Effect of supplementing heat stressed dairy cows with electrolytes on milk yield, composition, and blood metabolites

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Animal and Dairy Science
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

August 2014

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Title of Study: Effect of supplementing heat stressed dairy cows with electrolytes on milk yield, composition, and blood metabolites

Pages in Study: 57

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This study was done to examine the effects of supplementing electrolytes to heat stressed transitioning cows on dry matter intake (DMI), milk yield (MY), and blood metabolites. Overall, 104 Holstein and Jersey cows were utilized from August to September, 2012, and from August to November, 2013. Control (CON) cows were fed standard TMR and E+ cows received the same TMR plus 170 g of Bovine BlueLite. The DMI, MY and composition, rectal temperature, and respiration rate were monitored daily; while blood metabolites, body weight, condition score and frame size (withers height, hip height, and heart girth) were measured weekly. The DMI, MY and composition were not different among treatments. Health condition, body change, and blood chemistry were not affected by treatment. Electrolyte supplementation did not have any negative effects on performance of dry and lactating cows, but showed positive potential for alleviation of heat stress in the present study.

DEDICATION

The author would like to dedicate this work to Jennifer Marte-Cabrera, my support, my best friend, and my blessing, for being here and living this unforgettable experience with me, helping me through my up and downs. You do not understand how much I love you and appreciate you! To my little champion Mauricio José, for being our complement and blessing during this tough but wonderful stage of our lives. You have fulfilled our hearts and day to day life with your presence, and along with your mother, you are my main source of motivation and inspiration. I love you! To Juan and Elina Cabrera, for molding me into the man I am today, and always orient me through the right path. This is for you two! To my only brother Marcos, for always being there, and sharing your love to me. To Jesus and Fresa Marte, for their support and motivation during this journey. I hope that this achievement from all of us, could serve as an illustration that everything is possible and achievable if you trust in God, believe in yourself, and work hard. To all my relatives and family, I love you all!

ACKNOWLEDGEMENTS

The author would like to first thank God because without him and his blessing, nothing would be possible. To the Fulbright program, for sponsoring and giving me the opportunity to complete my master program in the United States, allowing me to learn more and grow personally, and professionally. The author would like to acknowledge Dr. Stephanie Hill-Ward, for trusting in me and giving me the opportunity I chased from back home, and even without knowing me, taking me as her master student, and for her patience and comprehension to me during some of the tough stretches I have had to go through while being here at Mississippi State. I really appreciate you very mucho, and would not get this far without your advice and help. To TechMix, for their interest in our research project and for sponsoring this studies. The Author also would like to acknowledge his lab-mates, Adam Geiger (former lab-mate), Kelly Kalletsch (former lab-mate), Steven Musupole, and Grace Phri their help provided to the completion of these experiments. In addition, the author would like to thank the other graduate students at the department, for their motivation and help whenever I asked for their assistance. The author would also like to especially thank Drs. Daniel Valerio, and Hector Sanchez, for always being disposed to help me and for their disinterested counseling and advising regarding this project, and my master work. Also, the author would like to thank the MSU dairy employees, especially, Kenneth Graves, J.B. Gardner, for their assistance and help with the logistics of these studies. I would also like to thank, the faculty members

and employees of the Animal and Dairy Science Department at Mississippi State Unviersity, especially, Dr. Peter Ryan, Dr. Brian Rude, Dr. John Blanton, Dr. Jean M. Feugang, Dr. Jamie Larson, Dr. Erdogan Memili, Dr. Trent Smith, Jessica Graves, and others, for always trying to get the best out of us as graduate students, and for motivating us, and providing us with their knowledge and insight. In especial, I would like to thank Dr. Brian Rude and Dr. Caleb Lemley, by along with Dr. Stephanie Hill-Ward serving as my committee members. I really appreciate you all for being part of my committee, and for your help and advice with my thesis project and during my defense process. I would also like to especially thank Karin Lee, for her unconditional help and advice throughout this journey, and for embracing and making us feel like home here at Mississippi State University, and Starkville. Finally, I want to thank my family, especially my wife and son, and my parents, for their unconditional support and for always believing in me no matter the obstacles. I love you and appreciate you all so much!

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CHAPTER I

INTRODUCTION

Prolonged periods of increased ambient temperature and relative humidity, coupled with a relatively poor ability of dissipating their heat load, compromises the productivity of dairy cows, primarily by a decreased intake of dry matter, and a subsequent decrease of milk yield. Although several alternatives have been investigated and adopted during the past decades, especially in the Southeastern United States, the negative impact of heat stress on animal health and performance remains a major concern for the dairy industry. Accordingly, the dairy industry continues to demand complementing alternatives to alleviate the negative impact on animal performance. Electrolyte supplementation, principally sodium (Na) and potassium (K), plays a vital role in the productive behavior of cows exposed to heat stress by maintaining osmotic pressure and acid-based stability. Because blood acid-base equilibrium is often altered in heat stressed cows, and relies on the constancy between anions and cations present in the blood, electrolyte supplementation may support or increase dry matter intake and milk production during periods of heat stress by improving mineral availability, or by controlling blood acid-base homeostasis via decreasing dietary cation-anion difference (DCAD).

CHAPTER II

LITERATURE REVIEW

Heat Stress and Dairy Cattle

The impact that heat stress has on dairy cattle is of relevant importance, more markedly in hot and humid environments such as experienced in the Southeastern United States. Heat stress is caused by a negative environmental condition that compromises the performance of dairy cattle, particularly lactating dairy cows, farmed in hot and humid climates. It originates from the junction of different environmental factors such as temperature, relative humidity, solar radiation, air movement (wind speed), and finally precipitation (Bohmanova et al., 2007).

In southern states such as Mississippi, dairy cows are exposed to prolonged periods of increased ambient temperature and relative humidity, and when these two factors along with solar radiation and wind speed exceed the thermal comfort zone of dairy cows, cows experience heat stress as they lack the ability to dissipate their heat load resulting from the increased amount of metabolic heat and, the accumulated heat from radiant energy (West, 2003: Hammami et al., 2013). While suffering this condition, dairy cows' performance is affected at first by a decrease of intake of dry matter and nutrient utilization(West, 1994; Wheelock et al., 2010) as a physiological means of regulating their own body temperature, by decreasing rumen fermentation and the metabolic rate. This reduction in DMI, results in a subsequent decrease in milk production (Bohmanova

et al., 2007; Boonkum et al., 2011) by a reduction in available nutrients for milk synthesis (Suadsong, 2012). In addition, a failure of reproductive performance mainly caused by an increase in days open, which led to increase reproductive culling rates (De Rensis and Scaramuzzi, 2003) has been reported, all of which simultaneously affect the farmer's economic returns (St-Pierre et al., 2003). The temperature humidity index (THI) is an indicator that has been established to express the combined effects of temperature and humidity has on dairy cattle's comfort (Ravagnolo and Mistzal, 2000: Suadsong, 2012). It has been reported that during a THI of 72 or greater, dairy cows begin to display signs of heat stress (Armstrong, 1994; West, 2003). In fact, Johnson et al., (1963), in a considered classical investigation, found that MY and DMI declined when maximum THI was 77, and then further research by Igono et al. (1992) established critical values for minimum, mean and maximum THI as 64, 72, and 76, respectively.

In the Southeastern US, the hot season is considerably long, and there is extreme radiant energy for the same period of time. In these prolonged periods of increased ambient temperature and relative humidity, cows are subject to overcome the effects of heat stress; however, normally the incidence and impact of heat stress translates into serious losses of production and returns of dairy farms due to the reduced performance of animals. In previous work, with no heat abatement being implemented, across animal classes in the United States, heat stress losses were reported to totally ascend to an average of nearly $2.4 billion annually, whereas, when optimum heat abatement was implemented, these total annual losses were reduced to about $1.7 billion (St-Pierre et al., 2003). From these figures, about $0.9 to $1.5 billion affect the dairy industry, $370 million for the beef industry, around $307.5 million to the swine industry, and $146.5

million for the poultry industry, with Texas, California, Oklahoma, Nebraska, and North Carolina accounting for up to a 43% (or $728 million) of the annual losses nationally across states. Clearly, it can be observed that dairy is the livestock industry being most economically affected by heat stress, as it accounted for approximately 63% of the total estimated losses, which makes the development of more improved and efficient heat abatement systems a priority to aid in the alleviation of the strains of heat stress by dairy cattle. Some of the factors believed to be behind these dairy industry losses involve: decreased milk yield, increased incidence of metabolic disorders and health issues (such as rumen acidosis and death), sluggish heifer growth, compromised quality of milk, and decreased reproductive performance (Collier et al., 1982; West, 1999).

As stated previously, the whole Southern US is exposed to long periods of increased hot weather and humidity, and certain Southern locations can experience heat stress for as much as nearly half of the year (West, 2003). Different management strategies have been determined and implemented in the United States to combat and alleviate the strain of heat stress. In a review, Beede and Collier (1986) identified three key techniques to reduce the impact of heat stress: 1) implementing shading and cooling systems to physically adjust the environment, 2) genetically developing heat-tolerant breeds, and 3) nutritional manipulation. As a matter of fact, the second strategy proposed in the previous mentioned review, could be complicated, as the majority of dairy cattle investigations has tended to be oriented on genetically improving milk production and on supplying nutrients to cows during early lactation, while short attention has been put to the improvement of the thermoregulatory capability of the cow, meanwhile her yield capacity has been increased (Ravagnolo and Mitsztal, 2000). Not to mention, that along

with this increase of MY, body heat production increases from metabolism, feed uptake, and digestive needs increase with yield, (West, 2003). Along with others, the use of shade, misters, foggers, and pad cooling, have been reported to help adjust the environment and ameliorate thermal heat load. By increasing sensible heat and/or increasing evaporative heat loss using sprinklers to wet the animals, the ability of the animals to dissipate their body heat could be improved, as ground and air temperature can be reduced. However, sensible and evaporative heat losses are interdependent, thus it is not effective if one is used without the other (Henry et al., 2012). Shading represents an easy and cost-effective method implemented to minimize heat coming from solar radiation, which should be included in the first steps to be taken when attempting to reduce the strains of a hot environment and to protect the cow from solar radiation (West, 2003). However, for dairy cows farmed in heat stress disposed environments, supplementary cooling is recommended as, though shade helps ameliorate the amassing heat from solar radiation, air temperature and relative humidity are not affected (West, 2003). In addition, St-Pierre and collaborators in 2003 provided a couple other strategies for decreasing heat stress to be applied regionally for dairy cattle. These strategies of heat abatement were: 1) moderate heat abatement (by forced ventilation using fans), 2) high heat abatement (with the effectiveness of this level coming from a combination of fans and sprinklers), and 3) intense heat abatement (due to the cooling properties of a high-pressure evaporative cooling system). Together, these heat abatement strategies have been widely used and demonstrated to have a positive effect for reducing the magnitude of the annual losses by the dairy industry.

Although several alternatives have been investigated and adopted during the last decades to negate the effects of heat stress, especially in the Southeastern United States, the negative impact of heat stress on animal health and performance remains a major concern for the dairy industry, as production continues to decrease during the summer (St-Pierre et al., 2003; Wheelock et al., 2010), and the dairy sector demands complementing alternatives to alleviate the negative impact of heat stress on animal performance.

