Impact of Non-Fiber Carbohydrates and Dietary Cation-Anion Difference on Milk Fat Yield in Lactating Cows: A Meta-Analysis

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Authorization to Submit Thesis

This thesis of Joshua Paul Peters, submitted for the degree of Master of Science with a Major in Animal Science and titled "Impact of Non-Fiber Carbohydrates and Dietary Cation-Anion Difference on Milk Fat Yield in Lactating Cows: A Meta-Analysis," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Recently, increasing demand for butter has resulted in milk fat accounting for 50% of the value of Class III milk, supporting a positive impact on profitability for dairies with greater milk fat yield. Nutritional strategies to accomplish greater milk fat yield may be multifactorial. Dietary factors such as non-fiber carbohydrates (NFC), lipids, polyunsaturated unsaturated fatty acids (PUFA), and rumen buffers can impact milk fat yield. The biology for reduction of milk fat yield includes high inclusion of dietary lipids, especially those containing PUFA, and is well established. The hallmark of milk fat depression is diets containing PUFA and high NFC. A quadratic response of milk fat yield to NFC has been found with maximal yield at approximately 36-38% diet DM supporting the risk of reduced milk fat yield in diets rich in NFC. The addition of rumen buffers, which increase the dietary cation-anion difference (DCAD) by providing the cations K and Na, is a method to minimize the risk associated with high NFC. Milk fat yield has been shown to have either a quadratic or positive linear relationship to DCAD. It is likely that both NFC and DCAD interact in regulating milk fat yield given both impact rumen pH, a factor affecting the production of milk fat synthesis inhibitors in the rumen. To address the possible interaction of NFC and DCAD on milk fat yield, a meta-analysis was conducted. We hypothesized that increasing DCAD would improve milk fat yield for all levels of NFC and the response to DCAD would be more impactful as NFC in diets became greater. Fifteen studies were identified from the literature, which reported adequate information for the analysis. Studies provided 62 observations, 60 diets, and 362 cows. Interactions were tested by binning NFC into Low NFC (mean= 38.6 % DM), Medium NFC (mean=42.7 % DM), and High NFC (mean=47.1% DM), while DCAD was a continuous variable. A difference in milk fat yield between the model intercept (Low NFC) and Medium NFC was not detected, but there was a reduction ($P < 0.01$) for High NFC (-0.242 ± 0.082) compared to the model intercept (Low NFC). Increasing DCAD had a positive linear effect ($P < 0.05$) on milk fat yield for all levels of NFC. The positive response to DCAD for all NFC levels and reduction in milk fat yield to High NFC suggests that both DCAD and NFC should be considered when formulating diets to increase milk fat yield.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

1.1 Introduction

Profitability in the commercial dairy industry can be challenging, and oftentimes, unattainable. Recently, an increase in the demand for butter has helped to counter low milk prices resulting in butterfat accounting for 50% of the value of Class III milk (Covington, 2017). This change in price has served as an indicator for dairymen to produce more milk fat. To help dairymen achieve this, nutritionists and researchers have focused on ways to economically and consistently increase milk fat yield. To achieve greater outputs of milk fat sustainably, the approach taken must be economical, feasible on commercial dairies, compatible with the promotion of cow health, and support for reproductive efficiency. The synthesis of milk fat is the leading energy requirement for milk production, such that increasing milk fat yield requires additional energy intake (Dado et al., 1993). One strategy used to provide more energy for milk fat production has been to increase the net energy density in lactating rations. This can be accomplished by incorporating more digestible fiber, increasing non-fiber carbohydrates (NFC), using high energy by-products, and adding fat sources such as tallow, prilled palm oil, or calcium soaps of palm fatty acids (Palmquist and Jenkins, 2017). The use of one or all of these strategies, however, does not always lead to increased milk fat yield. This is most likely related to the fact that milk fat production is impacted by many factors particularly in the disorder known as milk fat depression (MFD) tied directly to milk fat yield (Bauman et al., 2011b). The rumen biohydrogenation of polyunsaturated fatty acids (PUFA) found in many of these feeds can produce bioactive intermediates inhibitory to milk fat synthesis (Bauman and Griinari, 2003). Specific rumen microbes, which tend to proliferate in lower rumen pH environments or when high levels of PUFA are fed, utilize alternative biohydrogenation pathways that produce inhibitory intermediates (Troegeler-Meynadier et al., 2003; Harvatine and Allen, 2006). Ensuring the primary biohydrogenation pathway is used by rumen microbes can decrease the production of these bioactive intermediates. Many studies (Apper-Bossard et al., 2010; Harrison et al., 2012; Guiling et al., 2017) have examined the effects of using rumen buffers to stabilize rumen pH which correspondingly leads to an increased dietary cation-anion difference (DCAD). These studies showed a decrease in inhibitory intermediates with greater milk fat yield when buffers were fed. However, not all studies using rumen buffers have led to positive results in

increasing milk fat yield (Chan et al., 2005). Given the multifactorial relationships between diet and milk fat production, future research needs to focus on the interactions between and among dietary factors to increase milk fat yield on commercial dairies.

1.2 Milk fat synthesis and biohydrogenation

To better understand how to utilize different approaches to increase milk fat yield, it is first important to understand how *de novo* fatty acids are synthesized in the mammary gland, how dietary fat is incorporated in milk, and how rumen microbes impact fatty acid digestion.

1.2.1 De novo fatty acid synthesis

Ruminants ferment carbohydrates to volatile fatty acids (VFA) primarily acetate, propionate, and butyrate in the rumen, and the proportion of these VFA's are dependent on the components of the diet (Murphy et al., 1982; Bannink et al., 2008). Morvay et al. (2011) used a data set with 101 treatment diets to model the proportion of VFA produced in the rumen of Holstein cows. The mean proportion of VFA's observed was 62.5% for acetate, 22.4% for propionate, and 11.3% for butyrate. Acetate was the most abundant and unlike propionate is non-glucogenic. Acetate serves as the primary carbon source for fatty acid synthesis in adipose tissue, muscle tissue, and the mammary gland (Baldwin, 1968; Bauman and Griinari, 2003).

