

ABSTRACT

Title of Document: DETERMINATION OF OPTIMAL DCAD CONCENTRATION AND RELATIVE EFFECTIVENESS OF POTASSIUM VERSUS SODIUM CATION SUPPLEMENTATION FOR MAXIMAL FEED EFFICIENCY IN LACTATING DAIRY COWS

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The objective was to determine the optimal dietary cation-anion difference (DCAD) required to maximize 3.5% fat-corrected milk (FCM) and feed efficiency (FE; FCM per dry matter intake (DMI)) in lactating dairy cows. When potassium carbonate was added resulting in four dietary treatments: 250, 300, 350, and 400 meq/kg DCAD, increasing DCAD linearly increased FCM and FE suggesting an optimal DCAD of at least 400 meq/kg. In a subsequent study comparing the relative effectiveness of potassium versus sodium, cation source had no effect on DMI or FCM. However, milk fat percentage and FE were highest when sodium was used as the sole cation source. Finally, surface response equations developed from literature data showed that DMI, FCM, milk fat %, rumen pH, and fiber digestibility increased linearly with DCAD. This suggested that improved FE was a function of improved rumen function, energy availability, and partitioning of absorbed energy toward milk energy.

DETERMINATION OF OPTIMAL DCAD CONCENTRATION
AND RELATIVE EFFECTIVENESS OF POTASSIUM VERSUS SODIUM
CATION SUPPLEMENTATION FOR MAXIMAL FEED EFFICIENCY
IN LACTATING DAIRY COWS

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Dedication

This thesis is dedicated to my older brother, Dan, who has always inspired and encouraged me to pursue my dreams. I owe my successes to his constant guidance, support, and friendship.

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First and foremost, I would like to express my sincerest gratitude to my mentor, **Dr. Richard Erdman**, for his invaluable guidance and support throughout the completion of my Master of Science degree. Thank you for teaching me the fundamentals of research and enhancing both my personal and professional development. You are an incredible mentor and I am truly honored to be one of your students.

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Table of Contents

Dedication	ii
Acknowledgements	iii
Table of Contents	vi
List of Tables	viii
List of Figures	ix
List of Abbreviations	xi
Chapter 1: INTRODUCTION	1
Chapter 2: LITERATURE REVIEW	4
Dairy Feed Costs	4
Feed Efficiency	6
Feed Conversion Ratios	6
Dairy FE Equation	7
Equations Used to Standardize Milk Yields	8
Dry Matter Intake	13
Factors Affecting FE	15
Stage of Lactation	16
Parity and Milk Production Effects	16
Monensin	18
Dietary Protein	20
Dietary Fat	21
Dietary Cation-Anion Difference	21
Milliequivalent Conversions	22
DCAD Equations	23
Selecting a DCAD Equation	25
DCAD Affects FE	27
Mechanisms by which DCAD Improve Feed Efficiency	29
Cation Supplementation	31
Sodium	31
Potassium	32
Increasing DCAD	33
Benefits of Improved Feed Efficiency	33
Hypotheses and Study Objectives	36
References	37
Chapter 3: EXPERIMENT 1	43
INTERPRETIVE SUMMARY	44
ABSTRACT	45
INTRODUCTION	46
MATERIALS AND METHODS	47
Research Facility and Animals	47
Experimental Diets and Feeding	48
Measurements	49
Statistical Model	50

RESULTS	50
DISCUSSION	51
CONCLUSION	57
ACKNOWLEDGEMENTS	58
REFERENCES	59
Chapter 4: EXPERIMENT 2	65
INTERPRETIVE SUMMARY	66
ABSTRACT	67
INTRODUCTION	68
MATERIALS AND METHODS	69
Research Facility and Animals	69
Experimental Diets and Feeding	70
Measurements	71
Statistical Model	72
RESULTS	73
DISCUSSION	74
CONCLUSION	78
ACKNOWLEDGEMENTS	79
REFERENCES	80
Chapter 5: EXPERIMENT 3	87
INTERPRETIVE SUMMARY	88
ABSTRACT	89
INTRODUCTION	90
MATERIALS AND METHODS	91
Data Collection	91
Data Assembly	92
Missing Data Points	93
Surface Response Equations	94
RESULTS	96
DISCUSSION	99
CONCLUSION	104
REFERENCES	105
Chapter 6: COMPREHENSIVE LIST OF REFERENCES	135

List of Tables

Table 2.1 FCRs vary across species adapted from FAO (2010).....	7
Table 2.2 Average milk component analysis and heat of combustion values for Bos Taurus raw milk.....	9
Table 2.3 Unadjusted and adjusted 3.5% FCM and FE in response to increased feed intake adapted from Erdman (2011).....	15
Table 2.4 Effects of monensin on DMI, FCM, and FE adapted from Erdman (2011) and Symanowski et al. (1999).....	20
Table 3.1 Ingredient composition of experimental diets (DM Basis).....	62
Table 3.2 Chemical composition of experimental diets (DM Basis).....	63
Table 3.3 Effects of DCAD concentration on feed intake, milk production, milk composition, and feed efficiency.....	64
Table 4.1 Ingredient composition of experimental diets (DM Basis).....	82
Table 4.2 Chemical composition of experimental diets (DM Basis).....	83
Table 4.3 Relative effectiveness of cation supplementation on feed intake, milk production, milk composition, and feed efficiency.....	84
Table 5.1 Summary of the final selected literature studies on buffer effects of production responses.....	110
Table 5.2 Mean and range of variables within the database.....	111
Table 5.3 Regression relationships between DCAD and dependent variables.....	112
Table 5.4 Dependent variable response to a 100 meq/kg DCAD increase in DCAD.....	113

List of Figures

Figure 2.1 U.S. monthly dairy feed costs per cwt of milk sold in 2006, 2009, and 2012 (USDA-ERS 2012).....	4
Figure 2.2 Comparison of the U.S. domestic corn between 2001 and 2011 (figure derived from data provided by the ERS-USDA, 2013).....	5
Figure 2.3 Rumen biohydrogenation pathway of PUFAs to saturated fatty acids....	30
Figure 4.1 Change in rumen fluid concentrations of sodium and potassium as dietary potassium is increased.....	85
Figure 4.2 Change in rumen fluid Na:K ratio as dietary potassium is increased.....	86
Figure 5.1 Correlation between reported and NRC predicted values for dietary percentages of sodium.....	114
Figure 5.2 Correlation between reported and NRC predicted values for dietary percentages of potassium.....	115
Figure 5.3 Correlation between reported and NRC predicted values for dietary percentages of chloride.....	116
Figure 5.4 Correlation between reported and NRC predicted values for DCAD concentrations (meq/kg).....	117
Figure 5.5 Relationship between DCAD and study-adjusted DMI (kg/d).....	118
Figure 5.6 Relationship between DCAD and study-adjusted milk production (kg/d).....	119
Figure 5.7 Relationship between DCAD and study-adjusted 3.5% FCM production (kg/d).....	120
Figure 5.8 Relationship between DCAD and study-adjusted milk fat.....	121
Figure 5.9 Relationship between DCAD and study-adjusted milk fat yield (g/d)...	122
Figure 5.10 Relationship between DCAD and study-adjusted milk protein.....	123
Figure 5.11 Relationship between DCAD and study-adjusted milk protein yield (g/d).....	124
Figure 5.12 Relationship between DCAD and study-adjusted rumen pH.....	125

Figure 5.13 Relationship between DCAD and study-adjusted rumen acetate molar percentage.....	126
Figure 5.14 Relationship between DCAD and study-adjusted rumen butyrate molar percentage.....	127
Figure 5.15 Relationship between DCAD and study-adjusted rumen propionate molar percentage.....	128
Figure 5.16 Relationship between DCAD and study-adjusted ratio of rumen acetate to propionate molar percentages.....	129
Figure 5.17 Relationship between DCAD and study-adjusted rumen total volatile fatty acid (TVFA) production.....	130
Figure 5.18 Relationship between DCAD and study-adjusted dry matter digestibility.....	131
Figure 5.19 Relationship between DCAD and study-adjusted NDF digestibility (% of DM).....	132
Figure 5.20 Relationship between DCAD and study-adjusted ADF digestibility (% of DM).....	133
Figure 5.21 Relationship between DCAD and study-adjusted feed efficiency (3.5% FCM per unit of DMI).....	134

List of Abbreviations

ADF	acid-detergent fiber
Cl	chloride
CP	crude protein
CWT	centum weight
DCAD	dietary cation-anion difference
DHIA	Dairy Herd Improvement Association
DIM	days-in-milk
DM	dry matter
DMI	dry matter intake
FCM	fat-corrected milk
FCR	feed conversion ratio
FE	feed efficiency
K	potassium
K ₂ CO ₃	potassium carbonate
MUN	milk urea nitrogen
Na	sodium
NaHCO ₃	sodium bicarbonate
NDF	neutral-detergent fiber
NRC	National Research Council
OS	other solids-non-fat
S	sulfur
SCC	somatic cell count

Chapter 1: INTRODUCTION

Dairy feed costs represent the largest expense associated with milk production (Wolf, 2010). While in the past feed costs have accounted for approximately 40% to 50% of the cost of milk production, during the last five years feed costs have doubled and now account for nearly 70% of the cost of milk production (Bailey et al., 2009; Wolf, 2010; Mantysaari et al., 2012). Changes in feed costs are having a dramatic impact on dairy profitability including the minimal breakeven milk price for dairy producers. Thus, dairy producers are extremely interested in factors that will improve the efficiency of feed use for producing milk (Erdman, 2011). The most commonly used index of dairy feed efficiency (FE) is fat-corrected milk (FCM) per unit of dry matter intake (DMI) (Erdman, 2011). Previous work has shown that altering the dietary cation-anion difference (DCAD) can increase milk yield, milk fat yield, optimize dry matter intake, and improve dairy FE (Hu and Murphy, 2004; Sanchez et al., Beede, 1996; West et al., 1992).

Currently, the NRC (2011) recommends dietary concentrations of 0.22% Na, 1.06% K, and 0.28% Cl for Holstein cows averaging 90 days-in-milk (DIM) producing 45kg of milk that contains 3.0% milk fat and 3.0% protein. Based on these recommended requirements of Na, K, and Cl in the diet, the suggested minimal DCAD concentration ($\text{Na} + \text{K} - \text{Cl}$) for dairy cows would be 295 meq/kg of diet dry matter (DM) (NRC, 2001). However, a more recent meta-analysis of published research (Hu and Murphy, 2004) suggested that a higher DCAD concentration (400 to 450 meq/kg) might result in improved milk yield and milk fat production. Although

several studies have investigated the production responses to various DCAD concentrations, the optimal DCAD concentration for maximal dairy FE is unknown.

Supplementation with either Na or K carbonates and/or bicarbonates can be used to increase dietary DCAD concentration. However, increasing dietary DCAD with K supplements such as potassium carbonate (K_2CO_3) is 4-times more expensive (kg basis) than Na supplementation using sodium bicarbonate ($NaHCO_3$) such that the relative effectiveness of Na vs. K is economically important. Some previous research suggested that milk yield and milk composition were not affected by cation source and that the most important influence on production responses is the overall DCAD concentration (Tucker et al. 1988a; West et al. 1992; Hu and Kung, 2009). However, other studies have reported that there may be significant interactions affecting the milk yield and DMI response to DCAD when different ratios of Na:K are supplemented (Sanchez et al., 1994; Sanchez et al., 1997; Wildman et al., 2007). Because sodium and potassium are involved in numerous cellular functions such as osmotic balance and acid-base homeostasis, Hu and Kung (2009) suggested that altering Na:K ratios may beneficially impact physiological processes and result in improved production responses. However, the relative effectiveness of each cation has yet to be determined.

Finally, numerous studies with lactating dairy cows were conducted during the time period from 1960 to 1990 that examined the effects of dietary buffers such as $NaHCO_3$, K_2CO_3 , and others. In many of these studies, significant effects of dietary buffers were observed that resulted in changes in DMI, milk production, and dairy FE. However, these studies were published prior to the emergence of the DCAD

concept. Thus, the inherent DCAD effects of buffer supplementation on milk production were not studied nor reported. However, the literature data from these experiments could still be analyzed to determine the respective DCAD concentrations and the relationship between DCAD and intake, milk production, milk composition, rumen characteristics, and digestibility.

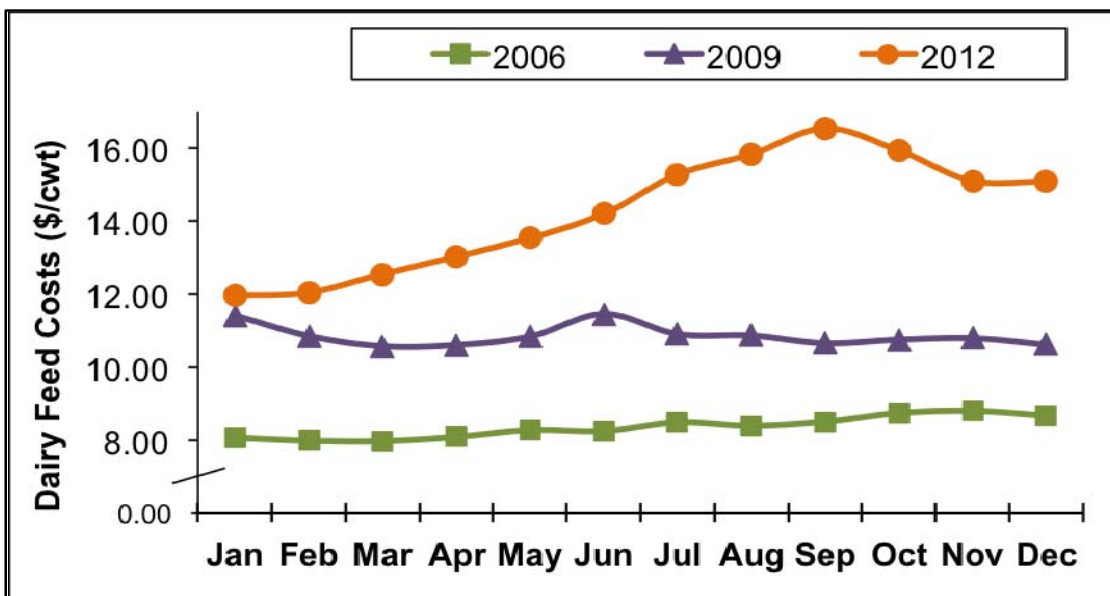
The central hypothesis of this thesis is that manipulating DCAD results in changes in intake, milk production, and milk composition. By altering production responses, it is possible that DCAD manipulation may be a potential mechanism to increase FE and improve dairy producer profitability. The first study objective was to determine the optimal DCAD concentration for maximal FE in lactating dairy cows. In addition, DCAD effects on production may vary depending on the source of cation used to increase DCAD concentration. Thus, the second study objective was to determine the relative effectiveness of sodium versus potassium as cation sources to increase DCAD and improve FE in lactating dairy cows. Finally, the last study objective was to perform a meta-analysis to determine the effect of DCAD on several dependent variables in lactating dairy cows using previously published journal articles.

Chapter 2: LITERATURE REVIEW

Dairy Feed Costs

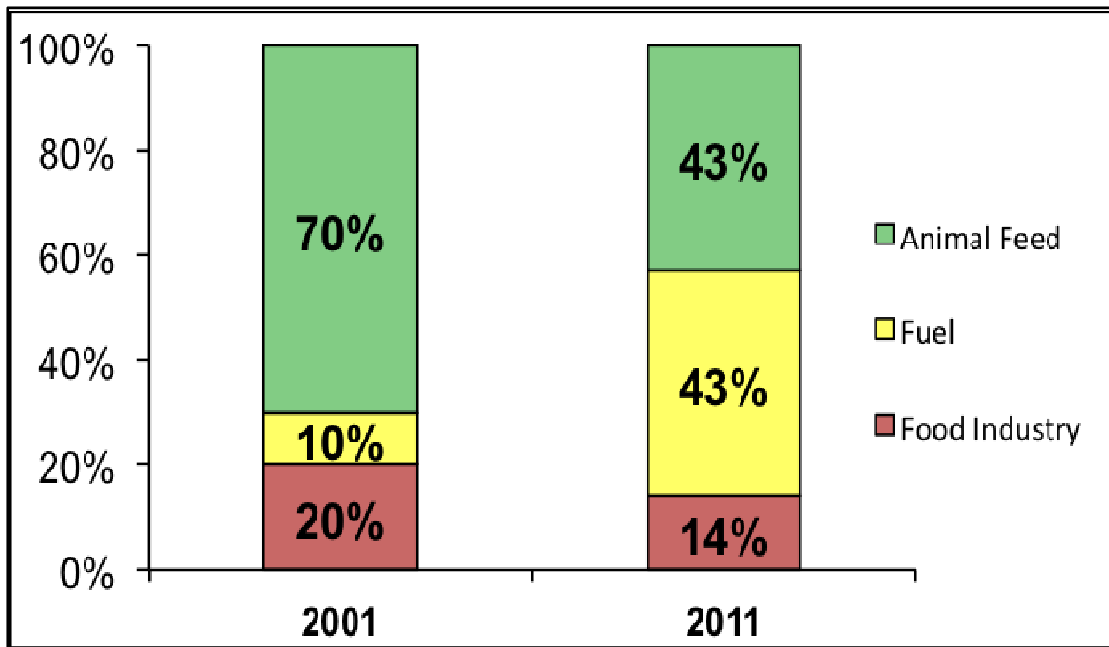
Dairy feed costs currently represent the single largest expense associated with producing milk on dairy farms (Wolf, 2010). Historically, feed has accounted for 40 to 50% of total dairy production costs. However, feed costs have doubled during the last five years such that feed now accounts for nearly 70% of total production costs (Bailey et al., 2009; Harrison et al., 2012; Mantysaari et al., 2012). United States Department of Agriculture statistics revealed that feed costs increased by 25% in 2011 alone (USDA-ERSa, 2012). Figure 2.1 illustrates the U.S. national monthly dairy feed costs per centum weight (cwt) of milk sold in the years 2006, 2009, and 2012. In particular, feed costs reached an all-time high in 2012 in part due to a historical drought (Hornby, 2013) that further drove up feed prices.

Figure 2.1 U.S. monthly dairy feed costs per cwt of milk sold in 2006, 2009, and 2012 (USDA-ERS 2013)



In addition to adverse weather conditions, dairy feed costs have also escalated due to an increased use of corn as a feedstuff for ethanol fuel production. As shown in Figure 2.2., the percentage of domestic corn used for fuel production has quadrupled within the past fifteen years (ERS-USDA, 2013), increasing from 10 to 43% of domestic corn usage. Conversely, the amount of corn used for animal feed has been greatly reduced from approximately 70% to 43% and for food use from 20 to 14%. This rapid increase in demand for corn for ethanol production has resulted in all-time high prices for corn and cereal grains (ERS-USDA, 2013). Because corn is a valuable, energy-dense feed for dairy cattle, the high price of corn has resulted in amplified dairy feed costs and decreased dairy producer profitability (Erdman, 2011). Due to the change in grain prices, dairy producers are keenly interested in techniques to improve feed utilization.

Figure 2.2 Comparison of the U.S. domestic corn between 2001 and 2011 (figure derived from data provided by the ERS-USDA, 2013)



Feed Efficiency

Feed Conversion Ratios

In animal production, feed efficiency equations are used to assess an animal's ability to convert feed into profitable products (FAO, 2010). Feed conversion ratios (FCR) are routinely used in the beef cattle, swine, and poultry industries and benchmarks for optimal FCRs have been established in each of these industries (Erdman, 2011). In most livestock species FCR are calculated by dividing the amount of feed consumed by the amount of body weight gained (product produced), as shown in Equation 1:

$$\text{FCR} = \frac{\text{Weight of Feed Consumed}}{\text{Body Weight Gained}} \quad (1)$$

A common FCR in the swine, poultry, and beef feedlots is feed per unit of gain (FPG) (FAO, 2010). A low FCR is preferred because it implies that the animal is utilizing the feed more efficiently (less feed per unit gain) (FAO, 2010). FCR values can vary depending on the age of the animal, environmental conditions, health conditions, the quality of feed, and numerous other factors (FAO, 2010). There are considerable differences in FCR values between different species (Table 2.1). Although feed efficiency equations and benchmarks have been determined for the beef, swine, and poultry industries, a standardized FE equation and benchmarks for FE have not yet been established for the dairy industry (Erdman, 2011).

Table 2.1 FCRs vary across species adapted from FAO (2010)

Animal Species	FCR
Broilers (poultry)	2.0
Turkeys (poultry)	2.5
Ducks (poultry)	2.5 – 3.0
Swine	3.5
Beef Cattle	≥ 8.0
Small Ruminants	7.0

Dairy FE Equation

In the dairy industry, various FE equations have been developed, but the most common equations focus on amounts of energy corrected milk produced per unit dry matter intake (DMI). While equations using either solids-corrected or energy-corrected milk have been developed, the most common method uses 3.5% fat corrected milk (FCM) per unit dry matter intake (DMI) to standardize milk production per unit feed intake on an equal milk energy basis (Erdman, 2011) as shown in Equation 2. Unlike FCRs used in other livestock, a higher FE value is preferred because it implies that more product (milk) is being produced for per unit feed reducing feed costs as a proportion of total production costs per unit of milk (Erdman, 2011). Dairy FE values should fall between 1.4 and 1.7 under normal conditions (Erdman, 2011).

$$\text{Dairy FE} = 3.5\% \text{ FCM (kg/day)} / \text{DMI (kg/day)} \quad (2)$$

Equations Used to Standardize Milk Yields

As suggested above, 3.5% FCM is the most common numerator used in the dairy FE equation. However, four different dairy FE equations exist. Each equation uses dry matter intake (DMI) as the denominator, but the numerator varies due to differences in standardizing milk production on an energy equivalent basis. The four potential numerators for the dairy FE equation include: 4.0% fat-corrected milk, 3.5% fat-corrected milk, energy-corrected milk, and solids-corrected milk.

4.0% Fat-corrected Milk

Milk has several components including water, fat, protein, sugars, vitamins, and minerals as shown in Table 2.2 (Field and Taylor, 2008). However, the relative amounts of the various milk components differ among species and even among breeds of dairy cattle (Ashworth et al., 1966). Although average dairy milk component percentages have been established as a standard reference, the actual milk component percentages vary between cows. Because each milk component has a different heat of combustion, different amounts of dietary energy are required to produce milks that vary in the individual milk components, especially milk fat (Friggens et al., 2007). In order to correct for energy differences in milk production, milk yields are standardized on an energy basis using formulas for FCM, SCM, and ECM (Erdman, 2011).

Table 2.2 Average milk component analysis and heat of combustion values for Bos taurus raw milk

Milk Component	Percentage in Milk ¹ (%)	Heat of Combustion ² (Mcal/kg)
Water	87.30	0.00
Milk protein	3.30	5.71
Milk fat	3.90	9.29
Lactose	4.80	3.95
Ash	0.70	0.00
Component Total	100.00	---

¹Values derived from Field and Taylor, 2008

²Values derived from NRC, 2001

The original FCM formula, standardized to 4% milk fat, was developed by Gaines and Davidson (1923) and was used to standardize lactation records among cows that produced milk with different fat concentrations, the primary factor affecting the energy content in milk. The 4.0% FCM equation used the heats of combustion of milk fat and solids-non-fat to predict the heat of combustion for milk (Gaines and Davidson, 1923). The equation was created using a heat of combustion of 9.28kcal/g for milk fat and 4.09kcal/g for solids-non-fat (Gaines and Davidson, 1923). The final equation for 4.0% FCM is shown in Equation 3:

$$4.0\% \text{ FCM} = (0.40 \times \text{kg milk}) + (15.00 \times \text{kg milk fat}) \quad (3)$$

There is perhaps one major flaw associated with the 4.0% FCM formula. Gaines and Davidson (1923) assumed that the milk protein, lactose, and ash that constituted solids-non-fat were always in a constant ratio; yet these ratios vary among individual breeds, herds and cows (Erdman, 2011), altering the energy content of the solids-non-fat component (milk production) in the 4.0% FCM equation. Due to this

misassumption, the original 4.0% FCM equation underestimated milk energy by 3.0% (Erdman, 2011). However, Gaines and Davidson (1923) recognized the error and increased their predicted energy value of one unit of 4% milk to account for the underestimation (Erdman, 2011). While the 4.0% FCM equation does not account varying ratios of milk protein, lactose, and ash in the milk solids-non-fat (SNF), the majority of the differences milk energy output are in fact related to differences in fat content which are accounted for using the 4% FCM equation (Erdman, 2011).

3.5% Fat-corrected Milk

For many years, 4.0% fat-corrected milk was the standard formula used in the dairy industry to standardize milk production to a constant fat content. However, as milk production per cow increased over time and the proportion of Holstein cows in the national dairy herd increased, there was a corresponding decrease in milk fat concentration. This occurred because increased milk production causes a decreased milk fat concentration and Holstein cows naturally have a milk fat concentration less than 4%. Therefore, the original 4% FCM formula was modified to 3.5% FCM which more closely reflects the current fat content of the U.S. Dairy Herd (Erdman, 2011). Using 3.5% FCM, fluctuations in milk fat composition, the principal milk component affecting variation in milk energy concentration, are still accounted for and milk yields are equalized on an energy concentration basis as shown in Equation 4:

$$3.5\% \text{ FCM} = (0.4318 \times \text{kg milk}) + (16.23 \times \text{kg milk fat}) \quad (4)$$

Like the 4.0% FCM equation from which it was derived, the 3.5% FCM equation does not account for varying proportions of milk protein, lactose, and ash in the solids-nonfat portion of milk (Erdman, 2011) such that the original inherent error in the Gaines and Davidson (1923) and Tyrell and Reid (1965) equations is still present.