Effect of Heat Stress on Dairy Cows Performance

Although dairy cows have several mechanisms to aid them with getting rid of their own body heat and maintain their body temperature, thermal stress reduces their overall performance primarily by a reduced DMI, and then subsequently a drop in MY, accompanied with failed reproduction and compromised health. In this particular study, the effects of heat stress will be discussed by physiological period, either during the gestational or dry period, and the lactating period.

As it is known, during the transition period of lactating cows from late gestation of dairy cattle, the fastest rate of fetal growth is experienced, amassing nearly 60% of the birth weight it will be born with, with two months of gestation remaining (Bauman and Currie, 1980), thus, reducing the impact of thermal stress during this period of time can have important benefits (Staples and Thatcher, 2011). In fact, cows that went into heat stress the last few weeks of gestation gave birth to calves with up to a 10% decreased weight (Collier et al., 1982b; West, 2003). As reported by Staples and Thatcher, 2011, no alleviation from heat stress conditions was observed during the last two months of gestation when cows were exposed to heat stress conditions, and significant alleviation

was observed when cows were provided relief from thermal stress, respectively. Although, upon parturition cows were managed the same, heavier calves (approximately 3 kg greater) were born from cows provided abatement from thermal stress, with more milk being yielded during the next lactation. Conversely, cows those cows that were unprotected from heat stress, had less DMI, which, as reported, was confirmed by greater plasma nonsterified fatty acids (NEFA), which also may have limited the growth of the fetus during the last three months of gestation, and lead to lighter calves at birth and decreased milk production. Along with that, the importance of this critical period is complemented with other factors that occur during the transition period such as the massive mammary gland growth, and cell turnover, which happens before calving, and has been claimed to have an effect on future milk yield of the cows (Capuco et al., 1997; Sorensen et al., 2006). Because dry cows generate less metabolic heat than lactating cows (West, 2003), they have a greater maximum critical temperature (Hahn, 1999), which is the temperature at which the animal starts to increase the production of heat as result of an increment in body temperature from inappropriate evaporative loss (Yousef, 1985). However, heat stress remains a critical threat to the performance of dairy cows during late gestation, and the negative consequences can carry over and affect the subsequent lactation and this factor has been further demonstrated (Wolfenson et al., 1988; do Amaral et al., 2009; Tao and Dahl, 2013).

In lactating cows, although the reduction of DMI resulting from heat stress has generally been claimed to be the original cause for the subsequent decline in milk production (Fuquay, 1981; Collier et al., 1982; West, 2003), the exact input of decreased feed intake to the total reduction in milk yield is unknown. To follow up this unknown

fact, Baumgard et al., (2007,) utilized a group of thermal neutral pair-fed animals in an attempt to separate the confounding effects of nutrient intake on milk yield. In this experiment, lactating Holstein cows at mid-lactation were managed either 1) cyclically heat stressed (THI ~ 80 for 16 hr/d) during 9 days, or 2) remained in constant thermal neutral conditions (THI ~ 64 for 24 hrs/d) but pair-fed with heat stressed cows to assure the maintenance of a similar nutrient intake by both groups. Cows were fed individually, and had adlibitum access to a TMR consisting primarily of alfalfa hay and steam flaked corn to either meet or surpass their nutrient requirements, (NRC, 2001). Heat stressed cows had an average rectal temperature of about 41°C during the afternoons that the treatment was applied. Immediately, heat stressed cows showed a reduction of ~5 kg/d in DMI. As designed, cows pair-fed and housed in a thermal-neutral environment had a feed intake pattern similar to heat stressed cows. Then, they calculated that heat stress reduced milk yield by approximately 14 kg/d and that production continuously declined during the first 7 days of treatment, after which it plateaued. Thermal neutral pair-fed cows also had a reduction of MY by approximately 6 kg/d. Based on these findings, they calculated that the reduction in DMI is responsible for about 40 to 50 % of the decrease in MY when cows are heat stressed. Therefore, 50 to 60 % of MY losses are due to other types of changes caused by the strain of heat stress. Rhoads et al., (2009,) showed similar results, when claiming that the reduction of nutrient uptake by the indirect effects of heat stress only accounts for 35 % of the decreased milk production influenced by heat stress. These authors also claimed that, to a considerable extent, the direct effects not brought about by decreased feed intake can be the consequence of changes in energy intake and independent factors in the partitioning of nutrients. As eluded before, the main strain of

heat stress is the reduction in DMI and MY provoked to dairy cows. In a report done by St-Pierre et al., (2003,) production losses of up to 2,072 kg/cow/year were calculated across states with the greater losses being caused in Southern states. These losses were experienced as cows in Southeastern states such as Florida, expend nearly half (or 50%) of their total annual hours under ambient conditions that result in thermal stress (St-Pierre et al., 2003). In fact, several reports have described that in hot and humid ambient conditions dairy cows decreased the amount of milk yielded by up to 40% when no heat alleviation strategies where utilized (Fuquay, 1981; Igono et al., 1992; Rhoads et al., 2009; Wheelock et al., 2010).

In their study, Wheelock et al., (2010,) combined thermal abatement and nutritional management strategies and studied the potential effects of heat stress on the metabolism of energy, dry matter intake, and milk production of lactating cows. They utilized 22 multiparous Holstein cows and assigned them to either: i) 7 days with ad-libitum feed intake and thermoneutral conditions (P1); ii) 7 days of either heat stress and ad libitum intake or they were pair-fed in thermoneutral conditions (PF); or finally iii) 7 days of both heat stress and ad libitum feed intake or being pair-fed in thermoneutral conditions with recombinant bovine somatotropin being administered on d 1 (P3). Findings from this trial demonstrated an increase in health condition parameters (rectal temperature and respiration rate) in stressed cows. Although it was noticed that milk yield was increased by approximately a 10% due to rBST supplementation, the overall DMI and milk yield decreased by 30% and 27.6%, respectively, for heat stress cows.

Milk composition is also compromised when cows experience heat stress. Bandaranayaka and Holmes (1976,) exposed two pairs of Jersey cows to either 15 or

30°C air temperatures, and found that the protein and fat concentrations of milk decreased at 30°C, with intake being maintained similar at the two temperatures. The authors claimed that this decrease in milk protein and fat was positively correlated to decreases in the amounts of acetate in the rumen, and also to a modest decline in ruminal pH when the air temperature was at 30 °C. Similar results were obtained in Central Italy by Bernabuccie et al., (2002,) when 40 Holstein cows in mid lactation (~141 DIM) were used to determine the effects of the hot season on milk protein fractions. Animals were balanced for parity; DIM, genetic index for milk production, and fed a total mixed ration, plus concentrate which was provided in self feeders. Of the total 40 cows, 20 were monitored for six weeks during Spring (from March to April), and the remainder 20 for six weeks during Summer (from June to August). During Summer cows had greater rectal temperatures than during the spring (39.8 vs. 39.0 ^{o}C, respectively), but decreased DMI than estimated during the spring (18.6 vs. 23.2 kg DM/d, respectively). It was also noted that Summer studied cows consumed less concentrate than cows in the spring (–0.96 kg/cow/d on average). Milk yield during the Summer was 10% less ($P < 0.01$) than during the spring (26.7 vs. 29.5 Liters/d, respectively), while milk protein percentages were 9.9% lower ($P<0.01$) in the summer than in the spring (3.01 vs. 3.31%, respectively). Along with that, milk from cows in the summer was reported to have less crude protein as well as casein. It was concluded that these results indicate that this reduction of milk protein in the milk of Summer cows, was the result of decreased casein content, which may affect the quality of elaborated milk products during the Summer months.

Blood chemistry can also be harmed by heat stress. Respiration is affected by blood pH, with 7.4 being a standard. If blood pH exceeds 7.4, then respiration is increased, whereas, when pH is below 7.4, respiration is decreased. Blood pH depends mainly in the relative concentration of carbonic acid and base bicarbonate in the blood (Coppock et al., 1982). In a study by Schneider et al., (1988,) blood pH was decreased in animals affected by thermal stress, as did blood pCO_2, which at a concentration under 40 mmHg, can stimulate respiration, while greater concentrations can inhibit it. Kadzere et al., (2002,) claimed that HCO_3^- and pCO_2 are at a constant ratio of 20:1 and that as pCO_2 concentrations are reduced due to heat stress, the kidney secretes HCO_3^- and HCO_3^- levels are reduced as pH in urine is incremented as result of increased bicarbonate secretion via urine. Moreover, in high ambient temperature, cows attempt to cool the body by evaporative cooling (panting respiration), with this rapid loss of CO_2 results in respiratory alkalosis. Then cows compensate by increasing urinary output of HCO_3^-, but, permanent replacement of this ion is vital to the adequate maintenance and management of blood acid-base balance. During periods of heat stress, dairy cow's dietary requirements of the vital electrolytes, Na^+, K^+ and $HCO3^-$ are considerably incremented (Kadzere et al., 2002), and the balance of dietary electrolytes is especially important in locations where environmental temperatures exceed 24 ºC and is exacerbated if relative humidity exceeds 50%. The term cation to anion balance is utilized to make reference to the physiological interrelationships among Na, K, and Cl (Leach, 1979). Heat stressed cows have been found to have reduced concentrations of electrolytes, especially Na and K, in the rumen and blood, reportedly as result of the increased loss of Na in urine and K as the form of sweat (Tucker et al., 1988; Schneider et al., 1988).

Electrolytes Supplementation and Heat Stressed Dairy Cows

The acid-base equilibrium of animals depends on the balance between anions and cations in the blood and can absolutely affect the performance of the animals (West et al., 1991). In hot and humid environments such as the Southeastern part of the United States, during heat stress the blood chemistry of the cow is influenced by distinct factors such as the incremented Na losses in urine, and the cost of dissipating heat, the sweat in which considerable amounts of K are lost (Schneider et al., 1988; Tucker et al., 1988). In addition, due to decreased feed uptake during heat stress, cows' activity is reduced, including the fact that they ruminate less and therefore generate less saliva. This drop in production of saliva and HCO3- content in saliva, along with decreased amount of saliva entering the rumen, dispose cows during heat stress to be way more sensible to sub-clinical and also acute rumen acidosis (Kadzere et al., 2002; Baumgard et al., 2007)

This situation reflects an opportunity for dairy cows taking advantage of positive effects of increased dietary cation-anion difference (DCAD) in their diets as confirmed by previous research (West et al., 1991; West et al., 1992). The DCAD for lactating cows have been established to be of more importance than individual ingredients (Delaquis and Block, 1995b), cation origin (West et al., 1992), or the individual concentration of the electrolyte (Tucker and Hogue, 1990). Studies have concluded that increased DCAD during times of elevated temperature can positively affect blood HCO_3^-, DMI, and milk yield (Sanchez et al., 1994; West et al., 1991). This further confirms the hypothesis that responses to DCAD may differ depending upon climatic and environmental conditions (Chan et al., 2005).

During hot weather, the reduced intake of dry matter and increased lactation demands require increased dietary mineral concentration in the diets. In spite of that, alterations in mineral metabolism are also present and affect the electrolyte status of the cow during hot weather (West, 1997). However, during heat stress, modifications in mineral metabolism also affect electrolyte status of the cow. Like eluded before, the main cation in bovine sweat is K, and sudden increments in the flow of K through sweat happens during hot weather conditions, as the cows are forced to get rid of their heat load by evaporative loss (Johnson, 1967; Jenkinson and Mabon, 1973; Mallonee et al., 1985). In addition, increased amount of Na is excreted with bicarbonates via urine to compensate for the respiratory alkalosis that can result during heat stress in cows (West, 2003). However, the absorption of these minerals, especially macrominerals, has also been affected as cows undergo thermal stress. A decline in the absorption of macrominerals, including Ca, P, and K, was reported during hot temperatures (Kume et al., 1987; Kume et al., 1989). In fact, trace element requirements may also increase in elevated temperature environments (Kume et al., 1986).