For ruminants to produce acetyl-CoA for fatty acid synthesis, acetate must be activated by acetyl-CoA synthetase to acetyl-CoA in the cytosol of mammary gland cells. It is important to note that B-hydroxybutyrate (BHBA) is also used for fatty acid (FA) synthesis by this same activation with CoA, but at a much lower rate than acetate, and only for the first initial 4 carbons of fatty acids (Bauman, 1969). Numerous studies demonstrated the process of fatty acid synthesis, starting with the formation of malonyl-CoA (Ganguly, 1960; Bauman, 1969)). Acetyl-CoA undergoes a carboxylation to malonyl-CoA, committing acetyl-CoA to fatty acid elongation. The acetyl group from acetyl-CoA, as well as the malonate group from malonyl-CoA, are both transferred to an acyl carrier protein within the fatty acid synthase enzyme. Fatty acid synthase is a multifunctional enzyme that catalyzes (1) condensation, (2) reduction, (3) dehydration, and (4) a second reduction, producing butyryl, the first 4 carbon skeleton of a fatty acid chain. Repeating this process six additional times will produce palmitic acid, the primary hydrocarbon produced. Many shorter-chain (≤14 C) fatty acids are also synthesized

in the mammary gland of ruminants (Lindmark Månsson, 2008). Fatty acids of greater length (≥18 C) are not produced by ruminants (Barber et al., 1997). Thioesterase I hydrolyze chains of fatty acids greater than sixteen C, giving it a specificity for palmitic acid (Smith and Abraham, 1971; Barber et al., 1997). The inability of ruminants to elongate palmitic acid to stearic acid means that all fatty acids of eighteen carbons or greater must be provided by the diet. The fatty acids produced in the mammary gland can be saturated or be desaturated by Δ9-desaturase (also called stearoyl-CoA desaturase; Mosley and McGuire, 2007). Most of these *de novo* fatty acids will be esterified to a glyceroal backbone to form triacylglycerides that are packaged into fat globules before being secreted into milk (Luick, 1961).

1.2.2 Milk fatty acids from dietary sources

Fatty acids for milk fat synthesis are provided by both *de novo* processes and dietary fat. The triglycerides in feeds consumed by ruminants are hydrolyzed to free fatty acids in the rumen, and released into the digesta (Hawke and Robertson, 1964; Lock et al., 2006). Once in the small intestine, bile salts form micelles which transport the fatty acids to the surface of the intestinal wall for absorption. The fatty acids are absorbed into the intestinal epithelium where they are esterified to form triglycerides and then packaged into very-low-density lipoproteins (VLDL) or chylomicrons. These lipid transport particles are exocytosed into the lymphatic system where they travel to the vena cava and enter the portal vein (Bauchart, 1993). In pre-ruminant calves, Bauchart et al. (1993) found that chylomicrons made up 76% of the lipoproteins exocytosed by the intestines, whereas VLDL was only 19%. Apoproteins embedded in these chylomicrons and VLDL act as receptors that bind to membrane-bound lipases found on capillaries in tissues such as the mammary epithelial cells. Lipases such as lipoprotein lipase hydrolyze triglycerides and allow free fatty acids to enter mammary gland cells (Lock et al., 2006). These fatty acids will be esterified, once again, to form a portion of triglycerides found in milk.

1.2.3 Biohydrogenation

It is important to note that most of the unsaturated fatty acids provided from dietary sources undergo biohydrogenation in the rumen, converting them to saturated fatty acids. Biohydrogenation of linoleic acid in rumen fluid cultures showed that after 6 h, more than 60% had been completely hydrogenated to stearic acid (Harfoot et al., 1973). This creates

challenges for providing specific unsaturated fatty acids to lactating dairy cows. The first challenge is that biohydrogenation reduces the amount of unsaturated fatty acids that reach the small intestine for absorption. Some PUFA such as omega-3 and omega-6 fatty acids are important for the production of many biochemical compounds that impact reproduction, fertility, and immune function (Staples et al., 1998; Calder et al., 2002; Lessard et al., 2004). Omega-3 fatty acids such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) can be synthesized by ruminants from alpha-linolenic acid at very low rates, however, most of the alpha-linolenic acid is hydrogenated in the rumen to stearic acid, leaving very little substrate for synthesis of these longer omega-3 fatty acids (Gulliver et al., 2012).

Supplying omega-3 or omega-6 fatty acids to the small intestine for absorption is difficult without protection from biohydrogenation. The limited delivery of these fatty acids to animal tissues after the extensive biohydrogenation of PUFA may lead to reduced reproductive capabilities of high producing cows (Gulliver et al., 2012). Secondly, unsaturated fatty acids have a bacteriostatic effect in the rumen, which can limit the growth of specific microbes (Kim et al., 2000). Maia et al. (2010) tracked the growth of *Butyrivibrio fibrisolvens* in the presence of PUFA in culture and their work showed that PUFA limited the growth of these bacteria. After hydrogenation of PUFA, the bacteria reinitiated growth. This work suggests that limiting PUFA supplied in the diet should be beneficial to microbial growth (Jenkins, 1994; Maia et al., 2010). Lastly, biohydrogenation of PUFA can also produce intermediates that cause MFD under certain rumen environments. Griinari et al. (1998) fed lactating cows diets that were high or low in fiber with either unsaturated fatty acid added in small or greater amounts (8 vs. 380 g/d). They found no significant difference in milk fat yield when unsaturated fatty acids were limited in diets or when diets were high in PUFA but coupled with high fiber. However, a significant reduction in milk fat yield occurred when the low fiber diet included unsaturated fatty acids. This suggested that a rumen environment shift increased the production of fatty acid intermediate's that cause MFD when low fiber diets are fed. Other research has shown that even under normal rumen environments (i.e., diets adequate in fiber), inhibitory intermediates are produced and MFD occurs when unsaturated fatty acids 565 g/d were fed (Harvatine and Allen, 2006). This observation further supports MFD as a problem caused by multiple interactive factors.

