ABSTRACT

Title of Document: **POTENTIAL INTERACTIONS OF DIETARY CATION-ANION DIFFERENCE AND MONENSIN WITH RESPECT TO FEED EFFICIENCY IN LACTATING DAIRY CATTLE**

Amy Elizabeth Weidman, MS, 2014

Directed By: Professor, Dr. Richard Erdman,
Department of Animal and Avian Sciences

The ionophore monensin improves feed efficiency (FE) by increasing sodium uptake in rumen bacteria, which alters rumen fermentation. Dietary cation-anion difference (DCAD) represents the balance between the dietary strong cations (Na and K) and strong anion (Cl) and increased DCAD also improves FE. This study tested the interaction of monensin and DCAD using sodium sesquicarbonate and potassium carbonate as the strong ion sources in 18 early to mid-lactation Holstein cows. Monensin, DCAD and the monensin-DCAD interaction had no effect on dry matter intake, milk production and milk composition, and FE. However, addition of dietary sodium and potassium increased rumen concentrations of those minerals and increased rumen acetate and decreased rumen propionate concentrations. The effect of sodium on rumen acetate and total VFA concentrations was more pronounced in the monensin diets suggesting an interaction between monensin and DCAD on rumen fermentation.

POTENTIAL INTERACTIONS OF DIETARY CATION-ANION DIFFERENCE AND MONENSIN WITH RESPECT TO FEED EFFICIENCY IN LACTATING DAIRY CATTLE

By

Amy Elizabeth Weidman

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2014

Advisory Committee:
Dr. Richard Erdman, Chair
Dr. Robert Peters
Dr. Rick Kohn

Acknowledgements

First, I would like to thank Dr. Richard Erdman for giving me the opportunity to pursue my Masters degree. Without his support and encouragement none of this would be possible. Secondly, I would like to thank my committee members, Dr. Rick Kohn and Dr. Robert Peters, for their time and willingness to serve on my committee. I would also like to thank the members of the Erdman laboratory, Dr. Beverly B. Teter, Marie Iwaniuk, Alzahraa Abdelatty, and Tracy Catterton for their support and assistance throughout my entire degree. Special thanks goes to Marie Iwaniuk for helping me to adjust to graduate school and never failing to give me an answer when I had a question. I would like to thank the staff at the Central Maryland Research and Education Center (CMREC): Tim Shellem, Michael Dwyer, Brian Spielman, Katie Albaugh, Dave Bowman, Albert Pickett, Jason Cline, Jorge Ovando, and Dolly Cline for assisting me with my research project and for having patience with me while I learned how to conduct research experiments.

I would not be where I am today without the love and support of my family. I would like to thank my parents for teaching me to never quit and for always coming to help when I needed it. Lastly, I would like to thank my fiancé for his unfailing love, support and encouragement while I have pursued this degree.

Table of Contents

Acknowledgements ii
List of Tables iv
List of Figures v
Chapter 1: Introduction 1
Chapter 2: Literature Review 3
Monensin 3
Discovery 3
Uses in Poultry, Beef and Dairy Cattle 3
Rumen Fermentation effects 4
Effects on Feed Efficiency and Performance 6
Dietary Cation Anion Difference ..... 8~~7~~
Background ..... 8~~7~~
Rumen Fermentation Effects 9
Acid Base Responses ..... 11~~10~~
Effects on Feed Efficiency and Production 11
Monensin DCAD Interaction 12
References 14
Chapter 3: EXPERIMENT ..... 19~~18~~
INTERPRETIVE SUMMARY ..... 20~~19~~
ABSTRACT ..... 21~~20~~
INTRODUCTION ..... 22~~21~~
MATERIALS AND METHODS ..... 24~~22~~
Research Facilities and Animals ..... 24~~22~~
Experimental Diets ..... 25~~24~~
Measurements ..... 26~~25~~
Statistical Analysis ..... 27~~26~~
RESULTS ..... 28~~27~~
DISCUSSION ..... 30~~29~~
CONCLUSIONS ..... 35~~34~~
ACKNOWLEDGEMENTS ..... 36~~35~~
REFERENCES ..... 37~~36~~
Chapter 4: Comprehensive List of References ..... 57~~56~~

List of Tables

Table 3.1 Ingredient composition of experimental diets (DM basis)……..45

Table 3.2 Chemical composition of experimental diets (DM basis)……...47

Table 3.3 Effect of monensin, DCAD and the monensin-DCAD interaction on feed intake, milk production and composition, and feed efficiency in mid-lactation dairy cows…….48

Table 3.4 Effect of monensin, DCAD and the monensin-DCAD interaction on rumen pH, ion concentrations and VFA concentrations in mid-lactation dairy cows……49

List of Figures

Figure 3.1 Change in rumen pH over time post-feeding……50

Figure 3.2 Change in rumen potassium concentration over time post-feeding……..51

Figure 3.3 Change in rumen sodium concentration over time post-feeding……...52

Figure 3.4 Change in rumen chloride concentration over time post-feeding……..53

Figure 3.5 Change in rumen acetate concentration over time post-feeding……54

Figure 3.6 Change in rumen propionate concentration over time post-feeding……..55

Figure 3.7 Change in rumen butyrate concentration over time post-feeding……..56

Figure 3.8 Change in rumen isobutyrate concentration over time post-feeding…….57

Figure 3.9 Change in rumen isovalerate concentration over time post-feeding…….58

Figure 3.10 Change in rumen valerate concentration over time post-feeding……59

Figure 3.11 Change in rumen total VFA concentration over time post-feeding…….60

Figure 3.12 Changes in rumen acetate:propionate ratio over time post-feeding……..61

Chapter 1: Introduction

During the last for four years (2010 to 2013) feed costs have accounted for between 77 to 82% of operating costs and 49 to 55% of the total cost of producing milk on U.S. dairy farms (USDA-ERS, 2014). The increased feed costs on dairy farms are in part due to the increased use of corn as a feedstock for ethanol production and also short-term shocks in feed supplies such as during the drought in the Midwest during the 2012 growing season. Because of the increase in average feed cost associated with producing milk, dairy producers have been keenly interested in improving the efficiency of converting feed into milk in their dairy herds. Typically, the most commonly used measure of feed efficiency (FE) in the dairy industry is fat-corrected milk (FCM) per unit of dry matter intake (DMI) (Erdman, 2011).

While there are multiple ways to improve the feed efficiency of lactating dairy cattle this paper investigates the effects of two dietary factors that are known to affect dairy feed efficiency: 1) monensin supplementation and 2) altering dietary cation-anion difference (DCAD) (Erdman et al, 2011). Monensin is an ionophore antibiotic that is approved as a feed additive in beef and dairy cattle. It functions by increasing the porosity of the cell wall of gram positive rumen bacteria to strong ions such as Na and to a lesser extent K. When fed to lactating dairy cows, monensin causes changes in the rumen bacterial population that shift the rumen volatile fatty acids (VFA) towards the production of propionate as opposed to acetate (Duffield et al, 2008). This alteration in the VFA ratio, among other effects of feeding monensin, leads to an improvement in feed efficiency.

Dietary cation anion difference is the sum of the dietary strong cations (Na^+ and K^+) minus the dietary strong anions (Cl^- and sometimes S^{2-}) and is typically expressed in millequivalents per kilogram feed dry matter (DM). Dietary cation anion difference can be

altered by either increasing or decreasing potassium and/or sodium or conversely increasing or decreasing the Cl content of the diet. Changes in DCAD can be achieved by either selection of feeds based on their Na, K, and Cl concentrations or through additions or subtractions of mineral supplements that are high in Na, K, or Cl. DCAD improves feed efficiency by increasing milk yield, milk fat content, and dry matter intake (DMI) by increasing rumen pH and improving the acid-base status of the cow (Erdman et al, 2011).

Although mechanistically different, increasing dietary concentrations of monensin and DCAD have been shown to alter the rumen bacterial environment which results in improved feed efficiency. Since monensin action depends on cation influx into rumen bacteria, it follows that there could be an interaction between monensin and DCAD with respect to dairy feed efficiency, the subject of this thesis.

Chapter 2: Literature Review

Monensin

Discovery

Monensin was first isolated from a strain of *Streptomyces cinnamonensis* in 1967 when Eli Lilly and Company was searching for new antibiotics. In that same year Agtarap et al (1967) described the structure of monensin. This was also the first detailed description of the structure of a polyether antibiotic, commonly referred to as ionophore. An ionophore is a lipid soluble molecule that causes the bacterial cell wall to be more permeable to certain ions such as sodium and potassium. Individual ionophores are different with respect to their ion it selectivity. Monensin, for example, shows preference to monovalent ions especially sodium followed by potassium, rubidium, and lithium (Chapman et al, 2010). Monensin's antibiotic properties allow it to select against gram positive bacteria, which in turn alters rumen fermentation. When monensin was discovered it was found to have anti-coccidial properties which lead to its original use in poultry for the treatment and prevention of coccidiosis (Chapman et al, 2010).

Uses in Poultry, Beef and Dairy Cattle

In the U.S. monensin was first approved by the U.S. Food and Drug Administration (FDA) as an antibiotic for the control of coccidiosis in the poultry industry in July of 1971 (Chapman et al, 2010). It is still in use today for that purpose under the trade name of Coban and it is distributed by Elanco (Greenfield, IN). After the success of monensin in the poultry industry, researchers began to investigate the effect of monensin on rumen fermentation in beef cattle. At that point it was found that monensin alters rumen fermentation such that there was an increase in propionate in relation to acetate production in the rumen which in turn improved the

feed efficiency of beef cattle (Raun et al, 1976; Richardson et al, 1976). This, among other rumen fermentation effects discovered, is what caused monensin to be approved by the FDA for use in beef cattle to improve feed efficiency. Monensin improves feed efficiency by increasing the efficiency of energy metabolism of rumen bacteria, improved nitrogen metabolism of rumen bacteria through decreased protein degradation, and retardation of digestive disorders resulting from abnormal rumen fermentation (Bergen and Bates, 1983). By using monensin, beef producers can increase the feed efficiency in their animals by 5 to 10 percent. Currently, monensin is the most universally used feed additive in beef cattle feedlot diets. In 2004, monensin became the first feed additive approved by the U.S. Food and Drug Administration for improving feed efficiency in lactating dairy cattle where it is used to increase milk production efficiency. Due to the changes in rumen fermentation through the feeding of monensin, a decrease in diseases related to abnormal rumen fermentation such as acidosis and bloat has been seen (Schelling, 1984; McGuffey et al 2001; Eastridge, 2006). This effect can also assist in improving milk production by improving overall animal health.

Rumen Fermentation effects

Gram negative bacteria have a three-layer cell wall which includes the cytoplasmic membrane space, a peptidoglycan layer and the outer membrane (Bauman, 2007). Comparatively, gram positive bacteria only have a two layer cell wall that is made up of the cytoplasmic membrane and a peptidoglycan layer (Bauman, 2007). Monensin functions by binding to bacterial cell membranes and creating a portal that facilitates the entry of hydrogen ions (H^+) into the cell and the exit of potassium (the main intracellular cation) from the cell. To counteract the potassium eflux, the bacterial cell expends a great deal of energy in an attempt to maintain cellular equilibrium (Russell and Strobel, 1989). Th increased energy expenditure

causes cell growth to decrease which can be followed by cell death (McGuffey et al, 2001). Since gram negative bacteria have a more complex cell wall they are less sensitive to monensin and therefore monensin inherently selects against gram positive versus gram negative bacteria in the rumen. Monensin effects on rumen fermentation are due to the nature of the bacteria it selects (gram negative) which causes an increase in energy metabolism and improves the nitrogen metabolism for the animal (Duffield et al, 2008). Monensin causes a change in the rumen flora that affects the ratio of volatile fatty acids (VFA) that are produced by the rumen.

When ruminants are fed high forage diets, the VFA in the highest proportion is acetate (typically 50-70% of total VFA production) (Sheperd and Combs, 1998). Comparatively, when ruminants are fed high grain diets such as in beef cattle fed feedlot diets, the molar proportion of acetate decreases and molar proportion of propionate increases and becomes the predominate VFA produced (Russell and Strobel, 1989). A similar shift in the rumen VFA toward increased propionate and decreased acetate occurs when monensin is added to the diet. Dairy cattle are usually fed high forage diets (50-70% forages). When dairy cattle are fed monensin in conjunction with high forage diets, the rumen environment changes causing a shift against acetate, increased propionate, and a decrease in the acetate-to-propionate ratio in the rumen (Richardson et al, 1976). These changes lead to a decrease in rumen hydrogen concentration which indirectly causes a decrease in rumen methane (CH_4) production.

The increase in rumen propionate production, and the subsequent effects on glucose metabolism may be partially responsible for the improved feed efficiency with monensin feeding. Ruminant animals derive the majority of their glucose from gluconeogenic precursors such as propionate and gluconeogenic amino acids that are absorbed from the diet. The liver is the primary site of gluconeogenesis and propionate is a primary precursor for gluconeogenesis in

ruminants; therefore, when more propionate is produced in the rumen it travels to the liver via the portal vein and liver glucose production is increased. In addition, propionate production by rumen bacteria is more energetically efficient than acetate production (Van Maanen et al, 1978). Thus by feeding monensin which increases propionate in the rumen, gluconeogenesis may be enhanced and the amount of energy available per unit of feed increases. Therefore, feed efficiency is improved (Van Maanen et al, 1978).

In addition to improving the energy metabolism in the rumen, monensin has also been shown to improve nitrogen metabolism. Bergen and Bates (1984) suggested that monensin may reduce feed protein degradation in the rumen, resulting in increased feed protein reaching the small intestine and the availability of amino acids for absorption. Monensin has also been shown to decrease rumen ammonia concentrations, an indicator of feed protein degradation in the rumen. Thus, part of the feed efficiency response to monensin could be due to reduced feed protein degradation in the rumen resulting in increased protein available for absorption in the small intestine. (Chen and Russell, 1991).

Effects on Feed Efficiency and Performance

Beef Cattle:

In beef cattle monensin is primarily used as a feed additive to improve feed efficiency, but it also has been shown to decrease the incidence of coccidiosis, acidosis, and bloat in feedlot cattle (Schelling, 1984; Eastridge, 2006). While many individual studies have demonstrated the effectiveness of monensin in increasing feed efficiency; Duffield et al (2012) published a meta-analysis that summarizes the feed efficiency and production response to monensin in growing and finishing beef cattle from the reports published over the last 40 years. In that summary, monensin reduced dry matter intake (DMI) by 0.27 kg per day and increased average daily gain

(ADG) by 0.029 kg/day (Duffield et al, 2012). The combination of reduced feed intake and increased rate of gain resulted in 0.53 unit decreased in feed per unit gain Duffield et al., (2012).

Dairy Cattle:

Since its approval as a feed additive for lactating dairy cattle, by the FDA in 2004 monensin has been primarily used to increase the efficiency of milk production. In a meta-analysis of published experiments with lactating dairy cows, Duffield et al., (2008) reported a 2% increase in milk yield, a 2% decrease in DMI and a 2.5% improvement in efficiency of milk production. Overall, monensin has no effect on the concentration of milk protein but increased milk protein yield by 1.9% due to its effect on total milk production. Milk fat yield was not affected but monensin was shown to decrease milk fat concentration by 3.1% (Duffield et al, 2008). Most likely, the change in milk fat concentration with monensin addition is caused by the changes in the microbial population of rumen bacteria that biohydrogenate dietary polyunsaturated fatty acids. Several double-bond containing (trans) fatty acid intermediates in the rumen biohydrogenation process, such as trans-10 18:1, trans-10, cis-12 conjugated linoleic acid, and others (Kadegowda et al., 2009) have been shown to inhibit milk fat synthesis (Kadegowda et al., 2009; Bauman and Griinari, 2003). Monensin addition has been shown to increase biohydrogenation intermediates in milk fat similar to the changes observed in dairy cows fed with a milk fat depressing diets (He et al., 2012). The monensin effect on milk fat concentrations, especially at higher concentrations of monensin in the diet (Symanowski et al., 1999), may reduce the overall feed efficiency effect of monensin by reducing milk fat concentration and therefore 3.5% fat-corrected milk yield, the numerator in the dairy feed efficiency calculation.