Studies supplementing K well more than the recommended by NRC, 1989, in hot environmental conditions, reported that lactating cows responded with greater milk yield (Schneider et al., 1984; Mallonee et al., 1985; West et al., 1987), while those supplemented with around 0.55% Na (total dietary sodium) during the hot period likely demonstrated improved feed intake and milk production when compared to those just receiving 0.18% Na (total dietary sodium) (Schneider et al., 1986). Providing diets high in Na and K with normal Cl, were found to result with increased DMI and MY, when correlated to diets high in Cl and normal for Na and K (Escobosa et al., 1984). The

improved intake or milk yield observed when more alkaline rations were offered to lactating dairy cows should be the consequence of more improved blood buffering or correction of mineral demands, and it is very difficult to separate both effects.

West et al., 1992, ran two separate but simultaneous 4 x 4 replicated Latin square studies, where 16 Holstein cows (8 cows per study) were used during the summer to assess the effects of dietary cation source (Na or K) and incrementing the dietary cation to anion balance (expressed as milliequivalents of Na + K - C1 / kilogram of feed DM) within source of cation (control = 120.4 mEq/kg of feed DM; Na source = 219.7, 347.8, 464.1 mEq/kg of feed DM; K source = 231.2, 352.6, 456.0 mEq/kg of feed DM). Maximum and minimum temperatures averaged 26.7 and 15.0 °C during the cool phase and 32.3 and 22.5 °C during the hot phase of the study. It was found that body temperatures were increased by environmental conditions but not by dietary cation-anion balance. They claimed that the differences found in body temperature were perhaps linked to observed differences in the body weight of cows rather than to dietary treatment, as during the Na source Latin square cows had greater body weights than in the K source Latin square. The DMI increased linearly, but no change was observed in milk yield and FCM with the increase of dietary cation to anion balance, nor any cation source influence. Milk fat and protein concentrations were not changed by dietary cation to anion balance. They concluded that the alterations in blood acid-base chemistry with increasing dietary cation to anion equilibrium were as expected, while greater blood buffering capability illustrated by the blood base excess and bicarbonate content should be responsible for the improvement in feed intake.

In another study, Chan et al., 2005, studied the effects of increasing DCAD (milliequivalents of (Na + K − Cl − S)/100 g of DM) on DMI, milk yield and composition, serum electrolytes, and blood chemistry to elucidate the optimal DCAD:S for early lactating cows during moderately cool weather. A total of 33 early lactation Holsteins cows (15 primiparous and18 multiparous) were fed rations with dietary cation-anion difference, containing 20, 35, or 50 mEq/100 g of DM from d 0 up to 42 d postpartum. For DCAD of 20, 35, and 50, the authors observed that when the DCAD was increased from 20 to 35 mEq/100 g of DM, there was no positive effect on DMI (3.30 vs. 3.38 kg/100 kg BW, respectively; $P > 0.10$), but a decreased DMI was observed when the DCAD of the diet ascended from 35 to 50 mEq/100 g of DM (3.38 vs. 2.96 kg/100kg BW, respectively; $P < 0.05$). Milk yields in this study were similar and not affected by DCAD (25.5, 24.2, and 22.4 kg/d, respectively; $P>0.10$). Similarly, no differences were encountered for yield or concentration of milk fat or protein ($P>0.10$). The authors concluded that a DCAD of between 23 and 33 mEq/ 100 g of DM seems to be appropriate to dairy cows during cool weather, with a DCAD of 50 mEq/100 g of DM being perhaps excessive and too alkaline or unpalatable to the animals, thus resulting in decreased DMI but, not in MY. This alkalinity of the 50 mEq/100 g of DM treatment was indicated by serum HCO_3^- (27.6 mEq/L), which was at the top end of the normal physiological range (21.5 to 27.7 mEq/L) described by Benjamin (1981).

In this same line of research, Sanchez et al., (1994,) found results to some extent contradictory. They found that that blood HCO_3^- was maximized when feeding diets with a DCAD of 38 mEq (Na + K − Cl)/100 g of DM, whereas a DCAD within 17 to 38 and 25 to 40 mEq/100 g of DM maximized DMI and milk yield, respectively. Hu et al.,

(2007,) used 6 lactating Holstein cows (~44 DIM) in a 6 x 6 Latin square design. Cows were fed diets with DCAD of -3, 22, or 47 mEq (Na + K – Cl – S)/100 g DM and the effect of this nutritional manipulation during early lactation was assessed. The DCAD was changed by just utilizing $CaCl_2$ (-3 mEq (Na + K – Cl – S)/100 g DM), or combining different concentrations of K_2CO_3, and $NaHCO_3$ (22, or 47 mEq (Na + K – Cl – S)/100 g DM) in the concentrate mix. As the DCAD of the diet increased, so did DMI linearly (24.4, 25.9, and 27.6 kg/d, respectively; $P < 0.01$). A similar effect was illustrated for FCM (30.7, 32.8, and 34.2 kg/d, respectively; $P < 0.01$). Likely, the fat (2.99 vs. 3.60%, respectively; $P < 0.02$) and protein (3.11 vs. 3.24%, respectively; $P < 0.01$) concentrations showed a rise when DCAD was increased from -3 to 47 mEq. These results illustrate some of the positive effects of increasing the diet DCAD during early lactation, as the DMI, MY and composition, were improved.

Summary and Objectives

Heat stress has a significant impact on dairy cattle particularly of those in hot and humid climates such as the Southeastern United States. It is a very costly issue that affects the dairy industry as a whole as it represents an economic burden to the industry. Although several alternatives have been investigated and adopted during the last decades to negate the effects of heat stress, and have been of very useful help to the dairy industry as a whole, by helping reduce the immense annual losses faced by the industry, particularly in the Southeastern United States, the negative impact of it on animal health and performance remains a major concern for the dairy industry, as production continues to decrease during the summer (St-Pierre et al., 2003; Wheelock et al., 2010). In fact, it has been stated that even on well managed and well cooled dairies, thermal stress can still

reduce feed intake by 10 to 15% with these numbers varying according to the severity of the hot season (Collier and Beede, 1985; Armstrong, 1994; West, 2003).

Thus, it is clear that the dairy sector demands complementing alternatives along with different heat abatement strategies and techniques to reduce and alleviate the negative impact of heat stress on animal performance. Electrolytes are a key element of acid-base chemistry and their supplementation during heat stress may be critical to aid the homeostatic mechanisms (West et al., 1997), but data establishing the potential effects of supplementing electrolytes to dry dairy cows and during the transition period on their performance is very scarce. Electrolytes, principally Na and K, play a vital role in the productive behavior of cows exposed to heat stress by maintaining osmotic pressure and acid-based stability. Thus, because blood acid-base equilibrium is often altered in heat stressed cows, and relies on the constancy between anions and cations present in the blood, we believe that electrolyte supplementation could support or increase DMI and MY during heat stress periods by improving mineral availability or, by maintaining blood acid-base stability improving the DCAD, which has been reported to be more important than single ingredients, cation derivation or, individual electrolyte density in the diet.

Therefore, our primary objective of this study was to evaluate the potential effect of supplying an electrolyte pelleted supplement (Bovine BlueLite, TechMix, Inc; Stewart, MN) to dry and lactating dairy cows on dry matter intake, blood chemistry, and milk production. A secondary objective was to evaluate the capability of the electrolyte supplementation to alleviate heat stress during the transition period of dairy cows.

CHAPTER III

MATERIALS AND METHODS

Between August and September, 2012, (67 dry cows (Control, n= 36; E+, n=31)), and August and November, 2013, (37 dry and lactating cows (Control, n=19; E+, n=18)) for a total of 104 Holstein and Jersey, cows and heifers, were housed in a free barn and maternity lot of the Mississippi State University Joe Bearden Dairy Research Center (JBDRC) to determine the effects of feeding Bovine BlueLite from -21 to 30 DIM on the DMI and MY of heat stressed dairy cows, during the transition period. Approximately three weeks prior to their expected calving date, cows were assigned to one of two treatments, which were: A standard dry cow ration balanced with 14 mEq (K + Na – Cl – S)/ 100g DM (CON); containing corn silage, ryegrass and alfalfa baleage, whole cottonseed, and a concentrate mix; or a treatment diet balanced with 17 mEq (K + Na – Cl – S)/100 g DM, using the same standard dry cow ration, with supplemental electrolytes which was dressed over the ration (E+; Bovine Bluelite Pellets). Prior to calving, all cows had access to a small exercise lot, and were group fed ryegrass baleage in the AM and TMR in the PM (CON) or the same base ration plus 170g/d of electrolyte (E+, Bovine Bluelite, TechMix, Inc; MN) providing balanced electrolytes (0.55% Ca; 0.30% P; 9.60% NaCl; 8.25% K; 0.14% Mg). Post-calving, CON cows were fed standard TMR and E+ cows received the same TMR plus 170g/d of Bovine Bluelite. Prior to

calving, cows were managed and fed as a group in a near-by free stall barn; meanwhile, post-calving lactating cows were managed and fed in groups but using Calan Gates® (American Calan©, Northwood, NH) where individual DMI was recorded. Subsequent to the first dry period, lactating cows remained on their treatments until 30 DIM, and then once they completed the trial, were moved and managed together with the rest of the herd animals and fed a standard herd diet.

Cows from both treatments received diets with similar dietary nutrient concentrations and composition during the dry and lactating periods of this study (Tables 3.1, and 3.2; respectively). However, as illustrated in Table 4.1, the forage concentration was decreased while the grain mix was augmented in the lactating cow ration, in order to account for the increased energy demand during early lactation.

Sample Collection and Analysis

Body weight (BW), condition score (BCS), and frame (wither height (WH), hip height (HH), and heart girth (HG)) were measured weekly before the pm milking. Health condition (rectal temperature, and, respiration rate and score) was monitored daily normally before the pm milking to determine any sign of heat stress. Respiratory condition was defined from 1 to 5, with: 1) Normal, 2) Runny Nose, 3) Heavy Breathing, 4) Moist Cough, and 5) Dry Cough. For the cows managed in Calan gates®, on a daily basis during the morning feeding, individual DMI was recorded both prior to calving and post-calving. Milk yields were obtained daily and once every week milk samples were taken and shipped to Mid-South Dairy Records (DHIA) to be analyzed for fat, protein, solids-not-fat, lactose, and somatic cell count. Weekly, feedstuffs and ort samples were taken and compiled by month, and by treatment and week, respectively, for later

subjection to proximate analysis. All feedstuffs and ort samples were ground to pass through a 2-mm screen using a Thomas Wiley Mill ® (Arthor H. Thomas, Philadelphia, PA) and analyzed for dry matter, ash, neutral detergent fiber, acid detergent fiber, and crude protein.

To determine the dry matter content, 2 g of the sample were placed in an aluminum pan (which was previously dried and weighted) and dried in a 100 °C oven for not less 24 hours and then weighed afterwards. Immediately after the dry matter analysis was finished, the ash content was determined by placing the same used sample in a muffle furnace set at 500° C for five hours, and subsequently the remaining samples were allowed to cool off to 100° C and then were weighed. To realize the fiber analysis, 0.5 g of sample were placed in an Ankom ® nylon bag which was heated and sealed.