Solids-corrected Milk

Based in part on the principles used to derive the 4.0% FCM equation, Tyrell et Reid (1965) developed a new equation was created to account for the differences in milk energy concentration that was based on the proportions of milk fat, milk protein, and solids-non-fat which they referred to as the solids-corrected milk (SCM). Tyrell and Reid (1965) measured the energy (heats of combustion) of milk from 42 cows that varied in composition. As expected, they found that milk energy fluctuated with changes in milk fat, protein, and lactose concentration. Subsequently, they developed a series of regression equations to predict milk energy concentrations based on the fat, protein, and lactose content (Tyrell and Reid, 1965). Tyrell and Reid (1965) reported that the variation between the measured FCM energy value and the predicted milk energy value was greatest at milk fat extremes: milks containing more than 4.0% milk fat also those containing or less than 3.0% milk fat. Tyrell and Reid (1965) found that especially in milks containing less than 3% milk fat percent, that the 4% FCM equation underestimated milk energy output by about 15.0% (18.0 kcal/kg) (1965). Differing milk ash contents did not affect the overall milk energy (Tyrell and Reid, 1965).

To correct this problem, they developed a new equation to predict milk energy that was based on milk yield, fat yield, and solids-non-fat (SNF) yield, which they referred to solids corrected milk (SCM) as shown in Equation 5:

$$\text{SCM} = (12.3 \times \text{lbs milk fat}) + (6.56 \text{ lbs SNF}) - (0.0752 \times \text{lbs milk}) \quad (5)$$

Energy-Corrected Milk

The last approach to correcting milk on an energy equivalent basis is energy-corrected milk (ECM). ECM is used by the Dairy Herd Improvement Association (DHIA) to equilibrate the national lactation records (Erdman, 2011). The ECM equation is used to adjust milk production to the energy content 3.5% milk fat and 3.2% milk protein which is shown in Equation 6 (DRMS, 2011) and is based on a regression equation for milk energy based on fat and protein reported by Tyrell and Reid (1965):

$$\text{ECM} = (0.327 \times \text{lbs milk}) + (12.95 \times \text{lbs milk fat}) + (7.65 \times \text{lbs milk protein}) \quad (6)$$

The ECM equation is likely somewhat better than FCM or SCM in predicting milk energy; however, it still assumes a constant energy for the lactose and ash concentration as proportion on non-fat, non-protein of milk solids. In other words, it assumes that the ratio of lactose to ash in milk is constant.

Although all four equations provide adequate predictions of milk energy, the most commonly used equation in the dairy industry is 3.5% FCM; thus, it is the equation that will be used for this thesis.

Dry Matter Intake

Dry matter intake is denominator in the dairy FE equation and feed dry matter is used for two primary functions: maintenance and production. Feed used for maintenance represents the portion of feed used by the dairy cow and other animal species to support body functions in the absence of milk production (or growth in other species). For example, in a dry non-pregnant mature dairy cow in a thermo-neutral environment feed is being used only for maintenance purposes. Feed can be used for other physiological functions besides maintenance and milk production. For example, dairy cows during their first lactation are still growing, having reached only 85% of their mature body weight (NRC, 2001), so a portion of first lactation cow's feed is being used for growth. Similarly, feed is used to support growth and development of the developing fetus (calf) in pregnant dairy cows, especially in the late stages of pregnancy. Another example could be the use of feed for maintenance of homeothermy during harsh climatic conditions. Finally, as cows mobilize body tissue to support milk production in early lactation, a portion of feed consumption is used in late lactation to replenish those reserves.

While feed is used for other functions, maintenance and production represent the majority of energy needs in high producing dairy cows. It is the partition of feed energy between production and maintenance that is a key driver of feed efficiency in the dairy cow. As cows consume more feed, they produce more milk, but the cow's maintenance requirement remains unchanged (NRC, 2001). Therefore, a larger portion of the cow's feed consumption is allocated for productive purposes and a

smaller portion of the feed is used to satisfy maintenance requirements (Erdman, 2011) and overall feed efficiency (FCM/DMI) increases.

In the dairy cow, increased consumption of feed also leads to a decrease in diet digestibility (NRC, 2001). According to the NRC, for each multiple (2X, 3X, 4X) of feeding above maintenance (X) consumed, diet digestibility decreases by approximately 3 percentage units of total digestible nutrients (TDN) (2001). For example, a diet that had an energy digestibility of 70% in a cow fed at maintenance would have an energy digestibility of 61% ($70 - (3 \times 3\% \text{ decline})$) at 4X maintenance feeding. Therefore, as more feed is consumed, fewer nutrients are absorbed per increment of feeding and this effect is known as maintenance dilution. Maintenance dilution is most likely responsible for the smaller than expected increase in 3.5% FCM per unit feed consumed as milk production increases (Erdman, 2011). Finally, the rate of decline in digestibility with increased feed intake is a function of a diet's digestibility at maintenance. So diets high digestibility at maintenance such as high grain diets also exhibit a more rapid decline in digestibility with increasing intake.

While feed efficiency (FCM/DMI) increases as feed intake increases, the rate of improvement is smaller than would be expected if diet digestibility was constant. The effect of the decline in digestibility is illustrated in Figure 2.3 where expected FE is illustrated under two scenarios: 1) constant diet digestibility (unadjusted); and 2) declining digestibility (adjusted) with increasing level of intake. In this example, diet digestibility was assumed to decrease by 3 digestibility units (0.03 Mcal/lb net energy of lactation (NE_L)) for each multiple of maintenance feed intake (Erdman, 2011; NRC 2001). Accounting for decreased diet digestibility results in vastly smaller increases

in FE: 1.60 and 1.62 as compared to 1.88 and 2.00, respectively (Figure 2.3). It is likely that the decline in digestibility with level of feeding is the reason that FE is not drastically improved by increased milk production and feed intake (Erdman, 2011).

Figure 2.3 Unadjusted and adjusted 3.5% FCM and FE in response to increased feed intake adapted from Erdman (2011)

Unadjusted 3.5% FCM and FE					
Maintenance (X)	1X	2X	3X	4X	5X
DMI, lb/d	10	20	30	40	50
Unadj. NE _L (Mcal/lb)	0.77	0.77	0.77	0.77	0.77
NE _L (Mcal)	7.7	15.4	23.1	30.8	38.5
3.5% FCM, lb/d	0	25	50	75	100
FCM/DMI	0.00	1.25	1.67	1.88	2.00

Adjusted 3.5% FCM and FE					
Maintenance (X)	1X	2X	3X	4X	5X
DMI, lb/d	10	20	30	40	50
Adj. NE _L (Mcal/lb)	0.77	0.74	0.71	0.68	0.65
NE _L (Mcal)	7.7	14.8	21.3	27.2	32.5
3.5% FCM, lb/d	0	22.5	44.5	64	81
FCM/DMI	0.00	1.13	1.48	1.60	1.62

Factors Affecting FE

Several factors are known to affect dairy FE including stage of lactation, parity, level of production, and dietary additions such as monensin, fat, and protein (Erdman, 2011).

Stage of Lactation

Lactation stage is one of the biggest factors that affect FE. Wood (1968) proposed an equation ($y_n = An^b e^{-cn}$) that predicted the lactation curve for dairy cows. This equation predicted average daily milk yield (y_n) by using the week of lactation (n) and three constants (A , b , c) that determine the shape of the curve (Wood, 1968; Nasri et al., 2008). The Wood equation has been used to predict milk yield, regardless of the parity (Nasri et al., 2008)

At the beginning of the lactation, FE is the highest (approximately 2.25) because cows are deriving a portion their energy required for milk production from tissue mobilization; reducing the energy required from feed (Erdman, 2011). Milk production peaks at approximately 4 to 8 weeks postpartum; however, DMI intake peaks at approximately 10 to 14 weeks postpartum (NRC, 2001). Therefore, dairy FE dramatically decreases as lactation progresses as DMI retroactively increases in order to support lactation energy needs and to replenish tissue reserves (NRC, 2001). After DMI peaks around 100 DIM, FE declines linearly. Thus, increasing DMI during mid-to-late lactation creates a larger denominator in the FE equation and reduces FE to approximately 1.30 by the end of lactation (Erdman, 2011).

Parity and Milk Production Effects

Multiparous cows have higher dairy FE (approximately 0.10 units) than primiparous cows because mature cows produce more milk, which reduces the proportion of feed used for maintenance (Erdman, 2011). Using the 2009 DHIA 350-day lactation records for Holstein herds in the 50th percentile for milk production,

Erdman (2011) estimated the FE of first parity, second parity, and third parity cows to be 1.44, 1.50, and 1.54, respectively. Typically, overall milk production of first lactation cows is about 85% of second lactation and greater parity cows (NRC, 2001). Lee and Kim (2006) confirmed that parity effects milk production and they reported that first lactation cows averaged 18,548 lbs milk/year whereas multiparous cows in lactations 2, 3, 4, and 5 averaged approximately 22,763 lbs milk/year. In addition to differences in total milk production, the lactation curve for primiparous cows is flatter; peak milk production is smaller and occurs later in the lactation cycle than in mature cows (Erdman, 2011). For example, reported peak of milk production for primiparous and multiparous was approximately 27 and 40 kg/day, respectively (Friggens et al., 1999).

While mature cows produce more milk, they also have a larger body size as body weight (BW) at first parity is generally about 85% of mature BW. The difference in BW would increase the amount of feed required for maintenance in mature cows resulting a reduced feed efficiency. However, since first lactation cows are still growing, the reduced feed required for maintenance is counterbalanced by feed required for growth such that differences in milk production account for majority of the parity effect (NRC, 2001) on feed efficiency. The NRC (2001) suggested that feed requirements equivalent to 20% of the cow's maintenance requirement were required to meet growth requirements in first lactation cows.

The effect of level of milk production was apparent when comparing herds with different productivities. Erdman (2011) simulated FE for U.S. Holstein Herds producing at the 30th, 50th, 70th, and 90th percentile for milk production (8,952 to

11,890 kg/lactation). Similar to the response seen with primiparous vs. multiparous cows, high producing herds would be expected to have greater FE (Erdman, 2011). The predicted 150-day FE for the 30th, 50th, 70th, and 90th percentile herds were 1.42, 1.49, 1.55, and 1.63, respectively (Erdman 2011). Erdman (2011) suggested that dairy FE would increase by 0.01 units for 0.45 kg per day increase in 3.5% FCM.

Monensin

Monensin is an ionophore that selectively inhibits gram-positive bacterial growth in the rumen, resulting in an altered rumen microbial population (NRC, 2001; Duffield et al., 2008a; Duffield et al., 2008b). Because of the change in the rumen bacterial environment, rumen fermentation patterns are altered such that the molar proportion of propionate is increased whereas the molar proportions of acetate and butyrate are decreased (NRC, 2001). Due to the increased production of propionate and decreased production of acetate and butyrate, more feed energy is conserved in VFA energy which is absorbed by the animal to be used for productive purposes by the cow (NRC, 2001). In addition, monensin may change nutrient partitioning to favor growth and production due to its ability to alter the hormonal status of lactating dairy cows (NRC, 2001).

Monensin has also been shown to help improve nitrogen and energy utilization in ruminants because it improves protein digestibility and promotes elevated blood glucose levels; thus, the animal spares some amino acids that would normally be involved in gluconeogenic pathways (NRC, 2001; Duffield et al., 2008b; Dubuc et al., 2010). Duffield et al. (2008a) reported that monensin increased glucose and urea

concentrations (blood, plasma, serum) and decreased acetoacetate, beta-hydroxy butyrate (BHBA), and non-esterified fatty acid (NEFA) concentrations in the blood; thus, monensin improved energy metabolism.

Although monensin has been as a feed additive to improve FE in beef cattle for several decades, it was only approved for use in lactating cows in 2005 (Erdman, 2011). Monensin can improve FE in lactating cows by altering rumen fermentation patterns such that energy that would normally be allocated for acetate, butyrate, and methane production is spared and can be used for milk synthesis (NRC, 2001). Also, increased blood glucose concentrations caused by monensin may result in an increased milk production because more glucose is available for milk lactose synthesis (NRC, 2001).

Using a meta-analysis of 36 journal articles consisting of 77 experimental trials, Duffield et al. (2008b) found that monensin increased MP by 0.7 kg/d and decreased DMI by 0.3 kg/d. However, the meta-analysis did not report monensin effects on FE as until recently there has not been a common method for reporting FE in dairy cattle (Erdman, 2011).

Symanowski et al. (1999) in a study that involved 9 university herds with 858 lactating dairy cows investigated monensin effects on FE and found that monensin improved FE by 0.06 units when cows consumed 300 mg per cow per day (Erdman, 2011). Dietary treatments consisted of the basal diet which contained 0 g/d monensin or the basal diet plus monensin supplementation of 238, 321, and 465 mg/day which resulted in FEs of 1.50, 1.54, 1.56, and 1.56, respectively (Symanowski et al., 1999; Erdman, 2011). Therefore, monensin is expected to improve FE by approximately

0.06 units when administered at the 300mg/day dosage rate (Erdman, 2011). A summary of the results is presented in Table 2.4.

Table 2.4 Effects of monensin on DMI, FCM, and FE adapted from Erdman (2011) and Symanowski et al. (1999)

Item	---- Monensin Supplementation (mg/cow/day) ----			
	0	238	321	465
DMI, kg /d	19.95	19.73	19.45	19.23
FCM, kg/d	30.05	30.36	30.32	30.00
FE	1.51	1.54	1.56	1.56

Dietary Protein

Early experiments with increased dietary protein showed an increase in diet dry matter and energy digestibility with increasing dietary crude protein (CP) concentration, especially in diets containing less than 15% crude protein (NRC, 2001; Holter et al., 1982). For example, the NRC (2001) reported that increasing dietary CP from 15 to 16 % would result in a 0.75 kg/d increase in milk production. Presumably this response was due inadequate rumen available protein for microbial fermentation. Kalscheur et al. (2006) tested the effects of rumen degradable protein (RDP) concentration on feed consumption, milk production, and FE and found that dairy FE increased as dietary RDP increased from 6.8% to 9.6% but did not increase further with 11.0% RDP. In summary, the authors reported that there is no effect of protein addition on diet digestibility or FE in diets containing more than 15.5% CP, but there was a marked improvement in diet digestibility and FE in diets containing less than 15.5% CP or 9.6% RDP (Erdman, 2011; Holter et al., 1982; Kalscheur et al., 2006).

Dietary Fat

Because dietary fat is more energy dense than carbohydrates (9.0 kcal/g vs. 4.0 kcal/g, respectively), the substitution of dietary fat for carbohydrates should increase FE because it would produce a diet with an increased dietary energy density (Maynard, 1944; Erdman, 2011). There are two proposed mechanisms by which increased dietary energy concentration would improve FE: 1) FCM could increase while DMI remains constant; or 2) DMI could decrease while FCM remains constant (Erdman, 2011). In simulating the effects of different fat supplements (calcium soaps, tallow and hydrolyzed tallow) the theoretical impact of fat supplementation was much greater (0.16 to 0.20 FE units) when increased energy was diverted to increased FCM as compared to when added fat reduced total feed intake (0.06 to 0.08 FE units) (Erdman, 2011). Likely the real response would be a mixture to the two mechanisms but the economic advantage of using fat to improve FE would have to be weighed against the increased cost of fat as compared to other energy sources in the cow's diet.

Dietary Cation-Anion Difference

Another potential means to improve dairy FE is through the manipulation of the dietary cation-anion difference (DCAD). Dietary cation-anion difference is a measure of the difference of the major dietary cations (Na and K) and anions (Cl). DCAD is the difference between the sum of the cations (sodium and potassium) and the anion (chloride), expressed in meq/kg DM as shown in Equation 7.

$$\text{DCAD (meq/kg)} = \text{Na} + \text{K} - \text{Cl} \quad (7)$$

Milliequivalent Conversions

Before the DCAD concentration of a diet can be calculated, the dietary concentrations of each of the three strong ions converted to their milliequivalent (meq/kg) basis, as shown in Equation 8.

$$\text{Meq/kg} = \left[\frac{\text{Ion (g)}}{\text{Molecular Weight (g)}} \right] * 1000 \quad (8)$$

The first step to calculate the DCAD of a diet is to convert the dietary percentages of each element to grams. For example, a diet that contains 0.10% Na, 0.65% K, and 0.20% Cl has 1 g Na, 6.5 g K, and 2 g Cl. Next, the milliequivalents of each ion are calculated by dividing the grams of each ion by its molecular weight (MW), as shown below.

$$\text{Na (meq/kg)} = \frac{(1\text{g Na})}{(23.0\text{g MW})} * 1000 = 43 \text{ meq/kg Na}$$

$$\text{K (meq/kg)} = \frac{(6.5\text{g K})}{(39.0\text{g MW})} * 1000 = 167 \text{ meq/kg K}$$

$$\text{Cl (meq/kg)} = \frac{(2\text{g Cl})}{(35.5\text{g MW})} * 1000 = 56 \text{ meq/kg Cl}$$

Finally, once each ion is converted from its dietary concentration to its milliequivalent form, the overall DCAD concentration can be calculated, as shown below using Equation 7.

$$\begin{aligned}\text{DCAD (meq/kg)} &= \text{Na} + \text{K} - \text{Cl} \\ &= 43 + 167 - 56 \\ &= 154 \text{ meq/kg DCAD}\end{aligned}$$

DCAD Equations

The relationship between acid-base homeostasis and dietary cation and anion balance was first discovered by Shohl (1923; 1939). Shohl (1923) showed that acid-base imbalances occurred when either excesses or deficiencies in cations or anions were present (1939; Block, 2011). Leach (1979) and Mongin (1980; 1981) confirmed this discovery and found that acid-base balance was directly related to the cation anion difference (Coppock et al., 1982; Block, 2011). In particular, Mongin (1980; 1981) found that Na, K, and Cl played major roles in acid-base balance in poultry (Coppock et al., 1982; Block, 2011). Based on his data, Mongin (1980, 1981), proposed that the net sum of these three major ions (Na + K – Cl) could be used to predict overall net acid intake (Sanchez, 1999; Block, 2011). Throughout the years, this equation has received several names; however, the acronym “DCAD” was first used by Sanchez and Beede (1991, as cited by Block, 2011).

Ender et al. (1971) proposed use of DCAD in prepartum dairy cow diets to prevent milk fever (Block, 1994; Dishington, 1975). Milk fever, or hypocalcaemia, is characterized by a sharp decrease in the blood calcium levels of postpartum cows and affects approximately 6% of U.S. dairy cows each year (NRC, 2001). After calving, the parathyroid hormone (PTH) is released to help cows adapt to lactation by increasing bone calcium resorption, reducing loss of calcium in the urine, and

increasing 1,25-dihydroxyvitamin D synthesis to help promote calcium transport in the intestines (NRC, 2001). Research has shown that milk fever is related to the acid-base balance of the cow prior to calving (NRC, 2001; Ender et al., 1971). The function of PTH is inhibited by high blood pH because alkalinity causes a conformational change in the PTH receptor (NRC, 2001). In order to maximize the functionality of PTH prior to calving and reduce the incidence of milk fever, an acidic blood pH is required (NRC, 2001). Ender et al. (1971) created the DCAD equation to assess the acid/base potential of the diet using the strong dietary ions ($\text{Na} + \text{K} - \text{Cl} - \text{S}$). Several studies reported reduced the incidence of milk fever by reducing DCAD it was proposed that lowering the DCAD to zero meq/kg promoted optimal acidification of the cow prior to calving (NRC, 2001; Giesy et al., 1997; Leclerc and Block, 1989).

Although the DCAD equation was created to combat milk fever in pre-partum cows, the concept was introduced into lactating cow research in 1988 by Tucker et al. (1988a; 1988b). Tucker et al. (1988a) reported that increasing DCAD ($\text{Na} + \text{K} - \text{Cl}$) from -100 to 200 meq/kg improved milk production by approximately 9.0%. As the interest in the effect of DCAD on production responses in lactating cows increased, two major DCAD equations emerged. As shown in Equation 7, the first major equation considers only Na, K and Cl because they are the three most highly absorbed (85 to 90% digestibility) and have been shown to play large roles in acid-base homeostasis within biological fluids (Block, 1994). Other equations were introduced that included other minerals such as calcium, magnesium, and phosphorus. However, meta-analysis results show that the most accurate dairy DCAD equation included Na,

K, Cl, and S (Block, 2011; Charbonneau, et al., 2006; Lean et al., 2006) as shown in Equation 9 where sulfur has a valence charge of -2.

$$\text{DCAD (meq/kg)} = [(\text{Na} + \text{K}) - (\text{Cl} - \text{S})] \quad (9)$$

Equation 9 is the other most commonly used DCAD equation and dietary sulfur is included as it has been shown to affect acid-base balance (Dishington, 1975; Tucker et al., 1991; Block, 1994; Goff et al., 2004).

Selecting a DCAD equation

There is much debate regarding which ions to include in the DCAD equation. Sodium, potassium, and chloride appear in all DCAD equations because these ions are considered “fixed ions” (Block, 1994). Fixed ions are not metabolized and directly affect the acid-base balance of the animal (Block, 1994). In some DCAD equations, only the fixed ions are present (Na + K – Cl). Sulfur is disregarded in this form of the DCAD equation because it is a multivalent mineral (S⁻²) that does not possess complete bioavailability (dissociation, solubility, and absorption) like the fixed ions: Na, K, or Cl (Sanchez, 2011).

Other researchers prefer to use a DCAD equation that involves dietary S (Na + K – Cl – S). Sulfur is included in some DCAD equations because, when fed in large amounts, it can affect the acid-balance balance (Block, 1994). Tucker et al. (1991) reported that when DCAD concentrations ranged from 0 to +30 meq/100g DM [(Na + K) – (Cl + S)], sulfur had a similar effect as chloride on acid-base balance. However,

Tucker et al. (1991) also reported that chloride absorption is much higher than sulfur absorption (95% for Cl vs. 51.8-60.8% for S); therefore, sulfur's effects on acid-base chemistry may actually be somewhat smaller than chloride's effects. Also, the effectiveness of sulfur may depend on the source of dietary sulfur used (Tucker et al., 1991). For example, sulfide anions (S^{2-}) tend to be better absorbed in the rumen as compared to sulfate anions (SO_3^{2-}), which are produced when sulfur-containing amino acids are oxidized (NRC, 2001). Despite its lower absorptivity, sulfate anions tend to affect acid-base status more than sulfide anions due to their three attached oxygen molecules (NRC, 2001). Elemental sulfur and sulfonates found in lignin tend to be poorly absorbed in the rumen because these forms possess low solubility; therefore, they may not be very effective in altering acid-base homeostasis (NRC, 2001).

When using sulfur in the DCAD equation, some have suggested a modifying coefficient for sulfur should be created to account for its relative effectiveness compared to chloride (Tucker et al., 1991). Goff et al. (2004) proposed an equation, $[(Na + K) - (Cl + 0.6S)]$ where the modifying coefficient for sulfur was based on its bioavailability and this modification more accurately predicted blood and urinary pH (Charbonneau et al., 2006).

When sulfur is included in the DCAD equation, the reported DCAD concentrations are lowered due to the subtracted sulfur milliequivalents from total DCAD. Therefore, it is imperative that the DCAD equation used to calculate the DCAD concentration of a diet is reported to avoid any potential confusion by the readers. With the exception of diets where anions such as Cl and S have been added

to reduce DCAD, the majority of the variation in DCAD is due to variation in dietary K and Na. In these circumstances, there is little advantage to using the more complex DCAD equations using S. In this thesis, DCAD concentrations were altered using either potassium carbonate, sodium bicarbonate, or a mixture of the two cations. Therefore, sulfur was not included in the DCAD equation because there was no manipulation of dietary anions (Cl and S).

DCAD Affects FE

The original use of DCAD manipulation in dairy cattle nutrition was in the prepartum feeding programs to prevent milk fever, a metabolic disease associated with hypocalcaemia in the dairy cow (Ender et al., 1971). Negative DCAD diets were found to prevent milk fever (Block, 1984; Hu and Murphy, 2004). However, more recent research has been focused on the production responses to altering dietary DCAD concentrations fed to lactating dairy cows (Tucker et al., 1988a; West et al., 1991; Sanchez et al., 1994; Sanchez et al., 1997; Roche et al., 2005; Wildman et al., 2007a; Wildman et al., 2007b). Several studies suggested that increasing dietary DCAD could increase milk yield and optimize dry matter intake (Wildman et al., 2007a; Hu and Murphy, 2004; Tucker et al., 1988b). Tucker et al. (1988a) reported that increasing DCAD concentrations (Na + K – Cl) from -100 to 200 meq/kg resulted in a 9% increase in milk yield.