Dietary Cation Anion Difference

Background

Dietary cation-anion difference (DCAD) has been shown to affect dairy feed efficiency (Erdman et al., 2011; Harrison et al., 2012). DCAD is the balance between the dietary strong cations and anions and is expressed in milliequivalents per kilogram of feed dry matter (DM) (Hu and Murphy, 2004). The strong ions are monovalent ions in the diet that generally have intestinal absorption of $\geq$ 90% (NRC, 2001). The strong cations include sodium and potassium and chloride is a major strong anion. In some instances sulfur, magnesium and phosphorous have been incorporated into DCAD equations (National Research Council, 2001). However, these elements vary in absorption rates which are typically much lower (40-60%) than those for sodium, potassium, and chloride. There have been several different DCAD equations suggested (Block, 1994), particularly ones that include sulfur as a strong anion, however, the equation that is most often used is the difference between the sum of sodium and potassium minus chloride (DCAD = Na + K – Cl) which is expressed on a milliequivalent (mEq) per kg or 100g diet dry matter. Balancing the strong ions in the diet in order to improve feed efficiency is not a new concept. It has been done for many years in monogastrics (Golz and Crenshaw, 1990; Mongin, 1981) and is now being applied to ruminants (Sanchez and Beede, 1996; Hu and Murphy, 2004; Hu et al, 2007; Erdman et al, 2011).

The DCAD can be altered by selection of feeds based on their strong ion concentrations or through the use mineral supplements such as potassium carbonate, potassium bicarbonate, sodium bicarbonate, and sodium sesquicarbonate. These provide increased strong cation concentrations without addition of a corresponding anion (Cl). Alternatively, supplements such as magnesium chloride and magnesium sulfate have been used to reduce DCAD.

Supplementations with salt (NaCl) or potassium chloride (KCl) are DCAD neutral since the milliequiqvalents of cations (Na or K) are balanced with anions (Cl). The first reported use of DCAD in dairy cows was for the prevention of milk fever (parturient paresis) or hypocalcaemia at the time of calving (Ender et al., 1971). In those studies, low DCAD (<10 meq/100g DM) diets fed to dry cows prior to calving was shown to prevent milk fever (Ender et al, 1971; Block, 1984). Tucker et al (1988) was the first to show that increasing DCAD improved milk production, milk fat percent, and dry matter intake (DMI) in lactating dairy cows. Many other studies have shown that high DCAD (30 to 50 meq/100g DM) diets can be used to increase milk fat, DMI, fat-corrected milk (FCM) and improve the acid-base status of lactating dairy cows (Hu and Murphy, 2004; Hu and Murphy, 2007). In part, high DCAD diets accomplish this by increasing the rumen pH by serving as buffers, which shift the volatile fatty acid (VFA) ratio in the rumen to favor acetate over propionate (Erdman, 1988).

Rumen Fermentation Effects

When DCAD is increased by adding potassium or sodium carbonates or bicarbonates to the diet, rumen pH is increased. The rumen pH in a lactating dairy cow varies with time after feeding, but can range from nearly 7 to as low as 5 depending on the diet fed and the time rumen pH is measured after feeding. However, normal rumen pH is between 5.5 and 7 (Erdman, 1988). Changes in the rumen pH result in shifts in the species of bacteria populating the rumen (Kalscheur et al, 1997). An increase in rumen pH results in more fiber digesting bacteria and fewer starch-digesting bacteria in the rumen. As rumen pH increases the acetate-to-propionate ratio increases (Erdman, 1988).

In addition to altering the VFA fermentation pattern, increasing DCAD alters rumen biohydrogenation of unsaturated fatty acids. The process of rumen biohydrogenation is essential

to milk fat synthesis and incomplete biohydrogenation can cause milk fat depression (Kalscheur et al, 1997). The rumen biohydrogenation process begins with the isomerization of dietary polyunsaturated fatty acids (PUFA). For example, oleic acid (*cis*-9, octadecenoic acid) isomerizes to form trans-double bond containing fatty acid intermediates such as elaidic (*trans*-10 18:1) or vaccenic (*trans*-11, 18:1) acids. Linoleic acid (*cis*-9, *cis*-12 18:1, octadecadienoic acid) is isomerized to form *trans*-10, *cis*-12 18:2 (conjugated linoleic acid). During complete biohydrogenation, these trans-double bond containing intermediates are subsequently fully saturated to form stearic acid (18:0). When the rumen pH is depressed, there is an increase in both trans 18:1 fatty acids and conjugated linoleic acids, which are intermediates resulting from the incomplete biohydrogenation of PUFA (Kalscheur et al, 1997, Piperova et al., 2002). Fatty acid intermediates such as trans 18:1 and trans-10, cis-12 conjugated linoleic acid when absorbed have been shown to interfere with *de novo* fatty acid synthesis in the mammary gland causing diet induced milk fat depression (Griinari et al, 1998). When the rumen pH is increased, there is a decrease in *trans* 18:1 fatty acids and conjugated linoleic acids (Kalscheur et al, 1997) which reflects a change in the rumen bacterial population associated with the later steps of the rumen biohydrogenation process. These microbes cause a more complete biohydrogenation, which allows for less of the fatty acid intermediates leaving the rumen and being absorbed in the small intestine and therefore removal of inhibitory effects of *trans* containing fatty acids and an increase in milk fat percentage. Kalscheur et al., (1997) and Piperova et al., (2002) demonstrated that buffer addition to the diet of cows fed high grain diets increased rumen pH, reduced the duodenal flow of rumen biohydrogenation intermediates and increased milk fat percent.

Acid Base Responses

Increasing DCAD not only affects the rumen environment but it also effects the cow's acid-base homeostasis. By increasing the concentrations of dietary Na and K, acid-base balance within the animal is altered such that body fluids become less acidic and more alkaline (Chan et al., 2005). The acid-base balance is important because the cow's normal blood pH is tightly regulated at pH 7.4 and small changes (0.02 to 0.04 units) in blood pH can have a profound impact on feed intake and milk production. Increasing DCAD has been shown to increase blood pH and bicarbonate (HCO^{3-}) levels which improve the buffering capacity of the blood helping to maintain a normal blood pH (Chan et al, 2005).

Effects on Feed Efficiency and Production

Several studies (Tucker et al, 1994; Delaquis and Block, 1995b; Sanchez et al, 1997; Hu and Murphy, 2004; Hu and Murphy, 2007) have examined the effects DCAD on the production responses in lactating dairy cattle. Hu and Murphy (2004) published a meta-analysis outlining the effect of DCAD on performance and acid-base status on lactating dairy cattle. Hu and Murphy (2004) showed that increasing DCAD resulted in increases in DMI, milk yield, and 4.0% fat-corrected milk (FCM) yield. Hu and Murphy (2004) also suggested that increasing DCAD will cause an increase in milk fat percentage due to the correlation of the milk fat percentage to the ruminal pH. However, milk protein percentage was unaffected by increasing DCAD (Hu and Murphy, 2004).

Increasing DCAD also helps to improve the acid-base status of high producing cows through increased blood pH, urine pH, and bicarbonate. Improved acid-base status is thought to be related to the increase in DMI, which is evident by the increase in blood pH and bicarbonate concentration–when DCAD is increased (Hu and Murphy, 2004). By increasing DMI, more of

the dietary nutrients are distributed to productive purposes so that fewer nutrients are used to satisfy maintenance requirements (Erdman, 2011). Thus DCAD increases feed efficiency by increasing DMI and milk production thereby reducing the proportion of feed used for maintenance.

Monensin DCAD Interaction

Feed efficiency responses from DCAD and monensin can be linked as the responses to both supplements depend upon potassium and sodium. In the case of DCAD, the diet is altered by using addition of strong cation sources such as sodium bicarbonate or potassium carbonate. As an ionophore, monensin has a preference to form complexes with both sodium and potassium, but primarily sodium due to its role as a sodium/hydrogen antiporter (Russell, 1987). The reason DCAD and monensin both depend upon sodium and potassium is because each plays a major role in cellular activity. Potassium, for example, is involved in acid-base regulation, water balance and osmotic pressure (NRC, 2001). Sodium bicarbonate is involved in rumen acid-base balance and is the major buffer in ruminant saliva (Erdman, 1988; Kohn and Dulap 1998). Sodium is the major cation in rumen fluid but the sodium and potassium concentration in rumen fluid varies with the amounts Na and K in the cow's diet (Bennink et al, 1978). When monensin forms complexes with strong cations it allows extracellular sodium to enter the cell and intracellular potassium to leave the cell. Since, potassium is the major intracellular cation this exchange results in energy expenditure by the bacteria sensitive to monensin eventually leading to cell death. This results in selection of bacteria that are less sensitive to monensin and changes in rumen fermentation. Even though sodium and potassium are crucial in the action of DCAD and monensin, there have been very few studies on the interaction between monensin,

sodium, potassium and DCAD with respect to the rumen environment and overall animal production performance.

The interaction between monensin, potassium, and sodium has been studied in beef cattle. However, those experiments (Rumpler et al, 1986) used chloride salts that are DCAD neutral and feed efficiency was not measured. Greene et al., (1986) conducted a study with lambs that were ruminally infused with potassium chloride to investigate the effect of monensin and potassium on the magnesium absorption in sheep. While the interaction between monensin and potassium in relation to the rumen environment or feed efficiency was not specifically studied, there was a significant interaction between monensin and potassium. With more potassium infusion, monensin addition resulted in a greater decrease in the rumen acetate to propionate ratio (Greene et al, 1986).

Dary et al., (2005) showed a possible interaction between monensin and sodium bicarbonate in lactating dairy cows. In that study, experimental diets included: 1) a control diet; 2) a diet with monensin supplement; and 3) a diet with supplements of monensin and sodium bicarbonate. While there were no significant effects of diet on milk production or the milk components, there was an increase in feed efficiency (FCM/DMI) comparing the control (1.23), monensin (1.31), and monensin plus sodium bicarbonate (1.40) treatments. This experiment suggested a possible interaction between sodium and monensin where the feed efficiency response to monensin was enhanced in cows fed sodium bicarbonate. While the interaction between sodium and monensin is important, it is essential to look at cation source to see the effects, if any, of sodium versus potassium in relation to DCAD concentrations and monensin in lactating dairy cows.

One additional study (Newbold et al, 2013) looked at how cation concentration affected the sensitivity of rumen bacteria to ionophores. In that study, high sodium media resulted in an

increased sensitivity of rumen bacteria to monensin whereas high potassium media had the opposite effect. Further, monensin decreased intracellular sodium and potassium in the most sensitive bacteria, *E. ruminantium* (Newbold et al, 2013). Based on that study, altering the rumen sodium and potassium concentrations could be used to alter "efficacy of monensin by increasing the rate of energy expenditure to maintain ionic homeostasis in bacteria that are sensitive to ionophores" (Newbold et al, 2013). Based on these results, we conclude that there was sufficient evidence to merit the conduct of studies that examined the interaction between monensin, DCAD concentration, and DCAD cation source.

References

Agtarap, A., J. W. Chamberlin, M. Pinkerton, and L. Steinrauf. 1967. The structure of monensin acid, a new biologically active compound. J. Am. Chem. Soc. 89:5737–5739.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23(1):203-227.

Bauman, Robert W. 2007. Microbiology: With Diseases by Taxonomy. San Francisco: Pearson/Benjamin Cummings, p65.

Bennink, M. R., T. R. Tyler, G. M. Ward, and D. E. Johnson. 1978. Ionic milieu of bovine and ovine rumen as affected by diet. J Dairy Sci. 61:315-323.

Bergen, W. G. and Bates D. B. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465–1483.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939−2948.

Block, E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. J. Dairy Sci. 77:1437-1450.

Chan, P.S., J.W. West, J.K. Bernard, and J.M. Fernandez. 2005. Effects of dietary cation-anion difference on intake, milk yield, and blood components of the early lactation cow. J. Dairy Sci. 88:4384-4392.

Chapman, H. D., T K Jeffers, R B Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788-1801.

Chen, G. C., and J. B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation and deamination by mixed ruminal microorganisms in vitro. J. Anim. Sci. 69:2196-2203.

Dary, C., M. Harris, N. Koppe, T. Ritson-Bennett, R. Khorasani, and L. Doepel. 2005. The effect of monensin and sodium bicarbonate on milk yield and composition. Advances in Dairy Technology. Abstract,17:359-359.

Delaquis, A. M., and E. Block. 1995b. Dietary cation-anion difference, acid-base status, mineral metabolism, renal function, and milk production of lactating cows. J. Dairy Sci. 78:2259–2284.

Duffield, T. F., J. K. Merrill, and R. N. Bagg. 2012. A meta-analysis of the effects of monensin in beef cattle on feed efficiency, gain and dry matter intake. J. Anim. Sci. 90:4583-4592.

Duffield, T.F., A. R. Rabiee, and I. J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347-1360.

Eastridge, M. L. 2006. Major advances in applied dairy cattle nutrition. J. Dairy Sci. 89:1311–1323.

Ender, F., I. W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemia paresis puerperalis. Zeitschrift Tierphysiologie Tierenahrung, Futtermittelkunde. 28:233-256.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: a review. J. Dairy Sci. 71:3246-3266.

Erdman, R.A. 2011. Benchmark dairy feed efficiency. *Feedstuffs* 82.28:16-19.

Erdman, R.A., L.S. Piperova, and R.A. Kohn. 2011. Corn silage versus corn silage: alfalfa hay mixtures for dairy cows: Effects of dietary potassium, calcium, and cation-anion difference. J. Dairy Sci. 94:5105-5110.

Golz, D. I., and T. D. Crenshaw. 1990. Interrelationships of dietary sodium, potassium and chloride of growth in young swine. J. Anim. Sci. 68:2736.

Greene, L. W., G. T. Schelling, and F. M. Byers. 1986. Effects of dietary monensin and potassium on apparent absorption of magnesium and other macroelements in sheep. J. Anim. Sci. 63:1960.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-octadecenoic acids and milk fat depression. J. Dairy Sci. 81: 1251–1261.

Harrison, J., R. White, R. Kincaid, E. Block, T. Jenkins, and N. St-Pierre. 2012. Effectiveness of potassium carbonate sesquihydrate to increase dietary cation-anion difference in early lactation cows. J. Dairy Sci. 95:3919-3925.

He, M., K.L. Perfield, H.B. Green, and L.E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95:1447–1461.

Hu, W., and M.R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. J. Dairy Sci. 87:2222-2229.

Hu, W., M. R. Murphy, and L. Kung, Jr. 2007. The relationship between dry matter intake and acid-base status of lactating dairy cows as manipulated by dietary cation-anion difference. Anim. Feed Sci. Technol. 136:216-225.

Kadegowda A. K. G., M. Bionaz, B. Thering, L.S. Piperova, R.A. Erdman, and J.J. Loor. 2009. Identification of internal control genes for quantitative polymerase chain reaction in mammary tissue of lactating cows receiving lipid supplements. J. Dairy Sci. 92:2007–2019.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-c18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80: 2104-2114.

Kohn, R. A. and T. F. Dunlap. 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. J Anim. Sci. 76:1702-1709.

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for dairy cattle: current status and future outlook. J. Dairy Sci. 84(E. Suppl.): E194-E203.

Mongin. P. 1981. Recent advances in dietary cation anion balance: applications in poultry. Nutr.Soc. 40:285.

National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Washington, D.C.: National Academy Press, p105-161.

Newbold, C. J., R. J. Wallace, and N. D. Walker-Bax. 2013. Potentiation by metal ions of the efficacy of the ionophores, monensin and tetronasin, towards four species of ruminal bacteria. FEMS Microbiology Letters 338(2):161-167.

Piperova, L. S., J. Sampugna, B. B. Teter, K. F. Kalscheur, M. P. Yurawecz, Y. Ku, K. M. Morehouse, and R. A.Erdman. 2002. Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J. Nutr. 132:1235-1241.

Raun, A.P., C.O. Cooley, E.L. Potter, R.P Rathmacher, and L.F. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43:670-677.

Richardson, L. F., A. P. Raun, E. L. Potter and C. O. Cooley. 1976. Effect of monensin in rumen fermentation in vitro and in vivo. J. Anim. Sci.43:657.

Rumpler, W. V., D. E. Johnson, and D. B. Bates. 1986. The effect of high dietary cation concentration on methanogenesis by steers fed diets with and without ionophores. J. Anim. Sci. 62:1737.

Russell, J.B. 1987. A proposed mechanism of monensin action in inhibiting ruminant bacterial growth: effects on ion flux and protonmotive force. J. Anim. Sci. 64:1519-1525.

Russell, J.B. and H.J. Strobel. 1989. Effects of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1-6.

Sanchez, W.K., and D.K. Beede. 1996. Is there an optimal dietary cation-anion difference for lactation diets? Anim. Feed Sci. Technol. 59:3-12.

Sanchez, W. K., D. K. Beede, and J. A. Cornell. 1997. Dietary mixtures of sodium bicarbonate, sodium chloride, and potassium chloride: Effects on lactational performance, acid-base status, and mineral metabolism of Holstein cows. J. Dairy Sci. 80:1207–1216.

Schelling, G. 1984. Monensin mode of action in the rumen. J. Anim.Sci. 58:1518–1527.

