP (6.0% RUP) diet in heat-stressed cows. The aforementioned results

further exemplify how reduced RDP and RUP increases nitrogen efficiency without negatively influencing production during heat stress.

A decrease in urinary nitrogen excretion may result in an increase in the amount of nitrogen captured in milk (Colmenero and Broderick, 2006). In the current study, feeding 8% RDP and 6% RUP both resulted in a 10.7% increase of nitrogen retained in milk. Reduction of nitrogen input by 55 g/d from 10% RDP to 8% RDP reduced the amount of urinary nitrogen excretion by 58 g/d. The reduction of nitrogen input by 86 g/d from 8% RUP to 6% RUP reduced the amount of urinary nitrogen excretion by 46 g/d. The reduction of urinary nitrogen excretion possibly resulted in a greater amount of that nitrogen being captured by mammary tissue as indicated by improvements in nitrogen efficiency and milk production for the 8% RDP with 6% RUP diet. Further improvements may be possible from the increase in nitrogen efficiency and the ability to maintain milk production in cows fed 8% RDP with 6% RUP compared to higher RDP and RUP diets.

Metabolism Parameters

Increased glucose concentrations by 5.0% may partially be explained by the 24% reduction in insulin concentrations in cows fed 8% RDP compared with 10% RDP (Table 8). The ≥ 400 g/d difference of CP intake between 8 and 10% RDP treatments may have resulted in a greater ability to maintain blood glucose levels (i.e. greater glucose homeostasis). The concentrations may also be indicative of a greater utilization of glucogenic precursors, which is in agreement with increased EAA concentrations in diets with lower RDP and RUP levels compared with 10% RDP and 8% RUP. There is lack of research looking into the effects of

varying RDP and RUP amounts fed to heat-stressed cows, which makes it difficult to determine the true reasoning behind these changes. However, the current results are in agreement with a 25% reduction of plasma insulin concentrations for cows fed a 14.7 or 14.9% CP diet compared with 18.3% CP diet (Bach et al., 2000). Reducing RDP may improve the partitioning of nutrients to benefit milk production during warm climates and the decreased insulin concentrations may explain the improvement in glucose homeostasis.

Feeding 8% RDP may have altered the secretion of insulin to prevent mobilization of adipose tissue and increase use of glucose to meet energy demands in heat-stressed cows (Rhoads et al., 2009a; Wheelock et al., 2010). We may speculate that insulin sensitivity was improved in insulin-sensitive tissues in cows fed low RDP in our study. Therefore, less insulin was needed to maintain blood glucose concentrations. The concentrations of insulin and glucose in cows fed low RDP are in agreement with those concentrations observed in early lactation cows with adequate glucose homeostasis (Bach et al., 2000).

In the current study, increasing the proportion of RDP in the 8% RUP diet reduced plasma concentrations of EAA by $\geq 12\%$ (Table 9). Reducing concentrations of EAA may indicate a greater removal and utilization to promote protein synthesis in the mammary gland (Broderick and Satter, 1990; Rius et al., 2010). Indeed, milk protein yield increased in cows fed 10% RDP with 8% RUP compared with those fed other treatments, which is in agreement with greater removal and utilization of EAA by the mammary gland. Cows fed high proportion of RDP in the high RUP diet produce ≥ 60 g of milk protein; however, these animals had ≥ 500 g of CP intake than their counterparts. Collectively, the reduction in concentrations of total EAA was associated with small improvement in milk protein yield.