When dietary Na and K are increased, the animal's acid-base balance is altered such that body fluids become alkalized (Chan et al., 2005; Block, 1984). Increasing DCAD has been shown to increase blood pH and bicarbonate (HCO_3^-) levels, which

improve the buffering capacity of the blood (Chan et al., 2005; Block, 1984; Tucker et al., 1988a). It is believed that the improved acid-base chemistry, along with changes in rumen pH, consequently result in improved production performance of dairy cattle (Kalscheur et al., 1997; Hu and Murphy, 2004).

In a meta-analysis conducted by Hu and Murphy (2004), increasing DCAD (Na + K – Cl) concentrations quadratically improved milk yield with the greatest milk yield achieved with a 340 meq/kg DCAD. They also reported that a DCAD concentration of approximately 490 meq/kg resulted in the highest 4.0% FCM yield. Increased DCAD concentrations resulted in increased blood pH and bicarbonate concentrations, which were greatest at DCAD concentrations of 350 and 470 meq/kg, respectively (Hu and Murphy, 2004). To optimize DMI, milk yield, and 4.0% FCM yield, Hu and Murphy (2004) suggested that the optimal DCAD concentration falls within the range of 340 to 490 meq/kg (Na + K – Cl). Like Hu and Murphy, Sanchez and Beede (1996) found that DCAD concentrations that exceeded NRC (2001) recommendations improved FE. Sanchez and Beede (1996) reported that milk yield, 4.0% FCM yield, and DMI were optimized when the DCAD concentration was approximately 380 meq/kg (Na + K – Cl).

In addition to improving 3.5% FCM and overall milk yields, increased DCAD also maximizes DMI (Hu et al., 2007; Tucker et al., 1991). Hu et al. (2007) reported that DMI increased linearly with increased DCAD concentrations. Hu et al. (2007) suggested that increased DMI was related to the improved acid-base status of the animals, which was indicated by elevated blood pH and bicarbonate levels. Generally, increased DMI results in increased milk production because a larger

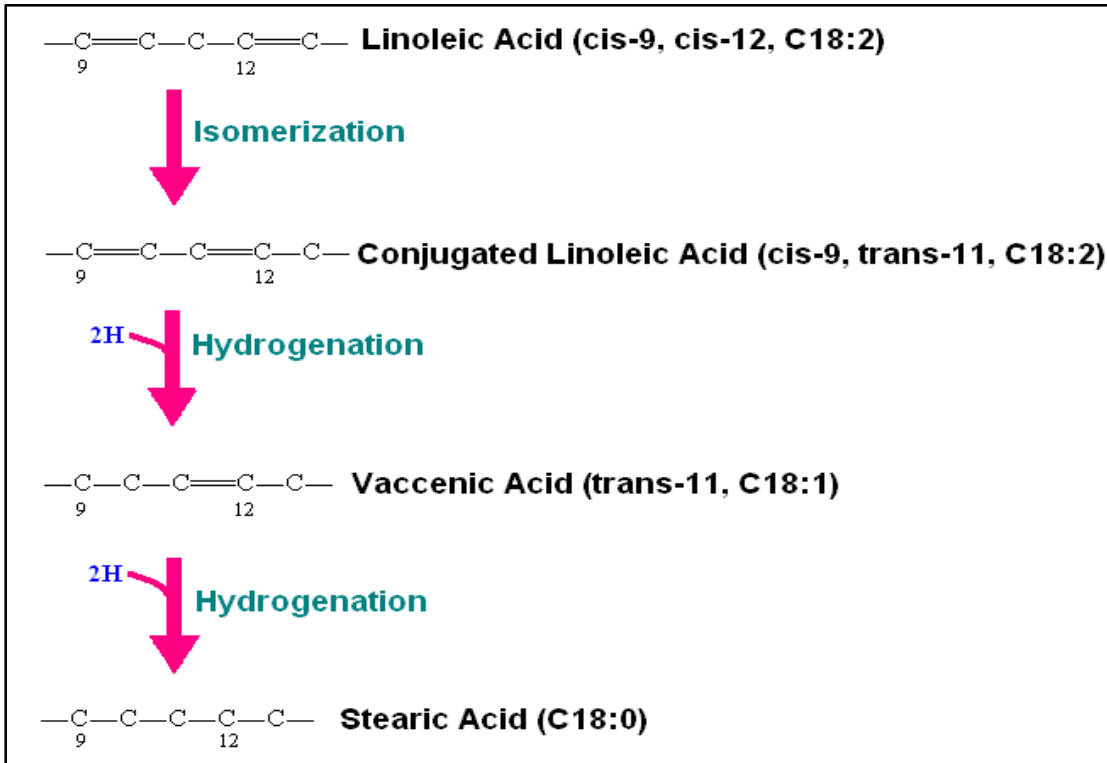
proportion of dietary nutrients are allocated to productive purposes and a smaller proportion is used to satisfy maintenance requirements (Erdman, 2011). By increasing milk production, increased DMI results in improved FE.

In summary, dietary DCAD has been shown to improve DMI, 3.5% FCM yield, and overall milk yield resulting in improved FE. Clearly, there is a potential to improve FE and reduce feed costs by increasing DCAD concentrations through cation supplementation.

Mechanisms by which DCAD Improves Feed Efficiency

Although the exact mechanism by which DCAD improves feed efficiency is not known, research has shown that increasing DCAD (through addition of Na and/or K) improves acid-base balance and increases rumen pH (Apper-Bossard et al., 2010; Hu et al., 2007a; Kalscheur et al., 1997, Sanchez and Beede, 1996). Increasing rumen pH results in the decrease of ruminal *trans* fatty acids, which are intermediates that produced during the incomplete biohydrogenation of poly-unsaturated fatty acids (PUFAs; Kalscheur et al., 1997; Wildman et al., 2007c). As shown in Figure 2.3, the basic mechanistic pathway of rumen biohydrogenation is as follows: 1) triglycerides are hydrolyzed to form glycerol and free fatty acids, 2) PUFAs are isomerized to form dienes that contain *trans*-double bonds such as conjugated linoleic acid (CLA; *cis*-9, *trans*-11 C18), 3) CLA is hydrogenated to form vaccenic acid (*trans* 11 C-18:1), and 4) vaccenic acid is hydrogenated to stearic acid (C18:0), which is a saturated fatty acid (NRC, 2001).

Figure 2.3 Rumen biohydrogenation pathway of PUFAs to saturated fatty acids



Low rumen pH results in increased amounts of trans-fatty acid intermediates due to incomplete (alternate) rumen biohydrogenation (NRC, 2001). Increased concentrations of ruminal trans fatty acids have been associated with diet induced milk fat depression in dairy cattle (Grinari et al., 1998; Wildman et al., 2007c; NRC, 2001). Thus, the reduction in ruminal *trans* fatty acids and *trans* double bond containing conjugated linoleic acid caused by the increased rumen pH may prevent milk fat depression and consequently explain the increased milk fat production associated with dietary cation supplementation. Because ruminal pH is positively correlated with milk fat percentage, the addition of dietary buffers increases the percentage of milk fat, resulting in an improved FE (Allen, 1997; Erdman, 1988; Hu and Murphy, 2004).

Cation Supplementation

Sodium

Sodium is a critical cation in the diet of lactating dairy cows (NRC, 2001). It is involved in numerous physiological functions such as saliva buffering, bone structure, and acid-base homeostasis (NRC, 2001). In addition, the ratio of sodium to potassium is also critical in physiological processes such as controlling extracellular fluid volumes, nerve impulses, heart function, and the transport of molecules across cellular membranes via the Na-K pump in eukaryotes (NRC, 2001). The renin-angiotensin pathway serves as an internal regulator of sodium concentrations and it also controls blood pressure, excretion/reabsorption of ions in the kidney, and potassium concentrations (NRC, 2001).

Research has shown that feeding diets with inadequate levels of sodium can reduce DMI and milk production in dairy cows after just one week (NRC, 2001). Other studies have suggested that increasing sodium (and increasing DCAD) can increase milk yield and improve DMI (NRC, 2001; Sanchez et al., 1994). The effect of sodium concentration on milk production and DMI was modeled using 15 experiments that had sodium concentrations that ranged from 0.11 to 1.20% DM (NRC, 2001). Milk production and DMI were maximized when the sodium concentration fell between 0.70 to 0.80% DM (NRC, 2001). However, other ions such as K, Cl, Ca, and P were not kept constant between experiments; thus, the effect of sodium on milk production and DMI is more likely a result of sodium interactions and ratios in relation to other ions (NRC, 2001).

Potassium

Like sodium, potassium is a dietary cation that serves a critical role in many cellular processes. Potassium is responsible for regulating water balance, acid-base homeostasis, osmotic pressure, and the exchange of oxygen and carbon dioxide (NRC, 2001). Potassium is also necessary for nerve impulses, heart function, kidney function, enzymatic activity, calcium/magnesium metabolism, protein synthesis, and carbohydrate metabolism (NRC, 2001). Because large quantities of potassium cannot be stored within the body, potassium must be supplemented daily and it has the highest dietary requirement compared to the other strong ions (NRC, 2001).

Similar to sodium, the effect of potassium concentration on milk production and DMI was modeled using 15 experiments that had potassium concentrations that ranged from 0.66 to 1.96% DM (NRC, 2001). A potassium concentration of 1.50% DM was shown to maximize milk production and DMI in lactating dairy cows (NRC, 2001). As discussed in the sodium model, other ions such as Na, Cl, Ca, and P were not kept constant between experiments; thus, the effect of potassium on milk production and DMI is more likely a result of potassium interactions and ratios in relation to other ions, especially sodium and chloride (NRC, 2001). Mallonee (1984) found that DMI and milk yield were not affected by increasing potassium from 1.07 to 1.58% DM; however, milk yield and DMI were affected by the interaction of potassium with sodium (NRC, 2001).

Increasing DCAD

Either sodium or potassium can be used to increase DCAD in lactating dairy cow diets. However, cation supplementation with potassium carbonate is currently 4-times more expensive (kg basis) than cation supplementation with sodium bicarbonate. Previous research suggested that milk yield and milk composition were not affected by type of cation supplementation (Tucker et al. 1988a; West et al. 1992; Hu and Kung, 2009). These results suggest that the most important influence on production responses is the overall DCAD concentration, not the concentrations of individual dietary ions. Of course, this can only be assumed in cases where each mineral in the diet is present at an appropriate biological concentration.

However, other studies have reported that there may be significant interactions between Na and K affecting milk yield and DMI response when DCAD is increased using different ratios of Na: K supplementation (Sanchez et al., 1994; Sanchez et al., 1997; Wildman et al., 2007a). Because sodium and potassium are involved in numerous cellular functions such as osmotic balance and acid-base homeostasis, Hu and Kung (2009) suggested that altering Na:K ratios may beneficially impact physiological processes and result in improved production responses. However, the relative effectiveness of each cation has yet to be determined.

Benefits of Improved Dairy Feed Efficiency

There are two primary benefits of improving FE in a dairy herd. First and foremost, improving FE will reduce feed costs per unit milk sold. A recently

published article by Erdman et al., (2011) illustrates this concept. Increasing DCAD from 251 to 336 meq/kg using K_2CO_3 supplementation in corn silage based diets fed to lactating dairy cows during the first 20 weeks postpartum increased dairy FE by 0.14 units and reduced feed costs by approximately \$1.00 per cow per day. (Erdman et al., 2011). The net savings to 100-cow dairy would be \$36,500 per year. Thus, improving feed efficiency can greatly improve annual profits for dairy producers.

The second benefit of improved feed efficiency is reduced nutrient excretion and potentially environmental pollution. When feed efficiency is increased, the animal is able to utilize more of the feed nutrients for productive purposes. If a higher percentage of the dietary nutrients are utilized, less undigested waste is produced per unit milk produced resulting in reduced excretion of wastes into the environment (Arriaga et al., 2009). Two major nutrients that are excreted in undigested waste (manure) are nitrogen and phosphorus (Arriaga et al., 2009). Approximately 65-75% of nitrogen consumed by dairy cows is excreted in urine and feces (NRC, 2001). In regards to phosphorous, dairy cows excrete the majority of any superfluous phosphorus (above requirement) provided in the diet (NRC, 2001). If excess excreted phosphorus and nitrogen accidentally contaminate local water sources, they can cause major environmental damage through the process of eutrophication (Arriaga et al., 2009). Reducing the amount of phosphorus and nitrogen excreted greatly reduces the potential for environmental pollution.

In addition to reducing nitrogen and phosphorous excretion, improved feed utilization will also reduce the use of valuable resources such as feed, water, land, fuel, and animals (Capper, 2011). Capper (2011) reported that U.S. dairy farms in

1944 produced 53 billion kilograms of milk using 25.6 million cows; however, U.S. dairy farms in 2007 produced 84.2 billion kilograms of milk using 9.2 million cows. This incredible transformation of the dairy industry was the result of improved productivity due to genetic, management, nutritional, and other advancements (Capper 2011). If productivity is further increased through improved feed efficiency, less cows will be required to produce the same amount of milk; thus, less feed, water, and land will be needed to support the U.S. dairy herds. In addition, less fuel will be burned to perform daily farm activities, fewer animals will be needed to support production (bulls, replacement heifers, etc.), and less waste (manure and greenhouse gases) will be produced (Capper, 2011). Therefore, improving feed efficiency would reduce the environmental impact of dairy farming.

Hypotheses and Study Objectives

Based on the previous literature, two hypotheses were investigated:

1. Increasing DCAD (meq/kg) will improve (FE) in lactating dairy cows and the optimal DCAD concentration for maximal FE is higher than the concentration recommended by the 2001 NRC
2. Potassium and sodium sources are equally effective when used as cation sources to increase DCAD to improve FE

To test these hypotheses, three study objectives were completed:

1. Determination of the optimal DCAD concentration to maximize FE in lactating dairy cows
2. Determination of the effectiveness of Na versus K as cation sources to increase DCAD to improve FE in lactating dairy cows
3. Perform a meta-analysis on previous research to create surface response equations in order to predict the effects of DCAD and cation source on milk production, 3.5% FCM, DMI, milk fat yield, and milk protein yield.

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Chapter 3: EXPERIMENT 1

Determination of Optimal DCAD Concentration for Maximal Feed Efficiency in Lactating Dairy Cows

INTERPRETIVE SUMMARY

Determination of optimal DCAD concentration for maximal feed efficiency in lactating dairy cows. *Iwaniuk et al., page 000.* Feed costs in the dairy industry have doubled during the last five years and dairy producers are interested in factors that will improve feed efficiency expressed as 3.5% fat-corrected milk per unit dry matter intake. Increasing dietary cation-anion differences (DCAD) has been shown to increase 3.5% fat-corrected milk, and feed efficiency; however, the optimal DCAD concentration has yet to be determined. In this study, cows were fed diets with DCAD between 277 and 407 meq/kg. Fat-corrected milk increased linearly with DCAD which increased feed efficiency. The optimal DCAD concentration could not be determined because maximal feed efficiency occurred at the highest treatment DCAD concentration (407 meq/kg). Therefore, it is possible that DCAD concentrations greater than 407 meq/kg may further improve FE.

RUNNING HEAD: OPTIMAL DCAD FOR MAXIMAL FEED EFFICIENCY

**Determination of optimal DCAD concentration for maximal feed efficiency in
lactating dairy cows**

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ABSTRACT

Feed costs in the dairy industry have doubled during the last five years and dairy producers are keenly interested in factors that will improve dairy feed efficiency (FE). The most commonly used index of dairy FE is fat-corrected milk (FCM) per unit of dry matter intake (DMI). Increasing dietary cation-anion differences (DCAD) has been shown to increase milk production, FCM, and FE while decreasing DMI. However, the optimal DCAD concentration for maximal FE has yet to be determined. The objective of this experiment was to determine the optimal DCAD concentration for maximal FE in early lactation dairy cows. Eight primiparous and 12 multiparous Holstein cows averaging 89 (\pm 25) days in milk at the start of the experiment were used. Cows were individually fed a basal diet consisting of 60% corn silage and 40% concentrate (dry matter basis). Experimental treatments consisted of 250 (basal), 300, 350, and 400 meq/kg DCAD which were applied in a 4 x 4 Latin square design with 3-week experimental periods. Potassium carbonate was added to the basal diet to provide the respective treatment DCAD concentrations. DCAD had no effect on milk production or DMI. However, milk fat percentage increased linearly ($P = 0.025$) with increasing DCAD resulting in an increased ($P = 0.048$) FCM. This resulted in a 0.06 unit increase in dairy FE ($P = 0.042$). The results of this experiment confirmed earlier studies suggesting that altering DCAD could be used to increase FE in dairy cows and reduce feed costs.

Key words: DCAD, 3.5% fat-corrected milk, dairy feed efficiency, potassium carbonate

INTRODUCTION

During the past five years, dairy feed costs have doubled and currently, dairy feed costs represent the largest expense associated with milk production (Bailey et al., 2009; Wolf, 2010; Mantysaari et al., 2012). One way that dairy producers can reduce feed costs and increase profitability is to improve the efficiency by which feed is converted to milk production in their herds. Erdman et al. (2011) and Harrison et al. (2012) demonstrated a potentially inexpensive way to improve dairy feed efficiency (**FE**) by increasing dietary cation-anion difference (**DCAD**) using K supplementation. Dairy FE was improved by 7.7% (0.14 units) when the DCAD concentration increased from 251 to 336 meq/kg using potassium carbonate (**K₂CO₃**) supplementation (Erdman et al., 2011). Similarly Harrison et al. (2012) reported a 6.7% increase (0.11 unit) increase in FE by increasing DCAD from 490 to 600 meq/kg. Erdman et al. (2011) reported that K addition reduced feed costs by approximately one dollar which for 100 cow dairy would save approximately \$36,500 each year. While these studies illustrate the potential for K supplementation to increase dairy FE, the optimal DCAD concentration for maximal FE has not been determined.

Using the minimal requirements for dietary K, Na, and Cl, (NRC, 2001) the suggested minimal DCAD concentration in dairy cow diets would be approximately 300 meq/kg diet DM. In a meta-analysis, Hu and Murphy (2004) reported that milk yield was greatest when the DCAD concentration was 340 meq/kg and dry matter intake (**DMI**) was maximized at 400 meq/kg DCAD. Earlier, Sanchez and Beede (1996) suggested that both milk yield and dry matter intake were highest when the

DCAD concentration was equal to 380 meq/kg. Optimal DCAD concentration should be determined in order to maximize FE and reduce feed costs.

Addition of dietary buffers (pH neutralizers) such as sodium and potassium carbonates, bicarbonates, and sesquicarbonates increases the strong ion content of the diet, thereby increasing DCAD concentration. Buffer addition has been shown to increase milk fat percentage, particularly in cows fed low forage, high starch diets that reduce rumen pH (Erdman, 1988; Kalscheur et al., 2006). Therefore, in addition to the effects on DMI and milk production, DCAD might also be expected to increase FCM and FE by increasing milk fat concentration. Therefore, the objective of this experiment was to determine the optimal DCAD concentration required to maximize feed efficiency. The results of this study will be used to help producers of dairy herds reduce feed expenses and maximize profitability.

MATERIALS AND METHODS

Research Facilities and Animals

The protocol 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. Experimental observations and corresponding cow numbers used in the study were determined by power analysis using the Analyst feature of Statistical Analysis Software (Version 9.2, SAS Institute, Cary, NC). Using an average standard error of the mean of 0.04 for FE taken from literature (Erdman et al., 2011 and Kalscheur et al., 2006), a required sample size of 20 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 (fat-corrected milk divided by dry matter intake, kg) in an experiment with 4 dietary treatments. Due to facility limitations, a 4x4 Latin Square design was selected to ensure that each treatment had 20 replicates.

Eight primiparous and 12 multiparous cows were used in the study. Cows averaged 40 kg/d milk production and 89 (± 25) days in milk (**DIM**), at the start of the experiment. Cows were housed and individually fed in tie-stalls fitted with water mattresses (Ryder Supply Company, Chambersburg, PA) and bedded with shavings. Lighting in the research barn was controlled such that the cows received twelve hours light and twelve hours darkness during the study. Cows had continuous access to water via shared drinking cups in their tie stalls. Cows were milked twice daily at approximately 0615 and 1600 h. This study was conducted from January until April.

Experimental Diets and Feeding

The basal diet was a TMR containing approximately 60% corn silage and 40% concentrate (DM basis) and was formulated to meet or exceed the NRC (2001) nutrient requirements for dairy cows producing 40 kg/d milk containing 3.7% fat and 3.1% protein (Table 3.1). The concentrate portion of the diet consisted of soybean meal (48% CP, As Fed Basis), a vitamin-trace mineral premix, and ground shell corn. Treatments consisted of the basal diet which contained approximately 250 meq/kg DCAD or the basal plus 50, 100, and 150 meq/kg added DCAD using added potassium carbonate sesquihydrate (DCAD Plus®, Church & Dwight Co., Inc., Piscataway, NJ) that resulted in final estimated DCAD of approximately 250, 300,

350, and 400 meq/kg diet DM (Table 3.2). Treatments were applied in a 4 x 4 Latin square design balanced for carryover effects with 3-week experimental periods. A basal TMR in sufficient quantity for all cows was mixed in portable mixer wagon once daily. In order to achieve the four experimental dietary treatments, potassium carbonate sesquihydrate was substituted for up to 4.0% of ground corn in basal diet that was mixed in advance. These mixes were then added to the basal TMR and mixed in a Calan Data Ranger[®] (American Calan, Northwood, NH) for cows within each treatment group prior to delivery to individual feed tubs. Amounts of feed offered and feed refusals were recorded once daily at the time of feeding at 0930h.

Measurements

Measurements included weekly individual cow BW and daily feed intake and feed refusals. Silage and concentrate samples were taken weekly for DM analysis to adjust the as fed TMR to maintain a constant forage-to-concentrate ratio and to measure feed DM such that daily DMI could be calculated for each cow. Milk production was recorded electronically at each milking. Milk samples were collected on consecutive milkings on d 7 and 14 and during the last 4 consecutive milkings of each experimental period (d 20 and 21) and analyzed for fat, protein, and somatic cell count (SCC).

Individual samples of the corn silage, ground corn, soybean meal-vitamin premix and the treatment K_2CO_3 were collected weekly and composited by experimental period 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). Actual DCAD was calculated based on the K, Na, and Cl concentrations of the

individual feeds and/or mixtures weighted proportionally to their contribution to the diet DM in the TMR.

Statistical Model

Mean data for DMI, milk production, fat, protein, and SCC, fat and protein yield, 3.5% FCM, and FE for each cow from the last week of each experimental period were used in the statistical analysis. Data were analyzed using the Mixed Procedure in SAS (Version 9.2, SAS Institute, Cary, NC) using the statistical model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where:

- Y_{ijk} = the response from the i^{th} Cow, the j^{th} Period, and the k^{th} Treatment
- μ = the grand mean
- C_i = the effect of the i^{th} cow
- P_j = the effect of the j^{th} period
- T_k = the effect of the k^{th} treatment level
- e_{ijk} = random error

Treatment was analyzed as a fixed effect while cow and period were analyzed as random effects in the Mixed Procedure of SAS. As the treatments were designed to provide 50 meq/kg DM increments in DCAD, the dose response to DCAD concentration was tested using linear and quadratic orthogonal contrasts. A probability of $P \leq 0.05$ was considered 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 K and DCAD. Dietary K increased evenly from 1.3 to 1.7% and the final DCAD (using the Na + K – Cl equation) was 277, 319, 368, and 406 meq/kg DM.

Treatment had no significant effect ($P > 0.05$) on DMI, BW, milk production, or milk protein yield (Table 3.3). However, there was a trend ($P = 0.063$) for reduced milk protein percentage with increased DCAD. DCAD had no effect on other solids-non-fat (OS) percentage, OS yield, or SCC ($P > 0.05$).

Milk fat percentage and fat yield increased linearly with increasing DCAD ($P < 0.05$) where fat percent and fat yield were greater (2.86% and 1108g/day) in cows fed the highest DCAD concentration (406 meq/kg DM). Because of the change in fat percent, 3.5% FCM was increased linearly ($P < 0.05$) with increasing DCAD.

While there was no change in DMI, the increase in 3.5% FCM resulted in a linear increase in FE ($P = 0.042$) with the greatest FE shown in the 406 meq/kg treatment.

DISCUSSION

Previous studies have reported variable DMI responses to increased DCAD concentrations. Hu et al. (2007a) reported that DMI increased linearly in response to increasing DCAD concentrations and similar DMI responses have been observed in several other studies (Apper-Bossard et al., 2010; Delaquis et Block, 1995; Tucker et al., 1991; West et al., 1991; Wildman et al., 2007b). However, not all DCAD experiments report a significant DMI response. Some recent studies have reported that increasing DCAD concentrations does not affect DMI (Roche et al., 2005; Hu et al., 2007b; Erdman et al., 2011; Harrison et al., 2012).

Although the discrepancy in DMI response has not been specifically investigated, it has been proposed that studies which used anionic salts to decrease DCAD may result in a significant DMI response due to decreased palatability and acidosis caused by anion supplementation (Charbonneau et al., 2006; Oetzel and Barmore, 1993; Vagnoni and Oetzel, 1998). It has also been suggested that stage of lactation has an effect on DMI where more variation in DMI occurs during the earlier stages of lactation compared with cows in mid and late lactation (NRC, 2001). Thus, studies using early lactation cows may show a significant effect of DCAD on DMI compared to studies that used mid-to-late lactation cows (NRC, 2001).