Sheperd, A.C. and D.K. Combs. 1998. Long-term effects of acetate and propionate on voluntary feed intake by midlactation cows. J. Dairy Sci. 81:2240-2250.

Tucker, W.B., G.A. Harrison, and R.W. Hemken. 1988a. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71:346-354.

Tucker, W. B., I. S. Shin, J. F. Hogue, M. Aslam, G. D. Adams, M. T. Van Koevering, R. K. Vernon, and K. R. Cummings. 1994. Natural sodium sesquicarbonate fed for an entire lactation: Influence on performance and acid-base status of dairy cows. J. Dairy Sci. 77:3111–3117.

USDA-ERSa. National Milk Cost of Production. *USDA Economic Research Service*. N.p., 2014. http://www.ers.usda.gov/data-products/milk-cost-of-production-estimates.aspx#.U4Y6U8Uxo7k. Accessed July 3, 2014.

Van Maanen, R.W., J.H. Herbein, A.D. McGilliard, J.W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002–1007.

Chapter 3: EXPERIMENT

Potential Interactions of Dietary Cation-Anion Difference and Monensin with Respect to Feed Efficiency in Lactating Dairy Cattle

INTERPRETIVE SUMMARY

Potential Interactions of Dietary Cation-Anion Difference and Monensin with Respect to Feed Efficiency of Lactating Dairy Cattle. *Weidman et al., page 000.* The ionophore monensin improves feed efficiency (FE) by increasing sodium uptake in rumen bacteria, which alters rumen fermentation. Dietary cation-anion difference (DCAD) represents the balance between the dietary strong cations (Na and K) and strong anion (Cl) and increased DCAD also improves FE. This study tested the interaction of monensin and DCAD using sodium sesquicarbonate and potassium carbonate as the strong ion sources in 18 early to mid-lactation Holstein cows. Monensin, DCAD and the monensin-DCAD interaction had no effect on dry matter intake, milk production and milk composition, and FE. However, addition of dietary sodium and potassium increased rumen concentrations of those minerals and increased rumen acetate and reduced rumen propionate concentrations. The effect of sodium on rumen acetate and total VFA concentrations was more pronounced in the monensin diets suggesting an interaction between monensin and DCAD on rumen fermentation.

Potential Interactions of Dietary Cation-Anion Difference and Monensin with Respect to Feed Efficiency in Lactating Dairy Cattle

A. E. Weidman, and R. A. Erdman*

Animal and Avian Sciences Department,

University of Maryland,

College Park, MD 20742

***Corresponding Author: erdman@umd.edu**

Richard A. Erdman

Department of Animal & Avian Sciences,

University of Maryland, College Park, MD 20742

Phone: 1-301-405-8340

ABSTRACT

The objective of this experiment was to determine if there was an interaction between monensin supplementation and DCAD concentration and DCAD source on milk production, feed efficiency, and rumen fermentation in lactating dairy cows. Eighteen early-to-mid lactation Holstein cows (6 primiparous and 12 multiparous, including 6 multiparous rumen fistulated cows) were used in the 11 wk study. Cows were individually fed a basal diet containing 66% forage and 34% concentrate (DM basis). Treatments consisted of two concentrations of monensin (0 or ~300 mg/d) that were fed continuously for 9 wk after a 2 wk preliminary period that was used as a covariate in the analysis of covariance. Within each monensin treatment cows were fed 0, 200 mEq/kg added DCAD using potassium carbonate or 200 mEq/kg added DCAD using sodium sesquicarbonate in a 3 x 3 Latin square design. Monensin and DCAD treatments had no effect on feed intake, milk production and composition, and feed efficiency. The lack of production and intake responses may have been due to the relatively small number of animals and the relatively short experimental periods used in the experiment. In the rumen fistulated cows, rumen pH declined with time post-feeding but there were no effects of DCAD or monensin. Rumen concentrations of K^+ and Cl^- were increased with K supplementation while rumen Na^+ and Cl^- were increased with Na supplementation. Monensin had no effect of rumen ion concentrations. Rumen propionate was decreased while rumen acetate:propionate was increased by both Na and K supplementation. There was an interaction between monensin and DCAD for rumen propionate and total volatile fatty acids where DCAD reduced propionate in the control but propionate and total VFA were was increased by K and especially Na in the monensin supplemented cows. These results demonstrated significant interactions between

DCAD concentration and cation source and monensin with respect to rumen fermentation in lactating dairy cows.

INTRODUCTION

Feed costs represent approximately 50% of the total cost of producing milk and approximately 70% of total operating costs in dairy herds (USDA-ERS, 2014). Because feed represents such a large portion of their total costs, dairy producers are keenly interested in improving the efficiency of feed utilization in their dairy herds. The most commonly used measure of feed efficiency (FE) used in the dairy industry is 3.5% fat-corrected milk (FCM) per unit of dry matter intake (DMI). Monensin has been used as a feed additive to improve feed efficiency in beef cattle since 1975 (Potter et al, 1984) and was approved for use as a feed additive to improve FE in lactating dairy cows by the FDA in 2004. Monensin feeding causes a shift in rumen fermentation resulting in increased rumen propionate concentration that increases the energy efficiency and a concomitant reduction in feed intake that results in improved feed efficiency (Duffield et al., 2008).

Improved FE in lactating dairy cows can be achieved by altering the dietary cation-anion difference (DCAD) of the ration (Erdman et al., 2011). Dietary cation-anion difference is the balance between the dietary strong cations (Na and K) and strong anions (Cl and S) and is expressed in mEq per kg feed dry matter (DM). It can be increased by adding cation sources such as sodium and potassium bicarbonates, carbonates, and sesquicarbontates to the diet which increase the cation in relation to the anion concentration. DCAD can improve feed efficiency by increasing milk yield and milk fat concentration and dry matter intake (DMI) due to an improvement in rumen pH and rumen fermentation along with the acid-base status of the cow (Erdman 1988, Erdman et al., 2011).

Gram positive rumen bacteria are sensitive to monensin addition to the diet. As an ionophore antibiotic monensin binds to the bacterial cell wall creating a portal for influx and efflux of intra and extracellular cations. Monensin forms complexes with both sodium and potassium ions but preferentially binds sodium due to its role as a sodium/hydrogen antiporter (Russell, 1987). This causes an influx of Na and an efflux of K, the main intracellular cation. Newbold et al, (2013) recently demonstrated increased monensin sensitivity of different strains of rumen bacteria by increasing Na as compared to K concentrations in the fermentation media.

Even though monensin's mode of action in the rumen is as an ionophore that facilitates movement of strong ions across the cell wall in rumen bacteria, little attention has been paid to the interaction of DCAD and monensin or the source of dietary cations (potassium vs. sodium) on feed efficiency responses to monensin. While Na is the main extracellular cation in rumen fluid, both Na and K concentrations in the rumen are affected by their concentrations in the diet (Bennick et al., 1978). Since monensin functions by increased Na influx into the cell, one could reason that diets that increase rumen Na concentration might enhance rumen fermentation responses to monensin. This suggests that feed efficiency responses to monensin could be modulated by both the DCAD concentration and the strong ion sources (Na, K, and Cl) of dietary cations. While the interaction of DCAD and monensin has received limited study (Dary et al., 2005), the effect strong ion source (Na vs. K) has not been considered. This is surprising since due to monensin's selective affinity for sodium is well know (Russell, 1987). Therefore, the objectives of this study were to determine the interaction between DCAD concentration and strong ion source (Na vs. K) and monensin and their effects on feed intake, milk production, feed efficiency and rumen fermentation responses in lactating dairy cows.

MATERIALS AND METHODS

Research Facilities and Animals

The protocol (R-13-39) for this experiment was reviewed and approved by the University of Maryland Institutional Animal Care and Use Committee. The experiment was conducted at the Clarksville Dairy Research Facility located in Ellicott City, Maryland. The number of experimental observations required for the study was determined by power analysis using the Analyst feature of Statistical Analysis Software (SAS). Using an average standard error of the mean of 0.165 for FE calculated from previous experiments conducted by this laboratory, a required sample size of 36 was calculated to be required to detect a significant difference (alpha = 0.05) with an 80% probability of detecting a 0.10 unit difference in dairy FE (3.5% fat-corrected milk divided by dry matter intake, kg) in an experiment with 6 dietary treatments. Even though the required sample size is 36, this experiment represents the first replicate that was conducted with 18 cows due to the limited availability of tie-stalls required for individually feeding. A second replication with 18 additional cows will be used to complete the study prior to submission for publication.

Six primiparous and 12 multiparous cows averaging 75 $\pm$ 38 days in milk and 38 kg/d milk at the start of the experiment were used in the study. Six of the multiparous cows were surgically fitted with rumen cannula (Perry and Macleod, 1969) prior to the start of the experiment to study the effects of dietary treatments on rumen fermentation. Cows were housed and individually fed in tie-stalls fitted with water mattresses and bedded with wood shavings. The photoperiod in the research barn was controlled such that the cows received 16 h of light and 8 h of darkness during the study. Cows had continuous access to water and were milked twice daily at approximately 0615 and 1600 h. Cows also had continuous access to their experimental

diets that were fed once a day as a TMR at approximately 0930 h. The study was conducted from March until May.

Experimental Diets

Cows were fed a control unsupplemented diet during a 2 week preliminary period prior to the start of the experiment. Preliminary period data were used as a covariate in the statistical analysis. The control diet contained approximately 58% corn silage 8% alfalfa hay and 34% concentrate (DM basis). The diet was formulated using the NRC 2001 software (NRC, 2001) to meet the nutrient requirements for lactating dairy cows producing 40 kg/d milk containing 3.7% fat and 3.1% protein (Table 3.1). Treatments consisted of 0 or 13.2 mg/kg DM monensin addition (Rumensin®, ELANCO Animal Health, Greenfield, IN) and DCAD which would supply 303 mg per cow per day monensin in a cow consuming 23 kg/d dry matter intake (DMI) and DCAD supplementation of 0, or 200 mEq/kg DM using either sodium sequicarbonate (S-Q810® ,Church & Dwight Inc., Piscataway, NJ)) or potassium carbonate sesquihydrate (DCAD Plus®, Church & Dwight Inc., Piscataway, NJ)) as the strong ion sources. Treatments were applied in a 2 x 3 factorial arrangement of treatments. At the end the preliminary period, monensin treatment began with 9 cows fed the un-supplemented diet and 9 cows fed the diet containing 13.2 mg/kg DM monensin. Cows remained on their respective monensin treatments for the remainder of the experiment. Superimposed on the monensin treatments were DCAD treatments including the basal diet containing approximately 300 mEq/kg DCAD and two diets containing added DCAD (+200 mEq/kg) using either Na or K as the strong ion sources. DCAD treatments were applied in replicated 3 x 3 Latin Squares within monensin treatment using 3 wk experimental periods. To summarize, the 6 treatment combinations consisted of: 1) Control (C0), un-supplemented basal diet; 2) Control plus K (200K), + 200 mEq/kg DCAD using

potassium carbonate sesquihydrate; 3) Control plus Na (200Na), + 200 mEq/kg DCAD using sodium sesquicarbonate; 4) Monensin (M0), 13.2 mg/kg added monensin; 5) Monensin plus K (M200K); 1.32 mg/kg added monensin + 200 mEq/kg DCAD using potassium carbonate sesquihydrate; and 6) Monensin plus Na (M200Na); 1.32 mg/kg added monensin + 200 mEq/kg DCAD using sodium sesquicarbonate.

The basal total mixed ration (TMR) for the all cows was mixed in a portable mixer wagon. The DCAD and monensin supplements were mixed with basal TMR using a Calan Data Ranger® (American Calan, Northwood, NH) for cows within each treatment group prior to delivery to individual feed tubs for each cow in order to minimize errors in applying the individual treatments.

Measurements

Measurements included twice daily individual milk weights recorded electronically at each milking and daily individual weights of feed offered and feed refusals to determine feed intake. Milk samples were collected during the last 4 milkings at the end of the covariate period and the third week each experimental period week and sent to be analyzed for fat, protein, SCC and MUN by infrared analysis (Lancaster DHIA, Manheim, PA). Cows were weighed on the 7th day of each week of the experiment. Weekly feed samples were retained and composited by experimental period (the preliminary period and Periods 1, 2, and 3 in the Latin squares) for analysis of diet DM, CP, ADF, NDF, Lignin, ether extract, Ca, P, Mg, Na, K, Cl and S by Cumberland Valley Analytical Services (Hagerstown, MD). Feed analyses were used to calculate the actual DCAD of each treatment. Weekly samples of corn silage were used for DM analysis to adjust the as fed TMR to maintain a constant forage-to-concentrate ratio and to calculate the DM of the TMR such that daily DMI could be calculated for each cow.

On the last day of each experimental period, rumen fluid samples were collected. Rumen fluid samples were taken just prior to feeding and at 3, 6, 9, and 12-h post-feeding using the 6 rumen fistulated cows in the study. Rumen fluid was collected using a rumen fluid sampling tube (Bar Diamond, Inc, Parma, ID) attached to a 60-mL syringe. Samples were collected in 10-mL increments from 5 locations including the atrial, dorsal, ventral, caudodorsal, and cauoventral sacks of the rumen. Rumen pH was measured immediately and recorded. A 10 mL subsample was acidified with 0.2 ml of 50% H_2SO_4 and frozen at -20C for subsequent VFA analysis by gas chromatography (Bennink, 1978; Erwin et al., 1961). The remaining 40 mL of sample was frozen at -20C for later analysis of Na, K, and Cl concentrations by selective ion probes (Cole Parmer, Thermo Fisher Scientific, Waltham, MA).

Statistical Analysis

Mean data for DMI, milk production, milk fat and protein percentage, and milk SCC along with milk fat and protein yields, 3.5% FCM, and FE were calculated for each cow during the 2-week covariate period as well as the last week of each experimental period. Data were analyzed by analysis of covariance using the Mixed Procedure in SAS (Version 9.2, SAS Institute, Cary, NC). The statistical model included the effects of the covariate, period, monensin, DCAD, and DCAD by monensin treatment interaction. DCAD, monensin, and DCAD by monensin interactions were analyzed as fixed effects while the covariate, cow within monensin treatment, and period effects were designated as a random effect in the statistical model. The main effects of monensin were tested using cow within monensin as the error term and cow as the experimental unit. DCAD treatment and monensin by DCAD treatment interactions were tested using residual error with cow within period as the experimental unit. A

probability of $P \leq 0.05$ was considered significant and a probability ($0.05 < P < 0.10$) was considered as a trend towards being statistically significant.

RESULTS

The chemical composition (DM Basis) of the dietary treatments is presented in Table 3.2. As expected, diets were similar in chemical composition (Table 3.2) except for Na and K. Calculated treatment DCAD concentrations (using the Na + K – Cl equation) were 313, 522, 520, 312, 521, and 520 mEq/kg for each of the 6 treatments (C0, C200K, C200Na, M0, M200K, M200Na, respectively. These values were consistent with the addition of 200 mEq/kg DM of Na or K to the basal diet which contained 312 mEq/kg DM DCAD. Neither Monensin nor DCAD had any significant effects on feed intake, milk production and composition, and FE (Table 3.3). Increasing DCAD with either K or Na tended to increase DMI ($P = 0.078$) where the DCAD response appeared to be greater in cows fed the control vs. cows fed the monensin diet. However, there was no monensin by DCAD interaction for feed intake, milk production and composition, and FE.

Rumen pH was not affected by monensin or DCAD treatment. However rumen pH declined significantly with time post-feeding ($P = 0.001$; Figure 3.1). DCAD treatment significantly increased the K and Na concentrations in the rumen ($P = 0.001$; Table 3.4). Specifically, the effect of using a K supplement ruminal K concentration ($P = 0.001$) whereas Na supplementation increased ruminal Na concentration ($P = 0.002$). Ruminal Cl was increased ($P = 0.017$; Table 3.4) by addition of either K or Na to the diet. However, the K supplementation appeared to have a greater impact on rumen Cl than Na supplementation. There were no monensin effects on rumen K, Na, or Cl concentrations. The ruminal K concentration increased with monensin supplementation until 6 h postfeeding where the concentration peaked and then

began to decrease (P = 0.046; Figure 3.2) thereafter. The monensin by time by DCAD interaction was significant for the ruminal Na concentration (P = 0.045; Figure 3.3). Rumen Na and Cl concentrations decreased with time post-feeding (P = 0.001; Figures 3.3 and 3.4) whereas rumen K concentration gradually increased (P = 0.001) up to 6 h post-feeding and then declined thereafter (Figure 3.5).