Concentrations of 3-methyl-histidine in plasma coincide with those of total EAA. Concentrations of 3-methyl-histidine were reduced by $\geq 37.5\%$ in cows fed 8% RDP with 6% RUP compared with the other treatment diets (Table 9). A reduction in 3-methyl-histidine may have been due to greater utilization of nitrogen indicating less AA were contributing towards the excretion of nitrogen from the liver. Sustained milk production in cows fed low RDP and RUP possibly resulted from AA requirements being met, and more AA being captured for milk protein synthesis in the mammary gland. Reduced 3-methyl-histidine is indicative of inhibition of muscle proteolysis (Nagasawa et al., 1996) when feeding low CP diets (Kamiya et al., 2006; Lamp et al., 2015). An explanation towards reduction in skeletal muscle catabolism may favor the sparing of glucose and AA for milk production. The 55 g/d reduction in offered nitrogen in the lowered RDP treatment reduced the requirements for energy to remove unused ammonia via urine; therefore, less AA may have been being catabolized to support the extra energy and nitrogen needed for ammonia removal and urea excretion. As a result, glucose and AA metabolism were improved for cows fed low proportions of RDP and RUP. Low proportions of RDP and RUP, providing increased EAA concentrations, may help improve milk protein concentrations and reduce muscle catabolism and utilization of AA for energy demand in heat-stressed lactating dairy cows.

The inhibitory neurotransmitter GABA, synthesized from glutamate, has been found to help regulate core body temperature and feed intake in various species (Seoane et al., 1984). Gamma-aminobutyric acid was found to influence the hypothalamic neural network that downregulates body temperature for both rats (Yakimova et al., 1996) and rabbits (Frosini et al., 2000). The current study reported increased GABA concentrations in cows fed 10% RDP with 8% RUP and 8% RDP with 6% RUP (Table 9). Previous research has reported that increased

plasma GABA concentrations help reduce core body temperature during periods of high environmental conditions in lactating cows (Cheng et al., 2014), pigs (Xu et al., 2009), and broilers (Chen et al., 2002). The present 0.40°C reduction in p.m. vaginal temperatures for cows fed high proportions of RDP in the 8% RUP diet are in agreement with increased plasma GABA concentrations and findings by Cheng et al. (2014). The greatest DMI from cows fed 10% RDP with 8% RUP may also have been influenced by increased GABA concentrations, effectually having a neural response to lower total body heat production, which is in agreement with Wang et al. (2013). The researchers reported that increased plasma GABA concentrations from the supplementation of dietary GABA induced feed intake for lactating cows experiencing negative energy balance. The present results indicate an opportunity for cows fed 8% RDP with 6% RUP to similarly reduce core body temperature and improve DMI in heat-stressed cows based on GABA concentrations. Comparatively, cows fed high proportions of RDP in the 6% RUP diet reported the lowest DMI and lowest GABA concentrations, which demonstrates the linear relationship between DMI and GABA concentrations that agree with Wang et al. (2013). The research provided herein for GABA concentrations on core body temperature and feed intake is novel research and further research needs explored to support our findings.

NRC Model Analysis

The NRC (2001) model predicted variable results when comparing allowable milk with actual milk yield (Table 4). Actual milk yields were greatly underpredicted by 35% for cows fed 8% RDP with 6% RUP (34.5 vs. 25.5 kg/d) and by 15% for cows fed 10% RDP with 6% RUP (31.5 vs. 27.5 kg/d) from the model, indicating an overestimation of requirements for cows fed

diets with 10 and 8% RDP with 6% RUP. For cows fed 8% RDP with 6% RUP, the predicted RUP supply was only 71% of the RUP required with a balance of -474 g/d. Therefore, the nutrient requirements for cows fed 8% RDP with 6% RUP were possibly lower than predicted due to improvement in nutrient utilization. Thus, the predictive ability of the NRC (2001) model is not well captured for improved nitrogen efficiency and nutrient partitioning in diets with lowered RDP and RUP. Inability to accurately predict milk production may be attributed to the lack of addressing the variability of AA capture in the mammary gland (Bequette et al., 2000). These observations are consistent with evaluations of the model presented by Cyriac et al. (2008) and Rius et al. (2010). However, an improved representative model for post-absorptive nitrogen partitioning would result in more accurate predictive measures for nitrogen requirements. Model adjustments would provide improved nutrient and protein management programs during warm climates and reduce nitrogen wasting to the environment.