In the present study, DMI was not affected by increasing DCAD concentration. This result most likely was due to the fact that DCAD concentrations were not altered using anionic salts, including the potential palatability effects of anionic salts. Secondly, the cows in this study were in mid-lactation; intake effects due to cation supplementation tended to be most pronounced in early lactation cows fed low forage and high concentrate diets (Erdman, 1988).

Previous work has shown that increasing DCAD concentration significantly improved milk production in lactating dairy cows (Hu and Murphy, 2004; Sanchez and Beede, 1996; Tucker et al., 1988a; Tucker et al., 1988b). The mechanism by which DCAD works to improve performance is still unknown. However, it has been suggested that milk production is increased as a result of rumen environmental changes and/or improved acid-base homeostasis (Apper-Bossard et al., 2010; Hu et al., 2007a; Sanchez and Beede, 1996). With regard to the rumen environment, it is understood that higher DCAD concentrations increase rumen pH and potentially alter

VFA production patterns (Apper-Bossard et al., 2010; Roche et al., 2005). A more alkaline rumen pH may provide a more suitable environment for rumen bacteria, improving rumen fermentation and digestibility which results in improved lactation performance (Apper-Bossard et al., 2010; Roche et al., 2005).

In the present study, DCAD had no effect on milk yield which has been reported in several other studies (Hu et al., 2007a; Hu et al., 2007b; Harrison et al., 2012). However, it has also been reported that DCAD affects milk production (Hu and Murphy, 2004; Sanchez and Beede, 1996; Tucker et al., 1988a; Tucker et al., 1988b). It is quite possible that the DCAD effect on milk production seen in other studies occurred as a result of larger increments in treatment DCAD concentrations (Delaquis and Block, 1995; Roche et al., 2005). For example, Wildman et al. (2007a) reported that increased DCAD concentrations improve milk yield; however, the DCAD increment between the two dietary treatments was 250 meq/kg DM. In a meta-analysis conducted by Hu and Murphy (2004), the authors reported a significant effect of DCAD on milk production, but the experimental DCAD concentrations ranged from -191 to 636 meq/kg DM. In the current study, treatment increments were only 50 meq/kg DCAD. Perhaps larger increments would have resulted in a significant increase in milk production. Indeed, it can be difficult to compare different DCAD experiments and results due the lack of similarity between DCAD concentration ranges, cation sources, basal diets, and experimental animals (parity, stage of lactation, breed, etc).

Both milk fat percentage and yield (g/day) increased linearly with increasing DCAD concentration. Fat percent and yield increased by 0.27 percentage units and

112 g/d, respectively by increasing DCAD between 277 and 406 meq/kg DM. Similar DCAD effects on milk fat have been reported in several other studies (Hu et al., 2007; Roche et al., 2005; Wildman et al., 2007a; Wildman et al., 2007b). The changes in milk fat would be expected considering the change in DCAD concentration across the treatments used in this experiment. However, milk fat percentage for all cows in the current study was low averaging only 2.74% across treatments. There is no clear explanation of the cause of milk fat depression in the current study. However, the Clarksville Herd in general (including cows not on experiment) had a low fat test (~3.0%) compared to the normal of 3.5 to 3.6% for the herd. Prior to the start of the experiment, a new corn silage trench was opened and the entire herd, experimental and non-experimental animals, received the new corn silage in their TMR. Shortly after the corn silage switch, milk fat tests for the entire Clarksville Herd dropped. Fatty acid analysis was performed on the corn silage to test for the presence of biohydrogenation intermediates linked to milk fat depression, but results indicated that the silage was normal. Upon further investigation, it was concluded that the corn silage contained abnormally low NDF content resulting a TMR NDF less than 25% and an abnormally high starch content (~40%) resulting in inadequate NDF from forage (NRC, 2001). It has been shown that inadequate levels of NDF can result in milk fat depression due to lowered rumen pH and decreased buffering capabilities (NRC, 2001). Because of the low milk fat, it might have been expected that milk fat response would have been even greater than observed in this study as compared with that suggested in a review of buffer effects on milk fat concentration (Erdman, 1988).

Originally, the DCAD effect on milk fat percentage was believed to be a result of the altered rumen environment causing changes in VFA fermentation patterns (NRC, 2001). However, previous work has shown that milk fat percentage is manipulated as a result of altered rumen biohydrogenation (NRC, 2001; Bauman and Griinari, 2003). In the rumen, bacteria saturate (hydrogenate) dietary polyunsaturated fatty acids (PUFA) to form saturated fatty acids in the process known as rumen biohydrogenation (NRC, 2001). In fact, rumen bacteria convert most of the PUFAs to saturated fatty acids such that very few unsaturated fatty acids escape the rumen, as shown in Figure 2.3 (NRC, 2001). Research has shown that trans-fatty acids in milk are increased during milk fat depression (NRC, 2001; Teter et al., 1990; Wonsil et al., 1994). These trans-fatty acid intermediates are the result of abnormal (alternate) rumen biohydrogenation and they reduce overall milk fat percentage by limiting de novo fatty acid synthesis (NRC, 2001). An increase in trans-fatty acid production in the rumen is the result of a low rumen pH (NRC, 2001). When a cow is fed a diet containing an inadequate amount of fiber, the rumen pH will be decreased, causing a change in rumen biohydrogenation that increases trans-fatty acid production (NRC, 2001). It is possible that the insufficient NDF present in the dietary treatments resulted in decreased rumen pH, altered biohydrogenation, and milk fat depression.

Dietary buffers have been shown to combat low rumen pH and milk fat depression (Erdman, 1988; Kalscheur et al., 1997). Buffers increase rumen pH which promotes normal rumen biohydrogenation of linoleic acid (unsaturated) to stearic acid (saturated) and reduces the amount of trans-fatty acid produced from an alternate biohydrogenation pathway (NRC, 2001; Harrison et al., 2012). Using artificial rumen

fermenters, Jenkins et al. (2010) found that the addition of dietary potassium, which increases DCAD, played a major role in rumen biohydrogenation, causing a reduction in trans-fatty acids and increased biohydrogenation of linoleic to stearic acid (Harrison et al., 2012). In the present study, increasing DCAD resulted in an increase in milk fat percentage. We speculate that by increasing the DCAD concentration, the rumen pH increased resulting in more complete biohydrogenation of PUFAs to saturated fatty acids. With a reduced amount of trans-fatty acid intermediates produced, de novo fatty acid synthesis increased which resulted in higher a milk fat percentage. Because of the increase in milk fat percentage, 3.5% FCM also increased linearly in response to increasing DCAD concentration.

Although increasing 3.5% FCM production is important from a production standpoint, in the present study, the key response variable to DCAD was dairy FE. Dairy FE expressed as 3.5% FCM per unit of DMI is an indicator of the relative proportion of feed DM used for production milk energy. In this study, DMI was not significantly affected by DCAD concentration. Therefore, the denominator of the dairy FE equation was similar between treatments. However, as DCAD concentration had a significant, linear effect on 3.5% FCM, FE increased 0.06 units with increasing DCAD and was maximized (1.58) at a DCAD concentration of 406 meq/kg DM. Harrison et al., (2012) also investigated the effects of DCAD on FE and reported that FE improved by 0.11 units when DCAD was increased from 490 to 700 meq/kg DM. In addition, calculated FEs from published treatment means show that increasing DCAD from 291 to 537 meq/kg DM resulted in a 0.09 unit change in FE and increasing DCAD from 310 to 550 meq/kg DM resulted in a 0.12 unit change in

FE in diets containing 15% and 17% CP, respectively (Wildman et al., 2007a). Therefore, the results from the present study confirm that increasing DCAD concentration can be an effective tool to improve dairy FE, resulting in reduced feed costs per unit milk energy produced and increased dairy herd profitability.

The main goal of this experiment was to determine the optimal DCAD concentration in order to maximize FE. However, the optimal DCAD concentration could not be determined because the maximum FE was observed at the highest DCAD concentration. Therefore, it is possible that higher concentrations of DCAD could further improve dairy FE. A follow-up study in our laboratory has been designed to determine the optimal DCAD concentration to maximize FE with DCAD ranging from 250 to 625 meq/kg DM. That study was designed with intent that the increased DCAD levels will create significant curvilinear responses in performance such that the optimal DCAD concentration can be determined.

CONCLUSION

While several studies have been conducted to test the effects of DCAD on production responses as well as acid base balance, this was the first study designed to determine an optimal DCAD concentration for maximal FE in lactating dairy cows. Our results indicate that increasing DCAD from 277 to 406 meq/kg linearly increased milk fat percentage, milk fat yield, 3.5% FCM production, and dairy FE. However, the optimal DCAD concentration for maximal FE could not be determined because the highest treatment DCAD concentration yielded the maximum FE. Therefore, we

concluded that at least 406 meq/kg DCAD is required and it is possible that even higher concentrations are required to maximize dairy FE.

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Table 3.1 Ingredient composition of experimental diets (DM Basis)

Ingredient	----- DCAD, meq/kg DM -----			
	250	300	350	400
Corn Silage	59.71	59.71	59.71	59.71
Ground Corn	17.7	17.3	16.89	16.49
Soybean Meal, 48%	18.63	18.63	18.63	18.63
DCAD Plus ^{®1}	0	0.4	0.81	1.21
Corn Gluten Meal, 60%	0.74	0.74	0.74	0.74
Limestone ²	0.79	0.79	0.79	0.79
Biophos ³	0.1	0.1	0.1	0.1
Dynamate ⁴	0.08	0.08	0.08	0.08
Salt-White	0.5	0.5	0.5	0.5
TM-433 ⁵	0.02	0.02	0.02	0.02
4-Plex ⁶	0.02	0.02	0.02	0.02
ADE Mix ⁷	0.03	0.03	0.03	0.03
Vitamin E ⁸	0.01	0.01	0.01	0.01
Selenium Premix ⁹	0.05	0.05	0.05	0.05
Megalac ¹⁰	1.41	1.41	1.41	1.41
Omigen-AF ¹¹	0.17	0.17	0.17	0.17
Rumensin-10g/lb ¹²	0.05	0.05	0.05	0.05

¹Contained 56% K (Church & Dwight Co., Inc., Piscataway, NJ)

²Contained 36% Ca and 0.02%P

³Contained 17% Ca and 21% P

⁴Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN)

⁵Contained 0.16% Co, 4.0% Cu, 3.0% Fe, 0.35% I, 15% Mn, and 16% Zn (Southern States Cooperative, Inc., Richmond, VA)

⁶Contained 0.20% Co, 0.99% Cu, 0.031% Fe, 1.57% Mn, and 2.83% Zn (Southern States Cooperative, Inc., Richmond, VA)

⁷Contained 5,454,545 IU/kg Vitamin A, 1,818,182 IU/kg Vitamin D, 9,091 IU/kg Vitamin E

⁸Contained 56,818 IU/kg Vitamin E

⁹Contained 0.3 IU/g Selenium; 28% Ca

¹⁰Contained 9% Ca; 84.5% Fat (Church & Dwight Co., Inc., Piscataway, NJ)

¹¹Contained 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 x 10¹⁰ 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)

¹²Contained 20% Monensin Na, 1% Mineral oil, and carriers such as rice hulls, limestone, and fermentation nutrients (Elanco, Greenfield, IN)

Table 3.2 Chemical composition of experimental diets (DM Basis)

Item	----- DCAD, meq/kg -----				SEM
	250	300	350	400	
DM, %	47.9	47.89	47.88	47.89	0.00
NEL, Mcal/lb	0.79	0.79	0.78	0.78	0.00
CP, %	16.30	16.27	16.24	16.21	0.00
NDF, %	26.71	26.70	26.62	26.62	0.02
ADF, %	15.32	15.3	15.3	15.29	0.01
Lignin, %	2.29	2.28	2.27	2.26	0.01
Ash, %	5.53	5.82	6.32	6.47	0.22
Fat ¹ , %	2.30	2.27	2.25	2.23	0.01
Na, %	0.24	0.24	0.25	0.25	0.00
K, %	1.30	1.46	1.64	1.79	0.11
Cl, %	0.57	0.57	0.57	0.57	0.00
S, %	0.18	0.18	0.18	0.18	0.00
Ca, %	0.75	0.75	0.75	0.75	0.00
P, %	0.38	0.38	0.38	0.38	0.00
Mg, %	0.20	0.20	0.19	0.19	0.00
DCAD, meq/kg DM ²	277	319	368	406	13.0
DCAD-S ³ , meq/kg DM ³	164	205	255	293	13.0

¹Measured as crude fat which would not include the 1.19% fatty acids (84.5% of 1.41% of diet DM) from Megalac[®]

²Dietary K + Na – Cl, meq/kg DM

³Dietary K + Na – Cl - S, meq/kg DM

Table 3.3 Effects of DCAD concentration on feed intake, milk production, milk composition, and feed efficiency

Item	----- DCAD, meq/kg -----				SEM	----- <i>P</i> < -----	
	250	300	350	400		Lin. ¹	Quad. ²
Observations	19	20	20	20			
BW, kg	615	610	614	607	18.5	0.208	0.876
DMI, kg/d	22.0	22.3	22.6	22.3	0.46	0.202	0.209
Milk, kg/d	39.4	39.0	39.6	39.3	1.28	0.937	0.979
SCC, linear score	4.06	3.62	3.73	3.79	0.941	0.753	0.627
Fat, %	2.59	2.77	2.72	2.86	0.181	0.025	0.758
Fat yield, g/d	996	1050	1070	1108	63.1	0.015	0.802
Protein, %	3.05	3.03	3.02	2.99	0.063	0.067	0.729
Protein yield, g/d	1192	1177	1191	1167	33.3	0.449	0.813
Other solids, %	5.64	5.65	5.67	5.65	0.064	0.685	0.650
Other solids, g/d	2220	2202	2244	2217	77.9	0.829	0.907
3.5% FCM, kg/d	33.2	33.9	34.5	34.9	1.16	0.048	0.836
FE ³	1.52	1.53	1.53	1.58	0.042	0.042	0.334

¹Linear orthogonal contrast

²Quadratic orthogonal contrast

³FE = 3.5% FCM/DMI

Chapter 4: EXPERIMENT 2

**Determination of the Relative Effectiveness of Potassium versus
Sodium as Strong Ions for Improving Feed Efficiency of Lactating
Cows**

INTERPRETIVE SUMMARY

Determination of the relative effectiveness of sodium bicarbonate versus potassium carbonate in improving feed efficiency of lactating cows. *Iwaniuk et al., page 000.* Increasing dietary cation-anion differences (DCAD) has been shown to increase feed efficiency (FE) dairy cows. Either sodium or potassium can be used to increase DCAD in dairy cow diets. However, supplementation with potassium carbonate is 4x more expensive than cation supplementation with sodium bicarbonate (kg basis). In this study, the relative effectiveness of sodium versus potassium was compared four milliequivalent ratios of K:Na 100:0, 67:33, 33:67, 0:100 in the supplemental cation used increase DCAD to 400 meq/kg. Milk fat percentage and feed efficiency increased linearly with increasing sodium. Therefore, these results suggested that sodium was not only more economical, but also was more effective than K as a cation source.

RUNNING HEAD: EFFECT OF K:Na RATIO ON LACTATING COWS

Determination of the relative effectiveness of sodium bicarbonate versus potassium carbonate in improving feed efficiency in lactating cows

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ABSTRACT

Increasing dietary cation-anion differences (DCAD) has been shown to increase milk production, 3.5% fat-corrected milk (FCM), and feed efficiency (FE) while optimizing dry matter intake (DMI) in lactating dairy cows. Either sodium or potassium can be used to increase DCAD in lactating dairy cow diets; however, cation supplementation with potassium carbonate (K_2CO_3) is 4X more expensive than cation supplementation with sodium bicarbonate ($NaHCO_3$) on a kilogram basis. The objective of this study was to determine the relative efficacy of K_2CO_3 versus $NaHCO_3$ for improving dairy FE. Eight primiparous and 12 multiparous Holstein cows averaging 40 kg/d milk and 95 (± 75) days in milk at the start of the experiment were used. Cows were individually fed a basal diet consisting of 65% corn silage and 35% concentrate (dry matter basis). Experimental treatments consisted of a basal diet containing 250 meq/kg DCAD, and the addition of 150 meq/kg DCAD using four ratios (meq/kg basis) of K:Na: 100:0, 67:33, 33:67, and 0:100 using K_2CO_3 and $NaHCO_3$, respectively. Treatments were applied in a 4 x 4 Latin square design with 3-week experimental periods. Cation source had no effect on milk production, DMI, or FCM. However, replacement of K with Na resulted in a linear increase in milk fat percentage ($P = 0.005$). Dairy FE, defined as FCM/DMI, was highest ($P = 0.04$) when Na was the sole cation source. This change was primarily a result of increased milk fat percent that was resulted in increased FCM. These results suggest that Na was more effective than K as a cation supplement to improve dairy FE.

Key words: **DCAD, feed efficiency, potassium carbonate, sodium bicarbonate**

INTRODUCTION

Either sodium or potassium can be used to increase DCAD in lactating dairy cow diets. However, cation supplementation with potassium carbonate sesquihydrate carbonate is currently 4-times more expensive than sodium sesquicarbonate as a cation supplement (kg basis). Previous research suggested that milk yield and milk composition were not affected by cation source (Tucker et al. 1988a; West et al. 1992; Hu and Kung, 2009). These results suggest that most important influence on production responses is the overall DCAD concentration, not the concentrations of individual dietary ions.

However, other studies have reported that there may be significant interactions affecting the milk yield and DMI response to DCAD when different ratios of Na:K are supplemented (Sanchez et al., 1994; Sanchez et al., 1997; Wildman et al., 2007). Because sodium and potassium are involved in numerous cellular functions such as osmotic balance and acid-base homeostasis, Hu and Kung (2009) suggested that altering Na:K ratios may beneficially impact physiological processes and result in improved production responses. However, the relative effectiveness of each cation has yet to be determined.

On a practical basis, if the effect of sodium is as effective as potassium as a cation source to increase DCAD and improve FE, dairy producers could reduce feed costs by using a cation source such as sodium bicarbonate rather than potassium carbonate since is currently 4-times less expensive. If the effects of sodium and potassium cation supplementation are different, then dairy producers could base their

decision on the source of cation supplement on both the supplement cost and its relative effectiveness.

We hypothesized that there was no difference in the relative effectiveness of sodium versus potassium as cations used to increase DCAD when used to improve FE in lactating dairy cows. We believed that the overall DCAD concentration is the most important influence on production responses, not the individual concentrations of the dietary strong ions. However, previous research has reported conflicting effects of Na:K ratios on production responses. Therefore, the objective of this study is to determine the relative effectiveness of potassium carbonate sesquihydrate ($K_2CO_3 \cdot 1.5 H_2O$; KCARB) versus sodium sesquicarbonate ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$; NaSCARB) on dairy FE.

MATERIALS AND METHODS

Research Facility and Animals

The protocol 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. Experimental observations and corresponding cow numbers used in the study were determined by power analysis using the Analyst feature of Statistical Analysis Software (Version 9.2, SAS Institute, Cary, NC). Using an average standard error of the mean of 0.04 for FE taken from literature (Erdman et al., 2011 and Kalscheur et al., 2006), a required sample size of 20 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 (fat-corrected milk divided by dry matter intake, kg) in an experiment with 4 dietary treatments. Due to facility limitations, a 4x4 Latin Square design was selected to ensure that each treatment had 20 replicates.

Eight primiparous and 12 multiparous cows were selected for the study. Cow selection was based on milk production and days-in-milk (DIM) that averaged 40 kg/d and ranged from 20 to 170 days postpartum, respectively at the start of the experiment. Cows were housed and individually fed in tie-stalls fitted with water mattresses (Ryder Supply Company, Chambersburg, PA) and bedded with shavings. Lighting in the research barn was controlled such that the cows received twelve hours of light and twelve hours of darkness during the study. Cows had continuous access to water via shared drinking cups in their tie stalls. Cows were milked twice daily at approximately 0615 and 1600 h. This study was conducted from May until July

Experimental Diets and Feeding

The basal diet was a TMR containing 65% corn silage and 35% concentrate (DM basis) formulated to meet or exceed the NRC (2001) nutrient requirements for dairy cows producing approximately 40 kg/d milk per containing 3/7% fat and 3.1% protein (Table 4.1). The concentrate portion of the diet consisted of soybean meal (48% CP As Fed), a vitamin-trace mineral premix, and ground shell corn. Treatments consisted of a basal diet, which contained approximately 250 meq/kg DCAD, and an addition of 150 meq/kg DCAD using four different ratios of potassium carbonate sesquihydrate (DCAD Plus, Church & Dwight Inc., Piscataway, NJ) and sodium sesquicarbonate (Church & Dwight Inc., Piscataway, NJ): 100:0, 66.7:33.3,

33.3:66.7, and 0:100 (meq/kg basis), respectively. Each treatment resulted in a final estimated DCAD of approximately 400 meq/kg diet DM (Table 4.2). Treatments were applied in a 4 x 4 Latin square design balanced for carryover effects with 3-week experimental periods. A basal TMR for the all cows was mixed in portable mixer wagon. In order to achieve the four experimental dietary treatments, mixtures KCARB and NaSCARB were substituted for corn as needed in 4.0% of ground corn in basal diet. These were mixed in advance. The treatment mixes were then added to the basal TMR and mixed in a Calan Data Ranger[®] (American Calan, Northwood, NH) for cows within each treatment group prior to delivery to individual feed tubs. Amounts of feed offered and feed refusals were recorded once daily at the time of feeding at 0930h.

Measurements

Measurements included weekly individual cow BW and daily feed intake and feed refusals. Silage and concentrate samples were taken weekly for DM analysis to adjust the as fed TMR to maintain a constant forage-to-concentrate ratio and to measure feed DM such that daily DMI could be calculated for each cow. Milk production was recorded electronically at each milking. Milk samples were collected on consecutive milkings on d 7 and 14 and during the last 4 consecutive milkings of each experimental period (d 20 and 21) and analyzed for fat, protein, other solids (lactose plus minerals; OS), SCC and MUN.

Individual samples of the corn silage, ground corn, soybean meal-vitamin premix and the treatment mixes were collected weekly and composited by

experimental period 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). Actual DCAD was calculated based on the K, Na, and Cl concentrations of the individual feeds and or mixtures weighted proportionally to their contribution to the diet DM in the TMR.

Statistical Model

Mean data for DMI, milk production, fat, protein, and SCC, fat and protein yield, 3.5% FCM and FE for each cow from the last week of each experimental period were used in the statistical analysis. Data were analyzed using the Mixed Procedure in SAS using the statistical model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

Where:

- Y_{ijk} = the response from the i^{th} Cow, the j^{th} Period, and the k^{th} Treatment
- μ = the grand mean
- C_i = the effect of the i^{th} cow
- P_j = the effect of the j^{th} period
- T_k = the effect of the k^{th} treatment level
- e_{ijk} = random error

Treatment was analyzed as a fixed effect while cow and period were analyzed as random effects in the Mixed Procedure of SAS. As the treatments were designed to provide equidistant ratios of K:Na (meq/kg), the production responses to the K:Na ratios (meq/kg) were tested using linear and quadratic orthogonal contrasts. A probability of $P \leq 0.05$ was considered statistically significant.

RESULTS

The chemical composition (DM Basis) of the dietary treatments is presented in Table 4.2. As expected, diets were similar in chemical composition (Table 4.2) except for Na and K. Calculated treatment DCAD concentrations (using the Na + K – Cl equation) were 417, 418, 447, and 457 meq/kg for the 100:0, 67:33, 33:67, and 0:100 K:Na treatments, respectively.

Treatment had no significant effect ($P > 0.05$) on DMI, BW, or milk production, (Table 4.3). As for milk composition, treatment had no effect on milk protein yield, protein percentage, OS yield, OS percentage, or SCC ($P > 0.05$).

Milk fat percentage and fat yield increased linearly with increased Na supplementation ($P < 0.05$) where fat percent and fat yield were greatest (3.36% and 1156 g/d) in cows fed the treatment in which sodium was the sole supplemental cation source used to increase DCAD. Despite a significant effect on milk fat percentage and yield, cation source did not affect 3.5% FCM ($P > 0.05$).

While there was no change in DMI or 3.5% FCM individually, the ratio of these two response variables (FE) was significantly different between treatments. As sodium supplementation increased and potassium supplementation decreased, mean DMI tended to decrease and 3.5% FCM tended to increase. Therefore, dairy FE was highest (1.67) when sodium was the only cation source used to increase DCAD ($P = 0.036$).

DISCUSSION

Treatment DCAD concentrations were higher than the intended DCAD (400 meq/kg) and they differed slightly between treatment groups (Table 4.2). This was due to a slightly greater than expected DCAD in the 33:67 and 0:100 K:Na treatments. The primary cause being a greater measured increase in Na compared with the 100:0 K:Na treatment which contributed 135 and 196 meq/kg as compared to the expected increases of 100 and 150 meq/kg in DCAD. As shown in the previous experiment, increasing DCAD by 50 meq/kg resulted in an average FE increase of only 0.02 units; thus, the 40 meq/kg DCAD difference between the highest and lowest DCAD treatments would not be large enough to cause a 0.11 unit change in FE, which was observed in this study.