Under normal conditions, linoleic acid (C18:2 *cis*-9 *cis*-12) is almost completely hydrogenated to stearic acid (C18:0). This pathway shifts (isomerizes) the double bond on C12 to C11, producing the isomer conjugated linoleic acid (CLA; C18:2 *cis*-9 *trans*-11), then reduces the double bond at C9, producing vaccenic acid (C18:1 *trans*-11), and finally reduces the double bond at C11 to form a fully saturated stearic acid (C18:0), (Figure 1.1; Harfoot et al., 1973). Under altered rumen conditions such as low pH, or the abundant presence of unsaturated fatty acids, another pathway of biohydrogenation can occur. Linoleic acid can be isomerized to a different isomer of CLA (C18:2 *trans*-10 *cis*-12), and it is this intermediate first identified to cause MFD (Baumgard et al., 2000b). The mechanism by which *trans*-10 *cis-12* CLA causes MFD is by a reduction in *de novo* fatty acid synthesis, as well as uptake of preformed fatty acids by the mammary gland (Bauman and Griinari, 2003). To better understand implications in the mammary gland, Baumgard et al. (2002) examined lipogenic enzymes in the mammary gland via biopsies. *De novo* synthesis was down-regulated with an associated decrease in mRNA for fatty acid synthase, acetyl CoA carboxylase, and Δ9-desaturase, which could decrease the translation and likely the abundance of all three enzymes. Uptake of preformed fatty acids was also decreased and thought to be related to the mRNA downregulation of lipoprotein lipase (LPL) and fatty acid binding protein (FABP). Although both *de novo* and preformed fatty acid pathways are reduced during MFD caused by *trans*-10 *cis*-12 CLA, a greater reduction for the de novo synthesis of short and medium-chain fatty acids (up to C16) originating from acetate is apparent (Harvatine and Allen, 2006).

The response of lipid synthesis during MFD may not be the same for all tissues throughout the body in lactating cows. Harvatine et al. (2009) showed that during CLA-induced MFD in dairy cows, the lipogenic enzymes down-regulated in the mammary gland are upregulated in adipose tissue. Much of the earlier research altered the energy density of treatment diets, not allowing for a very good measurement of energy intake and partitioning (Griinari et al., 1998; Harvatine and Allen, 2006). Harvatine et al. (2009) did not alter the energy density of the treatment diets, allowing better identification of energy balance and partitioning of that energy among body tissues. Other research using mice, and swine have shown that *trans-10 cis-12* CLA can reduce adipose tissue growth, although none of the animals in these studies were lactating (Dugan et al., 1997; West et al., 1998; DeLany et al., 1999). There is consensus, however, that CLA isomers such as *trans*-10 *cis*-12 CLA cause

MFD, and the mode of action is in the mammary gland. As proof, Baumgard et al. (2000b) abomasally infused cows with 1) skim milk as a control, 2) *cis*-9 *trans*-11 CLA, and 3) *trans*-10 *cis*-12 CLA. There was no change in the milk fat percentage or yield with *cis*-9 *trans*-11 CLA compared to the skim milk control, but *trans-10 cis-12* CLA reduced milk fat by 35%. The "biohydrogenation theory of milk fat depression" is now well accepted (Griinari and Bauman, 1999; Bauman et al., 2011a), and further research aims to better understand the energy partitioning that occurs during MFD, and what, and how many, additional biohydrogenation intermediates cause MFD.

1.2.4 Fatty acid mobilization

Another pathway for preformed fatty acids to be supplied to the mammary gland is through the mobilization of adipose tissue. During excess energy intake, fatty acids supplied from the diet, as well as from fermentation products such as acetate will be used for lipogenesis in adipocytes (Hanson and Ballard, 1967). These reserves are then mobilized during times of negative energy balance. If this mobilization occurs during lactation, the fatty acids released can be incorporated into milk fat. As explained by Gruffat et al. (1996), triglycerides in adipose tissue are mobilized by hormone-sensitive lipase to release non-esterified fatty acids (NEFA) and glycerol into circulation where they can be taken up by many tissues including the mammary gland. The contribution of NEFA to milk fat in early lactation until energy balance is reached can be substantial with estimates of NEFA supplying up to 40% of the fatty acids in milk (Bell, 1995).

In early lactation, a large amount of NEFA will also be taken up by the liver where it will be used for energy through beta-oxidation, stored as triglycerides, or converted to ketone bodies if in excess, as transport out of the liver is limited (Liu et al., 2014). Through beta-oxidation of NEFA in the liver, large quantities of the ketone, beta-hydroxybutyrate (BHBA), are produced, released into circulation, and taken up by tissues as an energy source. It is by this mechanism that the mammary gland is supplied with most of the BHBA that can be used as a substrate for fatty acid synthesis (Emery et al., 1992). Although a very important pathway to supply the mammary gland with fatty acid substrate, it is not one of the major pathways once energy balance has been restored after calving. If excess BHBA is produced, ketosis can occur. Estimates of the incidence of ketosis range from 7 to 14% in early lactation (Shaw,

1956; Gröhn et al., 1983; Herdt, 2020). Liver function is oftentimes impaired when high levels of NEFA are taken up by hepatocytes. Liu et al. (2014) treated bovine hepatocytes with a range of NEFA and showed that high levels of NEFA reduced the expression of apolipoprotein B100 (ApoB100), apolipoprotein E (ApoE), microsomal triglyceride transfer protein (MTP), and low-density lipoprotein receptor (LDLR) in hepatocytes which would lead to a decrease in VLDL and the export of triglycerides from the liver. Reducing the export of fatty acids from the liver can cause fatty liver, which impacts hepatic metabolism (Bobe et al., 2004). When NEFA accumulates in liver cells, incomplete oxidation produces ketones such as BHBA which can be released into the bloodstream leading to a greater risk of metabolic diseases such as ketosis, displaced abomasum, and metritis when serum BHBA concentrations reach ≥10 mg/dL (Ospina et al., 2010).