Ruminal acetate concentration decreased with time post-feeding (P = 0.0002; Figure 3.5). The monensin-DCAD interaction was significant for ruminal acetate concentration (P = 0.0275; Table 3.4). Specifically, the acetate concentration increased with monensin supplementation and when DCAD was increased using sodium bicarbonate (P = 0.0208). Ruminal propionate concentration decreased with increasing DCAD, especially when DCAD was increased using potassium carbonate (P = 0.0323; Table 3.4). The monensin-DCAD interaction was significant for the ruminal propionate concentration (P = 0.0008) where rumen proprionate decreased with DCAD in the Control diet but increased with DCAD in the monensin supplemented group. Ruminal butyrate concentration increased with increasing DCAD (P = 0.0001; Table 3.4), particularly when DCAD was increased using potassium carbonate (P = 0.0001). Ruminal isobutyrate concentration decreased over time after feeding (P = 0.0001; Figure 3.7). Ruminal isovalerate was highest 3 hours after feeding but decreased after that time (P = 0.001; Figure 3.8). The monensin-DCAD interaction was borderline significant for the ruminal isovalerate concentration (P = 0.0553; Table 3.4). Specifically, the isovalerate concentration increased with monensin supplementation and when DCAD was increased using sodium bicarbonate (P = 0.011). The Monensin-DCAD interaction was significant for the ruminal valerate concentration (P = 0.0002; Table 3.4). Specifically the rumen valerate concentration increased with monensin supplementation and when DCAD was increased using sodium bicarbonate (P = 0.011).

The total VFA concentration in the rumen decreased with time post-feeding (P = 0.0005; Figure 3.10). The Monensin-DCAD interaction was significant for the total ruminal VFA concentration (P = 0.018; Table 3.4). The total VFA concentration was highest with monensin supplementation and when DCAD was increased using sodium bicarbonate (P = 0.049). The acetate to propionate ratio (A:P) was increased when DCAD was increased using either potassium carbonate or sodium bicarbonate (P = 0.0001). The A:P ratio was lowest at 3 h post-feeding but the proceeded to steadily increase over time (P = 0.0383; Figure 3.11).

DISCUSSION

Previous work has shown the supplementation of monensin in the diet of lactating dairy cows decreases DMI (Duffield et al., 2008; Phipps et al., 2000; Symanoski et al, 1999). Conversely Hu et al, (2007), showed that increasing the DCAD increased DMI while in some studies increasing DCAD has had no effect on DMI (Erdman et al., 2011). The inconsistency of the feed intake response to DCAD addition might been attributed to stage of lactation when fed because cows tend to eat more in early lactation and there is an increase in the variability of DMI in early lactation cows as compared to mid and late lactation cows (NRC, 2001).

In this study, DMI was not affected by either monensin supplementation or increasing the dietary DCAD. However, there was a trend in the current experiment for increased DMI with DCAD, especially in the diets without monensin. This is may be due to the counteractive effects of DCAD and monensin on DMI or alternatively it could simply be the result of inadequate statistical power to determine the differences in feed intake. This experiment was the first 18-cow replication of an experiment that we determined required a minimum of 36 cows to test FE effects of DCAD and monensin. Because this study was done with half the amount of

observations needed it is very possible that there was not enough statistical power to detect a significant difference for the effect of the monensin-DCAD interaction on DMI.

Previous studies have shown monensin supplementation increases milk production but has no effect on milk composition (Duffield et al., 2008; Aguilar, 2005), especially at lower levels of monensin supplementation. However, monensin supplementation has sometimes been shown to decrease milk fat percentage, but this can be attributed to type of feed being fed while supplementing with monensin (Duffield et al., 2008). A decrease in milk fat percentage can be seen if the diet contains a large portion of unsaturated fats (Duffield et al., 2008). Increasing DCAD has also been shown to increase milk production, milk fat percentage, fat yield and protein yield while it did not have an effect on the other milk components (Hu and Murphy, 2004; Sanchez and Beede, 1996). In this study, milk production and the milk components were not affected by either monensin or DCAD nor were than any deceivable trends due to treatment. These results are consistent with those found by Dary et al., (2005) in their study investigating the effects of sodium bicarbonate and monensin supplementation on milk and milk composition. Ordinarily, an increase in milk production would be the expected from monensin (Duffield et al., 2008) and DCAD (Erdman et al., 2011, Harrison et al., 2011) supplementation. However, the lack of a production response again could be due inadequate statistical power. When the second repetition of this study is completed we should have enough observations to draw more concrete conclusions regarding the effects of DCAD and monensin and their interaction on milk production and composition.

In past experiments, monensin supplementation has been shown to increase dairy FE (Dary et al., 2005; Akins et al., 2013, Symanoski et al., 1999). However, the effects of DCAD on dairy FE however have been less consistent (Hu et al., 2007; Hu and Murphy, 2004; Clark et

al, 2009; Erdman et al., 2011; Harrison et al., 2012). In many of the previous studies done involving DCAD, dairy FE was not been reported (Hu et al., 2007; Hu and Murphy, 2004; Clark et al, 2009). In studies that reported FE, DCAD effects on FE tended to be significant, but if the effect was not significant there is a numerical increase in dairy FE (Erdman et al., 2011; Harrison et al., 2012). In this study, FE was not affected by monensin supplementation or increasing the dietary DCAD concentration. The lack of an effect on FE can be attributed to the lack of an effect seen on DMI and 3.5% FCM. In studies were FE was improved by DCAD (Erdman et al., 2011, Harrison et al., 2012), there was an significant increase in milk fat concentration which was the primary factor that increased 3.5% FCM. Since milk fat concentration and 3.5% FCM were not changed, and there were no effects of monensin or DCAD on DMI, FE was unaffected. While it is possible with more experimental units and more statistical power there might be differences in FE, the lack of any current trends suggests FE will not be significantly affected even with more cows in the completed experiment.

Previous work that monensin supplementation results either no change or a slight decrease in rumen pH (Russell, 1987; Schelling 1984; Lana and Russell 1997). Adding buffers such as sodium bicarbonate, which increase DCAD generally increase rumen pH (Erdman, 1988), especially in low forage diets. Some studies have reported that increasing DCAD causes an increase in pH (Tucker et al., 1988) while others have reported that increasing DCAD has no effect on rumen pH (Apper-Bossard et al., 2010). In this experiment neither monensin supplementation nor increasing the DCAD had any effect on the rumen pH. However, there was, a time effect such that rumen pH was the highest right before feeding then it decreased after feeding hitting its lowest point between 6 and 9 h post-feeding. By 12 h after feeding rumen had begun to increase. The pattern in the rumen pH post-feeding was typical of that normally seen

after the intake of a large meal in lactating dairy cows (Duffield et al., 2004; Nordlund and Garrett, 1994). There are few reports of monensin effects rumen pH (Duffield et al., 2008). However, buffer supplementation has been consistently shown to in increase rumen pH and reduce the degree of postprandial decline in rumen pH, especially in cows fed high grain diets (Erdman, 1988). Due to instrument malfunction, rumen pH before feeding could not be measured. Because of this missing data the covariate pH data was not used when performing the statistical analysis. However, this would not have any bearing on DCAD effects where variance due to individual cow effects could still accounted for in the Latin square design.

In this study, there was an inverse relationship between K and Na ion concentrations in the rumen. When potassium chloride was added to the diet of sheep, K concentrations in the rumen increased and the Na concentrations decreased (Warner and Stacey, 1971). Similarly Bennick et al. (1978) found an inverse relationship between rumen Na and K concentration in cattle fed diets that varied in K and Na concentrations. Our results agree with those of Warner and Stacey (1971) and Bennick et al. (1988) such that sodium concentration decreased in cows fed the diet with supplemental K (C200K and M200K diets). There was also a significant interaction of monensin and time on potassium concentration where the increase in rumen K concentration between 3 and 9 hours post-feeding was greater in the monensin diets than the Control. In part, the monensin by time interactions for rumen K might be due to the efflux of potassium from the rumen bacteria during the breakdown and absorption of feed (Russell, 1987; Newbold et al., 2013). Potassium concentration also tends to be the highest when hay is being fed. Therefore the increase in K concentration can be partially attributed to the diet being fed (Bennick et al., 1978). As stated previously, when the rumen K concentration is increased the Na concentration decreases. Warner and Stacey (1971) suggested that this was due to an

increase in the sodium absorption rate across the rumen wall. While absorption rates were not studied during this experiment it is possible that absorption rates influenced the monensin-DCAD-time interaction that was seen with respect to rumen Na concentration.

Tucker et al. (1988) found that rumen Cl concentration tended to decrease with increasing DCAD. In this experiment, however, rumen Cl increased only when DCAD was increased using potassium carbonate. Sanchez et al. (1994) suggested that increased rumen K with potassium bicarbonate supplementation resulted in an increased rumen Cl as a means to maintain the acid-base balance of the animal (Sanchez et al., 1994). The increased rumen Cl concentration with time after feeding that we observed is similar to the results found by Bennick et al. (1978) in cattle that were fed a concentrate-silage-hay diet.

One of the most consistent effects of monensin feeding is an increase in rumen propionate concentration and a decreased rumen A:P (Richardson et al., 1976; Schelling, 1984; Weimer et al., 2008; Lemenager et al., 1978). Conversely, increased DCAD typically decreases rumen propionate and increases rumen acetate and A:P (Erdman, 1988; Jenkins et al., 2014). Dietary cation anion difference has also been shown to increase the ruminal butyrate concentration (Wildman et al., 2007). The results of this experiment agree with previous DCAD studies. Acetate concentration increased with increasing DCAD, particularly when DCAD increased with sodium sesquicarbonate. Propionate concentration decreased and butyrate concentration increased with increasing DCAD. Rumen A:P increased with increasing DCAD due to the increase seen in acetate concentration and the decrease in propionate concentration. Each of these results coincides with previously mentioned studies (Erdman, 1988; Jenkins et al., 2014; Wildman et al., 2007).

There are some results that are not as easily explained. The monensin-DCAD interaction was found to be significant for acetate concentration, propionate concentration, and the total VFA concentration. In the case of the acetate concentration and the total VFA concentration, the DCAD effect was greater in the monensin supplemented diets, particularly when Na was supplemented. Most striking was the fact that rumen propionate decreased with either K or Na supplementation in the Control diets, while in the monensin supplemented diets, rumen propionate increased with Na supplementation. Further, total VFA concentration was either unaffected or slightly reduced in the Control diet by either K or Na but was increased by K and to an even greater extent by Na in the monensin supplemented diets. Rumen VFA account for 50% or more of the total energy supplied to the lactating dairy cow (Bergman, 1990). Data from this experiment suggest that the propionate and total VFA response to monensin was enhanced by Na addition. Since monensin preferentially binds sodium (Russell and Houlihan, 2003) and monensin sensitivity has been previously shown to be enhanced (Newbold et al., 2013) in the presence of higher Na concentrations in fermentation media, our rumen VFA data suggest that the rumen fermentation response to monensin can be altered by both dietary DCAD and strong ion source. In this case, added Na increases the VFA response to monensin.

CONCLUSIONS

With the limited number of animals in the study, intake, milk production and milk composition responses were not detected. Due to the lack of a significant effect on dairy FE it is difficult to draw conclusions regarding the influence of a DCAD-monensin interaction on FE. However, increasing DCAD with Na and K resulted in a corresponding increase in rumen Na and K concentrations while rumen Cl decreased when rumen K was increased. Increasing DCAD with potassium carbonate had more effects on rumen ion concentrations than rumen VFA. On

the contrary, increasing DCAD with sodium sesquicarbonate had a more significant impact on the VFA concentrations, particularly in cows fed the monensin diet suggesting that monensin responses in lactating dairy cows might be enhanced by supplementing sodium as the strong ion source to increase DCAD.

ACKNOWLEDGEMENTS

The authors would like to thank the entire staff at the Central Maryland Research and Education Center (CMREC), in particular Michael Dwyer, Brian Spielmann, and Katie Albaugh for their assistance in feeding, collecting samples, and care of the experimental animals throughout the course of this study. Also, the authors thank Tracy Catterton for her assistance on days which rumen samples were taken. Lastly, the authors would like to thank Dr. Rick Kohn and Latisha Judd for allowing use of their gas-liquid chromatograph, in addition to assisting with the analysis of rumen VFA concentrations.

REFERENCES

Aguilar, A. A. 2005. Milk production efficiency in dairy cows fed monensin. Proc. Southwest Nutr. Conf.: 192-196b.

Akins, M.S., K. L. Perfield, H. B. Green, S. J. Bertics, and R. D. Shaver. 2013. Effect of monensin in lactating dairy cow diets at two starch concentrations. J. Dairy Sci. 97:917-929.

Apper-Bossard, E., P. Faverdin, F. Meschy, and J. L. Peyraud. 2010. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regulation in dairy cows receiving two contrasting levels of concentrate in diets. J. Dairy Sci. 93:4196-4210.

Bennink, M. R., T. R. Tyler, G. M. Ward, and D. E. Johnson. 1978. Ionic milieu of bovine and ovine rumen as affected by diet. J Dairy Sci. 61:315-323.

Bergman, E. N. 1990. Energy contributions of volatile fatty-acids from the gastrointestinal-tract in various species. Physiol Rev. 70:567–590.

Clark, J.H., R.A. Christensen, H.G. Bateman II, and K.R. Cummings. 2009. Effects of sodium sesquicarbonate on dry matter intake and production of milk and milk components by Holstein cows. J Dairy Sci. 92:3354-3363.

Dary, C., M. Harris, N. Koppe, T. Ritson-Bennett, R. Khorasani, and L. Doepel. 2005. The effect of monensin and sodium bicarbonate on milk yield and composition. Advances in Dairy Technology. Abstract,17:359-359.

Duffield, T., J.C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59-66.

Duffield, T.F., A. R. Rabiee, and I. J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347-1360.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71:3246-3266.

Erdman, R.A., L.S. Piperova, and R.A. Kohn. 2011. Corn silage versus corn silage: alfalfa hay mixtures for dairy cows: Effects of dietary potassium, calcium, and cation-anion difference. J. Dairy Sci. 94:5105-5110.

Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768–1771.

Harrison, J., R. White, R. Kincaid, E. Block, T. Jenkins, and N. St-Pierre. 2012. Effectiveness of potassium carbonate sesquihydrate to increase dietary cation-anion difference in early lactation cows. J. Dairy Sci. 95:3919-3925.

Hu, W., and M.R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. J. Dairy Sci. 87:2222-2229.

Hu, W., M. R. Murphy, and L. Kung, Jr. 2007. The relationship between dry matter intake and acid-base status of lactating dairy cows as manipulated by dietary cation-anion difference. Anim. Feed Sci. Technol. 136:216-225.

Jenkins, T.C., W.C. Bridges Jr., J.H. Harrison and K.M. Young. 2014. Addition of potassium carbonate to continuous cultures of mixed ruminal bacteria shifts volatile fatty acids and daily production of biohydrogenation intermediates. J. Dairy Sci. 97:975-984.

Lana, R.P. and J.B. Russell. 1997. Effect of forage quality and monensin on the ruminal fermentation of fistulated cows fed continuously at a constant intake. J. Anim. Sci. 75:224–229.

Lemenager, R. P., F. N. Owens, B. J. Shockey, K. S. Lusby and R. Totusek. 1978. Monensin effects on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components, and cellulose disappearance. J. Anim. Sci. 47:255.

National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Washington, D.C.: National Academy Press, p105-161.

Newbold, C. J., R. J. Wallace, and N. D. Walker-Bax. 2013. Potentiation by metal ions of the efficacy of the ionophores, monensin and tetronasin, towards four species of ruminal bacteria. FEMS Microbiology Letters 338(2):161-167.

Nordlund, K.V. and E.F. Garrett. Rumenocentesis: A technique for the diagnosis of subacute rumen acidosis in dairy herds. 1994. Bovine Pract. 28: 109–112.

Perry, F. G. and G. K. Macleod. 1969. Permanent Rumen Cannula. J. Dairy Sci. 52: 264-265.

Phipps, R. H., J. I. D. Wilkinson, L. J. Jonker, M. Tarrant, A. K. Jones and A. Hodge. 2000. Effect of monensin on milk production of holstein-friesian dairy cows. J Dairy Sci 83:2789–2794.

Potter, E.L., R.L. VanDuyn, and C.O. Cooley. 1984. Monensin toxicity in cattle. J Anim. Sci. 58:1499-1511.

Richardson, L. F., A. P. Raun, E. L. Potter and C. O. Cooley. 1976. Effect of monensin in rumen fermentation in vitro and in vivo. J. Anim. Sci.43:657.

Russell, J.B. 1987. A proposed mechanism of monensin action in inhibition of ruminal bacterial growth: effects onion flux and proton motive force. J. Anim. Sci. 64:1519-1525.

Russell, J.B., and A.J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27:65-74.