In the present study, the source of cation did not affect DMI. These results support several other studies that showed that DMI is not affected by K:Na ratios (NRC, 2001; O'Connor et al., 1988; Sanchez et al., 1997, Tucker et al., 1988; Tucker et al., 1991; West et al., 1992; Wildman et al., 2007). However, some studies have reported that DMI is affected by cation supplementation (Hu and Kung, 2009; Sanchez et al., 1994). Using Na:K ratios of 0.21, 0.53, and 1.06, Hu and Kung (2009) reported that cation source quadratically affected DMI. However, they reported that DMI was 28.4 and 28.3 kg/d in treatments that contained Na:K ratios of 0.21 and 1.06, respectively. Therefore, DMI was not affected by cation source in treatments that contained a high amount of one cation and a low amount of the other. Hu and Kung (2006) reported that DMI was lowest when the Na:K ratio was 0.53 but DMI was unaffected by Na:K ratios of 0.21 or 1.06. In addition, Sanchez et al. (1994)

reported that cation source affected DMI only when concentrations of one cation were high while the other cation concentration was low.

In the present study, cation source had no effect on milk yield; similar to results have been reported in other experiments (Hu and Kung, 2009; NRC, 2001; O'Connor et al., 1988; Sanchez et al., 1997; Tucker et al., 1988; West et al., 1992). Wildman et al. (2007) reported a quadratic effect of Na:K ratio on milk production. At an average DCAD of 410 meq/kg, milk production was highest when the K:Na ratio was 4:1 (Wildman et al., 2007). Unlike the study by Wildman et al. (2007) which included high K:Na ratios of 2:1, 3:1, and 4:1, the present study included supplemental K:Na ratios of only 1:0, 2:1, 1:2, and 0:1. Perhaps a cation source effect on milk production may be visible only when the extremes K:Na or Na:K ratios are tested. It has been suggested the overall DCAD concentration is more important than individual ion concentrations in altering milk production responses (Tucker et al., 1988; West et al., 1992).

Unexpectedly, both milk fat percentage and fat yield (g/day) increased linearly with increasing Na. Fat percent and yield increased by 0.30 percentage units and 118 g/d, respectively, by increasing dietary Na from 0.26 to 0.71% and reducing dietary K from 1.79 to 1.19%. Several studies have reported that milk fat concentration and fat yield were not affected by cation source (Hu and Kung, 2009; O'Connor et al., 1988; Sanchez et al., 1997; West et al., 1991; West et al., 1992; Wildman et al., 2007). The NRC (2001) suggested that milk yield and DMI are not solely affected by individual dietary sodium or potassium concentrations. Instead, changes in these responses may be the result of the interactive effect of potassium

with sodium because a majority of physiological processes require a tightly regulated ratio of these cations (NRC, 2001). Therefore, if milk yield and DMI can be improved by manipulating Na:K ratios, it is quite possible that milk fat percentage and fat yield could also be increased by this method.

The dietary treatment that resulted in the highest milk fat production consisted of 1.19% K and 0.71% Na and a dietary K:Na ratio of 1.67 (1.0 on a milliequivalent basis). West et al. (1992) reported that cation source did not affect milk fat production but their treatment with the highest sodium percentage (0.87%) also contained 0.89% K resulting in a Na:K ratio of 0.98. A milk fat response to sodium in the West et al. (1992) study may not have been detected due to a low overall K:Na ratio. Therefore, the Na:K ratio may play a key role in altering the rumen environment and increasing milk fat production.

The cause of the increased milk fat production with increased Na is unknown; however, it could be speculated that this is a rumen fermentation response, especially because of the known effects of absorbed rumen biohydrogenation intermediates on mammary lipogenesis (Bauman and Griinari, 2003; NRC, 2001). It is possible that there is “sodium effect” in the rumen that may have been responsible for increased milk fat production when the dietary K:Na ratio is altered.

Lactating dairy cows are generally fed diets that are high in K yet relatively low in Na (NRC, 2001). Russell and Houlihan (2003) suggested that the rumen consistently has a “sodium-rich environment”. Figures 4.1 and 4.2, summarizes literature data on rumen Na and K concentrations in comparison with dietary K. (Bennink et al., (1978; Durand, 1980; Spears and Harvey, 1987; and Starnes et al.,

1984). As dietary K increases, the rumen K concentration (meq/L) increases and the rumen sodium concentration decreases. It is possible that rumen bacteria have grown accustomed to a potassium-rich rumen environment; therefore, increasing dietary potassium does not significantly affect rumen bacteria? For example, Wildman et al. (2007) reported that K:Na ratios did not affect milk fat percentage or yield. However, the dietary treatments consisted of K:Na ratios of 2:1, 3:1, and 4:1; thus, K:Na ratios in which sodium was the dominant cation were not investigated (Wildman et al., 2007). In the present study, milk fat percentage and fat yield were highest (3.36% and 1250 g/d) when sodium was the sole cation source supplemented. We speculate that the substitution of sodium for potassium may alter the rumen environment and it may have become more suitable for rumen bacteria that biohydrogenate PUFA. This results in a reduction of absorbed biohydrogenation intermediates which interfere with lipogenesis in the mammary gland.

Finally, it is possible that other studies did not detect a significant effect of cation source on milk fat production due the variation between treatments in overall DCAD concentration. Hu and Kung (2009) reported that the Na:K ratios of 0.21, 0.53, and 1.06 did not affect milk fat production. However, treatment DCAD concentrations did vary between treatment groups such that treatments containing Na:K ratios of 0.21, 0.53, and 1.06 had DCAD concentrations (Na + K – Cl – S) of 368, 320, 334 meq/kg DM, respectively (Hu and Kung, 2009). It is interesting to note that although milk fat percentage did not linearly increase in accordance with increasing sodium concentrations, it did increase linearly as DCAD increased. For example, milk fat percentage was 3.53%, 3.59%, and 3.68% when treatments that

contained 320, 334, and 368 meq/kg DCAD, respectively, were applied (Hu and Kung, 2009). As shown in the previous experiment, increasing DCAD concentrations resulted in a linear increase in milk fat percentage and yield. Perhaps DCAD concentration was confounded with the effect source which clouded any potential cation source effects in the study conducted by Hu and Kung (2009). Therefore, it is possible that some cation source effects on milk fat production have not been observed due to inflated experimental variation.

Our stated objective to determine the relative effectiveness of potassium carbonate sesquihydrate (KCARB) versus sodium sesquicarbonate (NaSCARB) on dairy FE measured in this study as 3.5% FCM per unit of DMI. Cation source had a significant effect on FE which was greatest (1.67) when sodium was the sole supplemental cation source. While neither 3.5% FCM ($P = 0.598$) nor DMI ($P = 0.903$) were significantly affected by cation source, the ratio of 3.5% FCM to DMI was significantly affected ($P = 0.036$) which was in contrast to our original hypothesis that there was effect of cation source. Thus, in this study Na was more effective than K as a cation source to increase DCAD as a means to increase dairy FE.

CONCLUSIONS

The main goal of this experiment was to determine the relative effectiveness of dietary K versus NA as cation sources used to increase DCAD and improve FE. At an average DCAD concentration of approximately 435 meq/kg DM, we found that dairy FE was highest when Na was the sole cation supplemented; thus, Na was more effective than K. Therefore in addition to the fact that sodium sesquicarbonate is

considerable less expensive than potassium carbonate sesquihydrate as a cation source, it is also more effective in improving feed utilization and feed efficiency.

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Table 4.1 Ingredient composition of experimental diets (DM Basis).

Ingredient	----- K:Na ¹ -----			
	100:0	67:33	33:67	100:0
Corn Silage	65.00	65.00	65.00	65.00
Ground Corn	11.50	11.44	11.37	11.31
Soybean Meal, 48%	18.63	18.63	18.63	18.63
Potassium Carbonate ²	0.91	0.60	0.30	0.00
Sodium Sesquicarbonate ³	0.00	0.37	0.74	1.10
Corn Gluten Meal, 60%	0.74	0.74	0.74	0.74
Limestone ⁴	0.79	0.79	0.79	0.79
Biophos ⁵	0.10	0.10	0.10	0.10
Dynamate ⁶	0.08	0.08	0.08	0.08
Salt-White	0.50	0.50	0.50	0.50
TM-433 ⁷	0.02	0.02	0.02	0.02
4-Plex ⁸	0.02	0.02	0.02	0.02
ADE Mix ⁹	0.03	0.03	0.03	0.03
Vit. E ¹⁰	0.01	0.01	0.01	0.01
Selenium ¹¹	0.05	0.05	0.05	0.05
Megalac ¹²	1.41	1.41	1.41	1.41
Omigen-AF ¹³	0.17	0.17	0.17	0.17
Rumensin-10g/lb ¹⁴	0.05	0.05	0.05	0.05

¹Each treatment contained an overall DCAD (Na + K – Cl) of 400 meq/kg DM

²Contained 56% K and 88% DM (Church & Dwight Co., Inc., Piscataway, NJ)

³Contained 27% Na (Church & Dwight Co., Inc., Piscataway, NJ)

⁴Contained 36% Ca and 0.02%P

⁵Contained 17% Ca and 21% P

⁶Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN)

⁷Contained 0.16% Co, 4.0% Cu, 3.0% Fe, 0.35% I, 15% Mn, and 16% Zn (Southern States Cooperative, Inc., Richmond, VA)

⁸Contained 0.20% Co, 0.99% Cu, 0.031% Fe, 1.57% Mn, and 2.83% Zn (Southern States Cooperative, Inc., Richmond, VA)

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

¹⁰Contained 56,818 IU/kg Vitamin E

¹¹Contained 0.3 IU/g Selenium; 28% Ca

¹²Contained 9% Ca; 84.5% Fat (Church & Dwight Co., Inc., Piscataway, NJ)

¹³Contained 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 x 10¹⁰ 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)

¹⁴Contained 20% Monensin Na, 1% Mineral oil, and carriers such as rice hulls, limestone, and fermentation nutrients (Elanco, Greenfield, IN)

Table 4.2 Chemical composition of experimental diets (DM Basis).

Item	----- K:Na ¹ -----				SEM
	100:0	67:33	33:67	100:0	
DM, %	52.89	52.81	52.70	52.67	0.05
NEL, Mcal/lb	0.77	0.77	0.77	0.77	0.00
CP, %	15.89	15.89	15.89	15.90	0.00
NDF, %	27.09	27.09	27.09	27.10	0.00
ADF, %	17.00	17.01	17.00	17.00	0.00
Lignin, %	2.37	2.37	2.37	2.37	0.00
Ash, %	7.13	7.12	7.21	7.23	0.03
Fat, %	0.02	0.02	0.02	0.02	0.00
Na, %	0.26	0.39	0.57	0.71	0.10
K, %	1.79	1.57	1.39	1.19	0.13
Cl, %	0.55	0.55	0.55	0.55	0.00
S, %	0.18	0.18	0.18	0.18	0.00
Ca, %	0.70	0.70	0.70	0.70	0.00
P, %	0.38	0.38	0.38	0.38	0.00
Mg, %	0.18	0.18	0.18	0.18	0.00
DCAD, meq/kg DM ¹	417	417	446	457	5.2

¹Na:K ratio of supplement cation. Each treatment was formulated to contained a total DCAD (Na + K – Cl) of 400 meq/kg DM

²Dietary K + Na – Cl, meq/kg DM.

Table 4.3 Relative effectiveness of cation supplementation on feed intake, milk production, milk composition, and feed efficiency.

Item	----- K:Na ¹ -----				SEM	----- <i>P</i> < -----	
	100:0	67:33	33:67	100:0		Lin. ²	Quad. ³
Observations	20.0	20.0	20.0	20.0			
DMI, kg/d	22.3	22.3	22.1	22.0	0.464	0.598	0.851
Milk, kg/d	37.5	37.3	36.3	37.9	1.28	0.903	0.219
SCC, linear score	4.63	5.57	4.95	4.79	0.980	0.960	0.430
Fat, %	3.06	3.20	3.20	3.36	0.169	0.005	0.885
Fat yield, g/d	1132	1173	1144	1250	49.1	0.041	0.354
Protein, %	2.99	2.99	3.01	3.07	0.065	0.181	0.476
Protein yield, g/d	1114	1106	1086	1156	33.9	0.332	0.114
Other solids, %	5.74	5.73	5.71	5.67	0.042	0.092	0.505
Other solids, g/d	2147	2141	2077	2151	74.2	0.786	0.349
3.5% FCM, kg/d	34.6	35.2	34.3	36.7	1.06	0.132	0.262
FE ⁴	1.56	1.58	1.55	1.67	0.040	0.036	0.125

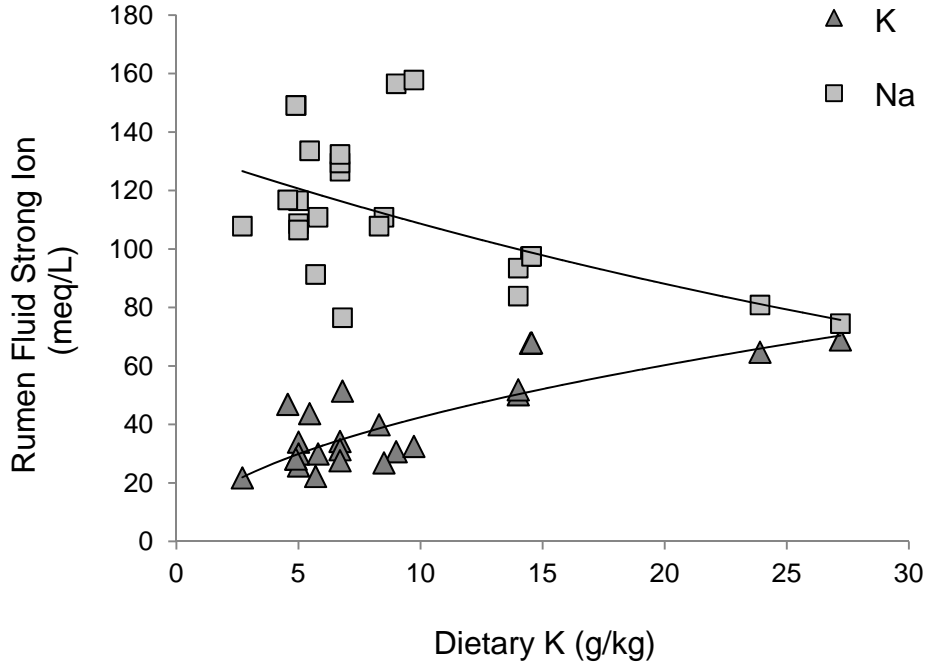
¹Each treatment contained an overall DCAD (Na + K – Cl) of 400 meq/kg DM

²Linear orthogonal contrast

³Quadratic orthogonal contrast

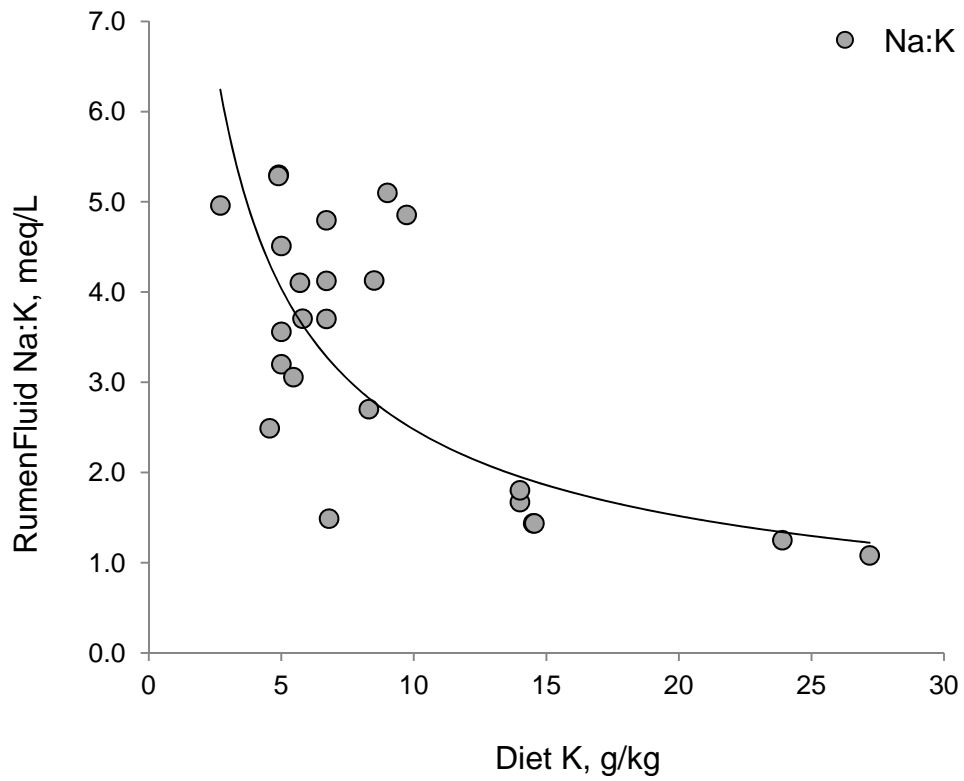
⁴FE = 3.5% FCM/DMI

Figure 4.1 Change in rumen fluid concentrations of sodium and potassium as dietary potassium is increased¹



¹Data adapted from Bennink et al., (1978), Durand, (1980); Spears and Harvey, (1987); and Starnes et al., (1984)

Figure 4.2 Change in rumen fluid Na:K ratio as dietary potassium is increased¹



¹ Data adapted from Bennink et al., (1978), Durand, (1980); Spears and Harvey, (1987); and Starnes et al., (1984)

Chapter 5: EXPERIMENT 3

Intake, Milk Production, Ruminal, and Feed Efficiency Responses to DCAD in Lactating Dairy Cow

INTERPRETIVE SUMMARY

Intake, Milk Production, Ruminal, and Feed Efficiency Responses to DCAD in Lactating Dairy Cows *Iwaniuk et al., page 000*. A meta-analysis was conducted to investigate the relationship between dietary cation-anion difference (DCAD) and production responses of lactating dairy cows. The database consisted of 34 published studies, 160 dietary treatments, and 74 treatment comparisons. Measured or when missing, 2001 NRC estimated dietary Na, K, and Cl concentrations were used to calculate DCAD. Increasing DCAD concentration resulted in a linear increase ($P < 0.05$) in several dependent variables, such as DMI, milk yield, 3.5% FCM, milk fat %, milk fat yield, rumen acetate molar %, rumen butyrate molar %, acetate to propionate ratio, DM digestibility, NDF digestibility, ADF digestibility, and dairy feed efficiency. Increasing DCAD most likely alters the rumen environment and acid-base balance which results in improved production responses.

RUNNING HEAD: META-ANALYSIS OF DCAD EFFECTS ON PRODUCTION RESPONSES

Intake, Milk Production, Ruminal, and Feed Efficiency Responses to DCAD in Lactating Dairy Cows

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ABSTRACT

A meta-analysis was conducted to investigate the relationship between dietary cation-anion difference (DCAD) ($\text{Na} + \text{K} - \text{Cl}$ (meq/kg)) and production responses in lactating dairy cows. The database consisted of 34 articles that were published between 1965 and 2007, 160 dietary treatments, and 74 treatment comparisons. If articles lacked information regarding dietary percentages of Na, K, or Cl, dietary ion percentages were estimated using the 2001 Dairy NRC software. The results suggested that production responses are linearly affected by DCAD concentration. Dry matter intake, milk production, and 3.5% fat-corrected milk production increased by 0.32, 0.23, and 0.71 kg, respectively, for each 100 meq/kg increase in DCAD. For each 100 meq/kg increase in DCAD, milk fat percentage and yield increased by 0.11% and 40g/d, respectively. DCAD also affected rumen characteristics and digestibility. Rumen acetate increased 1.49 molar percentage units for each 100 meq/kg incremental increase of DCAD. Also, the rumen acetate to propionate ratio increased 0.17 units per 100 meq/kg DCAD. Each 100 meq/kg increase in DCAD also increased dry matter digestibility, NDF digestibility, and ADF digestibility increased by 0.902, 1.87, and 3.22%, respectively. Finally, dairy feed efficiency increased by 0.02 units for each 100 meq/kg increase of DCAD. The dairy cow's positive responses to DCAD with respect to intake, milk production and composition, and rumen characteristics are most likely the result of an improved rumen environment and acid-base homeostasis.

INTRODUCTION

The original manipulation of DCAD in dairy cow diets was to combat milk fever in pari-parturient cows (Block, 1984; Delaquis and Block, 1995). More recent research has been focused on the productivity and intake responses to DCAD (Harrison et al., 2012; Hu and Murphy, 2004; Hu and Murphy, 2007b; Roche et al., 2005; Sanchez and Beede, 1996; Vagnoni and Oetzel, 1998; Wildman et al., 2007a). Several studies suggested that increasing the DCAD can increase milk yield, milk fat percentage, and optimize dry matter intake in the lactating cow (Apper-Bossard et al., 2010; Hu and Murphy, 2004; Tucker et al., 1988b; Wildman et al., 2007b; Wildman et al., 2007c).

Studies with dietary buffers, such as NaHCO_3 and K_2CO_3 , have been reported in earlier literature (1960-1990). Buffers were shown to increase dry matter intake, milk production, and feed efficiency, especially in low forage, high starch diets (Erdman, 1988). The addition of dietary buffers in the ration of lactating dairy cows undoubtedly altered the DCAD concentration; however, these data were published prior to the emergence of the DCAD concept and thus have not been included in previous meta-analyses of DCAD effects on dairy cow performance (Hu and Murphy, 2004). Further, many of these studies lacked complete chemical analysis of Na, K, and Cl in order to calculate DCAD using the simplest DCAD equation ($\text{Na} + \text{K} - \text{Cl}$). Although the DCAD effects were not originally reported or discussed in the earlier buffer literature, data reported has value in that it could be potentially used in a retrospective (meta) analysis to determine the effect of DCAD concentration and source of strong ion (Na vs. K) on dairy cattle performance.

We hypothesized that the addition of data from the dietary buffer literature could be used to enhance our understanding of dairy cow responses to altered DCAD concentrations. Therefore, the objective of this study combine earlier buffer feeding literature in lactating dairy cows with more recent studies on DCAD effects to build surface response equations which relate DCAD and dietary strong ion effects (Na and K) on DMI, milk production, milk composition, rumen characteristics, digestibility and FE.

MATERIALS AND METHODS

Data Collection

Results from 53 published journal articles involving the use of buffers in the diets of lactating dairy cows were reviewed for inclusion. Journal articles were selected from four primary journals: Journal of Dairy Science (JDS), Canadian Journal of Animal Science (CJAS), Journal of Animal Science (JAS), and the Journal of Animal Production (JAP). Each specific journal article was selected from the reference list of a review article that discussed the effects of dietary buffers (Erdman, 1988).

For a study to be included, treatment means for DMI, milk production and milk fat concentration had to be reported or be able to be calculated such that feed efficiency (**FE**) could be calculated. Feed efficiency was defined as 3.5% fat-corrected milk per unit DMI. The most common reason for the rejection of a study was the lack of treatment DMI or lack of milk fat concentration required to calculate 3.5% FCM such that FE could be calculated. In many instances, dietary Na, K, or most frequently Cl were not reported. In this case, dietary ingredient information was

used to estimate the missing strong ion. Thus, it was essential that specific ingredient information be included such the missing strong ion could be estimated using the diet evaluation software in the 2001 Dairy NRC (NRC, 2001). Journal articles were also rejected from the data set if diet information was unclear. For example, some papers did not provide the list of ingredients in a vitamin-mineral premix; therefore, it was unknown if specific DCAD-altering ingredients, such as potassium carbonate or sodium bicarbonate, were present in the vitamin-mineral mix. Some experiments reported ingredients that were not included in the Dairy NRC software and composition information was not available from published feed labels. After removing papers with insufficient animal performance information, feed ingredient, or ingredient composition data, 34 papers involving 74 treatment mean comparisons were used to compile the data set. A summary of the literature studies used in this meta-analysis are shown in Table 5.1.

Data Assembly

Several measurements, when available were collected from each journal article in order to compile the dataset. Data were collected from four general categories: 1) Diet composition: dietary CP, ADF, NDF, Na, K, Cl, S, Ca, Mg, P, and reported DCAD were either collected or calculated using the 2001 Dairy NRC Software; 2) Intake and milk production: daily milk production and fat concentration, and DMI were collected along with milk protein, lactose, and total solids concentrations when available; 3) Digestibility: digestibility of DM, ADF, NDF and starch were also collected when reported; and 4) Rumen characteristics: mean rumen

pH along with mean rumen acetate, propionate, butyrate molar percentages and total VFA (meq/L) were entered when reported. The number of observations, mean, standard deviation, and the minimum and maximum values each variable are presented in Table 5.2.