To determine neutral detergent fiber content, the sample bag was subjected to digestion for one hour in 2000mL of neutral detergent fiber solution (Goering and Van Soest, 1970) at 100° C, including 20 g of sodium sulfite and 4 ml of α – amylase (4.2 mg/mL). After one hour, samples were rinsed three times. The first two washes were made-up of 2000 mL of hot distilled water and 4 mL of α – amylase (4.2 mg/mL), and the last one was composed of 2000 mL of hot distilled water. After that, samples were rinsed once with acetone. Then, the samples were placed in a 100° C oven for not less than 24 hours and then weighed.

To obtain the acid detergent fiber, the same sample bags used for the neutral detergent fiber procedure were digested at 100° C in 2000 mL of acid detergent fiber solution (Goering and Van Soest, 1970) for one hour. After that time, just like for the NDF procedure, the sample bags were rinsed three times, but this time just with hot

distilled water and once with the acetone. Samples were then dried at 100° C in an oven for not less than 24 hours and weighed. The crude protein content was obtained using the Kjeldahl nitrogen method (AOAC, 2003). A 0.9 g portion of sample was weighted and placed on a FisherTab ™ paper (Thermo Fisher Scientific Inc., Waltham, MA), and along with 15 mL of H_2SO_4, were settled in glass tubes, and subsequently digested at 415° F (or 212.78 ° C) during 3 hours. After that, the actual crude protein content was obtained by distilling and titrating the digested samples using a distillation unit from Foss Kjeltec 1035 Analyzer ™ (Foss, Eden Prairie, MN). Because of high coefficient of variation error, some samples were re-run to have a more accurate and precise estimate of the nutrient content of the feedstuffs used in this study.

Blood Collection

Baseline blood samples were obtained prior to the beginning of each trial from all the cows that were going to be used. After that, blood samples were obtained once weekly immediately after body weight and frames were measured. During the 2013 trial, dry cows were not weighed during the last two weeks (-2, -1 wk) prior to their expected calving date, to prevent stress and early parturition incidence. Blood samples were taken from each cow via jugular venipuncture, using two evacuated tubes from 10 ml, a red-top tube with no anticoagulant (Fischer Scientific) and a green-top tube containing lithium heparin (from the same provider). Samples were kept cooled and immediately after the collection was finished, the heparin containing tubes were taken to an on-site lab at the JBDRC were they were analyzed for blood gas and electrolyte concentration (pH, HCO3, tCO2, pCO2, Anion Gap, Na+, K+, and Cl-) and for hematocrit values. Blood gas and electrolyte concentration was determined utilizing an IDEXX Blood Gas and Electrolyte

Analyzer (IDEXX Laboratories, Westbrook, ME), while the hematocrit values were determined by drawing a portion of the blood samples into micro-centrifuge tubes and by centrifuging them in a micro centrifuge for approximately two minutes. After that the hematocrit values were obtained. Blood samples collected in the red-top tube with no anticoagulant were centrifuged within no more than an hour of being collected at 1,057 x g at 4° C for 20 minutes and then stored at -20° C.

Weather Data Collection

A weather station (Hobo U30/NRC, Onset Computer, Pocasset, Mass.) was situated near by the freestall barns where the cows were housed during the time of the trial, to assess the change in environmental conditions by determining some of the ambient status parameters such as the temperature and the relative humidity, precipitation, solar radiation, and the speed of the wind. This data was attained with 4 h intervals, and was then compiled to obtain daily means. Then, using these means, the daily mean minimum and maximum temperature humidity index (THI) was worked out using the equation THI = 0.8*AT + ((RH/100)*(AT-14.3)) + 46.4, provided by Mader and Associates (2004), where RH= relative humidity (%) and AT= ambient temperature (° C).

Statistical Analysis

The data from this study was assayed using the MIXED procedure from SAS (Version 9.2, SAS Institute Inc, Cary, NC, 2004). The model statement was composed of cow id, the time measure (week or day), the treatment (CON or E+), the breed, the THI, and in some cases, the year and the parity, with all effects and interactions being

examined as well. The time measure, in some cases, week, or in others, day, should be interpreted as the time relative to calving. During the data analysis, according to needs, the time measure was computed as a repeated measure. Significance and trends were stated at $P < 0.05$ and $P>0.05$ but $P< 0.10$, respectively.

Table 3.1 Ingredient composition of diets fed to dry and lactating cows with or without electrolyte supplementation (DM basis).

Dry Cow Ingredient, %		
	Con	E+[1]
Ryegrass Baleage	27.20	26.78
Ryegrass Hay	0	0
Corn Silage	42.21	41.56
Whole Cottonseed	7.50	7.39
Grain Mix[2]	23.09	22.73
Bovine BlueLite	0	1.54

Lactating Cow Ingredient, %		
	Con	E+
Ryegrass Baleage	8.12	8.10
Ryegrass Hay	1.79	1.79
Corn Silage	38.56	38.50
Whole Cottonseed	5.46	5.45
Grain Mix[3]	46.06	45.48
Bovine BlueLite	0	0.68

[1] Received the Bovine Bluelite Supplement.
[2] Dry cow Grain Mix was composed of: Wheat midds, 21%; Soy hulls, 21%; Ground corn, 17%, Cottonseed meal, 13.5%, Soybean meal, 11.3%; Fish meal, 5.2%; Ca Carbonate, 4.83%; Magnesium oxide, 1.35%; Salt, 0.75%; Dical, 0.71%; Vit E 20,000 IU, 0.58%; Se, 0.34%; Zin pro 4 plex, 0.29%; Fat (grease), 0.25%.
[3] Lactating cow Grain Mix was composed of: Ground corn, 40.2%; Soybean meal 48%, 27.7%; Soybean hulls, 16.1%; Wheat midds, 4.71%; Ca Carbonate, 1.96%; Fish meal, 2.35%; Blood meal, 0.78%; Megalac, 0.98%; Poultry meal 0.71%; Salt, 0.57%; Animal Fat, 0.23%; K carbonate, 0.59%; MagOx 54%, 0.47%; Potash,0.39%; Zinpro 4 Plex, 0.11%; MTB-100, 0.13%; Se, 0.02%; Mn, 0.02%; Zn, 0.02%; Co, 0.01%; Cu, 0.01%; Vit A 325, 000 IU, 0.01%; Vit D3 200,000 IU, 0.01%; Vit E, 227,000 IU; 0.014%.

Table 3.2 Nutrient composition of the diets fed to dry and lactating cows with or without electrolyte supplementation[1].

Dry Cow Nutrient Composition, %		
	Con	E+
DM	50.17	50.51
CP	15.54	15.42
NDF[2]	50.23	49.60
ADF[3]	30.70	30.35
Ca	0.93	0.93
P	0.39	0.39
K	1.24	1.28
Cl	0.48	0.51
Na	0.20	0.26
S	0.21	0.21
DCAD, mEq/100g of DM	14	17
Lactating Cow Nutrient Composition, %		
	Con	E+
DM	55.04	55.13
CP	17.92	17.82
NDF	39.26	39.15
ADF	23.17	23.13
Ca	0.81	0.81
P	0.39	0.39
K	1.34	1.36
Cl	0.45	0.45
Na	0.44	0.46
S	0.25	0.24
DCAD, mEq/100g of DM	25	27

[1] DM basis.
[2] Neutral detergent fiber.
[3] Acid detergent fiber.

CHAPTER IV

RESULTS AND DISCUSSION

Environmental Conditions

As can be observed in Figure 4.1, during the 2012 trial although cows experienced 21 days of actual heat stress (mean THI >72), the mean minimum THI only exceeded 72 during 8 days, thus reflecting that cows were only stressed during 8 days of the trial. On the other hand, during the 2013 trial, cows experienced 28 days with mean THI greater than 72, but the minimum mean THI never exceeded the critical point of 72, thus reflecting that animals were not as challenged by heat stress during the 2013 trial (Figure 4.2), and to some extent potentially explaining our lack of treatment differences as neither CON or E+ cows may not have been severely heat stressed.

Previous reports have indicated that although mean THI may exceed the critical point of 72, if minimum mean THI is not above the critical point, it potentially does not contribute to heat stress, as the animal has the ability and opportunity to lose the heat gained from previous day (West et al., 1991; Silanikove, 2000). If fluctuations to cooler ambient conditions occur within the same day or previous day, potentially the effects of heat stress may not be felt by the animal. (Igono et al., 1992; Muller et al., 1994(a), Muller et al., 1994(b)). Therefore, the actual effect of heat stress is only felt if the capacity of the animal to completely dissipate heat gained during the day is severely prevented by the ambient conditions not dropping during cooler hours of the day.

Potentially, because majority of time during the present trials the mean minimum THI was below the critical point of 72, this indicates that although cows experienced THI above 72, they may have been able to dissipate the heat gained during the cooler hours of the day, thus minimizing the effect of heat stress.

Dry Matter Intake

Dry Cows

For the pre-calving (dry) period, total intake was not different by treatment (Table 4.1). Baleage intake was similar for both CON and E+ cows in 2013 (3.11 vs. 3.11 kg/d, respectively; P=0.99) and also similar amounts of TMR was consumed by dry cows within 2012 and 2013 (7.10 vs. 7.03 and 8.43 vs. 8.31 kg/d, respectively; P=0.44). However, overall dry cows consumed more dry cow ration in 2013 than in 2012 (8.37 vs. 7.07 kg/d, respectively; P<0.01), with CON cows consuming (8.43 vs. 7.10 kg/d, respectively) and E+ cows (8.31 vs. 7.03 kg/d, respectively). It is appropriate to mention that the baleage dry matter intake results are just from 2013 dry cows, as for the 2012 trial, dry cows had access to one bale (approximately 550 kg/bale), every other day provided in the exercise lot and both treatments had access to it, though no individual intakes were taken on baleage dry matter intake, unlike in 2013 when cows where offered approx. 6.80 kg/d (as fed) individualy. During the same dry period, although cows consumed similar amounts of individual nutrients from their diets, overall during 2013 dry cows consumed more CP from the dry cow TMR than in 2012 (1.56 vs. 1.09 kg/d, respectively; P<0.01); and more ADF from the dry cow TMR in 2012 than in 2013 (2.16 vs. 1.99 kg/d, respectively; P<0.01), but no treatment effects were observed. Dry cows'

baleage (3.09 vs. 3.12 kg/d, respectively; P=0.70) and dry cow TMR (7.69 vs. 7.74 kg/d, respectively; P=0.46) intake was not affected by mean daily THI.

Lactating Cows

Lactating cows consumed more dry matter than dry cows, as expected because they were fed more. Lactating TMR dry matter intake was similar for CON and E+ fed cows (20.9 vs. 21.3 kg/d, respectively; P=0.45), and was influenced by day (P<0.001) as also expected, by the sudden increase in milk yield directly incrementing the demands for nutrients. During the lactating period, individual nutrient intakes from the lactating TMR were not influenced by treatment. During the 2012 trial, upon calving, cows were managed and fed as a group in pens. Because only amount of feed offered was recorded, the 2012 lactating DMI intake was not included in these results.