1.3 Economics of milk fat production

1.3.1 Industry economics

Dairy products have been consumed by humans for 7,500 years (Itan et al., 2009). The dairy industry today is vastly different from its humble beginnings. In the United States, dairy products are a major part of the economy grossing more than $35 billion in revenue from about 9 million dairy cows (USDA, 2019). In 2018, people in the United States consumed 70.7 kg of fluid milk, 6.1 kg of yogurt, 2.6 kg of butter, 18.1 kg of cheese, and 9.8 kg of ice cream per capita (USDA, 2019). These dairy products are a staple in diets as they provide essential nutrients such as amino acids, minerals, and vitamins. As public and government agencies put more emphasis on human health, one concern with dairy products has been the greater concentration of saturated fatty acids (SFA) compared with many other food groups. The Dietary Guidelines for Americans (2015) recommended limiting SFA as evidence has shown that the consumption of SFA is linked to higher levels of low-density lipoprotein (LDL; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015; Nicolosi, 2018). An increased risk of cardiovascular disease (CVD), one of the leading causes of death in the United States, is associated with high levels of LDL (Lloyd-Jones et al., 2010). The perceived risk of SFA in butter and the availability of cheaper alternatives such as margarine led to a decrease in demand for butter following World War II (Bentley and Ash, 2016). However, a meta-analysis with results from over 600,000 subjects from 76 studies did

not detect a direct association between CVD and SFA intake (Chowdury et al., 2014). Although the meta-analysis is contrary to prior research showing that SFA increases LDL, there is recent evidence that public perception of dairy products is improving, possibly because of more positive news coverage since the results were provided to the public. The per capita consumption of butter has risen from 2.04 kg in 2003 to 2.63 kg in 2018 (USDA, 2019). With this increase in demand, the price dairy producers receive for milk fat is higher than ever before, with the federal milk marketing order price for butterfat per pound increasing from $1.68 in 2013 to $2.53 in 2018.

1.3.2 Economics of feedstuffs

Dairy farmers have adapted to the demand for more milk fat by taking several steps to increase production. These steps include improving genetics (Schennink et al., 2009), cow comfort (Krawczel and Grant, 2009), forage quality (Liu et al., 2016), and adjusting diets to meet requirements for milk and milk fat yield. Factors relating to the economics of the rations fed to dairy cattle, and how they may impact and improve milk fat yield, are of great concern. Feed cost requires great consideration as it is the single largest expense on dairy operations in the United States (Buza et al., 2014). To manage feed costs, the dairy industry has relied on the production of forages such as alfalfa and corn to meet most of the protein and energy requirements of dairy cattle (Martin et al., 2017). Depending on the quality of forage, additional protein and energy are supplemented to meet these nutrient demands. Common protein sources include soybean meal, canola meal, dried distillers grains, and blood meal, which are utilized to support the requirement of amino acids (Clark et al., 1987) Common energy sources include corn, wheat, barley, and sorghum (Eastridge, 2006). Other energy sources include lower protein by-products such as beet pulp, millrun, whole cottonseed, and corn gluten meal. Feeds with a high concentration of fat have become common in lactating diets, as fatty acids provide more than twice the energy as carbohydrates, allowing for increased milk and milk fat yield (Rabiee et al., 2012). These feeds include tallow (a product of the beef industry), prilled palm oils, and calcium salts of palm fatty acids (Palmquist and Jenkins, 2017). The prilled or calcium salt products have more consistently improved milk fat yield (Rabiee et al., 2012) than the other high fat feeds, but the price of these products can limit use. Additionally, feed additives such as buffers and direct-fed microbials can be used to improve rumen function.

1.3.3 Utilization of feed ingredients

Although forages are typically economical feed sources, the nutrients they provide can be found in other feeds. Effective fiber in forages is necessary as it supports rumen function and maintains rumen pH; forages can only be in part replaced by a feedstuff of comparable physical fiber content such as soyhulls, whole cottonseed, or almond hulls (Aguilar et al., 1984; Weidner and Grant, 1994; Allen, 1997; Firkins et al., 2002). Beauchemin et al. (2006) combined data from 23, studies which showed that when physically effective neutral detergent fiber (NDF) was reduced below 12.5% dry matter, rumen pH dropped below 6.0. Milk fat percent and rumen pH are highly correlated, with milk fat percent declining below 3.5 when rumen pH is below 6.0 (Allen, 1997). The physically effective fiber in forages encourages cud-chewing, which produces salivary buffers that neutralize fermentation acids in the rumen. The extent to which a diet stimulates cud-chewing is directly correlated to physically effective fiber intake, which is dependent on the particle size of the forage or fiber source (Grant et al., 1990). Grant et al. (1990) fed three diets that had either fine, medium, or coarse particle length (2.6, 2.8, and 3.0 mm, respectively) in diets with similar NDF resulting in mean rumen pH of 5.3, 5.9, and 6.0, respectively. Milk fat percent had a positive linear response (3.0%, 3.6%, and 3.8% for 2.6, 2.8, 3.0 mm particle length respectively) with no significant impact on milk yield (Grant et al. 1990). One of the challenges with feeding high levels of forage and physically effective fiber is the ability to meet the energy needs of high producing dairy cows. To increase production, dry matter intake (DMI) and energy density must be maximized (Selvaraj et al., 2007). The challenge of greater DMI and energy density is enhanced rumen acid load where as decreasing physically effective fiber limits the ability of cows to properly buffer the rumen. A balance among these two factors should have a greater impact on milk fat yield.

Forage, grains, and by-products are used to balance rations to economically optimize milk, fat and protein yield, reproduction, and cow health. How these ingredients are balanced provides specific nutrients that can be evaluated to optimize performance. Non-fiber-carbohydrates (NFC) consist of sugars, starches, oligosaccharides, fructans, and pectins and has been defined by Hall et al. (1999) as the whole minus the sum of crude protein, neutral detergent fiber, fat, and ash. These non-fiber carbohydrates represent the amount of soluble carbohydrate in the diet provided to rumen microbes to maximize VFA production and