Missing Data Points

One of the major problems associated with dataset assembly was the lack of measured concentrations for the minerals Na, K, and Cl. If one of dietary strong-ion values was missing, the DCAD concentration ($\text{Na} + \text{K} - \text{Cl}$) of that particular diet could not be calculated. Because these experiments were conducted prior to the emergence of the DCAD concept, several papers did not report any of the strong ion values. In order to overcome this obstacle, a preliminary study was conducted to determine whether or not the 2001 Dairy NRC Ration Evaluation Software could be used to estimate missing dietary ion concentrations. Journal articles, which measured the three dietary (Na, K, and Cl) ion concentrations, were used as “test articles.” The experimental dietary ingredient information for each treatment was extracted from each article and entered into the 2001 Dairy NRC ration evaluation software that was used to estimate the mineral concentrations based on either the software estimates or when measured the reported value for each feed ingredient. A strong correlation was observed between the estimated ion concentrations from by the NRC software and the measured ion concentrations reported in each paper as illustrated in Figures 5.1, 5.2, and 5.3. The respective R^2 and standard error of the estimate for Na, K, and Cl were 0.8580, 0.6105, and 0.6824 and 0.0665, 0.2105, and 0.2036. Where DCAD

concentrations were reported in a study, NRC software was used to estimate the concentration of each mineral and then DCAD was calculated. There was also good agreement between the NRC predicted and study reported DCAD with an R^2 of 0.9671 and a standard error of the estimate of 60.48 (Figure 5.4). Therefore, it was concluded that the NRC software accurately estimated mineral concentrations and the program was used to estimate the mineral concentration in instances where the study did not report them.

Surface Response Equations

In a recent meta-analysis, Hu and Murphy (2004) reported that DCAD had a quadratic effect on several production parameters such as milk yield, 4.0% FCM production, DMI, and milk fat yield (g/d). Upon visual examination of the data, it appeared that DCAD may actually have a curvilinear response with a plateau at higher DCAD concentrations on select responses such as DMI, 4.0% FCM, blood pH, blood HCO_3 , and urine pH (Hu and Murphy, 2004). Based on this published meta-analysis, a non-linear model was developed and, after the dataset was completed, the data was analyzed using the Non-Linear Mixed Procedure (NLMIXED) in Statistical Analysis Software (SAS). As the dataset was derived from 34 separate published reports, variance caused by experiment within these reports had to be accounted for and removed. Therefore, individual experiments were considered as random samples from the larger population (St-Pierre, 2001) and individual study effects were removed to reduce variance due to study effects. The NLMIXED procedure was used to test surface response models to evaluate the effects of DCAD on DMI and milk

production, digestibility, and rumen characteristics. Initially, we tested a nonlinear model with the following parameters:

$$Y = A_0 + B_0(1 - e^{-k_{DCAD} \cdot DCAD_{ij}}) + s_i + e_{ij}$$

Where:

A_0 = overall intercept across studies

B_0 = magnitude of the potential response to DCAD

k_{DCAD} = rate constant for DCAD effect

s_i = random study effects

e_{ij} = random error term, assumed $N(0, \sigma^2)$

However, the effects of DCAD on several response variables, such as fat yield and feed efficiency, were unable to fit the proposed model. Thus, a simpler linear model was developed and tested. The goodness of fit for both models was compared using Akaike's Information Criterion Correction (AICC) values. AICC values represent the amount of information lost based on the model and its parameters; thus, a smaller AICC value is best (Littell et al., 2006). In addition, model comparisons were performed using AICC values versus AIC values because AICC (AIC Corrected) tends to be more robust when working with smaller sample sizes (Hurvich and Tsai, 1988). After comparing AICC values of the nonlinear model above with a simpler linear model, it was concluded that a simple linear model was equivalent or superior in fitting the dataset. Such that the final model used was:

$$Y = A_0 + (kDCAD*DCAD_{ij}) + s_i + e_{ij}$$

Where:

A_0 = overall intercept across studies

$kDCAD$ = Rate constant for the effect of DCAD (slope of the predicted line)

$DCAD$ = Dietary DCAD concentration

s_i = random study effects

e_{ij} = random error term, assumed $N(0, \sigma^2)$

Because the model removed variance due to individual study effect, the study-adjusted values for each variable were used to create the linear equations. In the regression plots, the regression line (solid black line) represents the predicted values of the dependent variable in response to DCAD concentration. The study-adjusted values were also displayed on the plots and they demonstrate the pattern of the dependent variable in response to increased DCAD concentration.

RESULTS

The regression relationships between the dependent variables and DCAD concentration are presented in Table 5.3 and the unit increase for each dependent variable in response to a 100 meq/kg increase in DCAD concentration is presented in Table 5.4. Both overall DCAD effects and individual cation effects on production responses were investigated. However, individual cation effects (Na vs. K) could not be modeled due to insufficient numbers of experiments with for K supplementation.

Therefore, only the overall DCAD effect on each dependent variable will be discussed.

DMI increased from 18.24 to 20.04 kg/d as DCAD increased from 100 to 665 meq/kg of DM (Figure 5.5). Therefore, DMI increased 0.32 kg for each 100 meq/kg increase in DCAD concentration ($P < 0.0001$; $R^2 = 0.32$). Increasing the DCAD concentration also resulted in a linear increase in milk yield (Figure 5.6). Milk production increased from 26.41 to 27.71 kg/d as DCAD concentrations increased from 100 to 665 meq/kg of DM; thus, a 100 meq/kg increase in DCAD resulted in a 0.23 kg/d increase in milk yield ($P = 0.026$; $R^2 = 0.11$).

As for milk composition, milk fat percentage and fat yield increased linearly in response to increased DCAD. Milk fat percentage increased from 3.19 to 3.84% as DCAD concentration increased from 100 to 655 meq/kg of DM (Figure 5.8). This resulted in a 0.11% increase in milk fat percentage per 100 meq/kg increase in DCAD ($P < 0.0001$; $R^2 = 0.46$). Similarly fat yield increased from 842 to 1068 g/d which translated into a 39.9 g increase per 100 meq/kg increase in DCAD concentration ($P < 0.0001$; $R^2 = 0.51$; Figure 5.9).

Because both milk production and milk fat yield increased in response to increased DCAD concentration, it was no surprise that 3.5% FCM production also increased with increasing DCAD concentration. As shown in Figure 5.7, 3.5% FCM increased from 24.86 to 28.88 kg/d as DCAD increased from 100 to 665 meq/kg of DM. This resulted in a 0.71 kg/d increase in 3.5% FCM per 100 meq/kg increase in DCAD concentration ($P < 0.0001$; $R^2 = 0.45$). DCAD concentration did not affect

milk protein percentage or protein yield ($P > 0.05$) (Figures 5.10 and 5.11, respectively).

In addition to milk composition, DCAD concentration also affected rumen characteristics. Both rumen acetate and butyrate molar percentages were linearly increased as a result of increased DCAD concentration. As shown in Figure 5.13, rumen acetate increased from 52.2 to 60.4 molar percentage units as DCAD increased from 100 to 665 meq/kg ($P < 0.0001$; $R^2 = 0.54$). Rumen butyrate increased from 13.1 to 16.1 molar percentage units as DCAD increased ($P = 0.01$; $R^2 = 0.28$; Figure 5.14). Therefore, rumen acetate and butyrate increased by 1.49 and 0.65 molar percentage units, respectively, per 100 meq/kg DCAD. DCAD did not significantly affect molar percentages of propionate (Figure 5.15); however, DCAD concentration did affect acetate to propionate ratios (Figure 5.13). As DCAD increased from 100 to 665 meq/kg, the acetate to propionate ratio increased from 1.88 to 2.84 which translated into a 0.17 unit increase per 100 meq/kg DCAD ($P < 0.0001$; $R^2 = 0.54$; Figure 5.16). Total volatile fatty acid concentration (meq/L) was not affected by DCAD (Figure 5.17). Lastly, rumen pH tended ($P = 0.051$) to increase in response to increasing DCAD concentration (Figure 5.12). As DCAD increased from 100 to 665 meq/kg, rumen pH increased from 6.32 to 6.51 which resulted in a 0.03 unit increase per 100 meq/kg DCAD ($R^2 = 0.20$).

DCAD effects on digestibility were evaluated. As shown in Figure 5.18, increasing DCAD from 100 to 665 meq/kg resulted in an increased DM digestibility (67.5 to 70.35%). DM digestibility increased 0.90% per 100 meq/kg increase in DCAD ($P < 0.0001$; $R^2 = 0.62$). Similarly, NDF and ADF digestibility were also

improved by increased DCAD concentrations. NDF digestibility increased from 46.9 to 52.7% as DCAD increased from 100 to 665 meq/kg which resulted in a 1.87% increase in NDF digestibility per 100 meq/kg increase in DCAD concentration ($P = 0.0014$; $R^2 = 0.53$; Figure 5.19). ADF digestibility improved from 35.2 to 45.3 % as DCAD increased from 100 to 665 meq/kg which resulted in a 3.23% increase in ADF digestibility per 100 meq/kg increase in DCAD concentration ($P < 0.0001$; $R^2 = 0.62$; Figure 5.20). DCAD effects on starch digestibility were not reported due to insufficient raw data.

Finally, the results from this meta-analysis demonstrated that DCAD concentration affected dairy feed efficiency. As DCAD increased from 100 to 665 meq/kg, FE increased from 1.36 to 1.46 units which resulted in a 0.02 unit increase per 100 meq/kg increase in DCAD concentration (Figure 5.21). As previously mentioned, DCAD caused a 0.32 and 0.71 kg/d increase in DMI and 3.5% FCM, respectively. Thus, the larger response to DCAD shown by 3.5% FCM as compared to DMI (0.39 kg/d difference) resulted in a larger value for 3.5%FCM in the numerator of the FE equation. Therefore, the results from this meta-analysis suggest that dairy FE can be improved by increasing the DCAD concentration in the diets of lactating dairy cows.

DISCUSSION

In the present meta-analysis, DMI increased linearly in response to DCAD concentration. Similar reported effects of DCAD on DMI have been reported in several studies (Apper-Bossard et al., 2006; Apper-Bossard et al., 2010; Hu et al.,

2007a; Wildman et al., 2007b; Wildman et al., 2007c). For example, Apper-Bossard et al. (2006) reported DMI values of 22.8, 23.7, and 24 kg for DCAD concentrations of 4, 156, and 306 meq/kg of DM (Na + K – Cl – S), respectively. Linear regression analysis on this data indicates that DMI increased 0.40 kg per each incremental increase of 150 meq/kg DCAD ($R^2 = 0.93$). The results are similar to the results in the present study in which DMI increased 0.32 kg per 100 meq/kg increase in DCAD concentration. Therefore, the results of this meta-analysis confirm earlier reports that increasing the DCAD concentration linearly increases DMI.

Milk yield increased linearly with increasing DCAD concentrations. However, the effect of DCAD on milk production was smaller than the effect of DCAD on DMI. Milk production only increased 0.23 kg/d per 100 meq/kg increase in DCAD concentration. Some studies have reported that milk yield can be improved by increasing DCAD concentration (Apper-Bossard et al., 2010; Tucker et al., 1988a; West et al., 1991; Wildman et al., 2007b). Wildman et al. (2007b) reported that milk yield increased linearly from 24.0 to 25.7 kg/d as DCAD (Na + K – Cl – S) increased from 291 to 537 meq/kg of DM in diets that contained 15% CP. In the same article, Wildman et al. (2007b) also reported that milk yield increased linearly from 23.8 to 26.6 kg/d as DCAD (Na + K – Cl – S) increased from 310 to 500 meq/kg of DM in diets that contained 17% CP. Because the results from the meta-analysis indicated that DCAD concentration linearly increased DMI, it is possible that the increased milk production was a result of increased intake and, thus, more energy and nutrients were available to support production.

In regards to milk composition, milk fat percentage and fat yield increased linearly as a result of increased DCAD concentration. Milk fat percentage increased 0.11% per 100 meq/kg increase in DCAD. Similar effects of DCAD on milk fat percentage have been reported in several other studies (Apper-Bossard et al., 2006; Apper-Bossard et al., 2010; Hu et al., 2007a; Roche et al., 2005; West et al., 1991; Wildman et al., 2007b). For example, Hu et al. (2007a) reported milk fat percentages of 3.12, 3.27, and 3.57% for DCAD (Na + K – Cl – S) concentrations of -30, 220, and 470 meq/kg, respectively, in diets that contained 16% CP. Regression analysis of this data suggests that milk fat percentage increased approximately 0.09% per 100 meq/kg increase in DCAD. In the same study, Hu et al. (2007a) reported milk fat percentages of 2.85, 3.46, and 3.62% for DCAD (Na + K – Cl – S) concentrations of -30, 220, and 470 meq/kg, respectively, in diets that contained 19% CP. Regression analysis of this data suggests that milk fat percentage increased approximately 0.15% per 100 meq/kg increase in DCAD. The increases of 0.09% and 0.15% milk fat per 100 meq/kg DCAD observed by Hu et al. (2007a) is very similar to the 0.11% milk fat increase per 100 meq/kg DCAD reported in this meta-analysis. In addition, milk fat yield (g/d) was also linearly increased with DCAD. The effect of DCAD on milk fat yield was a result an increase in both milk yield and milk fat percentage.

Because milk yield and milk fat yield linearly increased with DCAD, 3.5% FCM also increased in response to increased DCAD concentration. Increasing DCAD by 100 meq/kg resulted in a 0.71 kg increase in 3.5% FCM. Other studies have reported that DCAD increased milk fat yield (g/d) which resulted in an increase in 4.0% FCM (Hu et al., 2007a; Roche et al., 2005; West et al., 1991).

Although the exact mechanism by which DCAD affects production responses is unknown, it is believed that manipulating DCAD can alter acid-base homeostasis and the rumen environment (Apper-Bossard et al., 2010; Hu and Murphy, 2005; Hu et al., 2007a; Roche et al., 2005; Sanchez and Beede, 1996). In the present meta-analysis, acid-base parameters were not recorded; thus, the effect of DCAD on acid-base balance was not investigated. However, the effects of DCAD on select rumen characteristics were determined. Results indicated that the rumen pH tended ($P = 0.0509$) to increase as the concentration of DCAD increased and similar results have been reported in other studies (Apper-Bossard et al., 2006; Roche et al., 2005). Increased rumen pH has been shown to alter both rumen VFA fermentation patterns and biohydrogenation (Apper-Bossard et al., 2006; Apper-Bossard et al., 2010; Hu et al., 2007a; Roche et al., 2005; Sanchez and Beede, 1996; Wildman et al., 2007b). In the present meta-analysis, molar percentages of acetate and butyrate increased linearly in response to DCAD. This change in VFA pattern most likely occurred due to increased rumen pH. However, DCAD did not have an effect on the molar percentage of propionate or total VFA production (meq/L). Due to an increase in acetate, the acetate to propionate ratio also increased linearly in response to DCAD concentration. This meta-analysis indicated that increasing DCAD can alter rumen characteristics such as pH and VFA production.

In addition to altering rumen VFA production, an increase in rumen pH has been shown to increase milk fat percentage (Allen, 1997; Hu et al., 2007a; Roche et al., 2005). Roche et al. (2005) suggested that an increase in rumen pH may cause increased de novo fatty acid synthesis due to elevated biohydrogenation. In this

meta-analysis, it was previously mentioned that milk fat percentage and yield were linearly increased as a result of increased DCAD concentration. This result most likely occurred as a result of increased rumen pH and improved biohydrogenation of unsaturated fatty acids (Bauman and Griinari, 2003; NRC, 2001).

DCAD concentration clearly affects feed digestibility which may explain some of the response in total milk and fat yield in addition to DMI effects. Dry matter digestibility increased 0.90% per 100 meq/kg increase in DCAD concentration. Fiber digestion as indicated by both NDF and ADF digestibility increased linearly and at a more rapid rate than DM digestibility, 1.87 and 3.22%, respectively versus 0.90% with increasing DCAD. Studies have suggested that changes in fiber digestibility occur as a result of changes in rumen pH (Erdman et al., 1982; Rogers et al., 1982; Rogers et al., 1985b; West et al., 1987). Therefore, the increase in fiber digestibility observed in the present meta-analysis was most likely the result of increased rumen pH caused by increased DCAD concentration.

Finally, the last dependent variable that was investigated was dairy FE. Results of this meta-analysis indicated that FE was linearly increased as DCAD increased. FE increased 0.02 units per 100 meq/kg increase in DCAD concentration and FE improved from 1.36 to 1.46 units as DCAD increased from 100 to 665 meq/kg. The increase in FE was likely due to both increased DMI and an improvement in dietary energy availability. Increasing DCAD resulted in an increase in both DMI and 3.5% FCM, which are both components of the FE equation. Although both variables increased in response to increasing DCAD concentration, the response rate (prediction slope) of 3.5% FCM (0.0071) was more than double the

response rate of DMI (0.0032). The intercept values for DMI and FCM were 17.9 and 24.1, respectively ratio of 1.35. This suggests that the major factor affecting FE was the increase in FCM with increased DCAD was responsible for the majority the increase in FE with increasing DCAD.

CONCLUSION

The results of this meta-analysis suggested that DCAD has a significant effect on a variety of performance indicators including DMI, milk production and milk composition, and FE. Changes in rumen pH and VFA concentrations suggest that some of the intake and production responses to DCAD are a result of improved rumen fermentation. Fiber digestibility is markedly increased with increased DCAD concentration resulting in increased DM digestibility and likely energy supply to the cow. Although the mechanism(s) still remains unclear, DCAD most likely alters production and digestibility by changing the rumen environment improving acid-base homeostasis in the dairy cow.

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Table 5.1 Summary of the final selected literature studies on buffer effects of production responses

Paper No.	Treatment Comparisons ¹	Cation Sources	Reference
1	1	NaHCO ₃	Coppock et al. (1986)
2	2	NaHCO ₃	DePeters et al. (1984)
3	1	NaHCO ₃	Edwards and Poole (1983)
4	1	NaHCO ₃	Eickelberger et al. (1985)
5	2	NaHCO ₃	English et al. (1983)
6	2	NaHCO ₃	Erdman et al. (1980)
7	4	NaHCO ₃	Erdman et al. (1982)
8	1	K ₂ CO ₃	Erdman et al. (2011)
9	1	NaHCO ₃	Escobosa and Coppock (1984)
10	1	NaHCO ₃	Fisher and MacKay (1983)
11	4	NaHCO ₃	Harris et al. (1983)
12	4	NaHCO ₃	Harrison et al. (1986)
13	1	K ₂ CO ₃	Harrison et al. (2012)
14	2	NaHCO ₃ + K ₂ CO ₃	Hu et al. (2007a)
15	4	NaHCO ₃ + KHCO ₃	McKinnon et al. (1990)
16	2	NaHCO ₃ + KHCO ₃	Miller et al. (1965)
17	1	NaHCO ₃ + KHCO ₃	Mooney and Allen (2007)
18	1	NaHCO ₃	Rogers et al. (1982)
19	2	NaHCO ₃	Rogers et al. (1985a)
20	2	NaHCO ₃	Rogers et al. (1985b)
21	1	NaHCO ₃	Sanchez et al. (1997)
22	4	NaHCO ₃ , NaCl, KCl	Schneider et al. (1986)
23	2	NaHCO ₃	Snyder et al. (1983)
24	4	NaHCO ₃	Stanley et al. (1969)
25	1	NaHCO ₃	Stokes et al. (1986)
26	3	NaHCO ₃	Teh et al. (1985)
27	2	NaHCO ₃ + KHCO ₃	Tucker et al. (1988a)
28	1	NaHCO ₃	Tucker et al. (1994)
29	6	NaHCO ₃	Van Horn et al. (1984)
30	3	NaHCO ₃ + K ₂ CO ₃	West et al. (1987)
31	2	NaHCO ₃ + KHCO ₃	West et al. (1986)
32	2	KHCO ₃	West et al. (1991)
33	2	NaHCO ₃ + KHCO ₃	West et al. (1992)
34	2	NaHCO ₃ + K ₂ CO ₃	Wildman et al. (2007a)

¹There were 74 total treatment comparisons

Table 5.2 Mean and range of variables within the database

Factor	No.	Mean	SD	Minimum	Maximum
DCAD, meq/kg of DM ¹	160	347	108.3	101	665
Diet					
Na, % of DM	160	0.42	0.20	0.06	1.25
K, % of DM	160	1.28	0.34	0.69	2.54
Cl, % of DM	160	0.57	0.26	0.23	1.25
S, % of DM	160	0.22	0.05	0.14	0.37
Ca, % of DM	107	0.80	0.21	0.44	1.35
Mg, % of DM	107	0.29	0.12	0.15	0.74
P, % of DM	101	0.48	0.08	0.30	0.67
CP, % of DM	118	16.82	1.25	13.90	19.90
NDF, % of DM	65	35.90	7.95	26.30	60.90
ADF, % of DM	113	18.42	3.41	13.00	24.60
DMI, kg/d	160	19.00	3.198	13.50	28.20
Milk					
Yield, kg/d	160	27.00	6.274	16.42	41.60
3.5% FCM, kg/d	160	26.63	6.615	15.86	46.70
Fat, %	160	3.48	0.475	2.40	5.10
Fat yield, g/d	160	940	264	511	1822
Protein, %	118	3.23	0.217	2.76	3.80
Protein, yield, g/d	118	860	182.3	530	1186
Lactose, %	23	4.84	0.085	4.69	5.04
Other Solids, %	51	12.05	1.002	8.82	14.60
Rumen					
Rumen pH	73	6.42	0.323	5.61	7.07
Acetate, molar %	93	55.98	6.353	40.60	66.40
Butyrate, molar %	80	14.52	4.133	9.40	27.80
Propionate, molar %	93	26.36	6.587	14.23	43.80
FE, 3.5% FCM/DMI	160	1.41	0.302	0.95	2.30

¹DCAD (meq/kg) = (Na + K - Cl)

Table 5.3 Regression relationships between DCAD and dependent variables

Variable	No.	Int	SE	<i>P</i>	kDCAD ¹	SE	<i>P</i>	R ²
DMI ²	160	17.9	0.434	< 0.05	0.0032	0.001	< 0.05	0.32
Milk yield ²	160	26.2	0.804	< 0.05	0.0023	0.001	0.03	0.11
3.5% FCM ²	160	24.1	0.859	< 0.05	0.0071	0.001	< 0.05	0.45
Milk								
Fat ³	160	3.08	0.078	< 0.05	0.0011	0.000	< 0.05	0.46
Fat yield ⁴	160	802	35.753	< 0.05	0.3990	0.061	< 0.05	0.51
Protein ³	118	3.22	0.029	< 0.05	0.0000			0.00
Protein yield ⁴	118	840	28.591	< 0.05	0.0473	0.036	0.19	0.05
Rumen								
Rumen pH	73	6.28	0.079	< 0.05	0.0003	0.000	0.05	0.20
Acetate ⁵	93	50.7	1.344	< 0.05	0.0149	0.003	< 0.05	0.54
Butyrate ⁵	80	12.4	0.995	< 0.05	0.0065	0.002	0.01	0.28
Propionate ⁵	93	26.3	0.936	< 0.05	0.0000			0.00
Ace:Prop	93	1.71	0.164	< 0.05	0.0017	0.000	< 0.05	0.54
Total VFA ⁶	69	82.8	6.158	< 0.05	0.0098	0.013	0.47	0.01
Digestibility								
DM ⁷	42	65.8	1.069	< 0.05	0.0090	0.002	< 0.05	0.62
NDF ⁷	32	43.3	3.059	< 0.05	0.0187	0.005	0.00	0.53
ADF ⁷	40	29.1	2.858	< 0.05	0.0323	0.005	< 0.05	0.71
Feed Efficiency ⁸	160	1.34	0.042	< 0.05	0.0002	0.000	0.01	0.28

¹The rate at which the response variable is affected by increasing the DCAD concentration (Na + K – Cl) by 100 meq/kg.

²Variables are expressed on a kg/d basis

³Variables are expressed as a percentage of milk composition

⁴Variables are expressed on a g/d basis

⁵Variables are expressed as a molar percentage

⁶Variables are expressed as meq/L

⁷Variables are expressed as percentages

⁸FE = 3.5% FCM per unit of DMI

Table 5.4 Dependent variable response to a 100 meq/kg increase in DCAD

Dependent Variable	Unit Increase	<i>P</i>	R ²
DMI, kg/d	0.32	< 0.05	0.32
Milk yield, kg/d	0.23	0.026	0.11
3.5% FCM, kg/d	0.71	< 0.05	0.45
Milk			
Fat, %	0.11	< 0.05	0.46
Fat yield, g/d	39.9	< 0.05	0.51
Protein, %	0.00		0.00
Protein yield, g/d	4.73	0.192	0.05
Rumen			
Rumen pH	0.03	0.051	0.20
Acetate, molar %	1.49	< 0.05	0.54
Butyrate, molar %	0.65	0.01	0.28
Propionate, molar %	0.00		0.00
Ace:Prop	0.17	< 0.05	0.54
Total VFA, meq/L	0.9771	0.471	0.0126
Digestibility			
DM, %	0.9016	< 0.05	0.62
NDF, % of DM	1.870	0.001	0.53
ADF, % of DM	3.225	< 0.05	0.71
FE, 3.5% FCM/DMI	0.02	0.012	0.28

Figure 5.1 Correlation between reported and NRC predicted values for dietary percentages of sodium ($\text{Na \%} = 0.9833x + 0.0466$; Intercept $P = 0.004$; Intercept SE = 0.019; Slope $P < 0.001$; Slope SE = 0.042; $R^2 = 0.8579$; Reg SE = 0.0665; $n = 94$).