Sanchez, W.K., D.K. Beede and J.A. Cornell. 1994. Interactions of sodium, potassium, and chloride on lactation, acid-base status, and mineral concentrations. J. Dairy Sci. 77:1661-1675.

Sanchez, W.K., and D.K. Beede. 1996. Is there an optimal dietary cation-anion difference for lactation diets? Anim. Feed Sci. Technol. 59:3-12.

Schelling, G. 1984. Monensin mode of action in the rumen. J. Anim.Sci. 58:1518–1527.

Symanowski, J. T., H. B. Green, J. R. Wagner, J. I. D. Wilkinson, J. S. Davis, M. R. Himstedt, M. S. Allen, E. Block, J. J. Brennan, H. H. Head, J. J. Kennelly, J. N. Nielsen, J. E. Nocek, J. J. van Der List and L. W. Whitlow. 1999. Milk production and efficiency of cows fed monensin. J. Dairy Sci. 82(Suppl 1):75. (Abstr.)

Tucker, W.B., G.A. Harrison, and R.W. Hemken. 1988a. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71:346-354.

USDA-ERSa. National Milk Cost of Production. *USDA Economic Research Service*. N.p., 2014. http://www.ers.usda.gov/data-products/milk-cost-of-production-estimates.aspx#.U4Y6U8Uxo7k. Accessed July 3, 2014.

Warner, A.C.I. and B.D. Stacy. 1971. Intraruminal and systemic responses to variations in intake of sodium and potassium by sheep. Q. J. Exp. Physiol. C.M.S. 57:89-102.

Weimer, P. J., D.M. Stevenson, D.R. Mertens and E. E. Thomas. 2008. Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. Appl. Microbiol. Biotechnol. 80:135-145.

Wildman, C.D., J.W. West, and J.K. Bernard. 2007. Effect of dietary cation-anion difference and dietary crude protein on milk yield, acid-base chemistry, and rumen fermentation. J. Dairy. Sci. 90:4693-4700.

Table 3.1 Ingredient composition of experimental diets (DM basis)

	Treatment[1]					
Item	**C0**	**C200K**	**C200Na**	**M0**	**M200K**	**M200Na**
Corn Silage	58.00	57.12	57.06	57.89	57.09	57.02
Alfalfa Hay	7.86	7.75	7.74	7.85	7.74	7.73
Ground Shell Corn	10.81	10.65	10.64	10.79	10.64	10.63
Soybean Meal (48% CP)	15.23	15.01	14.99	15.21	15.00	14.98
Soyplus	3.44	3.39	3.38	3.43	3.39	3.38
Corn Gluten Meal	0.61	0.60	0.60	0.61	0.60	0.60
Dyna-mate[2]	0.13	0.13	0.13	0.13	0.13	0.13
Biophos[3]	0.43	0.42	0.42	0.43	0.42	0.42
Limestone[4]	0.61	0.60	0.60	0.61	0.60	0.60
Mag Oxide	0.34	0.33	0.33	0.34	0.33	0.33
Salt	0.49	0.48	0.48	0.49	0.48	0.48
ADE mix[5]	0.03	0.03	0.03	0.03	0.03	0.03
Vit. E[6]	0.01	0.01	0.01	0.01	0.01	0.01
Megalac[7]	1.47	1.45	1.45	1.47	1.45	1.45
Selenium (0.06%)[8]	0.06	0.06	0.06	0.06	0.06	0.06
Omigen[9]	0.20	0.20	0.20	0.20	0.20	0.20
TM-433[10]	0.03	0.03	0.03	0.03	0.03	0.03
4-Plex C[11]	0.01	0.01	0.01	0.01	0.01	0.01
Dia. V. Yeast XP 2 oz[12]	0.24	0.24	0.24	0.24	0.24	0.24
Rumensin 10g/lb[13]	0.00	0.00	0.00	0.06	0.06	0.06
Sodium Bicarbonate[14]	0.00	0.00	1.60	0.00	0.00	1.60
DCAD Plus[15]	0.00	1.49	0.00	0.00	1.49	0.00

[1]Treatments C0, C200K, C200Na, M0, M200K, M200Na correspond to Control no added DCAD, Control plus 200 mEq/kg added K, Control plus 200 mEq/kg added Na, +300 mg/cow added monensin no added DCAD, +300 mg/cow added monensin plus 200 mEq/kg added K, +300 mg/cow added monensin plus 200 mEq/kg added Na treatments, respectively

[2]Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN)

[3]Contained 17% Ca and 21% P

[4]Contained 36% Ca and 0.02%P

[5]Contained 5,454,545 IU/kg Vitamin A, 1,818,182 IU/kg Vitamin D, 9,091 IU/kg Vitamin E

[6]Contained 56,818 IU/kg Vitamin E

[7]Contained 9% Ca; 85% Fat (Church & Dwight Co., Inc., Piscataway, NJ)

[8]Contained 0.3 IU/g Selenium; 28% Ca

[9]Contained 0.41 mg/kg Biotin, 15 mg/kg Choline, 31 mg/kg d-Pantothenic Acid, 1.4 mg/kg Folic Acid, 3.2 mg/kg Menadione, 102 mg/kg Niacin, 30 mg/kg Riboflavin, 4.5×10^{10} CFU/kg *Saccharomyces cerevisiae*, 15.5 mg/kg Thiamine, 8.2 mg/kg Vitamin B-6, and 41 mcg/kg Vitamin B-12 (Prince Agri Products, Inc., Quincy IL)

[10]Contained 0.16% Co, 4.0% Cu, 3.0% Fe, 0.35% I, 15% Mn, and 16% Zn (Southern States Cooperative, Inc., Richmond, VA)

[11]Contained 0.20% Co, 0.99% Cu, 0.031% Fe, 1.57% Mn, and 2.83% Zn (Southern States Cooperative, Inc., Richmond, VA)

[12]Contained *Saccharomyces cerevisiae* yeast (Diamond V, Cedar Rapids, IA)

[13]Contained 20% Monensin Na, 1% Mineral oil, and carriers such as rice hulls, limestone, and fermentation nutrients (Elanco, Greenfield, IN)
[14]Contained 27% Na (Church & Dwight Co., Inc., Piscataway, NJ)
[15]Contained 56% K and 88% DM (Church & Dwight Co., Inc., Piscataway, NJ)

Table 3.2 Chemical composition of experimental diets (DM basis)

	Treatment[1]						
Item	**C0**	**C200K**	**C200Na**	**M0**	**M200K**	**M200Na**	**SEM**
DM, %	56.50	56.98	57.20	56.41	56.94	57.16	0.136
CP, %	16.13	15.89	15.87	16.11	15.88	15.86	0.051
ADF, %	19.90	19.60	19.58	19.87	19.59	19.57	0.063
NDF, %	32.46	31.97	31.94	32.40	31.95	31.92	0.103
Lignin, %	3.11	3.07	3.06	3.11	3.06	3.06	0.010
Fat, %	2.96	2.92	2.91	2.96	2.92	2.91	0.009
Ash, %	6.58	6.48	6.47	6.57	6.48	6.47	0.021
Ca, %	0.76	0.75	0.74	0.76	0.75	0.74	0.002
P, %	0.46	0.45	0.45	0.46	0.45	0.45	0.001
Mg, %	0.33	0.32	0.32	0.33	0.32	0.32	0.001
K, %	1.45	2.26	1.42	1.44	2.26	1.42	0.174
S, %	0.21	0.20	0.20	0.21	0.20	0.20	0.001
Na, %	0.28	0.28	0.76	0.28	0.28	0.76	0.102
Cl, %	0.64	0.63	0.63	0.64	0.63	0.63	0.002
NE_L, Mcal/kg	1.61	1.61	1.61	1.61	1.61	1.61	0.000
DCAD, mEq/kg[2]	313	522	520	312	521	520	43.9
DCAD-S, mEq/kg [3]	248	458	457	248	458	457	44.1

[1]Treatments C0, C200K, C200Na, M0, M200K, M200Na correspond to Control no added DCAD, Control plus 200 mEq/kg added K, Control plus 200 mEq/kg added Na, +300 mg/cow added monensin no added DCAD, +300 mg/cow added monensin plus 200 mEq/kg added K, +300 mg/cow added monensin plus 200 mEq/kg added Na treatments, respectively

[2]DCAD= (%K / 0.00391) + (%Na / 0.00229) - (Cl% / 0.00355), DM basis

[3]DCAD-S= (%K / 0.00391) + (%Na / 0.00229) - (Cl% / 0.00355) + (%S / 0.003207) , DM basis

Table 3.3 Effect of monensin, DCAD and the monensin-DCAD interaction on feed intake, milk production and composition, and feed efficiency in mid-lactation dairy cows

	Treatment[1]						SEM		P =		
Item	**C0**	**C200K**	**C200Na**	**M0**	**M200K**	**M200Na**	**Mon[2]**	**DCAD[3]**	**Mon**	**DCAD**	**Mon X DCAD[4]**
N	18	18	18	18	18	18					
BW, kg	674	669	677	680	673	684	7.1	4.9	0.175	0.312	0.861
DMI, kg/d	22.8	23.3	23.7	23.2	23.8	23.5	0.55	0.38	0.534	0.078	0.233
Milk, kg/d	34.4	34.3	34.9	35.2	34.4	34.7	0.87	0.60	0.697	0.500	0.230
3.5%FCM, kg/d	34.0	34.4	34.8	35.1	34.1	34.6	0.97	0.67	0.754	0.590	0.123
Fat, %	3.45	3.52	3.49	3.50	3.44	3.47	0.106	0.072	0.742	0.778	0.434
Fat yield, kg/d	1180	1207	1215	1225	1182	1203	44.2	30.3	0.886	0.645	0.180
Protein, %	2.99	3.01	3.01	3.01	3.01	3.02	0.049	0.034	0.631	0.938	0.944
Prot. yield, kg/d	1021	1029	1046	1053	1036	1046	25.6	17.7	0.337	0.508	0.317
OS[5] yield, kg/d	1941	1946	1984	1975	1960	1977	54.8	38.0	0.684	0.388	0.486
OS, %	5.64	5.67	5.69	5.63	5.70	5.69	0.036	0.025	0.821	0.414	0.603
SCC	4.48	4.78	4.71	4.74	5.05	4.89	0.745	0.509	0.625	0.703	0.973
Feed efficiency[6]	1.50	1.47	1.47	1.52	1.44	1.48	0.036	0.025	0.935	0.534	0.192

[1]Treatments C0, C200K, C200Na, M0, M200K, M200Na correspond to Control no added DCAD, Control plus 200 mEq/kg added K, Control plus a 200 mEq/kg added Na, +300 mg/cow added monensin no added DCAD, +300 mg/cow added monensin plus 200 mEq/kg added K, +300 mg/cow added monensin plus 200 mEq/kg added Na treatments, respectively
[2]Monensin effect
[3]DCAD effect
[4]Monensin by DCAD interaction
[5]Lactose plus minerals
[6]3.5% FCM/DMI

Table 3.4 Effect of monensin, DCAD and the monensin-DCAD interaction on rumen pH, ion concentrations and VFA concentrations in mid-lactation dairy cows

	Treatment[1]						--------SEM------		---------- P = ----------		
Item	**C0**	**C200K**	**C200Na**	**M0**	**M200K**	**M200Na**	**Mon[2]**	**DCAD[3]**	**Mon**	**DCAD**	**Mon X DCAD[4]**
N	6	6	6	6	6	6					
pH	5.98	6.08	5.93	5.88	5.97	5.99	0.085	0.067	0.688	0.182	0.177
Rumen, mEq/L											
K,	24.7	35.3	25.1	26.5	37.9	24.8	0.53	0.64	0.146	0.001	0.297
Na	107	100	109	104	97	111	1.8	1.95	0.702	0.002	0.483
Cl	32.8	37.5	35.8	29.8	37.0	32.2	3.18	2.48	0.634	0.017	0.722
Rumen volatile fatty acids, mEq/L											
Acetate	83.7	86.3	83.4	81.2	83.5	88.2	1.58	1.43	0.946	0.088	0.028
Propionate	33.3	29.2	28.7	30.9	30.8	32.8	1.71	1.27	0.661	0.039	0.008
Isobutyrate	3.00	3.60	3.07	3.73	4.33	3.99	0.240	0.171	0.089	0.001	0.629
Butyrate	9.5	9.6	10.2	10.1	11.9	10.7	0.47	0.45	0.170	0.290	0.240
Isovalerate	2.41	2.33	2.25	2.51	2.50	2.66	0.060	0.055	0.062	0.750	0.055
Valerate	2.91	3.22	3.04	2.71	2.70	3.26	0.299	0.216	0.715	0.004	0.002
Total VFA	135	134	130	131	135	141	1.6	1.9	0.283	0.456	0.018
A:P[5]	2.75	3.17	3.09	2.66	2.86	2.77	0.312	0.221	0.618	0.001	0.099

[1]Treatments C0, C200K, C200Na, M0, M200K, M200Na correspond to: Control no added DCAD, Control plus 200 mEq/kg added K, Control plus a 200 mEq/kg DM added Na, +300 mg/cow added monensin no added DCAD, +300 mg/cow added monensin plus 200 mEq/kg added K, +300 mg/cow added monensin plus 200 mEq/kg added Na treatments, respectively
[2]Monensin effect
[3]DCAD effect
[4]Monensin by DCAD interaction
[5]Acetate:propionate

Figure 3.1 Changes in rumen pH with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.925), Monensin by Time (P = 0.143), and Monensin by Time by DCAD (P = 0.274)

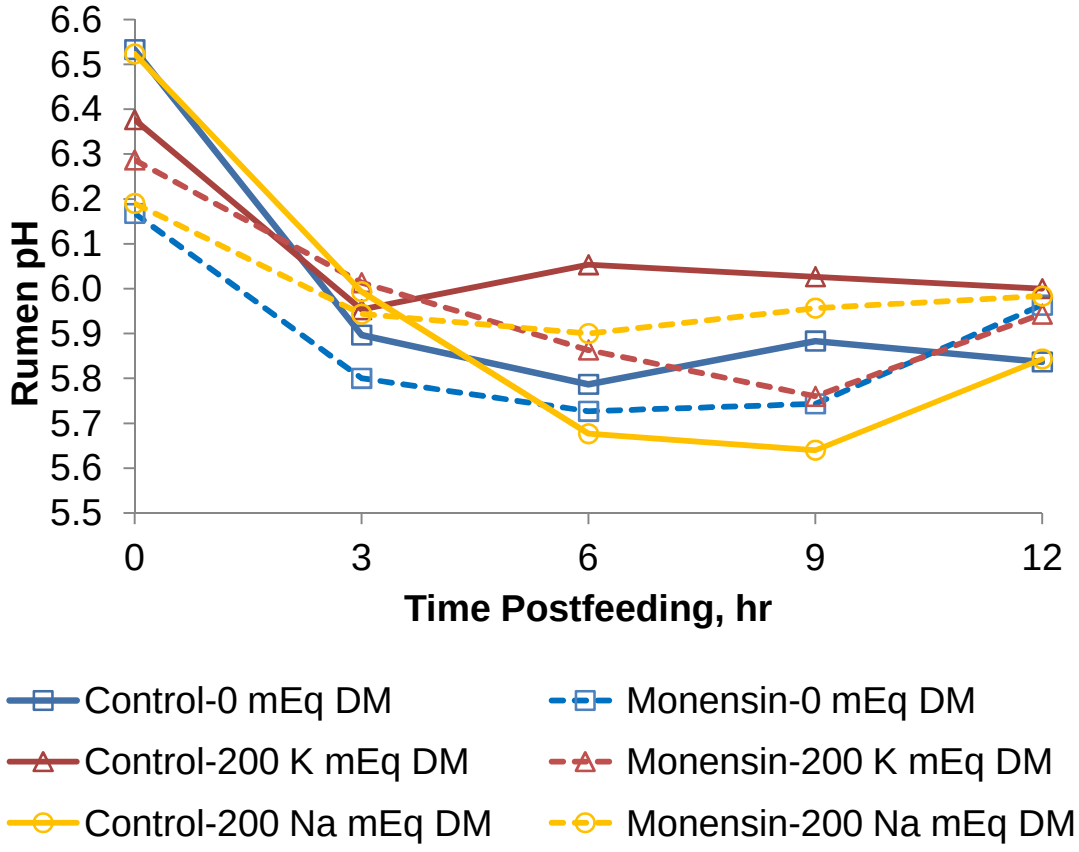


Figure 3.2 Changes in rumen potassium concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.368), Monensin by Time (P = 0.046), and Monensin by Time by DCAD (P = 0.404)