The DMI results from this study agree with those found by Schneider et al., (1988,) who found no differences in DMI (21.3 vs. 21.0 kg/d, respectively), resulting from feeding electrolyte greater than recommended (NRC, 1989). But disagree with results from West et al., (1991), who fed 4 different electrolyte balances during either a cool or hot phase (Cool phase: 1 = -79.4; 2 = 47.2; 3 = 166.6; 4 = 324.4. Hot phase: 1 = -166.6; 2 = 191.4; 3 = 180.0; 4 = 312.4 mEq Na + K - Cl / kg of DM). In this study, these authors observed a quadratic increase of DMI (P<0.05) when the DCAD of the diet increased in both phases (Cool: 13.0, 15.2, 18.9, and 18.7 kg/d, respectively; Hot: 10.7, 16.4, 15.8, and 15.9 kg/d, respectively). However, although DMI was decreased from phase to phase, yet increasing the DCAD of the diet resulted in an increase in DMI in the hot phase when cows were really challenged by heat stress, which not only illustrates the positive effect of increasing electrolyte balance on DMI, but also the reduction of DMI in

the hot phase when the even the mean minimum THI was above the critical comfort zone of 72, whereas in the cool phase, although mean THI was above the critical comfort zone (THI= 74), the mean minimum THI was well below the critical point 72. Thus, this indicates that cows were not really challenged during the cool phase of their study as cows were able to dissipate some of their heat load during the cool hours of the day.

Body Weight, Frame, and Condition Score

Dry Cows

No differences were observed for body weight (BW), condition score (BCS), and frame of dry cows in the present study (Table 4.2; See Figure 4.3 (from week -3 to week -1)).

During 2012, body weight was similar for dry cows fed the CON and E+ diets (556.8 vs. 546.9 kg, respectively; P=0.27), as was during 2013 (568.7 vs. 552.3 kg, respectively; P=0.0.27). Heights at the withers of dry cows were also not affected by treatment (P=0.12), but where different by year (P=0.02), as overall cows had greater measures in 2012 compared to 2013 (131.5 vs. 130.3 cm, respectively, P=0.02). A similar effect was noticed in hip height, as it did not change by treatment (P= 0.65), but was lower in 2013 dry cows compared to 2012 (136.0 vs. 137.4 cm, respectively; P=0.007). Heart girth was not affected by treatment (P=0.42), but a treatment by year interaction (P=0.04) showed that E+ cows had greater heart girth values in 2012 compared to 2013 (192.4 vs. 190.5 cm, respectively). No changes were noted for the body condition score of dry cows in this study (P=0.69), however, dry CON and E+ cows had decreased BCS in 2013 than in 2012 (3.43 vs. 3.21 and 3.44 vs.3.32, respectively; P<0.01). This effect may be explained by a difference of ADG observed for dry cows during the 2012 and

2013 year. During 2012, both CON and E+ cows gained weight during the dry period (1.88 vs. 1.90 kg/d, respectively) but then in 2013 negative values were observed as CON and E+ dry cows lost weight during the last three weeks prior to calving (-0.79 vs. -2.33 kg/d, respectively), which coupled younger animals' bodies, could explain the decreased BCS recorded in 2013 cows.

Lactating Cows

Both CON and E+ 2012 lactating cows had similar body weights (518.7 vs. 502.2 kg, respectively; P=0. 27), and although not different, E+ cows had less body weight than CON during the 2013 lactating period (492.7 vs. 516.3 kg, respectively; P=0.27). No major changes were observed in lactating cows' body frames and condition scores, however, for heart girth a treatment by year interaction (P=0.04) showed that while CON cows maintained their heart girth measures going from 2012 to 2013 (190.8 vs. 190.1 cm, respectively), E+ cows had a decrease in heart girth measure in the same time frame (192.3 vs. 184.5 cm, respectively). Although not different by treatment, the BCS of lactating cows was affected by period, as lactating cows had a lower BCS than did dry cows (3.24 vs. 3.35, respectively; P=0.002). Unlike dry cows, lactating ADG values for both years and treatments were negative.

Health Condition

Dry Cows

No treatment differences were observed in respiration rate (P=0.18; Table 4.3) during the period of this study. In 2012, as well as in 2013, dry CON and E+ cows had similar respiration rates. Similarly rectal temperature was not influenced by treatment

during the dry period (P=0.17), but was overall greater in 2013 than in 2012 overall (38.95 vs. 38.65 °C). Overall, dry cows had similar respiration rate (55.5 vs. 56.27 bpm, respectively) and rectal temperature (38.8 vs.38.7 °C, respectively), than lactating cows.

Lactating Cows

Similarly, no treatment influence was noticed during the lactating period in the respiration rate (P=0.18) and rectal temperature (P=0.17) of CON and E+ cows (P=0.17). However, just like in dry cows, in lactating cows greater rectal temperatures were measured during the 2013 year compared to the 2012 (P<0.01).

As expected due to the higher body frame and capacity of the Holstein cow compared to the Jersey cow, a breed effect showed that Holstein cows had greater (P<.0004) respiration rates than Jerseys (57.7 vs. 54.1 bpm, respectively), and this effect was expected due to a attributed improved ability of Jerseys to get rid of heat (Kadzere et al., 2002), and may also be explained by the more corpulent body of Holsteins, which during times of heat stress represents a greater heat load to get rid of. In addition, a treatment by period tendency illustrated that during the lactating period, CON cows tended to have more breaths per minute than E+ Cows (57.91 vs. 54.6 bpm, respectively), which evidences a potential positive effect of the addition of electrolytes to the diets of transitioning dairy cows, particularly during the lactating phase. Overall, the respiration rate tended to be greater (P=0.68; Table 4.5) during the 2012 trial than in during the 2013 one (56.87 vs. 55.55 bpm; respectively), which was to some extent unanticipated since the 2012 study was carried out under more mild climate conditions, compared to the 2013 environmental conditions. Unlike for respiration rate, rectal temperature was similar (P=0.39) for both Holstein and Jersey cows (38.8 vs. 38.8°C, respectively).

Blood Metabolites

Dry Cows

No differences were observed between CON and E+ dry cows' blood metabolites (Table 4.4). Blood pH was not affected by treatment (P=0.21), but, overall, dry cows had decreased blood pH compared with lactating cows (7.44 vs. 7.45, respectively; P<0.01). Similarly, HCO_3^- concentration was not different in dry cows fed CON and E+ diets (P=0.92), and overall dry cows had less HCO_3^- concentration than lactating cows (26.8 vs. 27.7 mmol/L, respectively; P=0.003). A treatment by period interaction showed that E+ cows had different HCO_3^- concentrations by period, with less concentration being observed in the dry period than in the lactating (26.6 vs. 27.9 mmol/L, respectively; P=0.02), but this effect did not held true for CON cows (27.0 vs. 27.4 mmol/L, respectively). Concentrations of pCO_2 were also not different by treatment during the dry period (P=0.76). Concentrations of tCO_2 were not influenced by treatment in dry cows (P=0.89), but, overall decreased concentrations were observed in dry cows compared to lactating cows (28.1 vs. 29.0 mmol/L, respectively; P=0.004). Anion gap concentrations were not different by treatment (P=0.86), though, overall dry cows had greater concentrations than lactating cows (14.5 vs. 13.3 mmol/L, respectively; P<0.01). Similarly, treatment did not influence the Na^+ concentration in dry cows blood (P=0.98), but dry cows had increased concentrations compared to lactating cows (144.6 vs. 142.0 mmol/L, respectively), and overall, greater Na^+ concentrations were observed in blood from 2013 dry cows, than 2012 (P<0.01). Chloride concentrations were not affected by treatment (P=0.71), but were greater for dry cows compared to those lactating (107.2 vs. 104.9 mmol/L, respectively). No treatment differences were noticed in K^+ concentrations

(P=0.89), and dry cows had similar concentrations in year 2012 and 2013 (P=0.29). Hematocrit % was not affected by treatment (P=0.11), nor by year (P=0.11). It was overall greater in dry cows compared to lactating cows (53.2 vs. 50.9 %, respectively; P=0.03).

Lactating Cows

No treatment differences were observed in blood metabolites between cows fed the CON or E+ diet during the lactating period either. Blood pH recorded in 2013 lactating cows was greater than those observed in 2012. In 2013, lactating E+ cows had greater blood pH than lactating CON cows (7.47 vs. 7.46, respectively; P=0.0004), but this effect was not observed in 2012. Concentrations of HCO_3^-, pCO_2, and tCO_2 were similar for both treatments during the lactating period, and both 2012 and 2013 year. In a like manner as observed in dry cows, anion gap concentrations during the lactating period of 2012 and 2013 years were different, as overall cows had increased concentrations in 2013 than in 2012 (P=0.02). Levels of Na+ were not different by treatment either (P=0.98), but similarly were higher for lactating cows managed in 2013, than in 2012 (P<0.01). Concentrations of Cl^- and K^+ were not altered by treatment during the lactating period of this study. Hematocrit during the lactating period was not affected by treatment (P=0.11), and nor by year (P=0.11).

Blood pH (P=0.01), HCO_3^- (P=0.008), pCO_2 (P<0.01), tCO_2 (P=0.01), anion gap (P=0.01), K^+ (P=0.05), and hematocrit (P=0.02) concentrations, were affected by mean daily THI, but Na^+, and Cl^-, concentrations were not. As the mean daily THI exceded the critical comfort zone of 72, pH, anion gap, and hematocrit concentrations were increased (7.44 to 7.45, 13.5 to 14.3 mmol/L, and 50.2 to 53.9 %, respectively); while

concentrations of HCO_3^- (27.7 to 26.8 mmol/L, respectively), pCO_2 (44.0 to 41.4 mmHg, respectively), tCO_2 (29.1 to 28.1 mmol/L, respectively), and K^+ (3.97 to 3.85 mmol/L, respectively), were overall decreased, but no treatment by mean daily THI interaction was observed for any of the blood measures.

Blood pH was also influenced by parity (P=0.001), as overall primiparous cows had decreased blood pH than multiparous cows (7.44 vs. 7.45, respectively). It was also noticed that Jersey multiparous cows had greater blood pH than primiparous Jerseys (7.46 vs. 7.43, respectively; P=0.004), with this effect just holding true as a tendency in Holstein cows (7.45 vs. 7.44, respectively). Concentrations of HCO_3^- tended to be greater in Jersey cows compared to Holsteins (27.6 vs. 26.9 mmol/L, respectively; P=0.09), as so did tCO2 levels (29.0 vs. 28.2 mmol/L, respectively; P=0.07). Levels of Na^+ were greater in Jersey cows (143.7 vs. 142.9 mmol/L, respectively; P=0.03), but a treatment by breed interaction was not associated to this effect (P=0.57). Hematocrit was greater in primiparous cows than in multiparous cows (55.1 vs. 49.0 %, respectively; P<0.01), and tended to be less in E+ primiparous compared to CON primiparous (53.0 vs. 57.2%, respectively; P=0.09), but this effect did not held true for multiparous cows (48.6 vs. 49.4%, respectively).