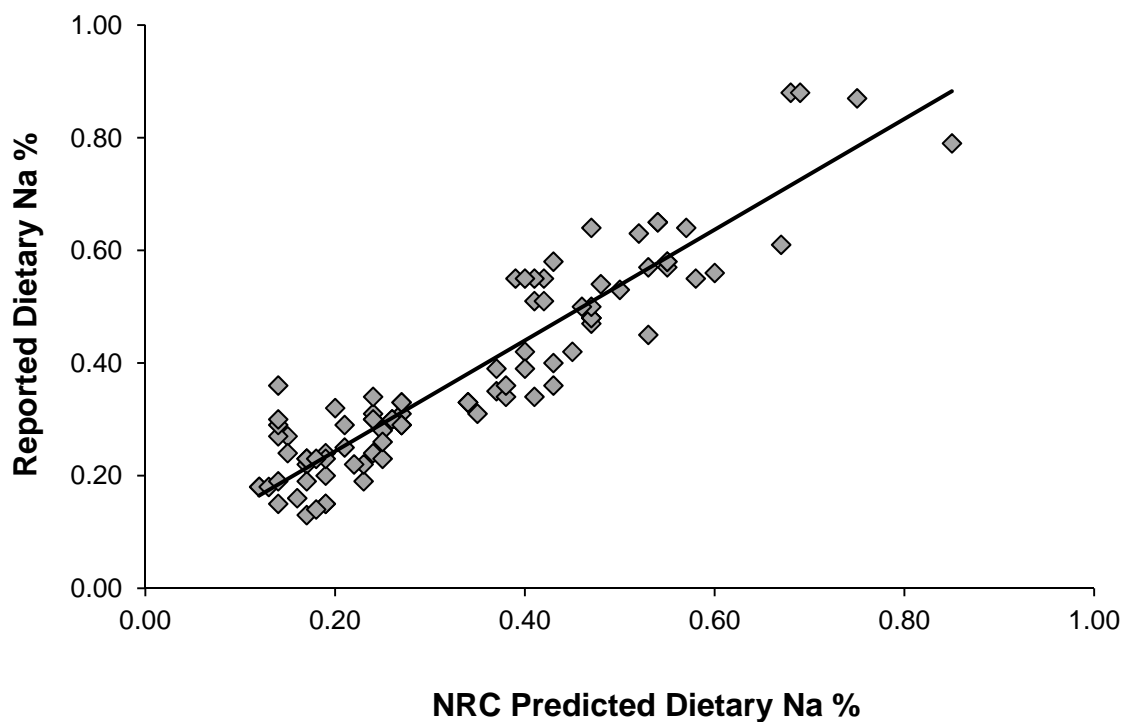


Figure 5.2 Correlation between reported and NRC predicted values for dietary percentages of potassium ($K \% = 0.7904x + 0.2734$; Intercept $P = 0.003$; Intercept SE = 0.090; Slope $P < 0.001$; Slope SE = 0.063; $R^2 = 0.6105$; Reg SE = 0.2105; $n = 102$).

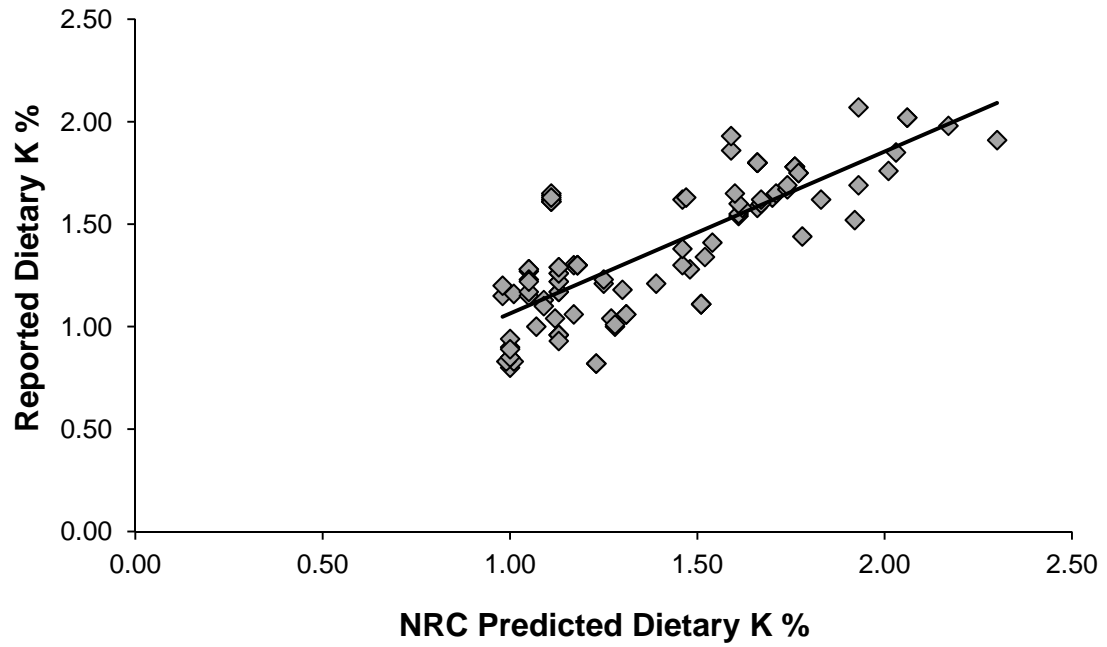


Figure 5.3 Correlation between reported and NRC predicted values for dietary percentages of chloride ($Cl \% = 0.7325x + 0.2029$; Intercept $P = 0.001$; Intercept SE = 0.058; Slope $P < 0.001$; Slope SE = 0.070; $R^2 = 0.6823$; Reg SE = 0.2036; $n = 53$).

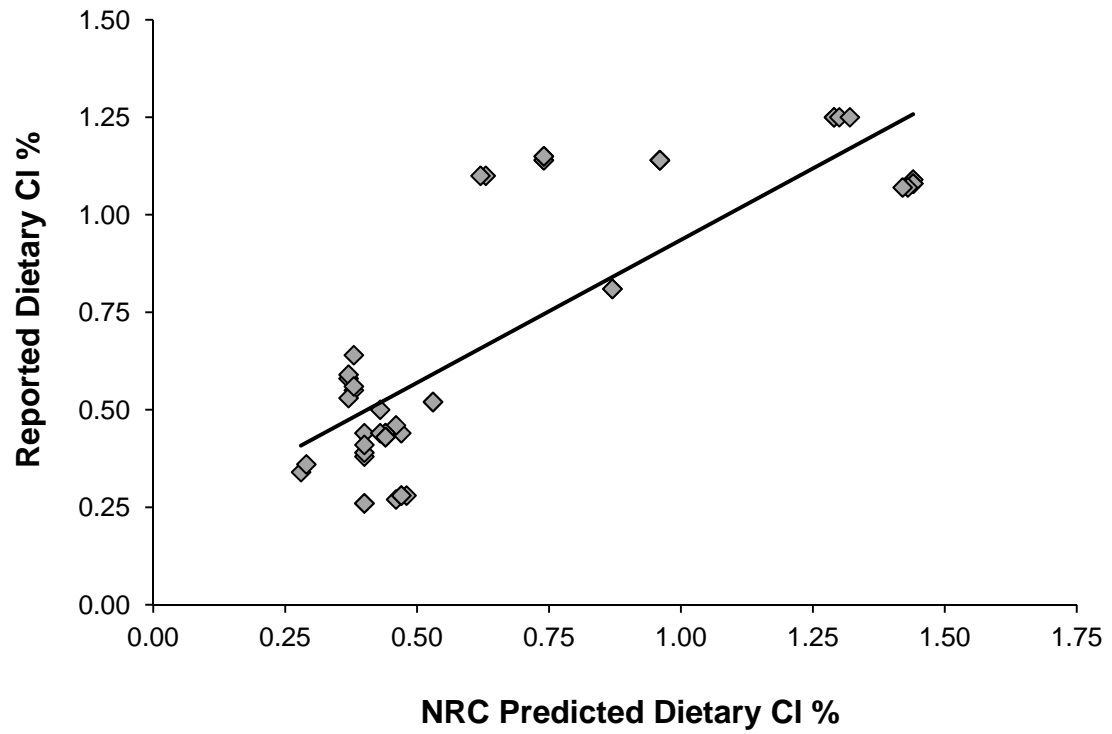


Figure 5.4 Correlation between reported and NRC predicted values for DCAD concentrations (meq/kg). The intercept (7.2825 meq/kg) was not different from 0 ($P = 0.8107$). Therefore it was set to 0 such that the final equation was: (DCAD (meq/kg) = 0.8803x; Slope $P < 0.001$; Slope SE = 0.026; $R^2 = 0.9671$; Reg SE = 60.48; $n = 41$).

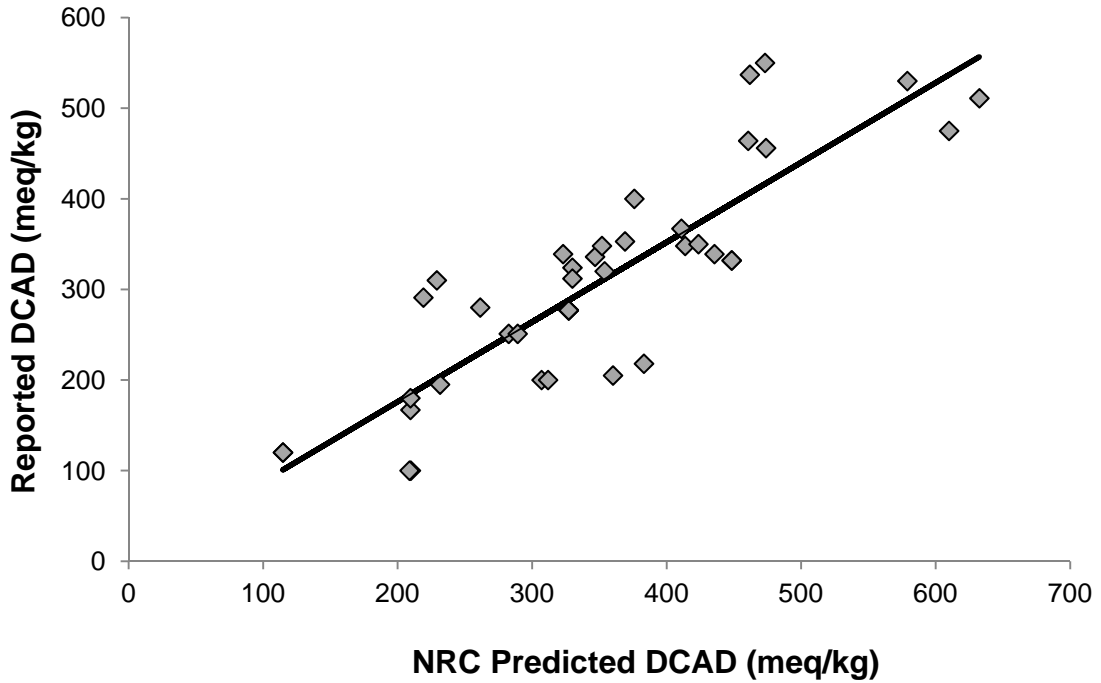


Figure 5.5 Relationship between DCAD and study-adjusted dry matter intake (kg/d). (Study-adjusted DMI (kg/d) = 0.0032 x DCAD (meq/kg of DM) + 17.918; Linear $P < 0.0001$; $R^2=0.3157$; $n=160$).

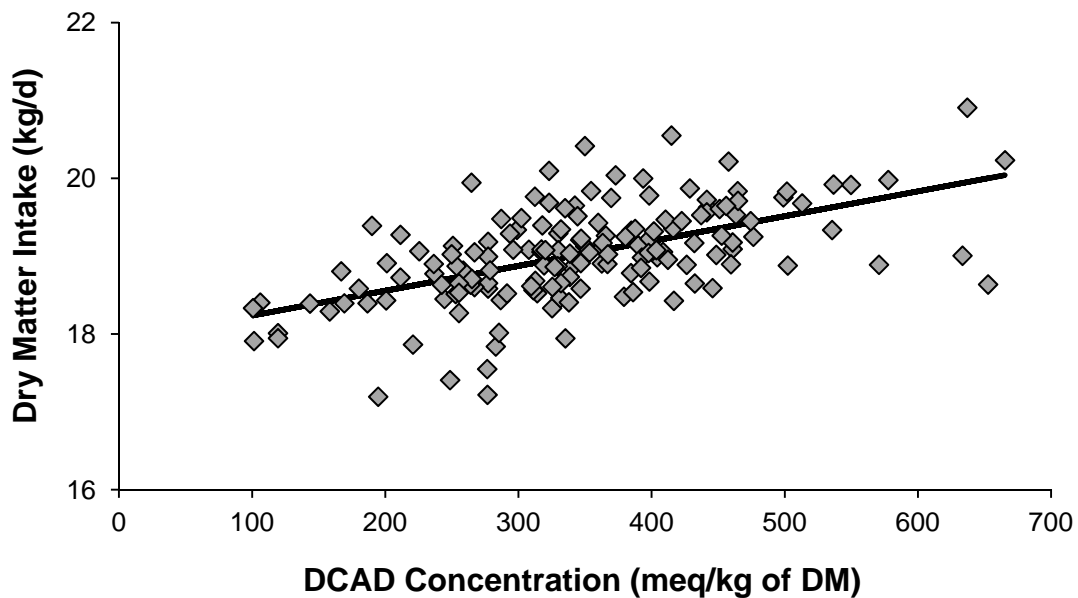


Figure 5.6 Relationship between DCAD and study-adjusted milk production (kg/d). (Study-adjusted milk yield (kg/d) = 0.0023 x DCAD (meq/kg of DM) + 26.187; Linear $P = 0.0260$; $R^2=0.1067$; $n=160$).

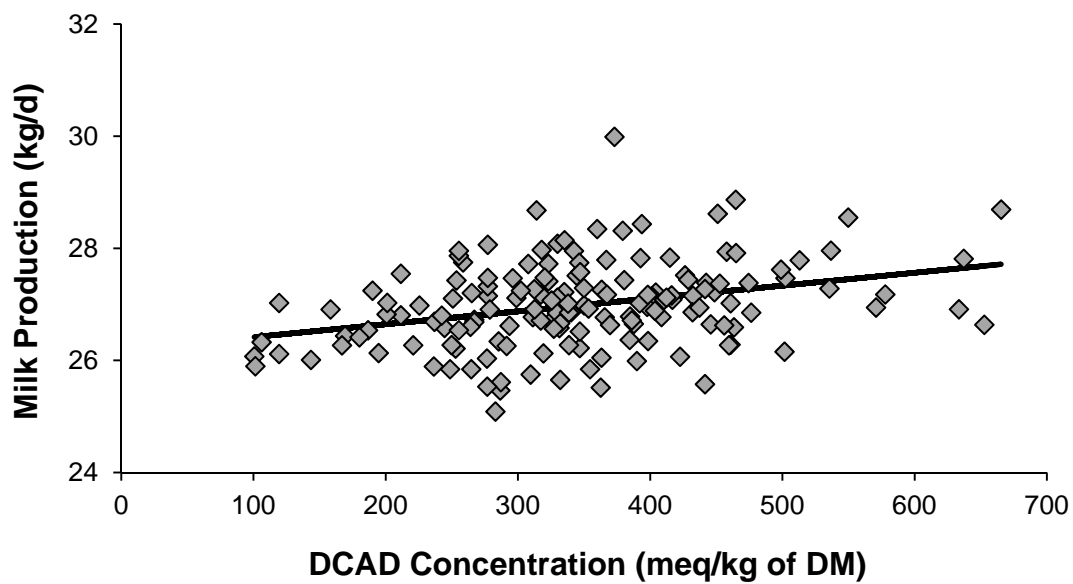


Figure 5.7 Relationship between DCAD and study-adjusted 3.5% FCM production (kg/d). (Study-adjusted 3.5% FCM (kg/d) = 0.0071 x DCAD (meq/kg of DM) + 24.139; Linear $P < 0.0001$; $R^2=0.4463$; $n=160$).

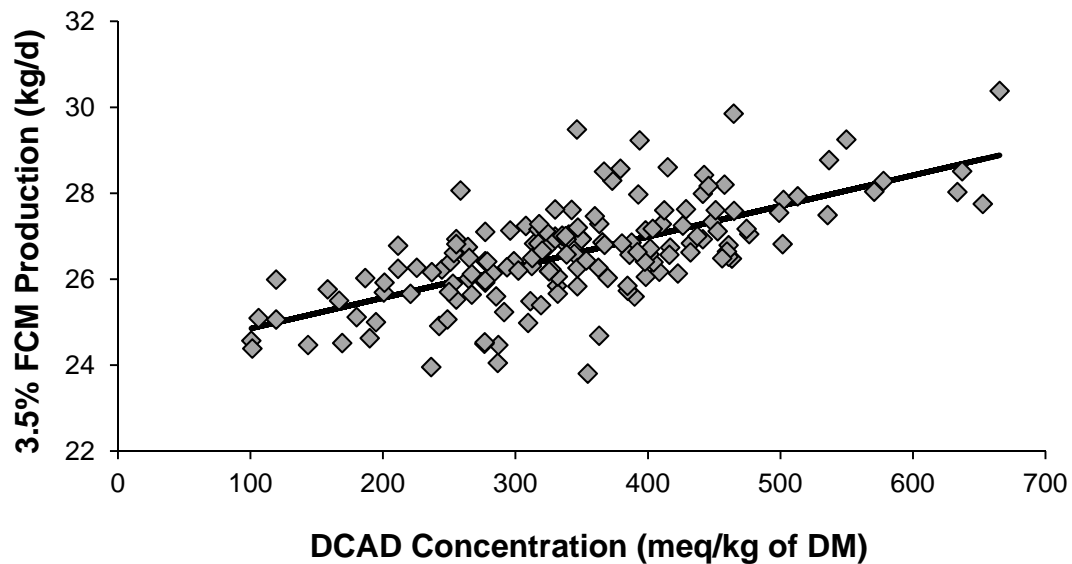


Figure 5.8 Relationship between DCAD and study-adjusted milk fat. (Study-adjusted milk fat % = $0.0011 \times \text{DCAD (meq/kg of DM)} + 3.0781$; Linear $P < 0.0001$; $R^2=0.4616$; $n=160$).

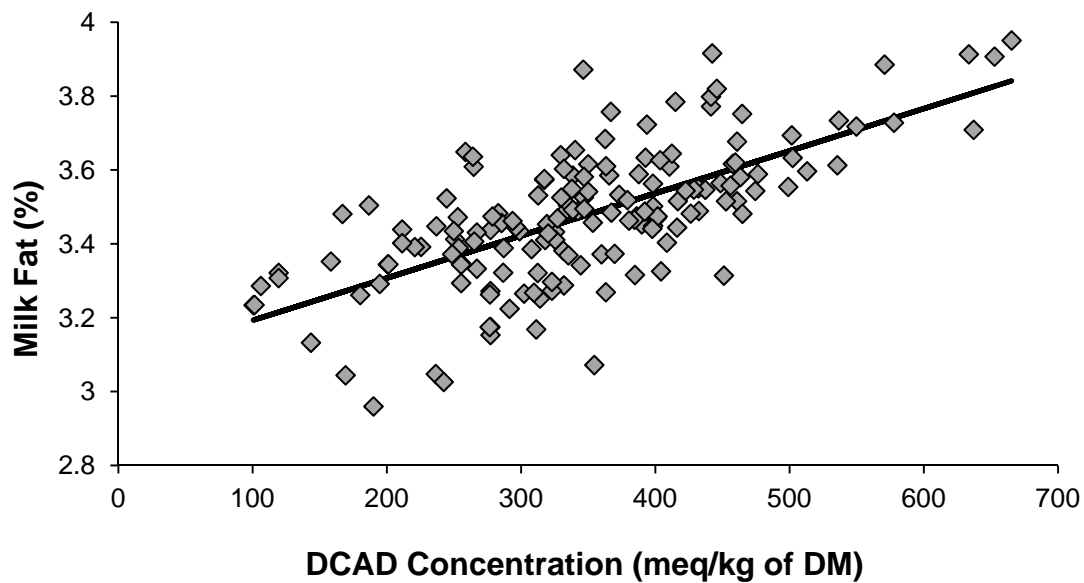


Figure 5.9 Relationship between DCAD and study-adjusted milk fat yield (g/d). (Study-adjusted milk fat yield (g/d) = $0.399 \times \text{DCAD (meq/kg of DM)} + 802.05$; Linear $P < 0.0001$; $R^2=0.5124$; $n=160$).

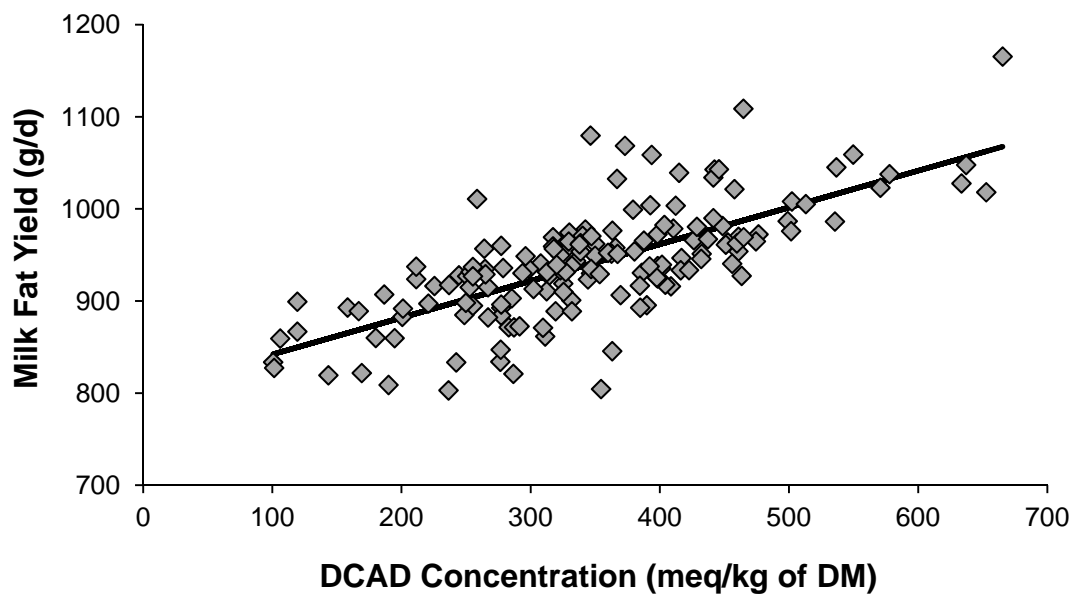


Figure 5.10 Relationship between DCAD and study-adjusted milk protein. (Study-adjusted milk protein % = $0.0 \times \text{DCAD (meq/kg of DM)} + 3.2223$; Linear $P > 0.05$; $R^2=0.0$; $n=118$).

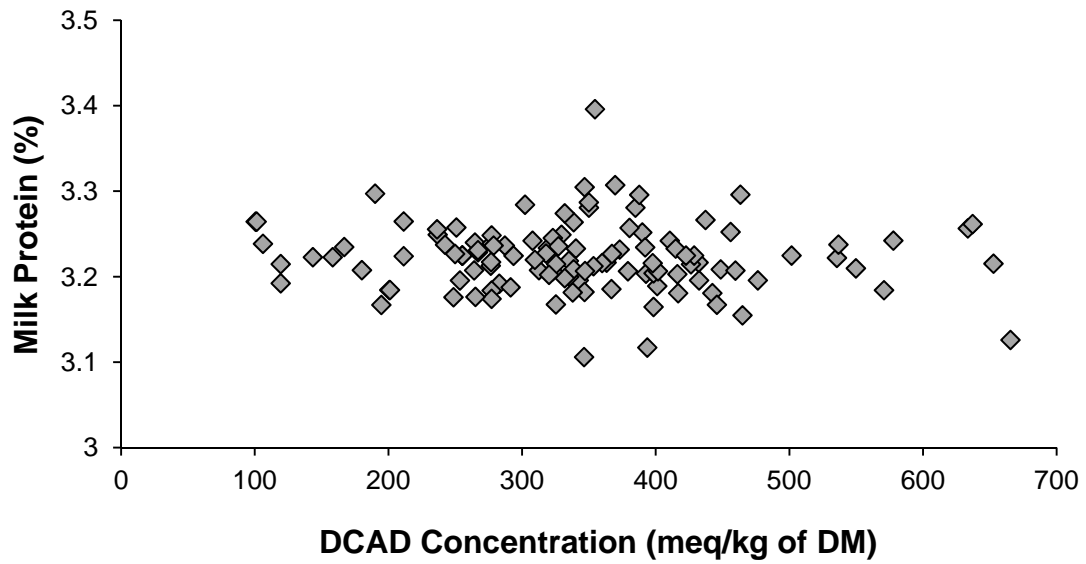


Figure 5.11 Relationship between DCAD and study-adjusted milk protein yield (g/d). (Study-adjusted milk protein yield (g/d) = $0.0473 \times \text{DCAD (meq/kg of DM)} + 840.1$; Linear $P = 0.1923$; $R^2=0.0517$; $n=118$).