Conclusion

Dietary reduction of RDP and RUP contributes toward sustaining milk production for lactating cows during warm climates. Feeding reduced RDP and RUP may improve nutrient partitioning and reduce catabolism of muscle, while supporting the synthesis of milk protein. Additionally, feeding high CP diets of 10% RDP and 8% RUP results in greater opportunistic loss of recycled nitrogen that could be used toward milk protein. Nitrogen efficiency was highest in lactating cows when feeding 8% RDP and 6% RUP in the diet, and the diet minimizes dietary nitrogen input and nitrogen output to the environment without compromising milk production. Formulating diets for heat-stressed cows using the NRC (2001) model overestimates

the requirements for lactating dairy cows. Therefore, the NRC model should be reevaluated and adjusted for feeding reduced RDP and RUP during warm climates to better predict nitrogen efficiency and milk production.

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APPENDIX

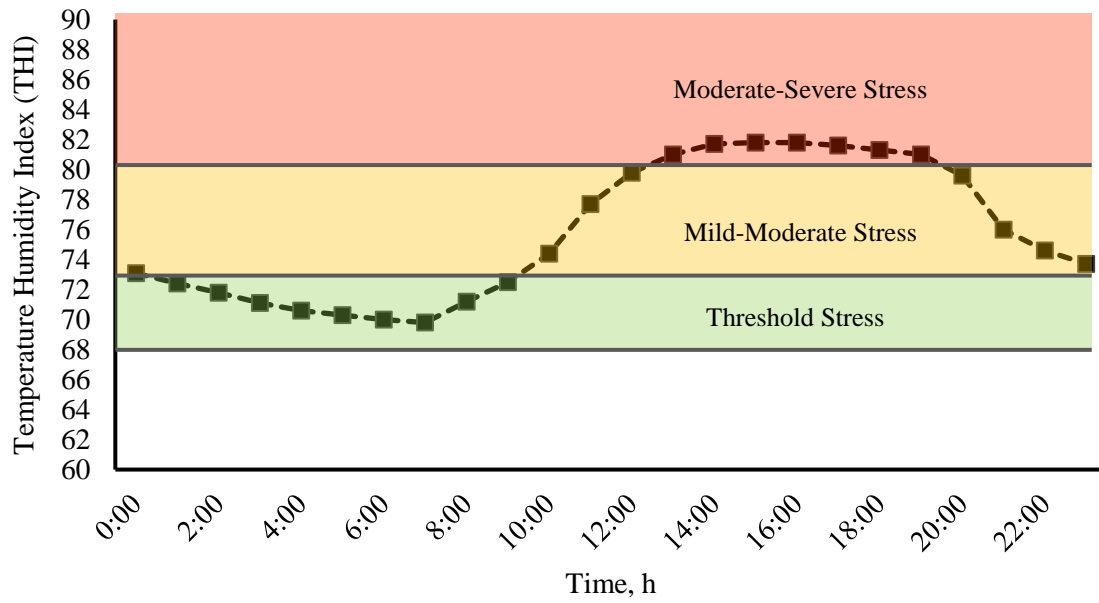


Figure 1. All cows experienced a circadian pattern of daily summer temperatures and relative humidity resulting in temperature-humidity index (THI), to mirror daily variation ranging from 21.2 to 31.5°C (79.8% humidity and 10 h of summer temperatures).

Table 1. Predicted nutrient requirements as determined from NRC (2001) on % of DM basis

Item	Experimental diet			
	10% RDP		8% RDP	
	8% RUP	6% RUP	8% RUP	6% RUP
Corn silage	45.0	45.0	45.0	45.0
Wheat silage	1.50	1.50	1.50	1.50
Clover hay	3.50	3.50	3.50	3.50
Corn grain, ground, dry	27.0	27.0	27.0	27.0
Soybean meal, solvent (48% CP)	12.1	5.60	2.70	5.80
Soybean hulls	-	6.40	2.40	6.60
Protected soybean meal ¹	5.60	2.60	11.9	4.00
Urea	-	0.75	-	-
Blood meal, ring dried	1.00	1.00	1.00	0.50
Fish meal, menhaden	0.80	0.70	0.50	0.40
MetaSmart, dry powder ²	0.35	0.35	0.35	0.35
BergaFat ³	0.30	2.50	1.10	2.30
Salt	0.35	0.35	0.35	0.35
Calcium phosphate (mono-) ⁴	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.70	0.70	0.70	0.70
Potassium carbonate	0.50	0.50	0.50	0.50
Calcium carbonate	0.60	0.60	0.60	0.60
Magnesium oxide	0.15	0.15	0.15	0.15
Calcium propionate	0.01	0.01	0.01	0.01
Trace mineral and vitamin mix ⁵	0.45	0.45	0.45	0.45

¹SoyPLUS, West Central Cooperative (Ralston, IA).