Milk Yield and Composition

Overall, there were no treatment differences in MY (P=0.14; Table 4.5), and overall cows were not really challenged by heat stress as MY was not influenced by mean THI. These findings agree with those found by Schneider et al., (1988,) who observed no change in MY (18.3 vs. 17.4 kg/d) after feeding greater than recommended electrolyte concentrations to heat stressed dairy cows. In a similar manner, Chan et al., (2005,) found

no differences in MY (3.5% FCM) when cows received diets going from 20 to 35 mEq (27.8 vs. 25.6 kg/d, respectively; P>0.10), nor with diets going from 35 to 50 mEq (25.6 vs. 25.7 kg/d, respectively; P>0.10). However, the lack of differences by treatment disagrees with previous findings from Hu et al., (2007,) who reported a 3.5 kg/d linear increment in MY when cows were supplemented with electrolytes. In addition, West et al., (1991,) fed 4 different electrolyte balances (Cool phase: 1 = -79.4; 2 = 47.2; 3 = 166.6; 4 = 324.4. Hot phase: 1 = -166.6; 2 = 191.4; 3 = 180.0; 4 = 312.4 mEq Na + K - Cl / kg of DM). During the cool phase mean maximum and minimum THI were 74.8 and 59.0, respectively, while during the hot phase mean maximum and minimum THI were 84.2 and 72.5, respectively. These authors observed a linear (P<0.05) response of MY to increasing the DCAD of the diet in both phases (Cool: 16.7, 18.6, 19.6, and 20.4 kg/d, respectively; and Hot: 16.4, 19.7, 19.2, and 19.7 kg/d, respectively). What is important to know is that, during the hot phase both the mean minimum and maximum THI exceeded the critical point of 72, indicating that cows were exposed to a THI capable of inducing heat stress, yet MY was positively affected by DCAD treatment. As a matter of fact, although the mean maximum THI did exceeded the critical point of 72 during the cool phase, minimum and the mean of maximum and minimum THI were well below the critical point and probably did not contribute to induce heat stress. These findings may potentially help explain the lack of differences in DMI and MY in our trial, as cows were not really heat stressed.

During year 2012, CON cows produced more milk than did 2013 CON cows (31.8 vs. 29.7 kg/d, respectively, P=0.0009). A similar effect was noticed in E+ cows, as in year 2012 yields of milk averaged greater than in 2013 (31.4 vs. 25.4 kg/d,

respectively; P=0.0009). Overall, MY was affected by year, as cows produced less milk in 2013 than in 2012 (27.6 vs. 31.6 kg/d, respectively; P<0.01), and was different by day (P<0.01), breed (P<0.01), and parity (P<0.01). As expected, Holstein cows produced more milk than Jerseys (34.0 vs. 25.16 kg/d, respectively), and first calving heifers produced less milk than multiparous cows (21.9 vs. 37.3 kg/d, respectively). A positive effect was noticed in Jersey cows, as E+ Jersey cows had greater milk yields than CON Jersey cows (25.8 vs. 24.5 kg/d, respectively; P=0.02), however, an opposite effect was observed in Holsteins, as milk yield was less in E+ Holsteins compared to CON Holsteins (31.0 vs. 37.0 kg/d respectively; P=0.02). This effect could be associated with the differences in body weight and corpulence of Holstein compared to a smaller Jersey cow.

Overall, no treatment differences were observed in milk composition (Table 4.5). Milk fat concentration was similar for both treatment groups (P=0.64), but was different by year, as CON and E+ cows had less fat concentration in 2012 compared to 2013 (3.23 vs. 3.90 and 3.40 vs. 4.03 %, respectively; P=0.0009). Overall fat concentration was also affected by week, as it increased linearly weekly from week 1 until week 4 (3.01 to 4.54 %, respectively; P<0.01). Surprisingly, milk fat did not differ between breeds, as Holsteins and Jerseys had similar fat concentration in their milk (3.59 vs. 3.68 %, respectively; P=0.76).

Milk protein was not affected by treatment (P=0.74), but overall was greater in 2013 than in 2012 (3.67 vs. 3.48 %, respectively; P=0.02), and was affected by breed, as Jersey cows had greater protein concentration in their milk than Holsteins (3.77 vs. 3.39 %, respectively; P=0.006). Milk protein concentration also was affected by week as going from week 1 to week 4 it plummeted (4.47 to 3.13 %, respectively; P<0.01). In addition,

a (P=0.05) treatment by week interaction showed that at week 1, CON cows had a greater protein concentration in their milk (Figure 4.4).

These results agree with those found by Chan et al., (2005), when they fed cows with diets containing 20, 35, and 50 mEq, and found no differences (P>0.10) in milk fat and protein concentration. Milk fat was not affected in cows receiving diets from 20 to 35 mEq had similar milk fat % (4.14 vs. 3.97 %, respectively), while those eating diets from 35 to 50 mEq had (3.97 vs. 4.31%). Likewise, protein % was not affected in cows consuming diets from 20 to 35 mEq (2.89 vs. 2.95 %, respectively), or from 35 to 50 mEq (2.95 vs. 2.91 %, respectively).

Milk lactose concentration was not affected by treatment but was overall decreased by year, as cows had less concentration in 2013 than in 2012 (4.52 vs. 4.66 %, respectively, P=0.002). Overall, it linearly increased after parturition (P<0.0001) going from 4.28 to 4.67 for week 1 and 5, respectively. Milk lactose was also similar for Holstein and Jersey cows (4.62 vs. 4.56%, respectively; P=0.44).

Total milk solids non-fat concentration was not influenced by treatment (P=0.69), however, a treatment by year interaction showed that in year 2013, milk from CON cows tended to have a greater Solids non-fat concentration in their milk, compared to CON cows from 2012 (9.21 vs. 8.98 %, respectively; P=0.04), while E+ cows tended to have greater in 2012 (9.10 vs. 8.97%, respectively). Overall, solids non-fat concentration was different by week (P<0.01), and by breed (P=0.02). Overall, total solids content decreased linearly from week 1 to 4 (9.63 to 8.70%, respectively), and a treatment by week interaction revealed that CON cows tended to have greater solids non-fat concentrations than E+ cows at week 1 after calving (P=0.05). This same interaction

showed that despite the overall plummeting week effect on total solids non-fat observed, the decrease was more severe for CON cows than for E+ cows, particularly going from week 1 to 2 (9.80 to 9.07 vs. 9.47 to 9.13%, respectively). Jersey cows had a greater total solids non-fat concentration than Holsteins (9.23 vs. 8.90%, respectively), which perhaps could be explained by the greater protein concentration as mentioned previously. Raw somatic cell count (P=0.75), and the somatic cell score (P=0.36) were not influenced by the addition of electrolytes to the diets of lactating cows.

Table 4.1 Dry matter and nutrient intake of dry and lactating cows fed diets with or without electrolyte supplementation.

	2012			2013			P<		
	Con	E+	SEm	Con	E+	SEm	Trt	Year	Trt*Year
DMI intake, kg/d									
				Dry Cow					
Baleage[1]	N/A	N/A	N/A	3.11	3.11	0.06	0.99	N/A	N/A
CP	N/A	N/A	N/A	0.44	0.42	0.01	0.45	N/A	N/A
NDF	N/A	N/A	N/A	1.69	1.69	0.03	0.91	N/A	N/A
ADF	N/A	N/A	N/A	1.02	1.02	0.01	0.81	N/A	N/A
Dry Cow TMR	7.10	7.03	0.09	8.43	8.31	0.14	0.44	0.01	0.82
CP	1.10	1.09	0.01	1.57	1.55	0.02	0.43	0.01	0.83
NDF	3.56	3.51	0.04	3.54	3.54	0.06	0.63	0.97	0.62
ADF	2.18	2.15	0.02	1.98	2.00	0.03	0.82	0.01	0.39
				Lactating Cow					
Lactating TMR[2]	N/A	N/A	N/A	20.9	21.3	0.37	0.45	N/A	N/A
CP	N/A	N/A	N/A	3.41	3.49	0.04	0.25	N/A	N/A
NDF	N/A	N/A	N/A	7.48	7.57	0.08	0.51	N/A	N/A
ADF	N/A	N/A	N/A	4.41	4.47	0.06	0.48	N/A	N/A
Feed Efficiency[3]	N/A	N/A	N/A	0.73	0.74	0.05	0.83	N/A	N/A

[1] Baleage was fed as a group with a bale being offered each other day, thus 2012 baleage intake was not recorded.
[2] Lactating intake in 2012 was not recorded
[3] Feed Efficiency was calculated daily individually (Total kg of dry matter intake / Total kg of milk produced)

Table 4.2 Body weight, frame and condition score changes of dry and lactating cows fed diets with or without electrolyte supplementation.

	2012			2013			P<		
	Con	E+	SEm	Con	E+	SEm	Trt	Year	Trt*Year
					Dry Cow				
BW, kg	556.8	546.9	11.9	568.7	552.3	12.8	0.27	0.81	0.54
WH, cm	132.5	130.7	0.85	130.5	128.6	1.08	0.12	0.02	0.84
HH, cm	137.8	137.1	0.76	135.7	135.7	0.99	0.65	0.007	0.67
HG, cm	192.2	192.4	1.61	193.2	190.5	2.31	0.42	0.04	0.04
BCS	3.43	3.44	0.03	3.21	3.32	0.07	0.69	0.01	0.88
ADG, kg/d	1.88	1.90	0.49	-0.79	-2.33	0.89	0.98	0.01	0.59
					Lactating Cow				
BW, kg	518.7	502.2	12.0	516.3	492.7	12.6	0.27	0.81	0.54
WH, cm	132.3	130.6	0.86	131.6	130.5	0.93	0.12	0.02	0.84
HH, cm	137.6	137.0	0.77	136.6	136.2	0.85	0.65	0.007	0.67
HG, cm	190.8	192.3	1.64	190.1	184.5	1.84	0.42	0.04	0.04
BCS	3.23	3.28	0.03	3.28	3.20	0.06	0.69	0.01	0.88
ADG, kg/d[1]	-1.23	-0.77	0.53	-1.60	-0.49	0.63	0.98	0.01	0.59

[1] Average Daily Gain, kg/d, was calculated using the weekly body weight measures (Initial weight (kg) – Final weight / number of days in between)

Table 4.3 Respiration rate and rectal temperature of dry and lactating cows fed diets with or without electrolyte supplementation.

	2012			2013			P<		
	Con	E+	SEm	Con	E+	SEm	Trt	Year	Trt*Year
				Dry Cow					
Respiration rate, bpm	55.6	55.3	1.22	56.4	54.8	1.54	0.18	0.68	0.97
Rectal temperature, °C	38.6	38.6	0.06	39.0	38.8	0.07	0.17	0.01	0.11
				Lactating Cow					
Respiration rate, bpm	58.6	54.6	1.52	57.2	54.6	1.42	0.18	0.68	0.97
Rectal temperature, °C	38.7	38.6	0.07	39.0	38.8	0.07	0.17	0.01	0.11

Table 4.4 Blood metabolite parameters of dry and lactating cows fed diets with or without electrolyte supplementation.

	2012			2013			P<		
	Con	E+	SEm	Con	E+	SEm	Trt	Year	Trt*Year
				Dry Cow					
pH	7.43	7.44	0.003	7.44	7.44	0.005	0.21	0.002	0.98
$HCO3^-$, mmol/L	26.6	26.3	0.34	27.6	26.9	0.54	0.92	0.19	0.89
pCO2, mmHg	42.4	41.4	0.55	43.9	43.6	0.91	0.76	0.68	0.95
tCO2, mmol/L	27.8	27.5	0.36	28.9	28.2	0.58	0.89	0.18	0.99
Anion Gap, mmol/L	14.2	14.8	0.30	14.4	14.6	0.49	0.86	0.02	0.71
Na^+, mmol/L	143.9	143.8	0.30	145.4	145.1	0.49	0.98	0.01	0.90
Cl^-, mmol/L	107.2	106.9	0.30	107.6	107.5	0.45	0.71	0.26	0.99
K^+, mmol/L	3.95	3.93	0.08	3.91	3.88	0.08	0.89	0.29	0.97
Hematocrit, %	55.9	51.2	1.38	53.3	52.2	2.39	0.11	0.11	0.46
				Lactating Cow					
pH	7.44	7.45	0.003	7.46	7.47	0.004	0.21	0.002	0.98
$HCO3^-$, mmol/L	27.5	27.9	0.36	27.3	27.9	0.42	0.92	0.19	0.89
pCO2, mmHg	43.0	43.6	0.59	41.9	41.7	0.68	0.76	0.68	0.95
tCO2, mmol/L	28.8	29.3	0.38	28.6	29.3	0.44	0.89	0.18	0.99
Anion Gap, mmol/L	12.9	12.7	0.32	14.0	13.7	0.37	0.86	0.02	0.71
Na^+, mmol/L	141.5	141.7	0.32	142.3	142.6	0.37	0.98	0.01	0.90
Cl^-, mmol/L	105.0	104.8	0.31	104.9	104.7	0.36	0.71	0.26	0.99
K^+, mmol/L	3.90	3.94	0.05	3.84	3.89	0.06	0.89	0.29	0.97
Hematocrit, %	53.3	51.3	1.48	50.6	48.4	1.72	0.11	0.11	0.46

Table 4.5 Milk yield and composition of lactating cows fed diets with or without electrolyte supplementation.