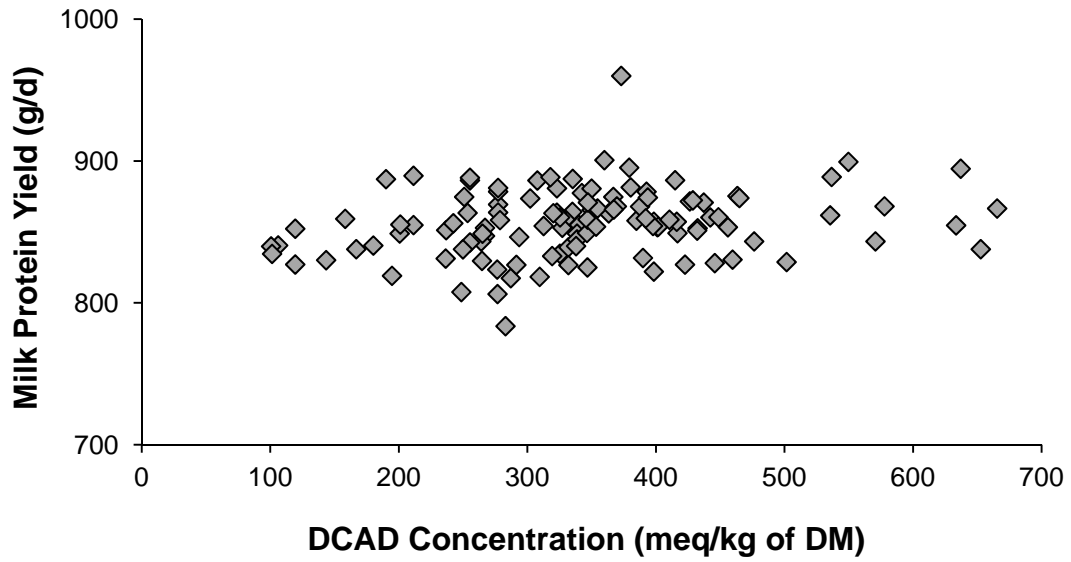


Figure 5.12 Relationship between DCAD and study-adjusted rumen pH. (Study-adjusted rumen pH = $0.0003 \times \text{DCAD (meq/kg of DM)} + 6.2839$; Linear $P = 0.0509$; $R^2=0.1877$; $n=73$).

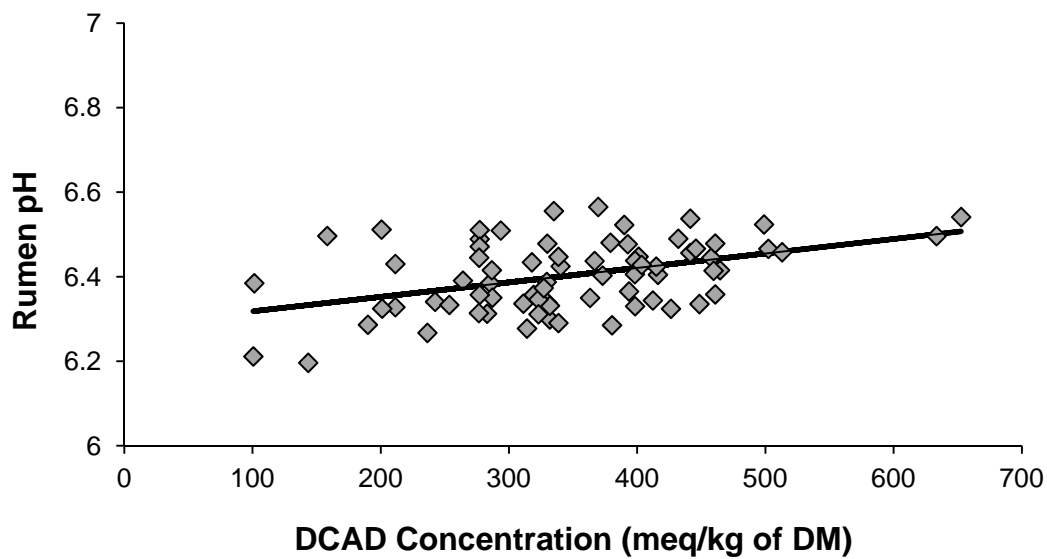


Figure 5.13 Relationship between DCAD and study-adjusted rumen acetate molar percentage. (Study-adjusted rumen acetate (molar %) = $0.0149 \times \text{DCAD (meq/kg of DM)} + 50.653$; Linear $P < 0.0001$; $R^2=0.5437$; $n=93$).

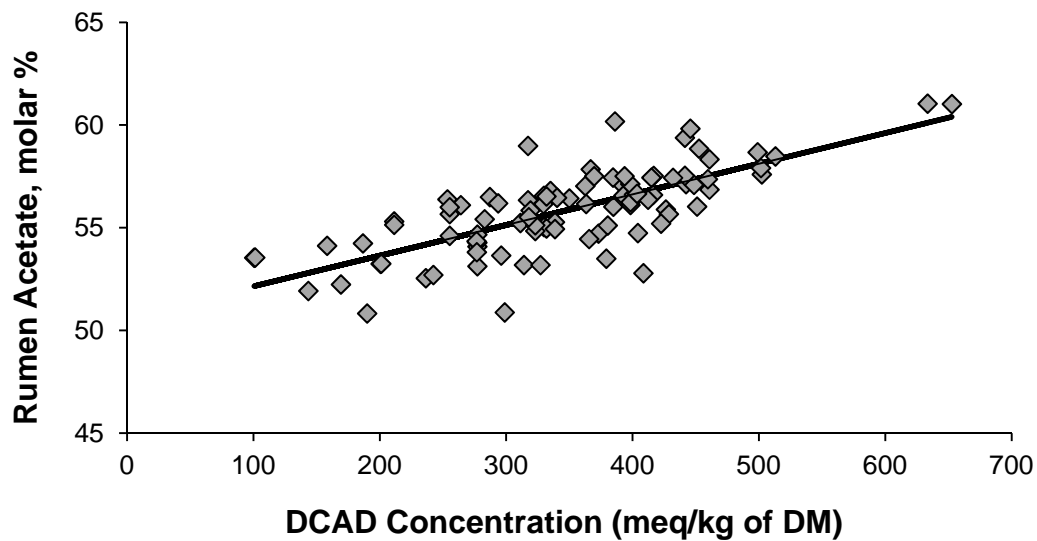


Figure 5.14 Relationship between DCAD and study-adjusted rumen butyrate molar percentage. (Study-adjusted rumen butyrate (molar %) = $0.0065 \times \text{DCAD (meq/kg of DM)} + 12.399$; Linear $P = 0.007$; $R^2=0.2822$; $n=80$).

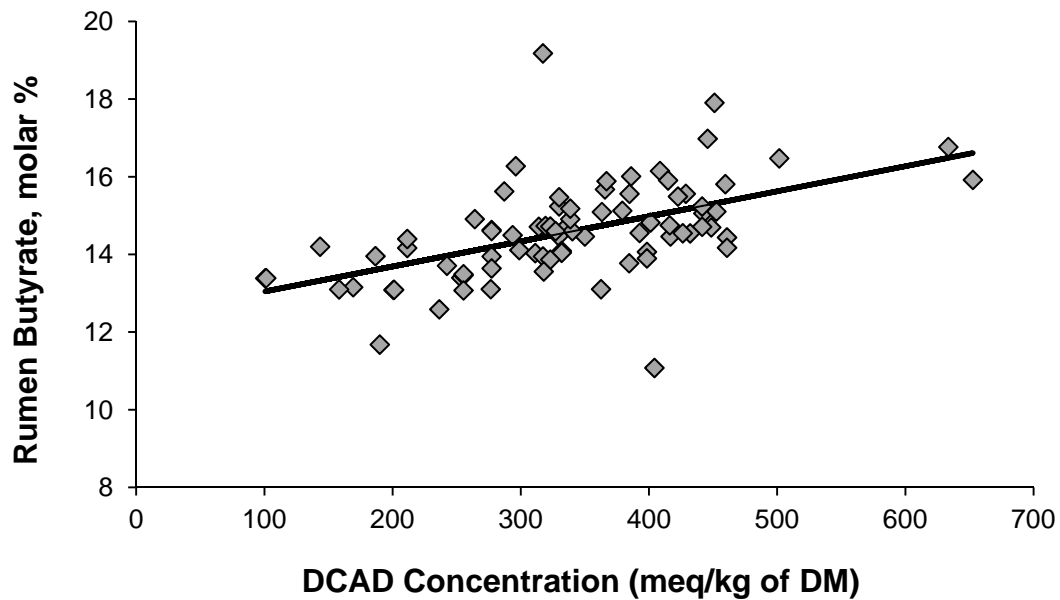


Figure 5.15 Relationship between DCAD and study-adjusted rumen propionate molar percentage. (Study-adjusted rumen propionate (molar %) = $0.0 \times \text{DCAD (meq/kg of DM)} + 26.2804$; Linear $P > 0.05$; $R^2=0.0$; $n=93$).

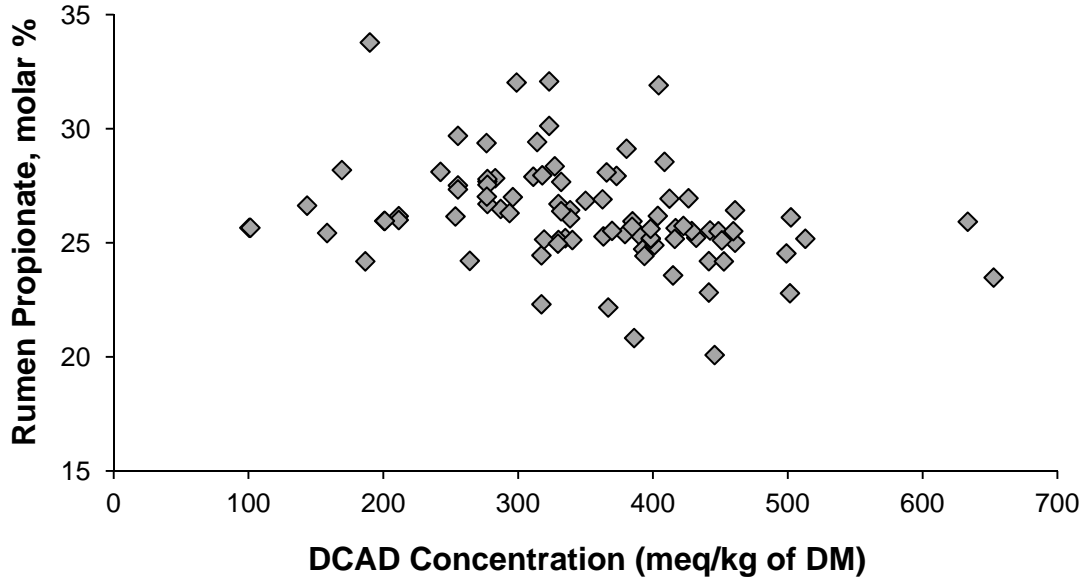


Figure 5.16 Relationship between DCAD and study-adjusted ratio of rumen acetate to propionate molar percentages. (Study-adjusted rumen acetate to propionate ratio (molar %) = $0.0017 \times \text{DCAD (meq/kg of DM)} + 1.7059$; Linear $P < 0.0001$; $R^2=0.5426$; $n=93$).

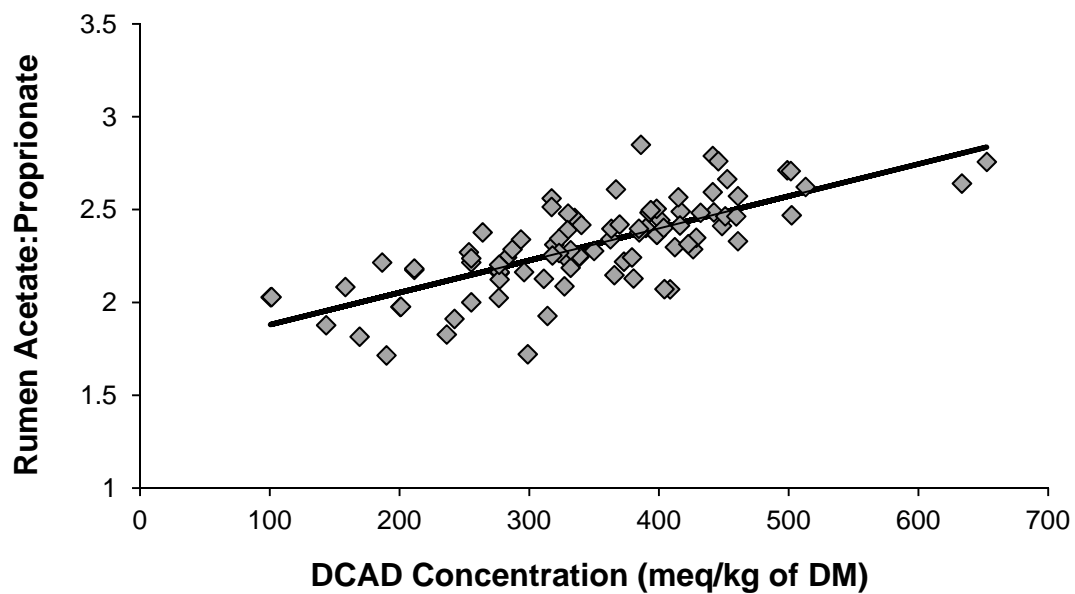


Figure 5.17 Relationship between DCAD and study-adjusted rumen total volatile fatty acid (TVFA) production. (Study-adjusted rumen TVFA = 0.0098 x DCAD (meq/kg of DM) + 82.7897; Linear $P = 0.4706$; $R^2=0.0126$ n=69).

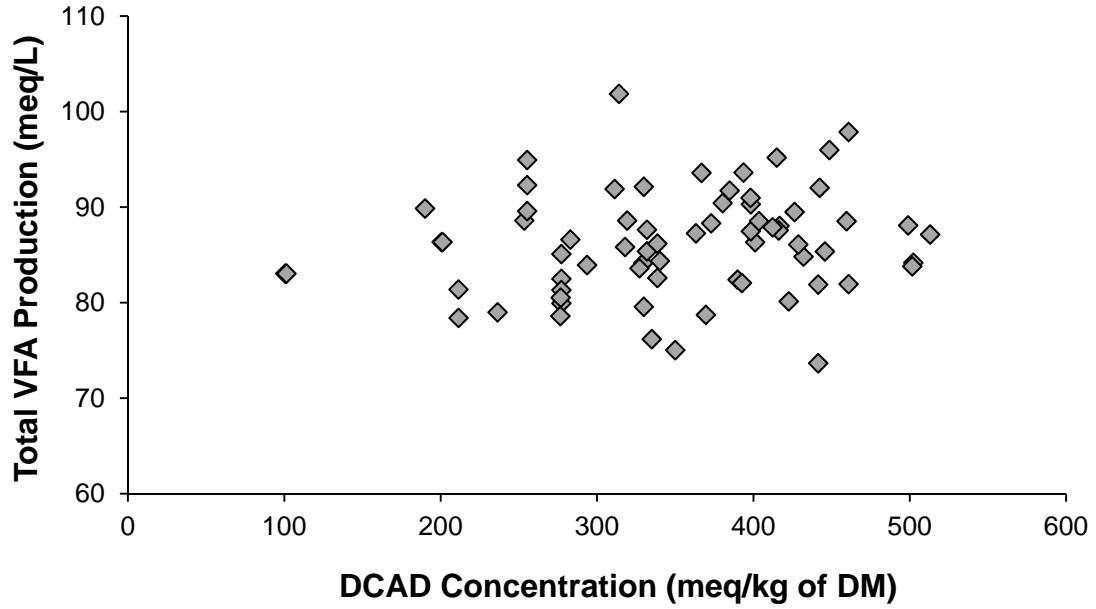


Figure 5.18 Relationship between DCAD and study-adjusted dry matter digestibility. (Study-adjusted DM digestibility = $0.00902 \times \text{DCAD (meq/kg of DM)} + 65.7512$; Linear $P < 0.0001$; $R^2=0.6232$ $n=42$).

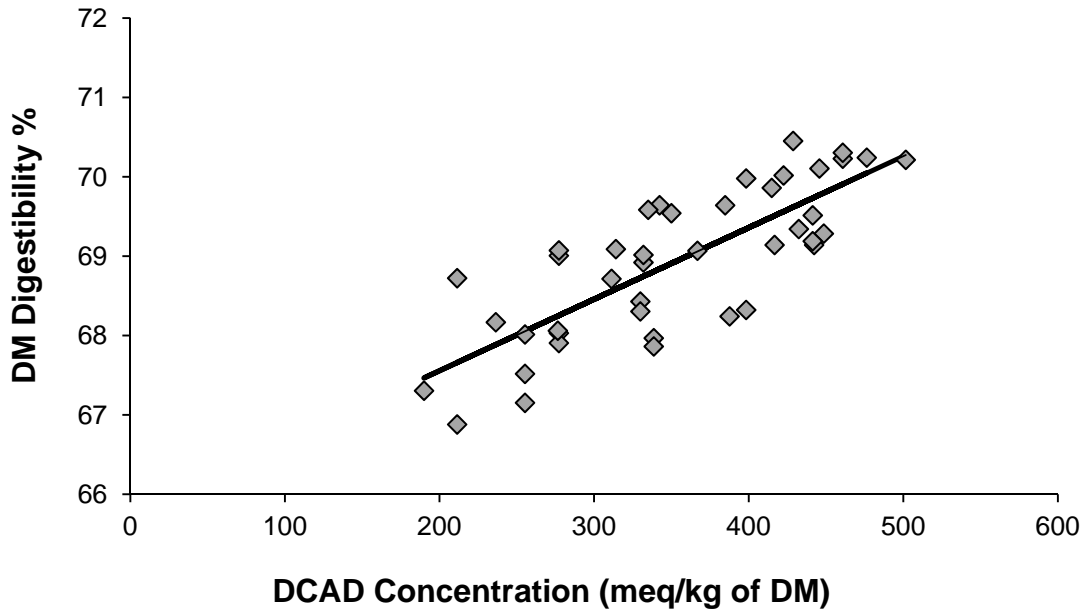


Figure 5.19 Relationship between DCAD and study-adjusted NDF digestibility (% of DM). (Study-adjusted NDF digestibility = 0.0187 x DCAD (meq/kg of DM) + 43.332; Linear $P = 0.0014$; $R^2=0.5318$ n=32).

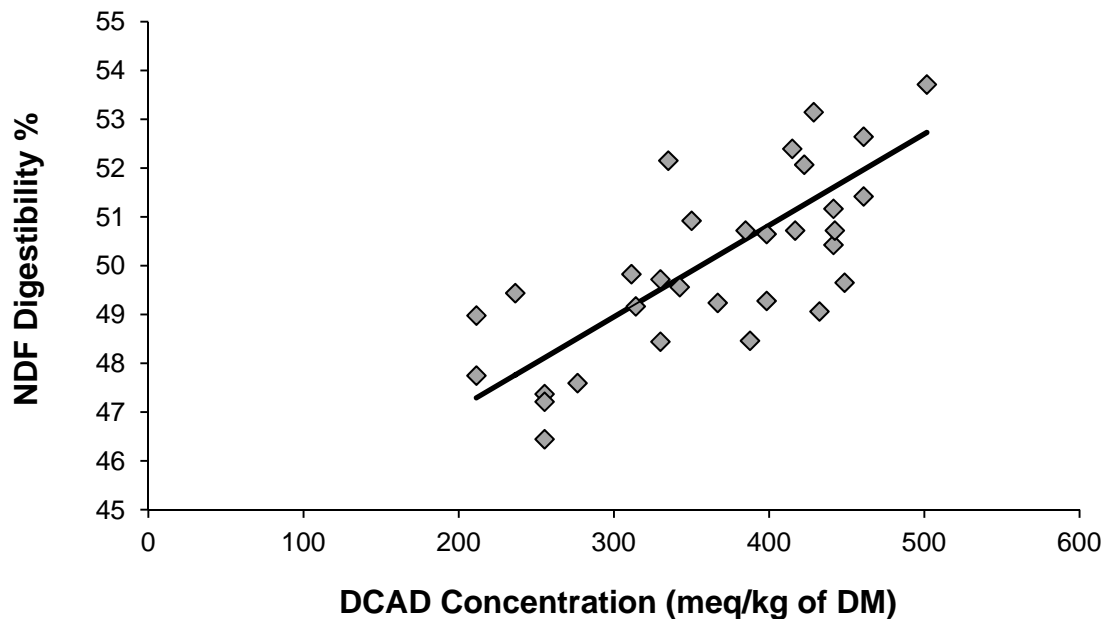


Figure 5.20 Relationship between DCAD and study-adjusted ADF digestibility (% of DM). (Study-adjusted NDF digestibility = $0.0322 \times \text{DCAD (meq/kg of DM)} + 29.1058$; Linear $P < 0.0001$; $R^2=0.707$; $n=40$).

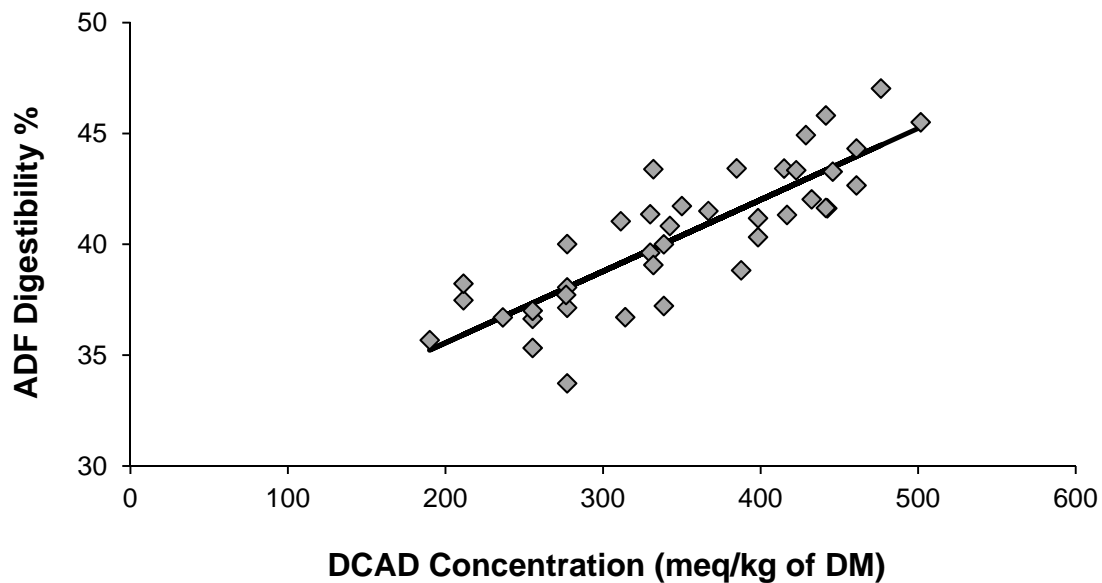
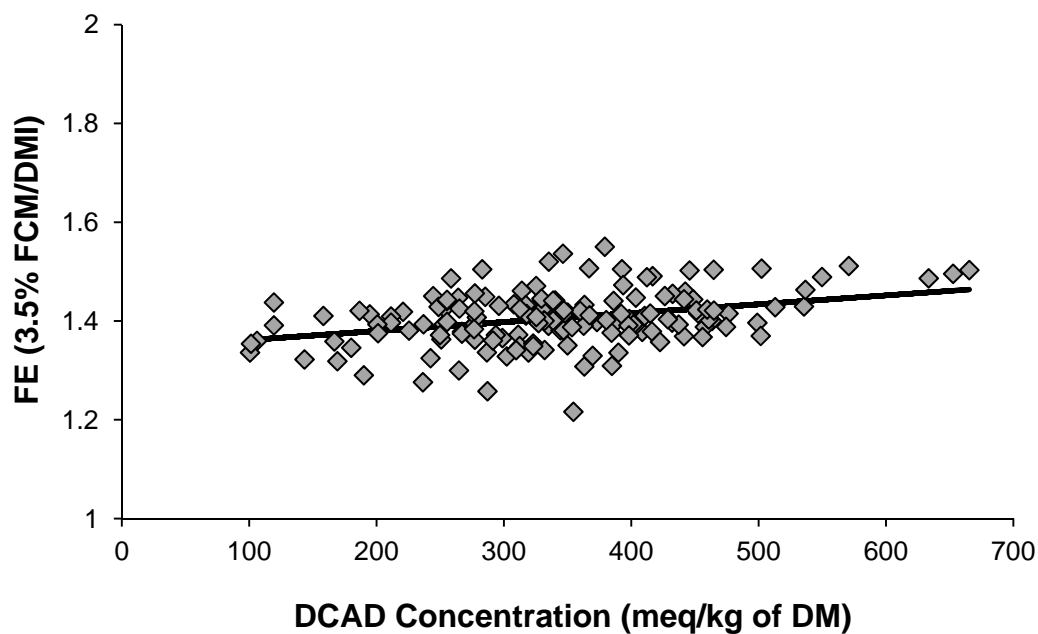


Figure 5.21 Relationship between DCAD and study-adjusted feed efficiency (3.5% FCM per unit of DMI). (Study-adjusted feed efficiency (3.5% FCM per DMI) = $0.0002 \times \text{DCAD (meq/kg of DM)} + 1.3436$; Linear $P = 0.0116$; $R^2=0.2822$; $n=160$).



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