microbial protein yield. It also helps determine the percentage of the diet not rapidly fermentable. The use of NFC in ration formulation is not clear as several factors including energy and protein source, forage type, particle size of forages and fiber sources, DMI, and milk and component yield, need consideration. Batajoo and Shaver (1994) fed varying levels of NFC (24, 30, 36, and 42%) to multiparous Holstein cows and found that decreasing NFC had a positive linear relationship with milk fat percent, but not yield. Hoover and Stokes (1991) reported that microbial efficiency and microbial protein yields were greatest at 56% NFC, well above the recommended NFC concentration for lactating dairy cows (Weiss, 2002). Varying NFC by manipulating forage type and grain sources in lactating diets influences milk and milk fat yield. Valadares Filho et al. (2000) fed four diets to Holstein cows (24.5, 29.3, 36.2, and 42.8% NFC) by substituting alfalfa silage with high moisture corn. Increasing NFC was related to a positive linear response in milk yield, where as milk fat yield had a quadratic response, with the highest milk fat yield estimated to occur with diets containing 38% NFC. Supporting evidence from Robinson and McQueen (1997) also showed that milk fat yield had a quadratic relationship to increasing NFC. This could be due to the fact that higher NFC diets increase the risk of MFD and rumen acidosis (Grant, 1993). Milk fat depression which occurs when NFC is high was made evident by Griinari et al. (1998). Their research showed that increasing NFC from 31 to 47% DM reduced milk fat yield from 1.05 kg when saturated fat was fed to 0.68 kg when unsaturated fat was fed. The interaction between high concentrate (high NFC) and unsaturated fat decreased milk fat yield significantly because of the altered biohydrogenation pathway that produces *trans*-10 *cis*-12 CLA. As demonstrated by these studies, NFC alone cannot explain the variation in the milk fat yield by a particular diet, although there is evidence that high levels of NFC (>42% DM) to optimize rumen VFA production and microbial growth can be related to a reduction in milk fat yield.

Buffers can be incorporated in lactating diets when higher energy and lower fiber levels are fed to maintain rumen pH. Dietary buffers include magnesium oxide, potassium carbonate, potassium bicarbonate, sodium bicarbonate, and sodium sesquihydrate (Erdman, 1988). Sodium and potassium buffers have been studied more extensively (Iwaniuk et al., 2015), and the impact they have on milk fat yield has been variable (Chan et al., 2005; Harrison et al., 2012). Dietary buffers increase milk fat yield when cows are fed diets at the

lower end of requirements for NDF and effective fiber (NRC, 2001), as well as when diets contain high levels of corn silage (Erdman, 1988). Both of these scenarios are common on dairies in the United States, where corn silage represents 22.4% of diet DM (Martin et al., 2017). Apper-Bossard et al. (2010) used a split-plot design to feed varying levels of starch and buffer. Cows fed high starch (35.2% DM) with high buffer (1% DM) had a higher yield of milk, fat, and protein compared to low starch (31%) and high buffer (1%). Each one of these dietary factors, whether it be an ingredient, formulation of ingredients (i.e., NFC), the buffering capacity of the diet, or the use of buffers, has been shown to alter milk fat yield alone under specific situations. Likewise, the economic return must be considered when evaluating the use of any feedstuff. By evaluating the relationship among these factors, we may be better able to improve economically beneficial production of milk fat when feeding specific feedstuffs in certain types of diets to lactating cows.

1.4 Dietary cation-anion balance

1.4.1 Dietary cation-anion difference (DCAD) and buffers

The role of cations and anions in metabolism is well established (Block, 1984). By definition, DCAD consists of the cations, potassium (K) and sodium (NA), and anions, chloride (Cl) and sulfur (S). These four macro-minerals have the greatest impact on acid-base balance in dairy cows (Block, 1984; Tucker et al., 1991). The most common DCAD equation used today is calculated as (K+Na)-(Cl+S) mEq/100 g DM. The cations are considered to be alkalogenic, where as the anions are acidogenic (Block, 1984). Buffers that stabilize rumen pH contain K and Na and increase DCAD. The products used to modulate DCAD contain both a buffering capacity related to the bicarbonate or carbonate concentration present with the cation, as well as the alkalogenic properties of K and Na. The most prominent buffers used to increase DCAD are sodium bicarbonate, sodium sesquicarbonate, potassium bicarbonate, and potassium carbonate (Erdman, 1988). These buffers have a pKa >6.0, allowing them to accept a proton at the desired pH in the rumen (Table 1.1). Erfle et al. (1982) manipulated the pH in artificial rumens to analyze the change in proteolytic enzyme activity. Production of VFA was decreased by 37.5% when pH was reduced from 7 to 5, and proteolytic organisms did not survive when the pH was below 6. This evidence suggests that maintaining a rumen pH >6.0 maximizes VFA production and enzymatic processes, so a

buffer with a pKa of 6.25 is most effective at buffering the rumen (Erfle et al., 1982; Erdman, 1988). Many studies have evaluated negative DCAD-balanced lactation diets that utilized acidogenic minerals (Tucker et al., 1988); however, this is not normal for diets fed on commercial dairies. Anionic salts is the common term for DCAD-reducing products such as calcium chloride, magnesium chloride, ammonium chloride, magnesium sulfate. All of these anionic salts, as well as elemental sulfur, are considered to be acidogenic and decrease blood and urine pH to varying degrees in ruminants (Goff et al., 2004).

Much of the focus using DCAD balancing has been directed toward pre-fresh cows to improve calcium status and ensuing milk production through a decrease in the incidence of milk fever and subclinical hypocalcemia during the transition period (Lean et al., 2019). When considering DCAD balancing for pre-fresh cows, diets balanced at -10 mEq/100 g DM will reduce blood pH which increases receptor sensitivity for calcium mobilization, leading to a reduction in the incidence of milk fever (Block, 1984; Goff et al., 1991). Using negatively balanced DCAD diets for pre-fresh cows has become common in the field, and the literature supporting the decrease incidence in milk fever is well accepted (Santos et al., 2019). Results are more variable when evaluating the impact of balancing DCAD in lactation diets. A DCAD meta-analysis by Hu and Murphy (2004) showed that increasing DCAD in lactation diets had a quadratic effect on milk yield, DMI, 4%-fat corrected milk, blood pH, blood bicarbonate and urine pH with maximums at 34, 40, 49, 35, 47, and 62 mEq/100 g DM, respectively. Milk fat yield also had a quadratic response which peaked at 55 mEq/100 g DM. Iwaniuk and Erdman (2015) also evaluated the response to DCAD by lactating dairy cattle using a meta-analysis. Their analysis showed a positive curvilinear relationship among DCAD and milk yield and DMI, and a linear relationship for milk fat yield, fat percent, and rumen pH. One criticism of these meta-analyses (Hu and Murphy, 2004; Iwaniuk and Erdman, 2015) is that they included studies with negative DCAD diets, known to decrease DMI and acidify the blood (Escobosa et al., 1984; Tucker et al., 1988; Apper-Bossard and Peyraud, 2004; Lean et al., 2019). Zimpel et al. (2018) were able to uniquely balance diets to 19 meq/100 g or -11.4 mEq/100 g DM, both containing an acidogenic chloride product (BIO-CHLOR^{TM}). They hypothesized that negative DCAD diets that decrease urine pH are responsible for reduced DMI. Their results supported this hypothesis, as DMI was not reduced when positive DCAD diets were fed but DMI was reduced when DCAD diets using the acidogenic product were