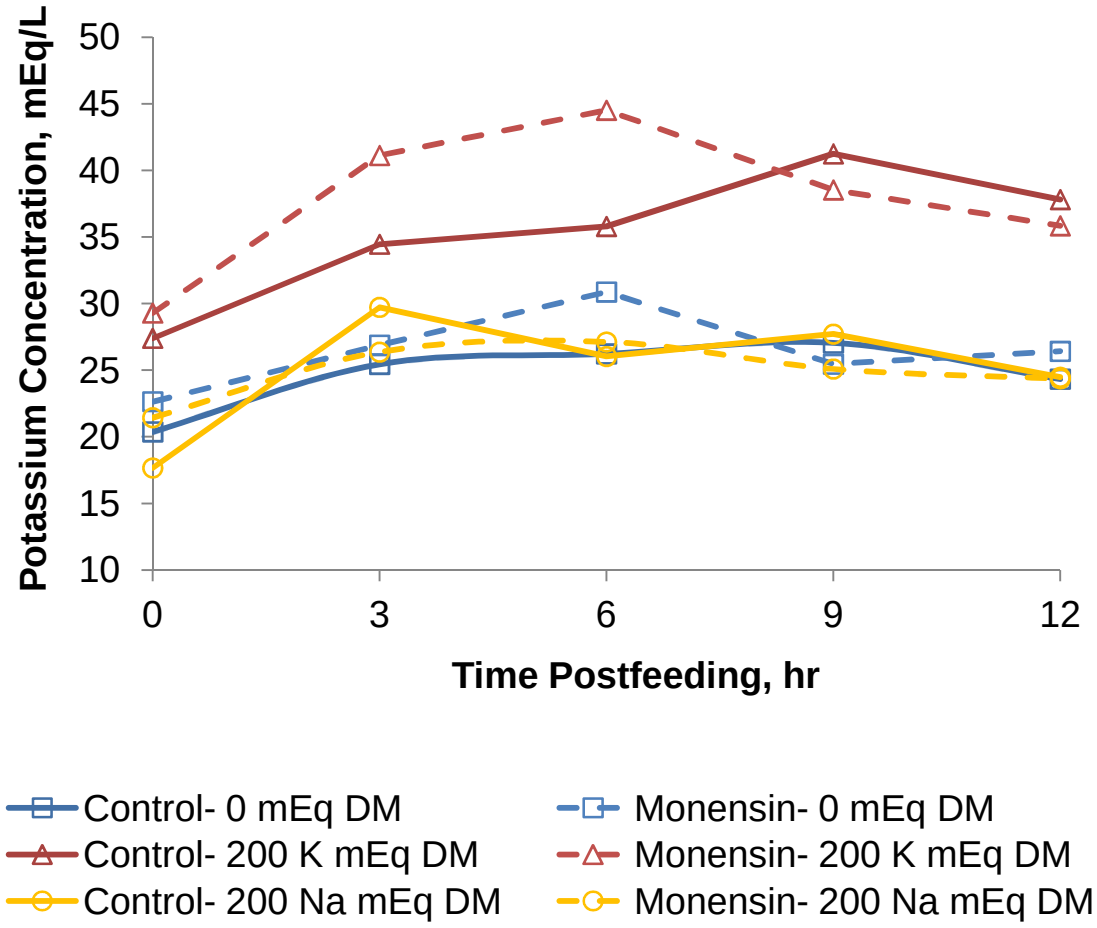


Figure 3.3 Change in rumen sodium concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.263), Monensin by Time (P = 0.461), and Monensin by Time by DCAD (P = 0.045)

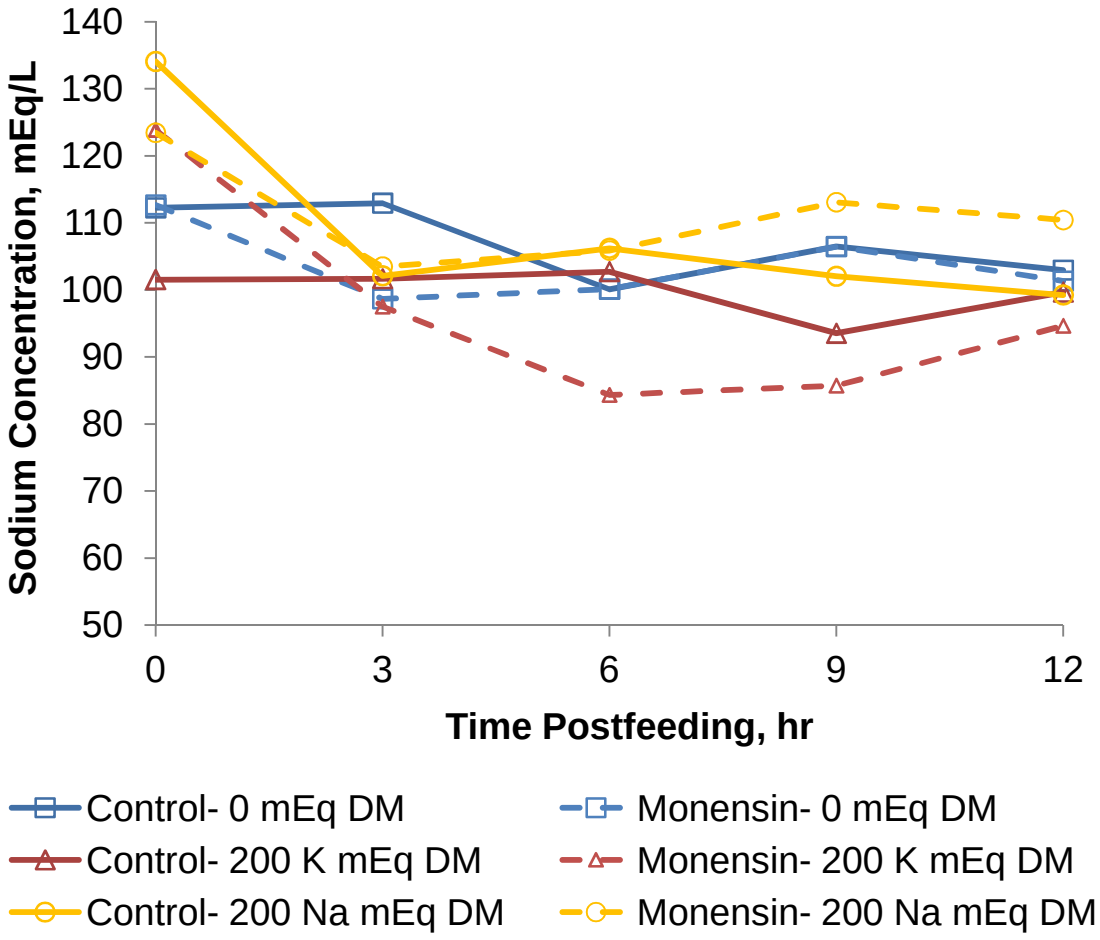


Figure 3.4 Changes in rumen chloride concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.721), Monensin by Time (P = 0.437), and Monensin by Time by DCAD (P = 0.317)

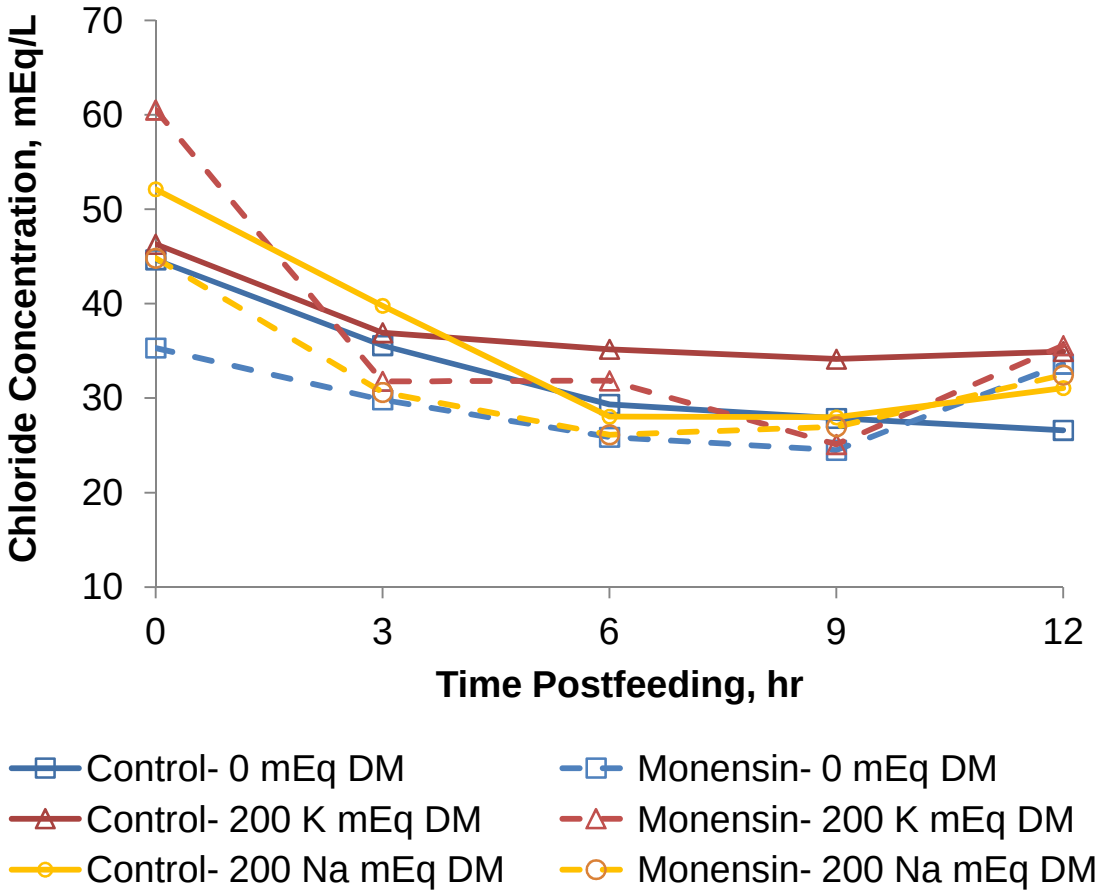


Figure 3.5 Changes in rumen acetate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.002), DCAD by Time (P = 0.947), Monensin by Time (P = 0.332), and Monensin by Time by DCAD (P = 0.909)

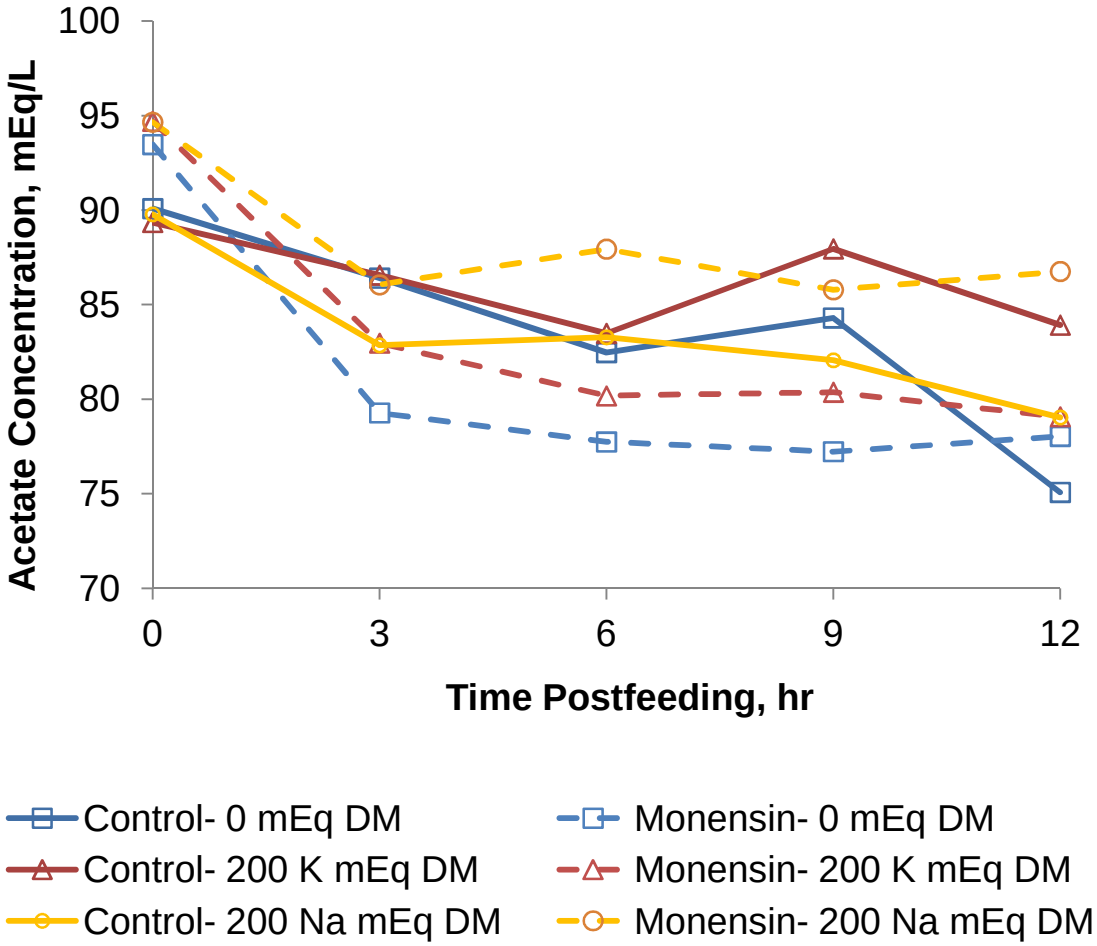


Figure 3.6 Changes in rumen propionate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.199), DCAD by Time (P = 0.726), Monensin by Time (P = 0.481), and Monensin by Time by DCAD (P = 0.924)

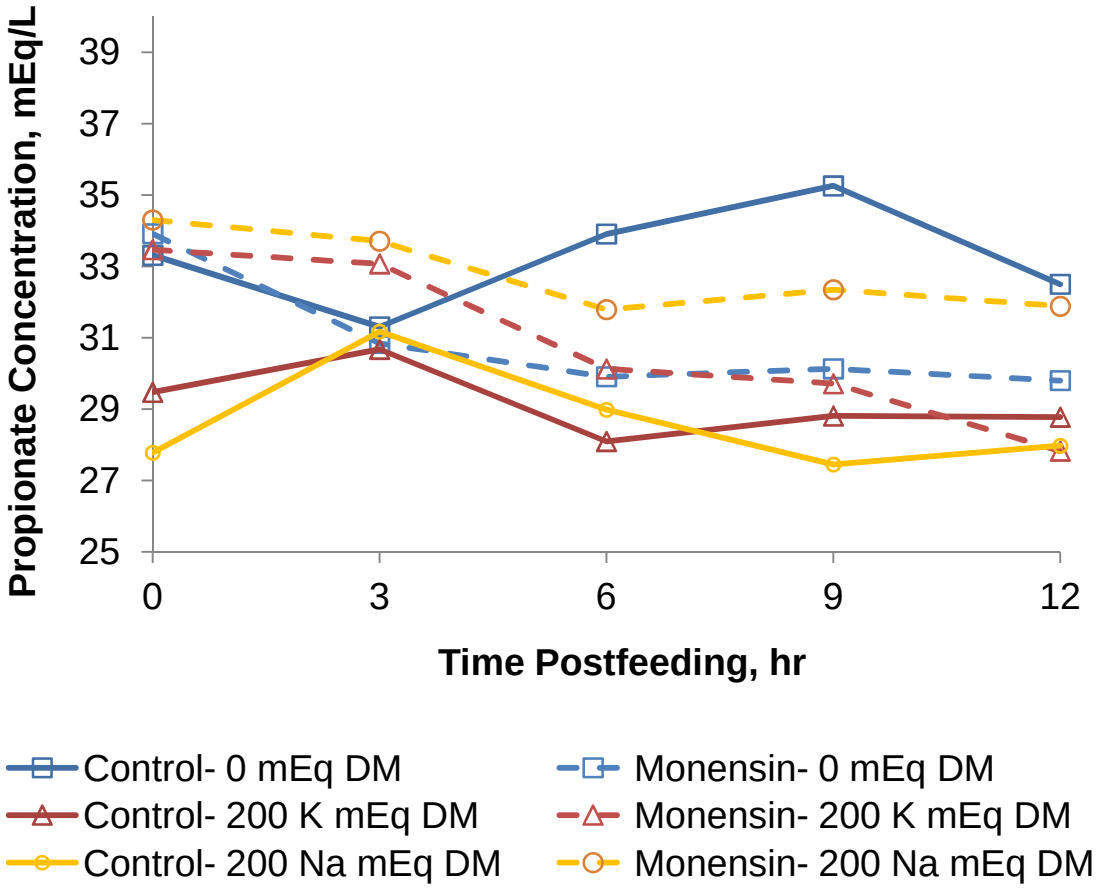


Figure 3.7 Changes in rumen butyrate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.306), DCAD by Time (P = 0.983), Monensin by Time (P = 0.273), and Monensin by Time by DCAD (P = 0.394)