²Adisseo (Alpharetta, GA); Brand Contains 50% RDP and 50% RUP as pelletable form of Methionine.

³Berg+Schmidt Feed (Libertyville, IL); Rumen-stable fat powder.

⁴Contained 16.4% Ca and 21.6% P.

⁵AgCentral Cooperative (Athens, TN); formulated to provide (per kg of dietary DM): 12.4 x 10⁶ IU of vitamin A, 3.1 x 10⁵ IU of vitamin D, and 26.7 x 10³ IU of vitamin E, 1200 mg of Co, 2.6 x 10⁴ mg of Cu, 2100 mg of I, 8.3 x 10⁴ of Fe, 1.2 x 10⁵ of Mn, 2.1 x 10⁵ mg of Zn, and 600 mg Se.

Table 2. Observed chemical composition of the feed ingredients used in the experimental diets (% of DM basis)

Item	Ingredient ¹						
	Corn silage	Wheat silage	Clover hay	Conc. mix A	Conc. mix B	Conc. mix C	Conc. mix D
DM, % of feed	33.2	23.3	82.6	88.6	88.4	89.4	88.8
NDF	39.7	60.8	55.2	9.47	12.7	14.2	16.7
ADF	24.9	37.1	32.2	4.03	6.05	7.70	9.50
CP	7.70	13.3	13.0	26.5	23.7	23.8	20.0
NFC	44.1	14.4	19.7	-	-	-	-
Calcium	0.17	0.31	0.66	1.39	1.46	1.47	1.42
Phosphorus	0.21	0.33	0.31	0.65	0.59	0.53	0.53
Magnesium	0.16	0.16	0.23	0.37	0.39	0.35	0.36
Potassium	0.88	3.09	2.41	1.62	1.50	1.38	1.37
Sodium	0.007	0.013	0.025	0.78	0.73	0.78	0.72

¹Concentrate mix A was used to formulate the 10% RDP and 8% RUP diet, concentrate B was used to formulate the 8% RDP and 8% RUP diet, concentrate C was used to formulate the 10% RDP and 6% RUP diet, and concentrate D was used to formulate the 8% RDP and 6% RUP diet.

Table 3. ANOVA table for the statistical model

Effect ¹	Type	df	ddfm ²
RDP (D)	Fixed	1	87
RUP (U)	Fixed	1	87
Parity (P)	Random	1	
Date (T)	Fixed	6	492
$\beta(\chi)$	Fixed	1	87
D x T	Fixed	6	492
U x T	Fixed	6	492
Animal (D x U x P)	Random	87	
Residual	Random	492	
Total		601	

¹The RDP treatment (D) effect (10% or 8% RDP), the RUP treatment (U) effect (8% or 6% RUP), the parity block effect (primiparous or multiparous), the effect of the regression analysis of the covariate $\beta(\chi)$ for the variable of interest, and the effect of date (d 1 through 7).

²Denominator degrees of freedom of *F*-test.

Table 4. Composition of experimental diets and observed nutrient requirements as predicted from the NRC (2001) model using the chemical analysis of feed¹