	2012			2013			P<		
	Con	E+	SEm	Con	E+	SEm	Trt	Year	Trt*Year
Milk yield, kg/d	31.8a	31.4a	1.24	29.7b	25.4c	1.38	0.14	0.01	0.0009
Fat, %	3.23	3.40	0.24	3.90	4.03	0.30	0.64	0.00	0.91
Protein, %	3.44	3.52	0.10	3.75	3.60	0.13	0.74	0.02	0.20
Lactose, %	4.64	4.67	0.05	4.54	4.49	0.07	0.88	0.00	0.33
SNF[1], %	8.98	9.10	0.11	9.21	8.97	0.13	0.69	0.50	0.04
SCS[2]	1.97	1.90	0.10	2.01	1.83	0.12	0.36	0.87	0.47
Raw SSC[3]	317.2	346.2	143.6	249.2	337.7	167.2	0.75	0.72	0.68

[1] Solids Non-Fat Concentration
[2] Somatic Cell Score
[3] Raw Somatic Cell Count

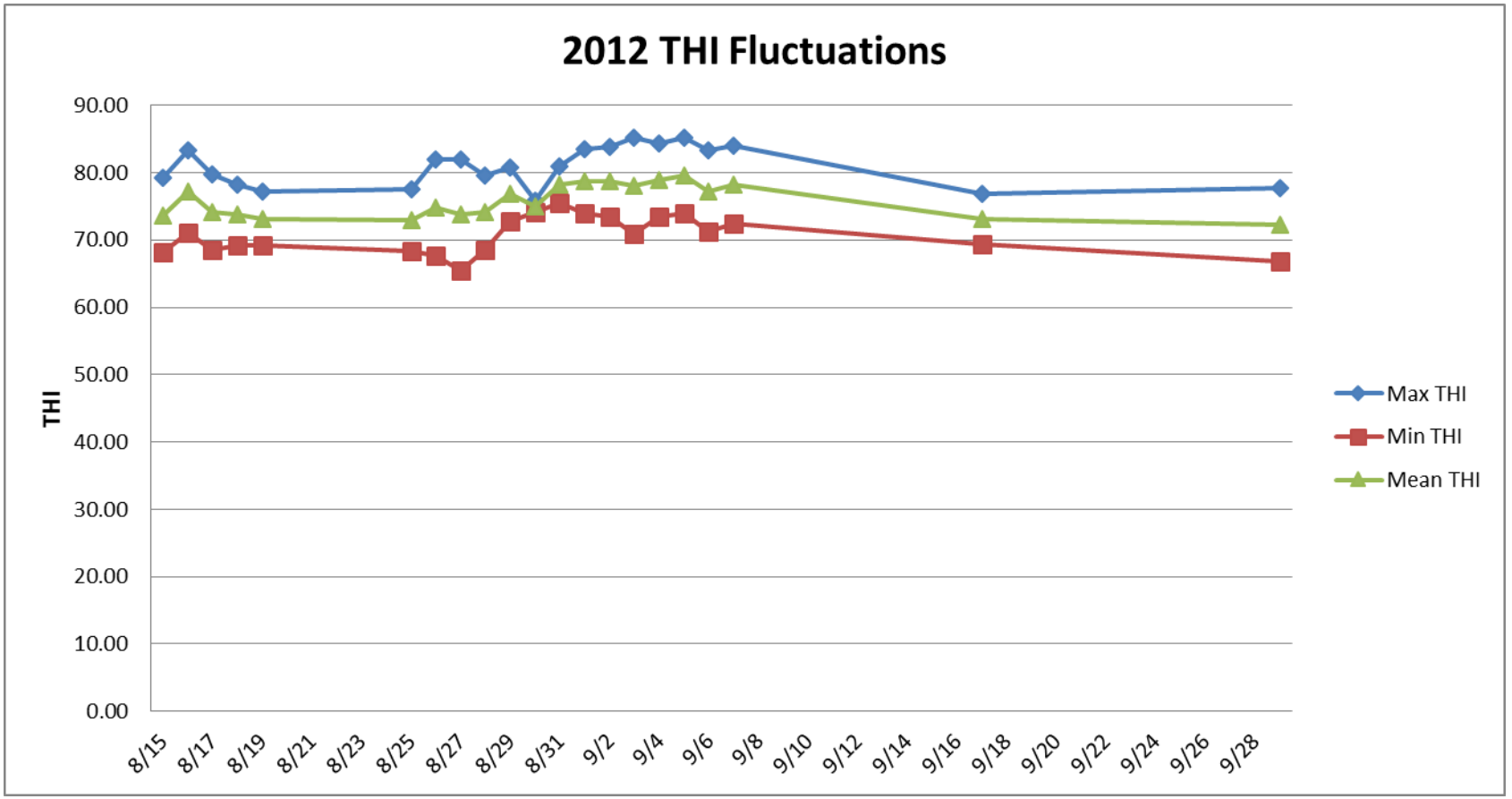

Figure 4.1 Maximum, Minimum, and Mean THI fluctuations by date during the 2012 trial.

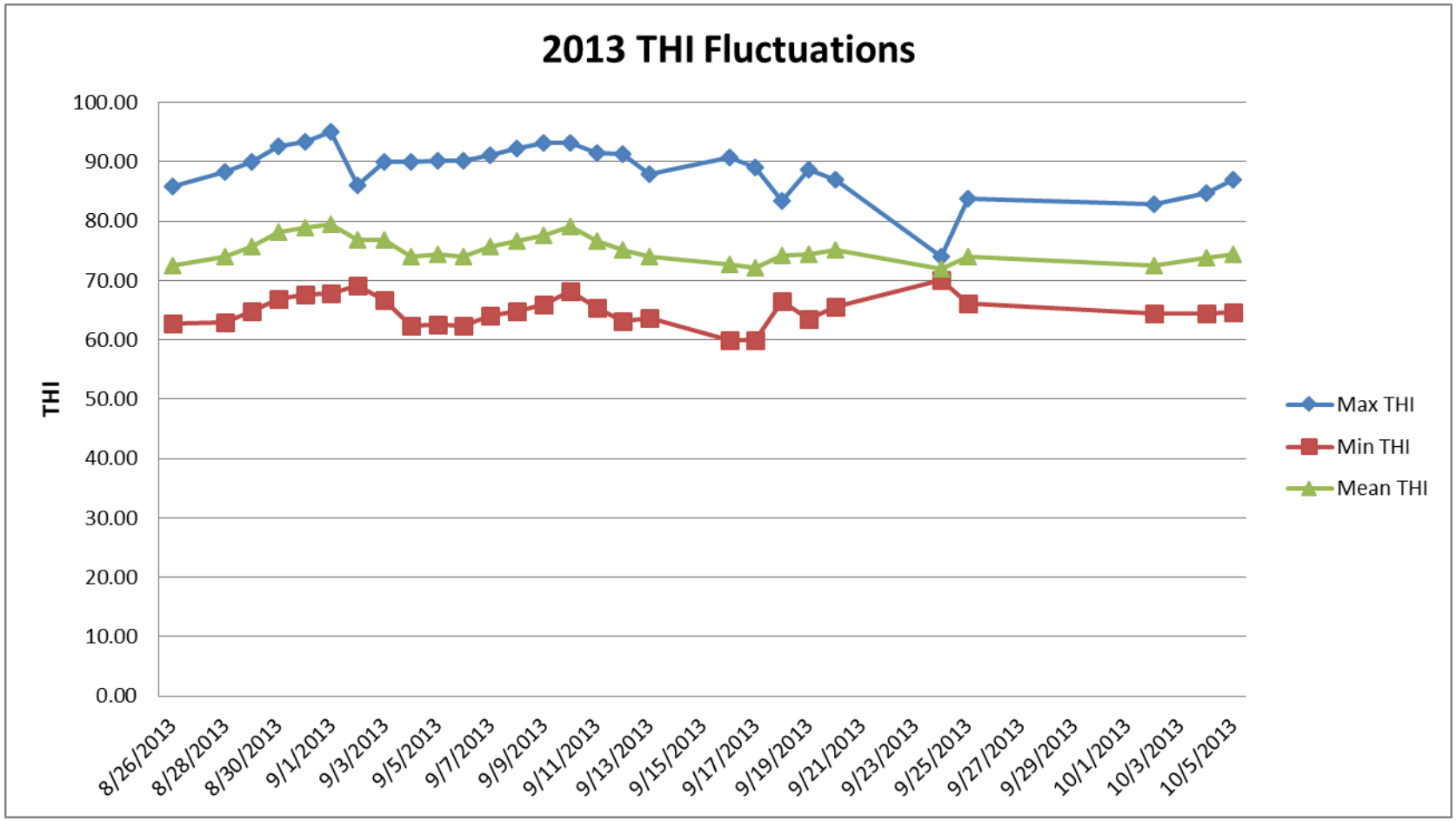

Figure 4.2 Maximum, Minimum, and Mean THI fluctuations by date during the 2013 trial.