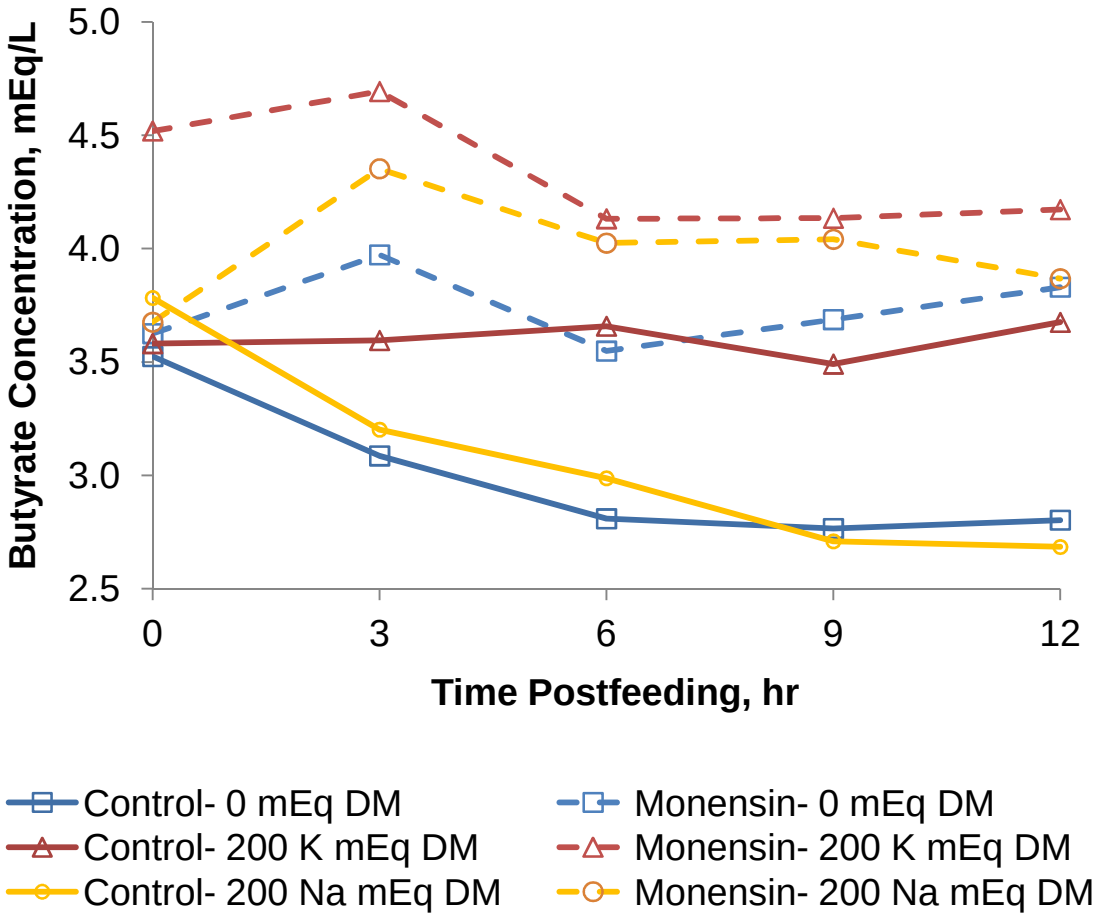


Figure 3.8 Changes in rumen isobutyrate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.999), Monensin by Time (P = 0.985), and Monensin by Time by DCAD (P = 0.155)

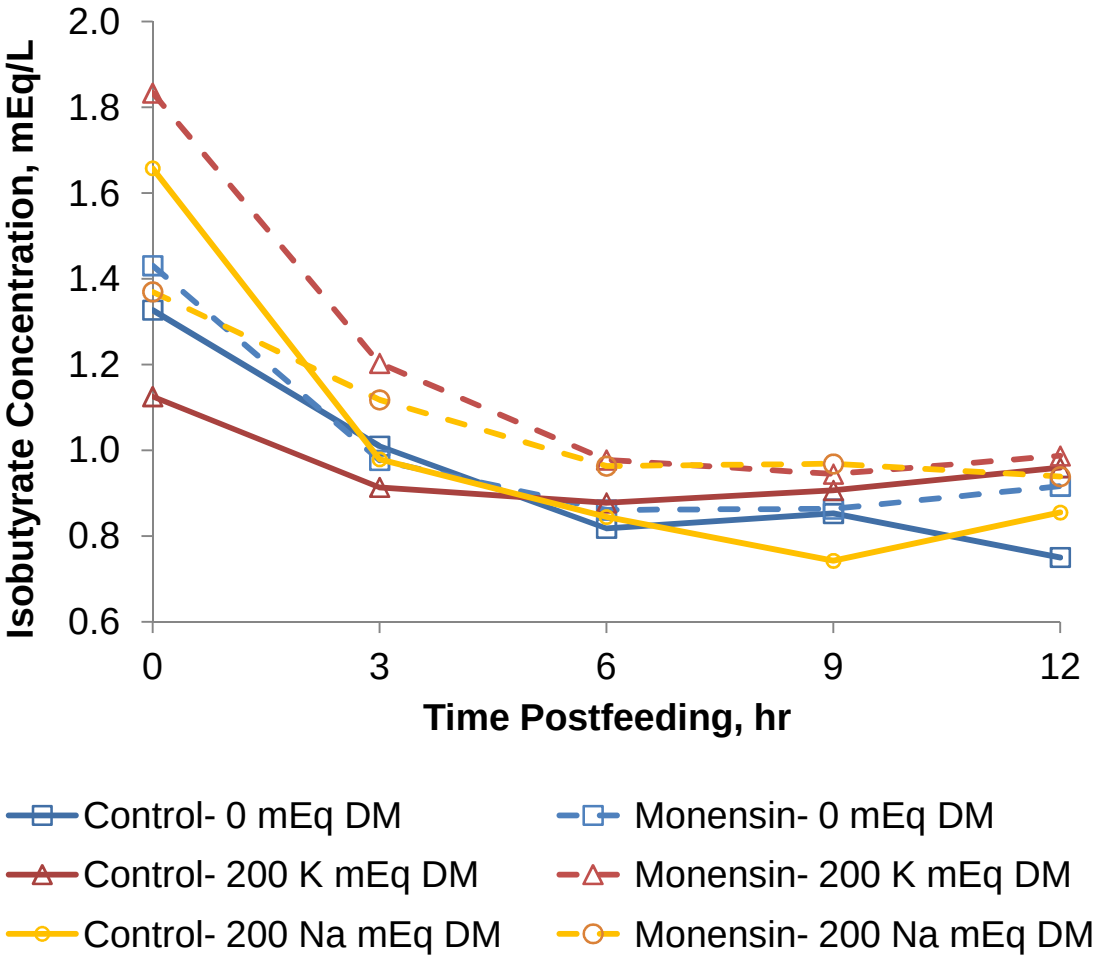


Figure 3.9 Changes in rumen isovalerate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.001), DCAD by Time (P = 0.917), Monensin by Time (P = 0.315), and Monensin by Time by DCAD (P = 0.982)

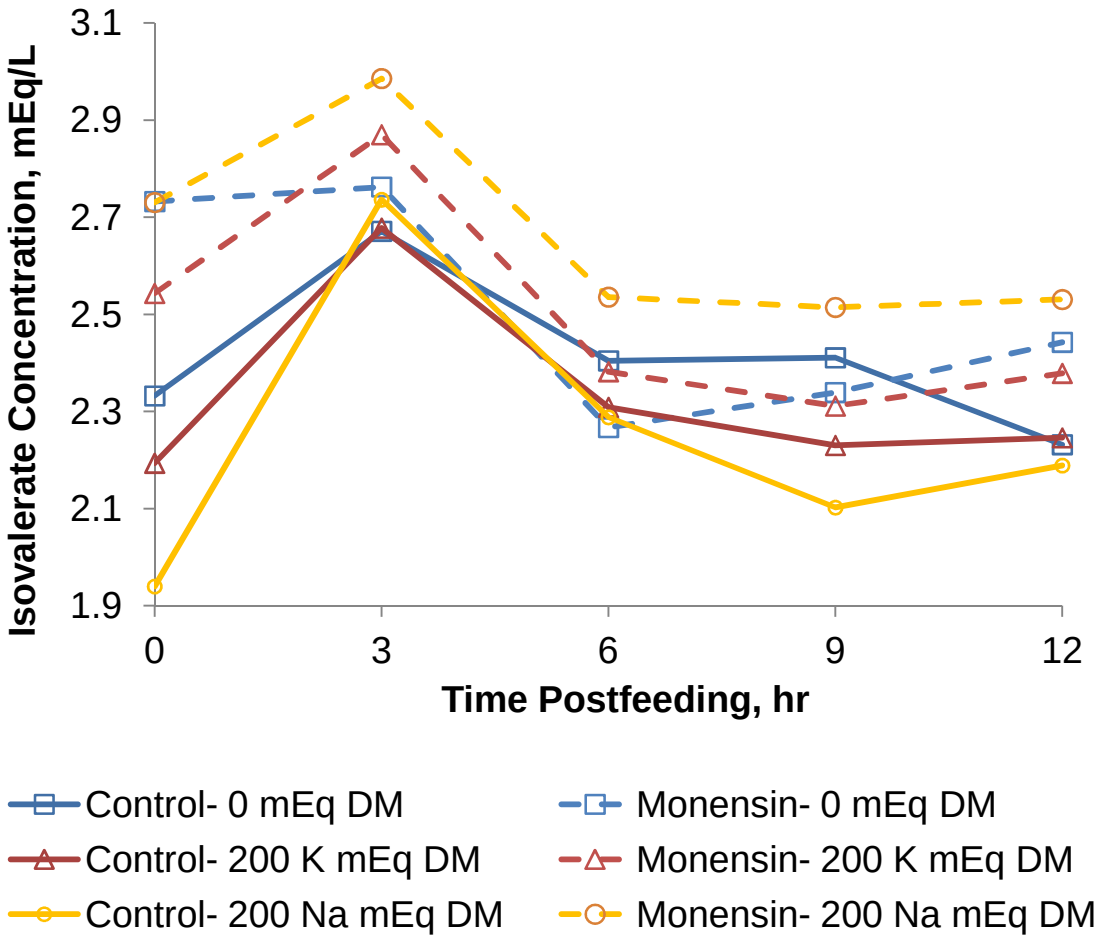


Figure 3.10 Changes in rumen valerate concentration with time post-feeding. Time and time by treatment interaction effects were respectively: Time (*P* = 0.414), DCAD by Time (*P* = 0.887),
Monensin by Time (*P* =0.794), and Monensin by Time by DCAD (*P* = 0.828)

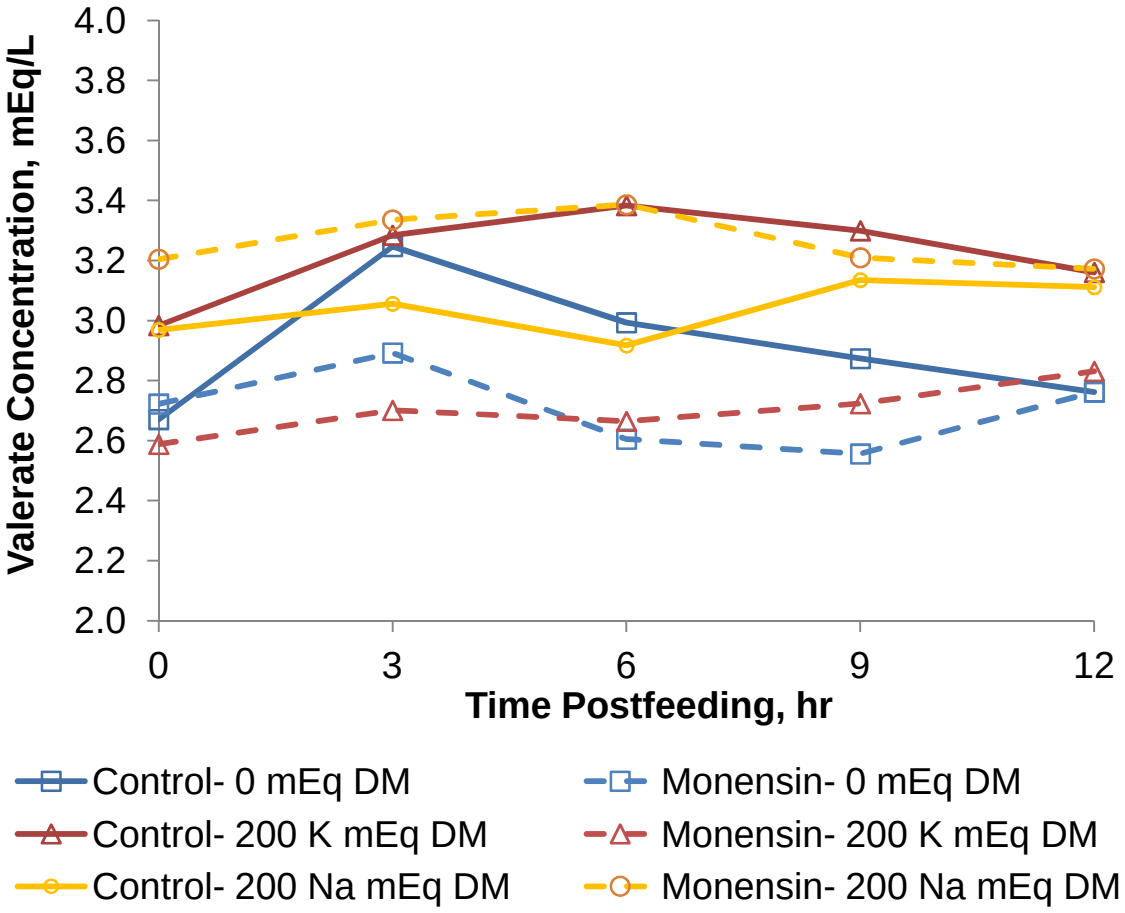


Figure 3.11 Changes in rumen total VFA concentration over time post-feeding. Time and time by treatment interaction effects were respectively: Time (P = 0.007), DCAD by Time (P = 0.980), Monensin by Time (P = 0.236), and Monensin by Time by DCAD (P = 0.896)