Item	Experimental diet			
	8% RUP		6% RUP	
	10% RDP	8% RDP	10% RDP	8% RDP
DM ² , %	48.1	47.8	48.7	48.1
CP, % of DM	17.6	15.9	15.6	13.8
RDP, % of DM	9.60	7.80	9.70	7.80
RUP, % of DM	8.00	8.10	5.90	6.00
NDF, % of DM	25.4	26.9	28.3	28.7
ADF, % of DM	15.2	16.1	17.0	17.2
NFC, % of DM	47.9	47.2	45.0	46.7
Crude Fat, % of DM	3.60	4.70	5.70	5.60
NE _L , Mcal/kg	1.67	1.70	1.70	1.70
RDP required, g/d	2071	2028	1929	1992
RDP supplied, g/d	1927	1516	1790	1473
RDP balance, g/d	-144	-512	-139	-519
RUP required, g/d	1416	1582	1312	1615
RUP supplied, g/d	1606	1572	1086	1141
RUP balance, g/d	191	-10.0	-227	-474
MP required, g/d	2381	2295	2161	2260
MP supplied, g/d	2548	2286	1971	1858
MP balanced, g/d	167	-9.00	-190	-402
MP allowable milk, kg/d	41.3	34.7	27.5	25.5
NE _L allowable milk, kg/d	38.6	35.6	33.0	34.9

¹Actual ingredient analysis from Dairy One chemistry and actual DMI, milk yield, and components were used for each treatment. NRC (2001) ingredient composition was adjusted to mirror the actual chemical values of CP, NDF, and ADF or, in the case of the concentrate mixes, the composition that would be required to achieve the observed mix values.

²Actual DM of TMR of each treatment.

Table 5. Body temperature variables in lactating Holstein cows fed varying amounts of RDP and RUP during summer conditions in a.m. and p.m. values¹

Item	Experimental diet				SEM	Effect (<i>P</i> -value)		
	8% RUP		6% RUP			RDP (D)	RUP (U)	D x U
	10% RDP	8% RDP	10% RDP	8% RDP				
Rectal, °C								
a.m.	38.8	38.9	38.9	38.8	0.14	0.90	0.72	0.57
p.m.	39.7	39.9	39.8	39.6	0.15	0.76	0.61	0.30
Vaginal, °C								
a.m.	38.7	39.0	39.0	39.0	0.17	0.19	0.29	0.22
p.m.	39.0 ^b	39.4 ^a	39.4 ^a	39.3 ^a	0.14	0.18	0.15	0.04
RR ² , breaths/min								
a.m.	62.3	66.1	63.8	63.8	2.7	0.49	0.89	0.49
p.m.	85.7	90.9	82.8	87.1	3.1	0.14	0.28	0.88

^{a-b}Values within a row with differing superscripts denote RDP by RUP interactions ($P < 0.05$; $P < 0.10$).

¹Differences amongst a.m. and p.m. body temperature variables were significant for rectal and vaginal temperatures and respiratory rate ($P < 0.01$).

²Respiratory rate.

Table 6. Least squares means of intake, milk production and composition, BW, BCS, and energy balance for lactating Holstein cows fed varying amounts of RDP and RUP during summer conditions

Item	Experimental diet				SEM	Effect (<i>P</i> -value)		
	8% RUP		6% RUP			RDP (D)	RUP (U)	D x U
	10% RDP	8% RDP	10% RDP	8% RDP				
Intake, kg/d								
DM	20.1 ^a	19.4 ^{ab}	18.4 ^c	19.0 ^{bc}	0.32	0.98	<0.01	0.03
CP	3.60 ^a	3.10 ^b	2.93 ^c	2.64 ^d	0.05	<0.01	<0.01	0.03
NDF	5.57	5.69	5.64	5.97	0.11	0.03	0.09	0.29
ADF	3.31	3.39	3.43	3.66	0.07	0.02	0.02	0.24
Milk production								
Milk yield, kg/d	37.5 ^a	34.9 ^b	31.5 ^c	34.5 ^b	0.64	0.92	<0.01	<0.01
Lactose, kg/d	1.76 ^a	1.63 ^b	1.46 ^c	1.60 ^b	0.04	0.90	<0.01	<0.01
True Protein, kg/d	1.08 ^a	1.02 ^b	0.96 ^b	1.02 ^b	0.02	0.93	<0.01	<0.01
Fat, kg/d	0.99	1.04	0.92	1.03	0.04	0.15	0.61	0.80
SNF, kg/d	3.15 ^a	2.95 ^b	2.69 ^c	2.91 ^b	0.06	0.92	<0.01	<0.01
Lactose, %	4.74	4.71	4.65	4.65	0.02	0.38	<0.01	0.62
True Protein, %	2.95 ^b	3.00 ^b	3.16 ^a	2.99 ^b	0.04	0.14	0.02	<0.01
Fat, %	2.71 ^b	3.01 ^a	2.99 ^a	3.00 ^a	0.10	0.24	0.16	0.04
SNF, %	8.54 ^b	8.57 ^{ab}	8.68 ^a	8.50 ^b	0.04	0.05	0.42	<0.01
MUN, mg/dL	11.5	8.13	8.84	5.59	0.39	<0.01	<0.01	0.90
SCC, x1,000 cells/mL	110	116	93.3	167	38.1	0.10	0.50	0.17
ECM ¹ , kg/d	33.0 ^a	32.5 ^a	29.4 ^b	31.7 ^{ab}	0.76	0.28	<0.01	0.07
BW, kg	623	629	614	621	5.41	0.70	0.95	0.75
BCS	2.50	2.32	2.35	2.35	0.07	0.26	0.45	0.21
EBAL ² , Mcal/d	-0.15	-1.96	-0.98	-2.63	1.00	0.09	0.45	0.94