offered. The implication is that a negative DCAD likely reduced DMI leading to decreased production in lactating cows. Anionic salts are also known to be less palatable than alkagenic salts (Oetzel et al., 1988; Oetzel and Barmore, 1993). Because feeding lactating cows a negative DCAD diet is not a common practice, the results are not reflective of what would occur in commercial dairy applications. Another criticism is that the DCAD equation used in many of these studies did not include sulfur. When the NRC (2001) requirement of 0.20% DM of sulfur is added into the equation, the peak DCAD for milk fat yield would have been closer to 43 mEq/100 g DM, not 55 mEq/100 g DM as Hu and Murphy (2004) reported.

Increasing DCAD to >40 mEq/100 g DM in commercial dairy rations is most commonly done by increasing Na and K while meeting requirements (NRC, 2001) for Cl and S. The recommendation (NRC, 2001) of lactating dairy cows for K is 0.75% of DM, with maximum performance observed around 1.5% DM. Sodium levels below 0.20% DM were deficient for lactating cows, while maximum performance was reported to be 0.70 to 0.80% of DM. The recommendation (NRC, 2001) for Cl is 0.25% DM, with toxicity at 1.2% DM. Sulfur is more tightly regulated with a requirement (NRC, 2001) of 0.20% DM, and a maximal tolerable level of 0.40% DM. The lowest NRC (2001) requirements for K, Na, Cl, and S would provide a DCAD of only 8 mEq/100 g DM when S is incorporated in the DCAD calculation. With limited research available evaluating DCAD, NRC (2001) identified 38 mEq/100 g DM without S would maximize milk yield and DMI. If S was included, the DCAD for maximal milk yield would be 25 mEq/100 g DM. If maximal response concentrations of K and Na are used however, a DCAD of 51 mEq/100 g DM using the full equation would be optimal. Sanchez et al. (1994) found that fat-corrected milk and DMI were maximized with a DCAD between 30 and 50 mEq/100 g DM, without S inclusion. The NRC recommendation (2001) and the meta-analyses conducted by Hu and Murphy (2004) and Iwaniuk and Erdman (2015) do not agree on the optimum DCAD for lactation to maximize milk fat yield. Since these reports, there have been several studies conducted to help identify the optimum DCAD, as well as identify if K or Na is more impactful.

To identify if maintaining rumen pH with DCAD balancing was impacting the biohydrogenation of PUFA, studies were conducted that measured *trans*-10 *cis*-12 CLA, the cause of MFD in milk. Harrison et al. (2012) fed two diets balanced at 32 and 52 mEq/100 g

DM using potassium carbonate sesquihydrate to increase the K from 1.28% to 2.07% DM for 15 wk to early lactation Holstein cows. Diets contained moderate levels of lipids (3.6% and 3.1%), with grass hay as the main forage providing NDF (35%) above NRC (2001) recommendation. These diets lacked two of the factors expected to be present during MFD (high PUFA and low fiber). The cows fed a DCAD of 32 mEq/100 g DM had a milk fat yield of 1.58 kg/d and a milk fat percentage of 4.01%, where as the cows fed 53 mEq/100 g DM had a milk fat yield of 1.77 kg/d and a percentage of 4.38%. Although the 53 mEq/100 g DM treatment had greater fat yield and percentage, cows in either treatment were not experiencing MFD. When evaluating fatty acids in milk, *trans*-10 *cis*-12 CLA was not reduced, but *trans*-10 C18:1 was reduced and C18:0 was increased for cows fed the diet with a DCAD of 53 mEq/100 g DM. This study was not conclusive about the role potassium carbonate has in terms of the biohydrogenation theory but may support that its use can reduce the altered biohydrogenation pathway of *trans*-10 C18:1 (Harrison et al., 2012). The result also suggests a higher requirement for K than is currently recommended by NRC (2001), specifically for early lactation cows (Harrison et al., 2012). To evaluate the timing in which the addition of potassium carbonate increased milk fat, Guiling et al. (2017) used a switchback design comparing a DCAD of 37.7 and 54.3 mEq/100 g DM with K at 1.74 and 2.33% DM. The cows fed greater DCAD and K had an increase in milk fat percent after 3 d. The rapidity in milk fat change is similar to the recovery of milk fat percent after MFD caused by the infusion of *trans*-10 *cis*-12 CLA (Baumgard et al., 2001; Rico and Harvatine, 2013). Guiling et al. (2017) reported an increase of *trans*-11 C18:1 and suggested the addition of potassium carbonate sesquihydrate promoted the normal pathway of biohydrogenation. Harrison et al. (2012) fed diets that would limit the risk of MFD, while Guiling et al. (2017) fed similar diets but with added soybean oil to induce MFD. Both hypothesized that K may play a role in biohydrogenation, not just increasing rumen pH from greater buffering. The challenge with testing this hypothesis is that it is hard to evaluate the relationship between K and milk fat yield independent of the DCAD effect, or without using potassium chloride (KCl), which would affect DCAD equally in a negative and positive direction. Studies have evaluated lactation performance feeding KCl with mixed results. Mooney and Allen (2007) fed potassium carbonate, potassium chloride, sodium chloride, and sodium carbonate to high producing Holstein cows. Both carbonate treatments yielded more milk fat than the chloride

treatments, suggesting the buffering capacity impacted rumen function (Table 1.1). However, K treatments (bicarbonate or chloride) tended to increase fat corrected milk and milk fat yield compared to sodium treatments (bicarbonate or NaCl). This supports the findings of Guiling et al. (2017) and Harrison et al. (2012) that K may play a bigger role in biohydrogenation than just providing bicarbonate, possibly also due to the alkalogenic properties of K.