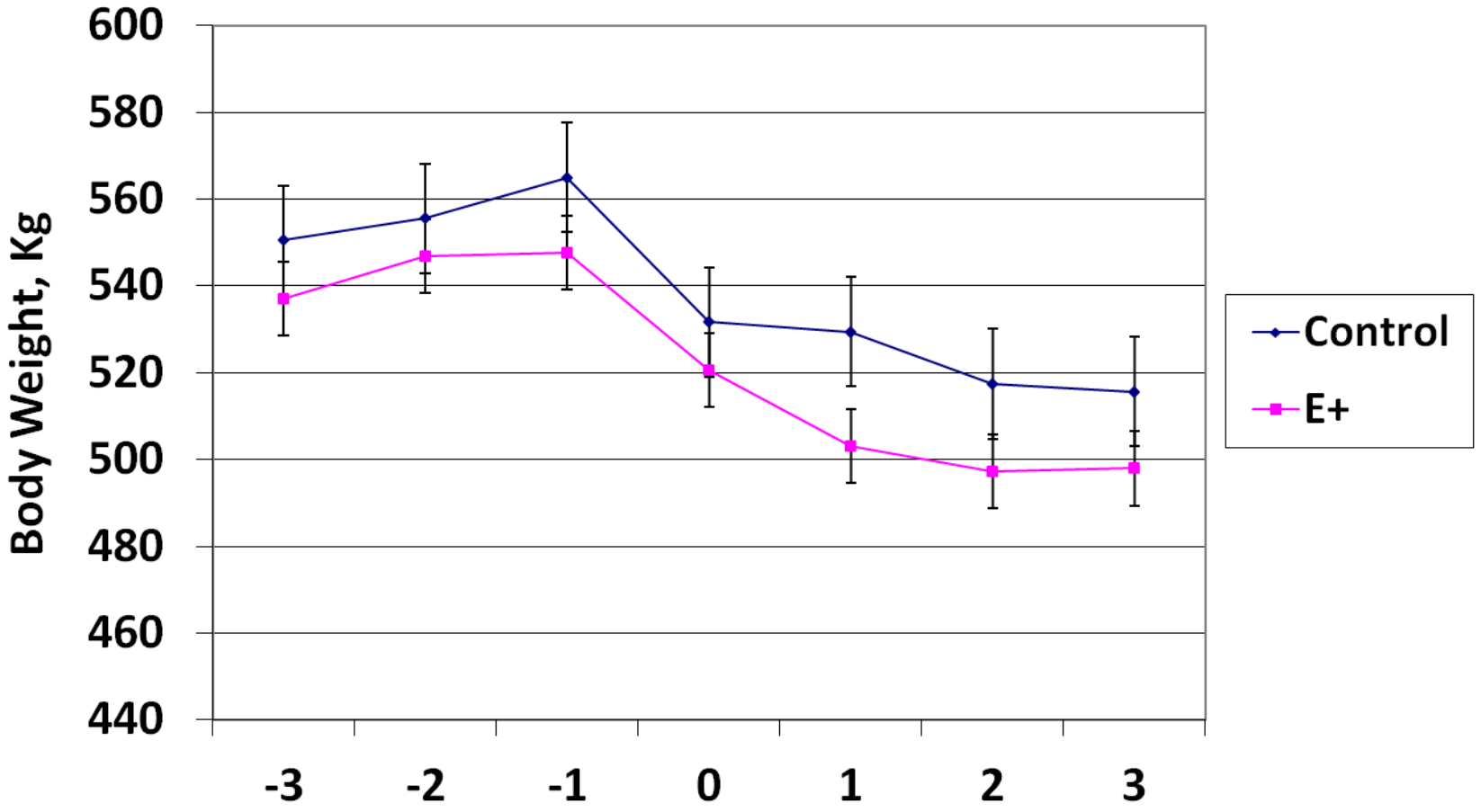

Figure 4.3 Weekly body weight change of cows fed diets with or without electrolyte supplementation.

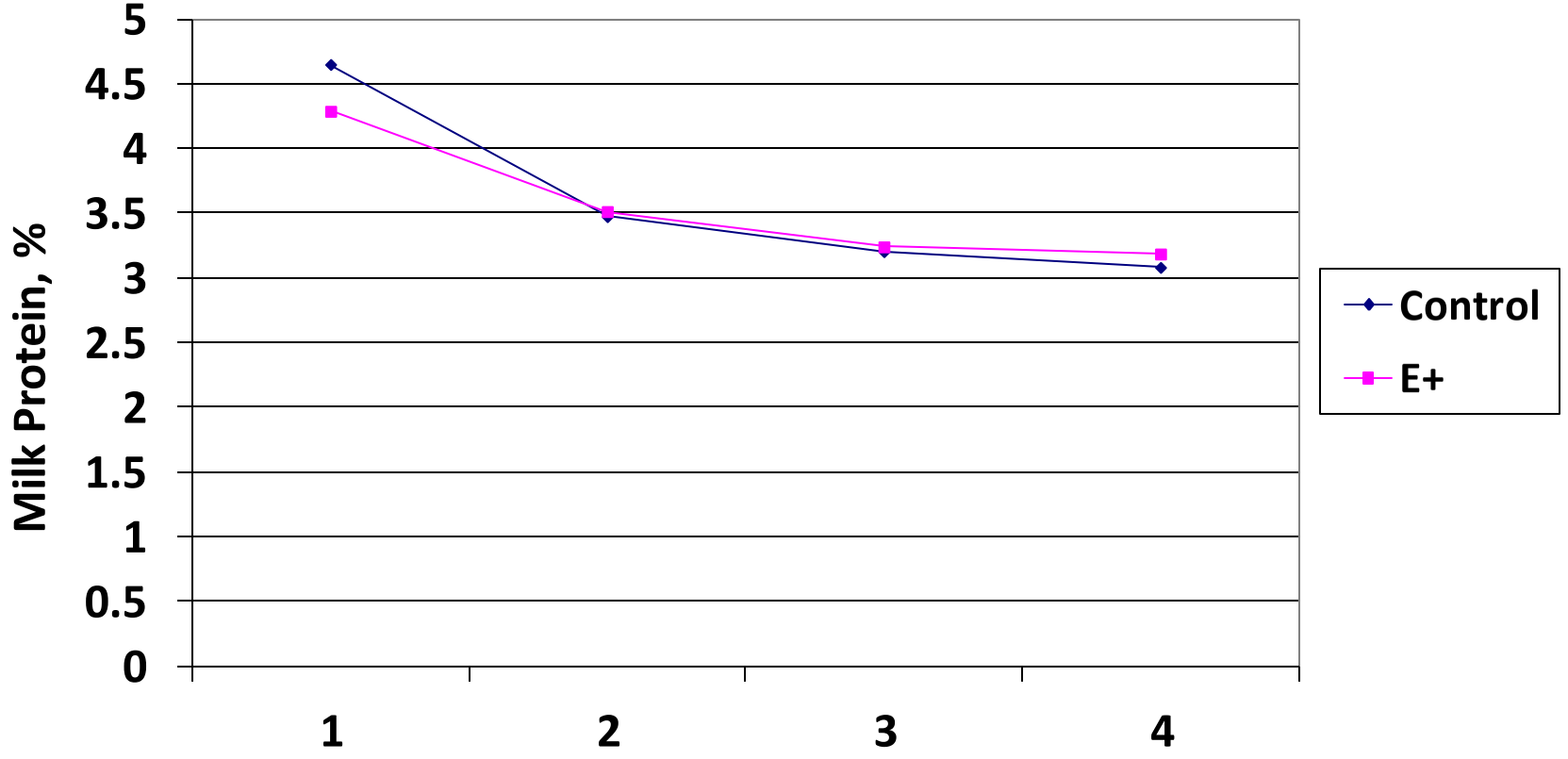

Figure 4.4 Weekly protein concentration of milk from cows fed diets with or without electrolyte supplementation.

CHAPTER V

SUMMARY

In this study, no negative effects of adding Bovine Bluelite to the diets of dry and lactating cows were noted. However, potential benefits of the electrolyte supplementation during the critical transition period from dry to the lactating state were evidenced. This potential and beneficial effect, was indicated more markedly in 2012, when cows fed the E+ treatment showed a better MY for almost the first week of lactation, as a result of being more disposed or ready to counteract the tough and stressful changes associated with the calving and lactating process, by being supplemented 21 days prior to and 30 post calving.

The addition of electrolytes did not affect DMI. During both periods, dry and lactating cows consumed similar amounts of dry matter and nutrients. Lactating cows consumed more dry matter than dry cows, but that was expected because nutritional requirements of dairy cows are greater in the lactating period. Overall, DMI was not affected by mean daily THI, which could have helped not to be a difference by treatment, as cows were not really challenged by heat stress. Overall, no treatment differences were observed for milk production and composition. As expected, Holstein cows had greater yields of milk than Jerseys, and MY was greater in 2012 than in year 2013. Parity also had an effect on MY, as primiparous cows had lower MY compared to their more adult counterparts. Overall, electrolyte supplementation was not detrimental to milk

composition and quality. Milk fat concentration was not affected by treatment, but in 2012, fat concentrations were lower than in year 2013, and although not expected, milk fat was not different between Jerseys and Holsteins. Although milk protein was not affected by treatment, milk from Jersey cows had increased protein % compared to milk from Holsteins. Milk lactose concentration was not influenced by treatment, was similar for both breeds, and in the present study, but was different by year, as in 2013 cows presented less concentration of lactose in their milk compared to 2012. Solids not-fat concentration was not affected by electrolyte supplementation, though during 2013, CON cows tended to have greater concentration of solids not-fat in their milk, compared to CON cows from 2012, along with milk from treated cows (E+) tending to have greater solids non-fat in 2012. The total solids content decreased linearly from week 1 until the end of the experimental period, and at week 1, CON cows tended to have greater solids not-fat concentrations than E+ cows, but in spite of this effect, the same interaction revealed that the plummet was more severe for CON cows than E+ cows, especially during the first two weeks after calving. Milk quality was not affected by supplementing cows with electrolytes, as raw somatic cell count and somatic cell score were not affected by treatment.

No treatment differences were observed for body weight, condition score, and frame measures during the dry and lactating periods in this study. However, average daily gains were different by year, as in 2012 dry cows from both treatment groups gained weight, whereas in 2013, negative values representing body weight loss were recorded, as dry cows lost weight during the last three weeks prior to calving, which may help explain the differences in milk yield noticed in 2013 versus 2012.

Overall, no differences were observed in blood chemistry due to the supplementation of Bovine Bluelite to transitioning dairy cows. Both CON and E+ cows had similar pH, $HCO3^-$, pCO2, tCO2, Anion Gap, Na^+, Cl^-, K^+, and Hematocrit, %. However, during both the dry and lactating period, hematocrit tended to be reduced in E+ cows compared to CON cows, and a parity effect showed that hematocrit % tended to be lower in E+ primiparous compared to CON primiparous, although this change was not observed in multiparous cows. Blood pH, HCO_3^-, pCO_2, tCO_2, anion gap, K^+, and hematocrit concentrations, were affected by mean daily THI, but Na^+, and Cl^-, were not influenced by a change in the mean daily THI. Increased mean daily THI values increased pH, anion gap, and hematocrit concentrations, while decreasing HCO_3^-, pCO_2, tCO_2, and K^+ concentrations.

Although no treatment differences were observed in the heat stress indicators respiration rate and rectal temperature, increased rectal temperatures were observed in 2013 compared to 2012, and a breed effect illustrated that Holstein cows had greater respiration rates than Jerseys, though this effect was to some extent expected as Holstein cows have a bigger body and thus face a greater heat load to get rid of. During 2012, E+ cows tended to have slower respiration rates than CON cows this influence was more marked in 2013, which was a more hot and humid year than 2012. In addition, there was a tendency for E+ cows to have decreased rectal temperature values in year 2013 than in 2012. Clearly, these effects illustrate the potential worth and impact of supplementing transitioning and lactating dairy cows with electrolytes to the abatement of heat stress, as it positively reduced the respiration rates and rectal temperature in E+ fed cows. Moreover, these changes also may confirm a tendency of the Bovine Bluelite supplement

to aid dairy cows in a better way in hotter environmental conditions such as those encountered in 2013 compared to 2012.

In conclusion, it was demonstrated in the present study that although DMI and MY were not increased, the supplementation of an electrolyte supplement (Bovine Bluelite Pellets) 21 days prior and 30 post partum, evidentially showed some positive effects. Transitioning dairy cows took advantage of important benefits to counteract the stressful and demanding process of calving, early lactation and being heat stressed. The relatively decreased severity of heat stress during both trials, may potentially help explain the lack of effect on DMI and MY to Bovine BlueLite supplementation shown by our transitioning dairy cows, as positive effects have been observed in previous research when cows were really challenged by heat stress.

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