^{a-d}Values within a row with differing superscripts denote RDP by RUP interactions ($P < 0.05$; $P < 0.10$).

¹Energy-corrected milk calculated in equation derived from Tyrrel and Reid (1965).

²Energy balance.

Table 7. Nitrogen efficiency of lactating Holstein cows fed varying amounts of RDP and RUP during summer conditions

Item	Experimental diet				SEM	Effect (<i>P</i> -value)		
	8% RUP		6% RUP			RDP (D)	RUP (U)	D x U
	10% RDP	8% RDP	10% RDP	8% RDP				
Intake N, g/d	571 ^a	503 ^b	471 ^b	431 ^c	11.2	<0.01	<0.01	0.03
Milk N, g/d	150 ^a	124 ^b	93.6 ^c	125 ^{bc}	10.1	0.80	<0.01	<0.01
Predicted urine N ¹ , g/d	203	141	153	99.2	7.17	<0.01	<0.01	0.57
Predicted fecal N ² , g/d	197	196	162	170	12.8	0.78	0.03	0.76
N efficiency ³ , %	30.7	32.6	32.6	37.6	1.05	<0.01	<0.01	0.28

^{a-c}Values within a row with differing superscripts denote RDP by RUP interactions ($P < 0.05$; $P < 0.10$).

¹Predicted urine N output = 0.0283 x milk urea N (mg/dL) x body weight (kg); (Wattiaux and Karg, 2004).

²Predicted fecal N output = N intake – predicted urinary N – milk N.

³N efficiency = 100 x milk N / intake N.

Table 8. Relative amount of plasma metabolites of lactating Holstein cows fed varying amounts of RDP and RUP during summer conditions

Item	Experimental diet				SEM	Effect (<i>P</i> -value)		
	8% RUP		6% RUP			RDP (D)	RUP (U)	D x U
	10% RDP	8% RDP	10% RDP	8% RDP				
Glucose, mmol/L	3.02	3.10	2.94	3.16	0.07	0.04	0.91	0.35
Insulin, μ U/mL	21.1	18.5	20.7	13.1	1.55	<0.01	0.08	0.12
NEFA, μ Eq/L	118	164	172	173	19.7	0.12	<0.01	0.16
BHBA, μ mol/L	407 ^a	251 ^b	173 ^b	190 ^b	26.5	0.02	<0.01	<0.01

^{a-b}Values within a row with differing superscripts denote RDP by RUP interactions ($P < 0.05$; $P < 0.10$).