The larger role in milk fat yield K may play compared to Na could be related to differences in concentration within and across diets. Sodium ranged between 0.2% and 0.79% DM in DCAD studies, while K ranged between 1.0% and 2.64% (Wildman et al., 2007; Apper-Bossard et al., 2010; Iwaniuk et al., 2015; Alfonso-Avila et al., 2017). Both cation sources have a similar buffering capacity (Erdman, 1988); however, K makes up a larger percentage of the diet than Na when balancing for high levels of DCAD. Potassium is also one of the most abundant minerals in milk, and that concentration does not decrease when high producing cows lack supplemental dietary K (Barry and Rowland, 1953; Pradhan and Hemken, 1968). The demand for potassium in early lactation could result in a need for higher supplementation, which could explain the added benefits, specifically the increase in milk fat yield, observed in recent studies.

1.5 Conclusion

Producers are required to be more competitive to stay profitable. One of the major factors influencing the value of milk is the price of butter (Covington, 2017). As demand for butter has increased, the willingness to take steps toward producing more milk fat is evident. Researchers and nutritionists have put effort towards accomplishing this goal. High levels of milk fat can be achieved through the meticulous balancing of dietary energy, fiber, protein, and minerals. Ensuring that biohydrogenation of PUFA is through the *trans*-11 rather than the *trans*-10 pathway can result in a greater milk fat yield. The biohydrogenation theory identifies the production of *trans*-10 *cis*-12 CLA by rumen microbes as extremely detrimental to milk fat production, as it can lower milk fat yield up to 50% (Griinari et al., 1998; Harvatine and Allen, 2006). Although limiting *trans-10 cis-12* CLA production is multifactorial, balancing diets with adequate effective fiber, NFC, PUFA, and buffers can help reduce the risk. Understanding how DCAD and NFC, or the interaction between the two influence milk yield and milk fat yield can help nutritionist's better balance rations to decrease the risk of MFD, as

well as optimize milk fat yield. The efficacy of DCAD balancing in lactating cow rations has been very impactful in certain situations, however, economics does not always support its use. Currently, milk fat price has sufficient value where increasing DCAD should have a positive economic return for producers. Our research will evaluate past studies to identify possible interactions between level of dietary NFC and DCAD. It is our goal to identify if increasing DCAD has a positive impact on milk fat yield at all levels of NFC and if there are differences between levels of NFC.

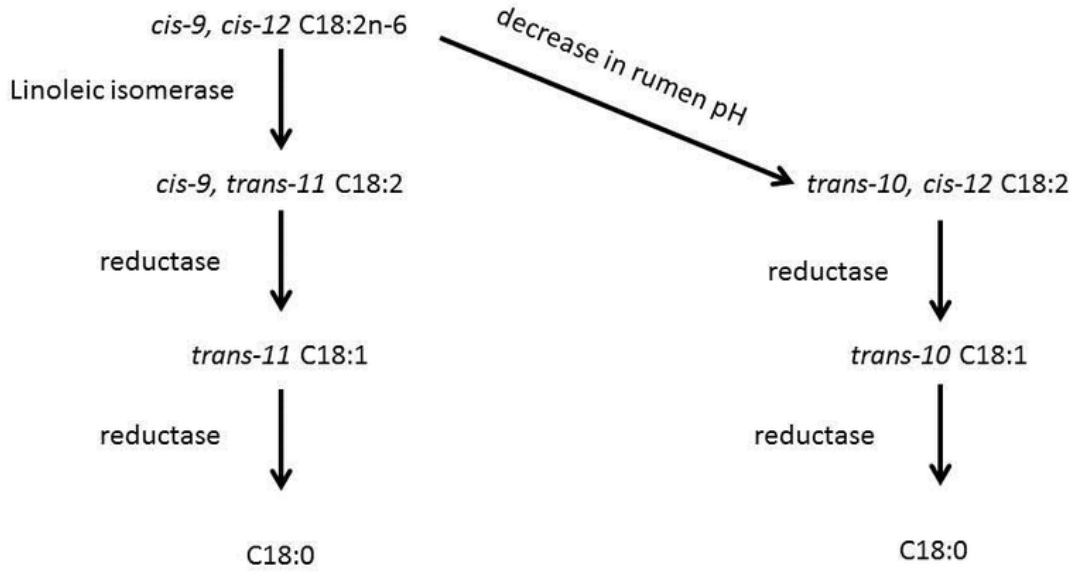

Figure 1.1: Primary and alternate biohydrogenation pathway for linoleic acid (adapted from Griinari and Bauman, 1999)

Table 1.1: Chemical properties of selected buffers	
Buffer	pKa
Potassium carbonate	6.25, 10.25
Potassium bicarbonate	6.25
Sodium bicarbonate	6.25
Sodium sesquicarbonate	6.25, 10.25

Chapter 2: Effects of DCAD and NFC on milk fat yield

2.1 Introduction

Milk fat has a large influence on the price dairy producers receive for their milk, such that formulating diets that improve rumen health and increase milk fat yield may improve dairy profitability. Recent research has shown that the use of buffers, which commonly increase the dietary cation-anion difference (DCAD), has a positive linear relationship with milk yield, milk fat yield, and rumen pH (Iwaniuk and Erdman, 2015). Enhancing the digestible energy density of the diet through greater inclusion of non-fiber carbohydrates (NFC), however, identified a quadratic relationship with milk fat yield with maximum yield at approximately 36 to 38% diet DM (Robinson and McQueen, 1997; Valadares Filho et al., 1999). Excess NFC can lead to a reduction in rumen pH, DM intake (DMI), and a disruption of bacterial and enzymatic processes (Erfle et al., 1982; Grant, 1993). Furthermore, low rumen pH can encourage an altered biohydrogenation pathway for linoleic acid to produce *trans-10 cis-12* CLA, which inhibits milk fat synthesis (Griinari et al., 1998; Baumgard et al., 2000a). The relationship among physically effective fiber, rumen pH, and milk fat percent is well established; increasing NFC is related to a reduction in all three (Allen, 1997; Beauchemin et al., 2006). Interactions among dietary factors can make it challenging to identify optimal levels of NFC and DCAD to maximize milk fat yield. Previous studies (Apper-Bossard et al., 2010; Erdman et al., 2011; Iwaniuk et al., 2015) have evaluated the effects of DCAD and NFC on lactation performance. Comparison among the studies is difficult due to differences in DCAD or NFC. The objective of this study was to evaluate the effect of DCAD within different levels of NFC on milk fat yield using data from the literature. We hypothesized that increasing DCAD would improve milk fat yield at all levels of NFC, and the response to DCAD would be more impactful as NFC in diets became greater.