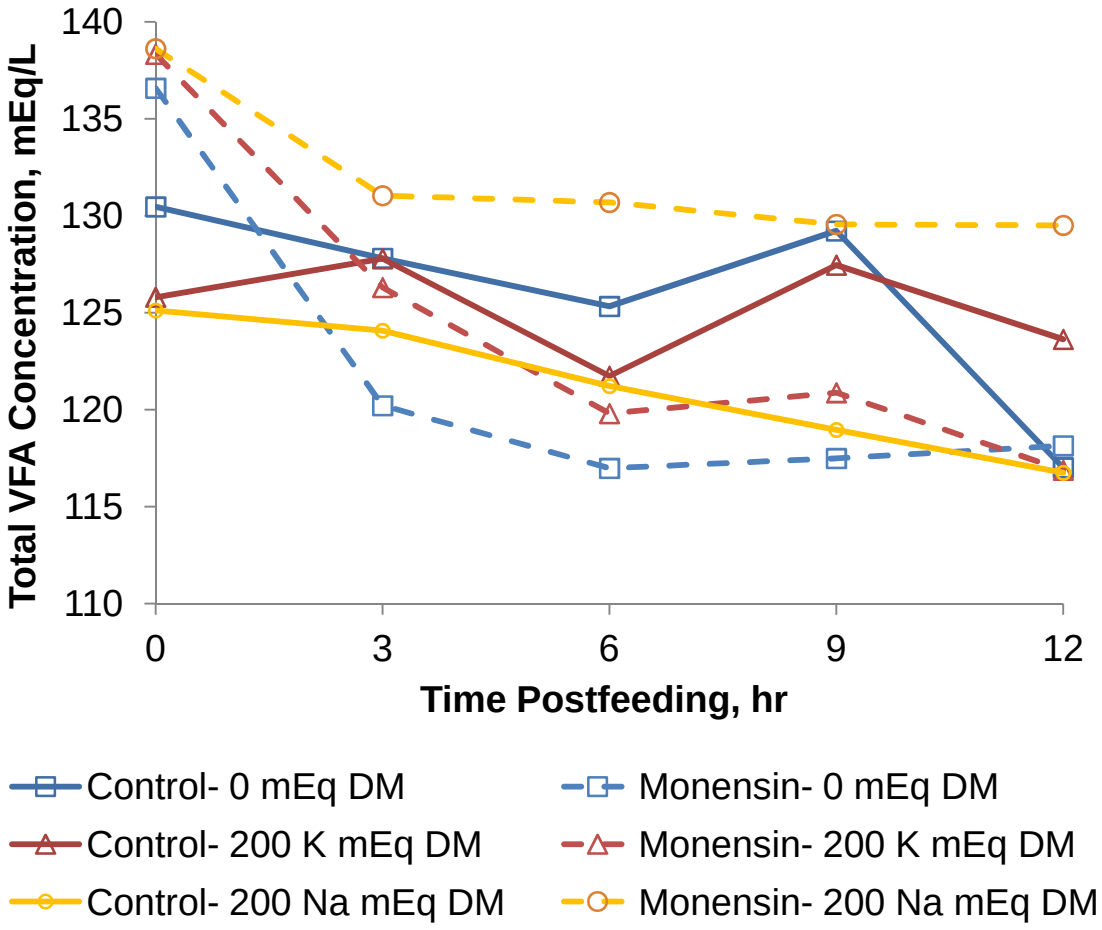
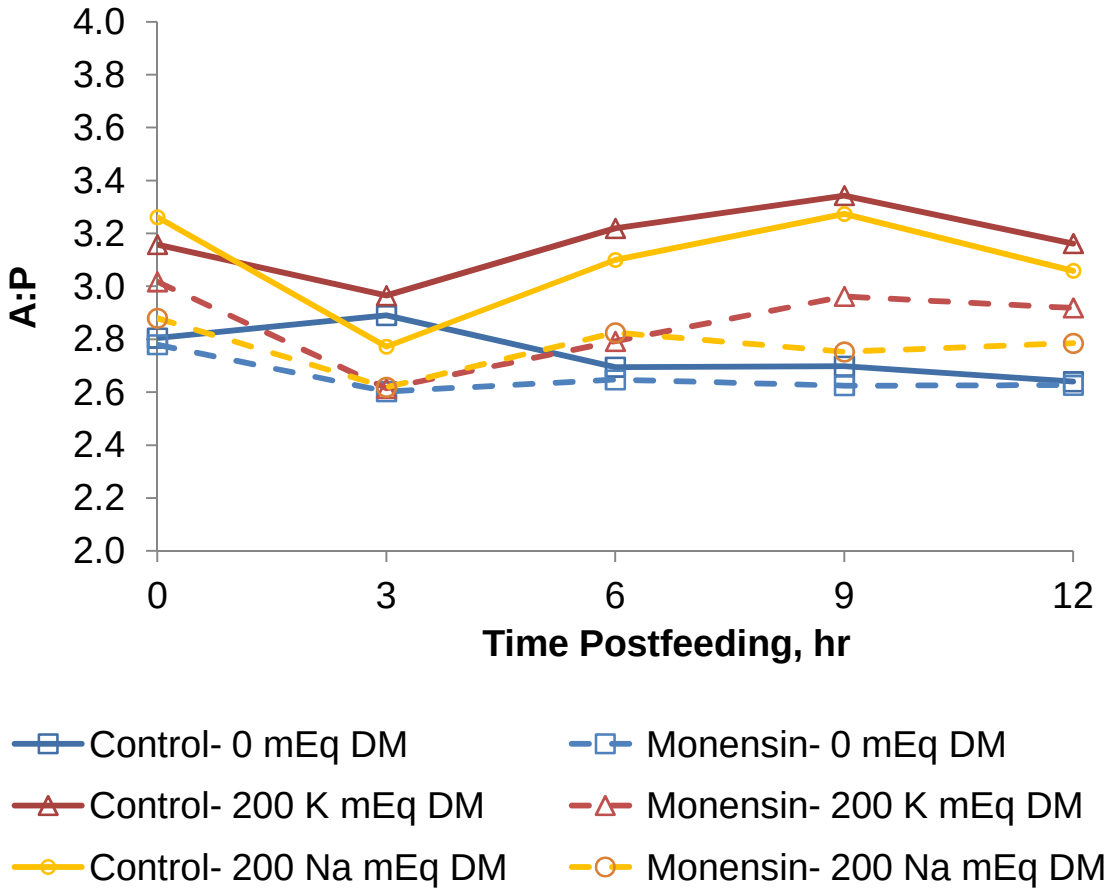


Figure 3.12 Changes in rumen acetate:propionate ratio (mEq/L) with time post-feeding. Time and time by treatment interaction effects were respectively: Time (*P* = 0.038), DCAD by Time (*P* = 0.357), Monensin by Time (*P* = 0.870), and Monensin by Time by DCAD (*P* = 0.859)



Chapter 4: Comprehensive List of References

Agtarap, A., J. W. Chamberlin, M. Pinkerton, and L. Steinrauf. 1967. The structure of monensin acid, a new biologically active compound. J. Am. Chem. Soc. 89:5737–5739.

Aguilar, A. A. 2005. Milk Production Efficiency in Dairy Cows Fed Monensin. Proc. Southwest Nutr. Conf.: 192-196b.

Akins, M.S., K. L. Perfield, H. B. Green, S. J. Bertics, and R. D. Shaver. 2013. Effect of monensin in lactating dairy cow diets at 2 starch concentrations. J. Dairy Sci. 97:917-929.

Apper-Bossard, E., P. Faverdin, F. Meschy, and J. L. Peyraud. 2010. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regularion in dairy cows receiving 2 contrasting levels of concentrate in diets. J. Dairy Sci. 93:4196-4210.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23(1):203-227.

Bauman, Robert W. 2007. Microbiology: With Diseases by Taxonomy. San Francisco: Pearson/Benjamin Cummings, p65.

Bennink, M. R., T. R. Tyler, G. M. Ward, and D. E. Johnson. 1978. Ionic milieu of bovine and ovine rumen as affected by diet. J Dairy Sci. 61:315-323.

Bergen, W. G. and Bates D. B. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465–1483.

Bergman, E. N. 1990. Energy contributions of volatile fatty-acids from the gastrointestinal-tract in various species. Physiol Rev. 70:567–590.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939−2948.

Block, E. 1994. Manipulation of dietary cation-anion difference on nutritionally related production diseases, productivity, and metabolic responses of dairy cows. J. Dairy Sci. 77:1437-1450.

Chan, P.S., J.W. West, J.K. Bernard, and J.M. Fernandez. 2005. Effects of dietary cation-anion difference on intake, milk yield, and blood components of the early lactation cow. J. Dairy Sci. 88:4384-4392.

Chapman, H. D., T K Jeffers, R B Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788-1801.

Chen, G. C., and J. B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation and deamination by mixed ruminal microorganisms in vitro. J. Anim. Sci. 69:2196-2203.

Clark, J.H., R.A. Christensen, H.G. Bateman II, and K.R. Cummings. 2009. Effects of sodium sesquicarbonate on dry matter intake and production of milk and milk components by Holstein cows. J Dairy Sci. 92:3354-3363.

Dary, C., M. Harris, N. Koppe, T. Ritson-Bennett, R. Khorasani, and L. Doepel. 2005. The effect of monensin and sodium bicarbonate on milk yield and composition. Advances in Dairy Technology. Abstract,17:359-359.

Delaquis, A. M., and E. Block. 1995b. Dietary cation-anion difference, acid-base status, mineral metabolism, renal function, and milk production of lactating cows. J. Dairy Sci. 78:2259–2284.

Duffield, T. F., J. K. Merrill, and R. N. Bagg. 2012. A meta-analysis of the effects of monensin in beef cattle on feed efficiency, gain and dry matter intake. J. Anim. Sci. 90:4583-4592.

Duffield, T., J.C. Plaizier, A. Fairfield, R. Bagg, G. Vessie, P. Dick, J. Wilson, J. Aramini, and B. McBride. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. J. Dairy Sci. 87:59-66.

Duffield, T.F., A. R. Rabiee, and I. J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. J. Dairy Sci. 91:1347-1360.

Eastridge, M. L. 2006. Major advances in applied dairy cattle nutrition. J. Dairy Sci. 89:1311–1323.

Ender, F., I. W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemia paresis puerperalis. Zeitschrift Tierphysiologie Tierenahrung, Futtermittelkunde. 28:233-256.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: a review. J. Dairy Sci. 71:3246-3266.

Erdman, R.A. 2011. Benchmark dairy feed efficiency. *Feedstuffs* 82.28:16-19.

Erdman, R.A., L.S. Piperova, and R.A. Kohn. 2011. Corn silage versus corn silage: alfalfa hay mixtures for dairy cows: Effects of dietary potassium, calcium, and cation-anion difference. J. Dairy Sci. 94:5105-5110.

Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768–1771.

Golz, D. I., and T. D. Crenshaw. 1990. Interrelationships of dietary sodium, potassium and chloride of growth in young swine. J. Anim. Sci. 68:2736.

Green, B.L., B.W. McBride, D. Sandals, K.E. Leslie, R. Bagg and P. Dick. 1999. The impact of a monensin controlled-release capsule on subclinical ketosis in the transition dairy cow. J. Dairy Sci. 82:333-342.

Greene, L. W., G. T. Schelling, and F. M. Byers. 1986. Effects of dietary monensin and potassium on apparent absorption of magnesium and other macroelements in sheep. J. Anim. Sci. 63:1960.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-octadecenoic acids and milk fat depression. J. Dairy Sci. 81: 1251–1261.

Harrison, J., R. White, R. Kincaid, E. Block, T. Jenkins, and N. St-Pierre. 2012. Effectiveness of potassium carbonate sesquihydrate to increase dietary cation-anion difference in early lactation cows. J. Dairy Sci. 95:3919-3925.

He, M., K.L. Perfield, H.B. Green, and L.E. Armentano. 2012. Effect of dietary fat blend enriched in oleic or linoleic acid and monensin supplementation on dairy cattle performance, milk fatty acid profiles, and milk fat depression. J. Dairy Sci. 95:1447–1461.

Hu, W., and M.R. Murphy. 2004. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: A meta-analysis. J. Dairy Sci. 87:2222-2229.

Hu, W., M. R. Murphy, and L. Kung, Jr. 2007. The relationship between dry matter intake and acid-base status of lactating dairy cows as manipulated by dietary cation-anion difference. Anim. Feed Sci. Technol. 136:216-225.

Jenkins, T.C., W.C. Bridges Jr., J.H. Harrison and K.M. Young. 2014. Addition of potassium carbonate to continuous cultures of mixed ruminal bacteria shifts volatile fatty acids and daily production of biohydrogenation intermediates. J. Dairy Sci. 97:975-984.

Kadegowda A. K. G., M. Bionaz, B. Thering, L.S. Piperova, R.A. Erdman, and J.J. Loor. 2009. Identification of internal control genes for quantitative polymerase chain reaction in mammary tissue of lactating cows receiving lipid supplements. J. Dairy Sci. 92:2007–2019.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of

dietary forage concentration and buffer addition on duodenal flow of trans-c18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80: 2104-2114.

Kohn, R. A. and T. F. Dunlap. 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. J Anim. Sci. 76:1702-1709.

Lana, R.P. and J.B. Russell. 1997. Effect of forage quality and monensin on the ruminal fermentation of fistulated cows fed continuously at a constant intake. J. Anim. Sci. 75:224–229.

Lemenager, R. P., F. N. Owens, B. J. Shockey, K. S. Lusby and R. Totusek. 1978. Monensin effects on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components and cellulose disappearance. J. Anim. Sci. 47:255.

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophores for dairy cattle: current status and future outlook. J. Dairy Sci. 84(E. Suppl.): E194-E203.

Mongin. P. 1981. Recent advances in dietary cationanion balance: applications in poultry. Nutr.Soc. 40:285.

National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Washington, D.C.: National Academy Press, p105-161.

Newbold, C. J., R. J. Wallace, and N. D. Walker-Bax. 2013. Potentiation by metal ions of the efficacy of the ionophores, monensin and tetronasin, towards four species of ruminal bacteria. FEMS Microbiology Letters 338(2):161-167.

Nordlund, K.V. and E.F. Garrett. 1994. Rumenocentesis: A technique for the diagnosis of subacute rumen acidosis in dairy herds. Bovine Pract. 28:109–112.

Perry, F. G. and G. K. Macleod. 1969. Permanent Rumen Cannula. J. Dairy Sci. 52: 264-265.

Phipps, R. H., J. I. D. Wilkinson, L. J. Jonker, M. Tarrant, A. K. Jones and A. Hodge. 2000. Effect of Monensin on Milk Production of Holstein-Friesian Dairy Cows. J Dairy Sci 83:2789–2794.

Piperova, L. S., J. Sampugna, B. B. Teter, K. F. Kalscheur, M. P. Yurawecz, Y. Ku, K. M. Morehouse, and R. A.Erdman. 2002. Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that postabsorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. J. Nutr. 132:1235-1241.

Potter, E.L., R.L. VanDuyn, and C.O. Cooley. 1984. Monensin toxicity in cattle. J Anim. Sci. 58:1499-1511.

Raun, A.P., C.O. Cooley, E.L. Potter, R.P Rathmacher, and L.F. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. J. Anim. Sci. 43:670-677.

Richardson, L. F., A. P. Raun, E. L. Potter and C. O. Cooley. 1976. Effect of monensin in rumen fermentation in vitro and in vivo. J. Anita. Sci.43:657.

Rumpler, W. V., D. E. Johnson, and D. B. Bates. 1986. The effect of high dietary cation concentration on methanogenesis by steers fed diets with and without ionophores. J. Anim. Sci. 62:1737.

Russell, J.B. 1987. A proposed mechanism of monensin action in inhibition of ruminal bacterial growth: effects onion flux and protonmotive force. J. Anim. Sci. 64:1519-1525.

Russell, J.B. and H.J. Strobel. 1989. Effects of ionophores on ruminal fermentation. Appl. Environ. Microbiol. 55:1-6.

Russell, J.B., and A.J. Houlihan. 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiol. Rev.* 27:65-74.

Sanchez, W.K., D.K. Beede and J.A. Cornell. 1994. Interactions of sodium, potassium, and chloride on lactation, acid-base status, and mineral concentrations. J. Dairy Sci. 77:1661-1675.

Sanchez, W.K., and D.K. Beede. 1996. Is there an optimal dietary cation-anion difference for lactation diets? Anim. Feed Sci. Technol. 59:3-12.

Sanchez, W. K., D. K. Beede, and J. A. Cornell. 1997. Dietary mixtures of sodium bicarbonate, sodium chloride, and potassium chloride: Effects on lactational performance, acid-base status, and mineral metabolism of Holstein cows. J. Dairy Sci. 80:1207–1216.

Schelling, G. 1984. Monensin mode of action in the rumen. J. Anim.Sci. 58:1518–1527.

Sheperd, A.C. and D.K. Combs. 1998. Long-term effects of acetate and propionate on voluntary feed intake by midlactation cows. J. Dairy Sci. 81:2240-2250.

Symanowski, J. T., H. B. Green, J. R. Wagner, J. I. D. Wilkinson, J. S. Davis, M. R. Himstedt, M. S. Allen, E. Block, J. J. Brennan, H. H. Head, J. J. Kennelly, J. N. Nielsen, J. E. Nocek, J. J. van Der List and L. W. Whitlow. 1999. Milk

production and efficiency of cows fed monensin. J. Dairy Sci. 82(Suppl 1):75. (Abstr.)

Tucker, W.B., G.A. Harrison, and R.W. Hemken. 1988a. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71:346-354.

Tucker, W. B., I. S. Shin, J. F. Hogue, M. Aslam, G. D. Adams, M. T. Van Koevering, R. K. Vernon, and K. R. Cummings. 1994. Natural sodium sesquicarbonate fed for an entire lactation: Influence on performance and acid-base status of dairy cows. J. Dairy Sci. 77:3111–3117.

USDA-ERSa. National Milk Cost of Production. *USDA Economic Research Service*. N.p., 2014. http://www.ers.usda.gov/data-products/milk-cost-of-production-estimates.aspx#.U4Y6U8Uxo7k. Accessed July 3, 2014.

Van Maanen, R.W., J.H. Herbein, A.D. McGilliard, J.W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002–1007.

Warner, A.C.I. and B.D. Stacy. 1971. Intraruminal and systemic responses to variations in intake of sodium and potassium by sheep. Quarterly Journal of Experimental Physiology 57:89-102.

Weimer, P. J., D.M. Stevenson, D.R. Mertens and E. E. Thomas. 2008. Effect of monensin feeding and withdrawal on populations of indivivual bacterial species in the rumen of lactating dairy cows fed high-starch rations. Appl. Microbiol. Biotechnol. 80:135-145.

Wildman, C.D., J.W. West, and J.K. Bernard. 2007. Effect of dietary cation-anion difference and dietary crude protein on milk yield, acid-base chemistry, and rumen fermentation. J. Dairy. Sci. 90:4693-4700.