Table 9. Plasma AA concentrations (μM) of lactating Holstein cows fed varying amounts of RDP and RUP during summer conditions

Item	Experimental diet				SEM	Effect (<i>P</i> -value)		
	8% RUP		6% RUP			RDP (D)	RUP (U)	D x U
	10% RDP	8% RDP	10% RDP	8% RDP				
Total essential AA	918 ^b	1188 ^a	1141 ^a	1054 ^a	68.7	0.47	0.15	0.03
Arg	143	116	128	96.0	17.3	0.45	0.22	0.53
His	42.7 ^b	78.6 ^a	78.2 ^a	67.0 ^a	9.21	0.14	0.16	<0.01
Ile	93.8	121	106	114	9.54	0.08	0.80	0.34
Leu	135	168	154	160	11.3	0.11	0.66	0.25
Lys	108 ^b	148 ^a	139 ^a	133 ^a	8.79	0.06	0.34	0.02
Met	20.9 ^b	23.8 ^{ab}	29.1 ^a	23.8 ^{ab}	1.69	0.50	0.03	0.03
Phe	48.1 ^b	72.5 ^a	65.0 ^a	62.8 ^a	4.39	<0.01	0.38	<0.01
Thr	66.4	124	95.5	85.5	26.6	0.31	0.94	0.07
Trp	40.1 ^b	65.1 ^a	63.1 ^a	64.7 ^a	4.67	<0.01	<0.01	<0.01
Val	194	231	203	233	21.0	0.13	0.82	0.88
Total nonessential AA	1415 ^b	1762 ^{ab}	1973 ^a	1565 ^b	152	0.52	0.04	<0.01
Ala	181	226	216	285	24.4	0.02	0.06	0.62
Asn	38.3 ^b	71.4 ^a	75.7 ^a	33.0 ^b	7.75	0.52	0.95	<0.01
Asp	5.80	6.30	7.84	8.30	0.55	0.37	<0.01	0.97
Cit	47.7 ^b	56.8 ^{ab}	63.6 ^a	49.2 ^{ab}	5.52	0.62	0.45	0.04
Glu	128	114	156	120	0.01	0.87	0.56	<0.01
Gln	124 ^c	168 ^b	231 ^a	135 ^{bc}	9.35	0.02	0.09	0.25
Gly	585	706	740	555	15.2	0.11	0.02	<0.01
Orn	54.3 ^b	72.9 ^a	85.4 ^a	70.4 ^a	123	0.93	0.46	0.27
Pro	95.3	134	134	144	0.79	0.70	0.16	<0.01
Ser	84.3 ^b	128 ^a	167 ^a	69.1 ^b	4.96	0.73	<0.01	<0.01
Tyr	55.0 ^b	79.2 ^a	73.0 ^a	76.4 ^a	8.45	<0.01	<0.01	0.11
3-methyl-histidine	4.00 ^{bc}	6.65 ^a	5.80 ^{ab}	2.50 ^c	14.3	0.08	0.43	<0.01
GABA ²	0.091 ^a	0.057 ^{bc}	0.053 ^c	0.084 ^{ab}	5.27	<0.01	0.15	0.05

^{a-c}Values within a row with differing superscripts denote RDP by RUP interactions ($P < 0.05$; $P < 0.10$).

² γ -aminobutyric acid.

VITA

Jeffrey Daniel Kaufman was born in Fremont, IN to Michael and Jinger Kaufman. Growing up he developed strong interests in boating, fishing, running, and more outdoor activities. The northeast region of Indiana allowed Jeff to develop a strong interest for the care and development of animals (cows in particular). After attending and graduating from Fremont High School, he started his college career at Indiana University-Purdue University of Fort Wayne in Fort Wayne, IN in the fall of 2007 for a B.S. in Biology. During his undergraduate career, he worked toward gaining experiences and education for veterinary school. After mentorship and maturity, he developed an interest in research and developing new ideas, and followed through with his love for biochemistry, nutrition, biology, and dairy cows. Therefore after graduation in 2012, he pursued a master's degree in dairy cattle nutrition, and accepted a graduate research assistantship at the University of Tennessee-Knoxville, TN in 2014. Studying dairy cattle nutrition allowed for peak interests in nitrogen metabolism, which is the topic of this thesis and environmental effects and concerns. Jeff graduated with a Master of Science degree in Animal Science in August 2016. He is continuing with his educational career with a Doctor of Philosophy in Animal Science at the University of Tennessee-Knoxville, TN.