2.2 Materials and methods

2.2.1 Data collection and assembly

A search for studies in Google scholar, PubMed, and the University of Idaho catalog database (over 15 publicly available databases) was performed using the keywords dietary cation-anion difference, non-fiber carbohydrates, and lactating dairy cows. Sixty-five journal articles were examined for completeness of information necessary for inclusion. All journal articles used were published in the *Journal of Dairy Science* except for Lamar et al. (2013), a thesis published at Ohio State University, and Zali et al. (2019) published in *Preventive Veterinary Medicine*. Of the 65 articles, 43 were used in a previous meta-analysis (Iwaniuk and Erdman, 2015) with the remainder published after that analysis. For each treatment diet, the concentration of DCAD (milliequivalents of (Na + K) - (Cl + S) per 100 g/DM) or the concentration of each mineral necessary to calculate DCAD was required. The concentration of NFC was calculated using (100 - (CP + NDF + fat + ash) if not provided. Three of the four components used in the calculation of NFC (CP, NDF, fat, and ash) were needed for the study to be included. However, studies that did not report CP or NDF were not included as those nutrients were considered too variable in feeds to use NRC (2001) values to generate a dietary concentration. The concentration of fat or ash was estimated using NRC (2001) values if one was not reported. Only studies that reported milk fat yield, or milk yield and milk fat percent so milk fat yield could be calculated were used. Data collected for cow performance included treatment means for milk yield, milk fat yield, milk fat percent, milk protein yield, milk protein percent, and DMI. Feed efficiency (FE) was calculated using the energy corrected equation [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)] divided by DMI (NRC, 2001).

2.2.2 Acceptable studies

Common reasons for rejection were the lack of both fat and ash concentration or the lack of S or Cl concentration. After the removal of articles with insufficient data, 15 studies with 62 observations for comparison were used in the data set (Table 2.1). Fat content was estimated for treatments in Borucki Castro et al. (2004) and Apper-Bossard et al. (2010). Iwaniuk et al. (2015) reported basal fatty acid diet concentration and fatty acid additions separately; the two were added together for total fatty acid concentration of the diets. Ash content was estimated and DCAD calculated for treatments in Wildman et al. (2007). Milk

protein percent was calculated using milk yield and milk protein yield for Zali et al. (2019), and milk fat yield was calculated for Erdman et al. (2011). Lamar et al. (2013) reported treatment means for all 3 wk of the study, as well as the last wk separately to evaluate response to treatment over time, which provided two different treatment means used in the analysis. Treatments within studies that had DCAD values below 0 were excluded from the dataset as those diets do not represent diets fed on commercial dairies. The final data set consisted of 60 feeding treatments with 362 cows. The cows in the study were 84.2% multiparous, 15.8% primiparous, 97.7% Holstein 2.3% Jersey, and days in milk ranged from 1 to 381. Diets all contained corn silage and most included some form of corn with NFC ranging from 33.3 to 49.5% DM and DCAD ranging from 9.8 to 54.6 mEq/100 g DM (Table 2.1).

2.2.3 Statistical Analysis

Seven performance variables were evaluated based on their response to NFC and DCAD levels. Milk yield, milk fat yield, milk protein yield, milk fat percent, milk protein percent, DMI, and FE were analyzed using the linear mixed model procedure with a normal distribution, $Y_{ijk} = i$ (NFC level) + i (NFC level) * j (DCAD) + k (study) + ijk (error). The fixed effect of three levels of NFC was used while DCAD was a continuous variable. The model included the random effect for study, and the studies were weighted by the inverse of the variance of the dependent variables (Borenstein et al., 2009). The NFC levels of Low (33.3 to 40.90% DM, n=21), Medium (40.97 to 44.82% DM, n=19), and High (45.3 to 49.5% DM, n=22) were selected using natural gaps in the NFC values that closely balanced the data among bins (Figure 2.1). The distribution of DCAD observations within each NFC level was plotted (Figure 2.2). Treatments with a DCAD of 2 mEq/100 g DM were all assigned to Low NFC. The absence of low (<9.8 mEq/100 g DM) DCAD values in Medium NFC and High NFC supported removal of 4 treatments in Low NFC from the analysis, providing a more similar minimum DCAD for each NFC level (Figure 2.2). Not all studies reported FE so no variance was recorded. Therefore, the regression analysis for FE did not have the studies weighted by the inverse of their variance. Residual plots were examined for homogeneity of variance and normality of error terms. Comparisons among levels of NFC and DCAD within level of NFC were conducted by ANOVA. All models met assumptions of independently and identically distributed error terms. Significance was declared at $P < 0.05$. All statistical

analyses were performed using R version 3.6.1 (R Development Core Team, 2019) using packages ‘lme4’ for mixed model analysis (Bates et al., 2015). Estimated marginal means were calculated using the package ‘emmeans’ and ‘emtrends’ for pairwise comparison (Length, 2019).

2.3 Results and discussion

The NFC concentration (mean ± SE) differed ($P < 0.001$) among Low NFC, Medium NFC, and High NFC, and were 38.6 ± 0.43, 42.7 ± 0.26, and 47.1 ±0.26, respectively (Figure 2.1). The NFC levels were higher than expected as research (Batajoo and Shaver, 1994; Valadares Filho et al., 1999) has shown that milk fat yield or percent is decreased with NFC levels above 42% DM. Some of the studies included examined how DCAD and high concentrate diets affected performance, and therefore had an expanded range of NFC (Apper-Bossard et al., 2010; Iwaniuk et al., 2015). The DCAD concentration did not differ ($P = 0.34$) among levels of NFC with values (mean ± SE) of 32.0 ± 2.81, 26.5 ± 2.82, and 30.8 ± 2.44 mEq/100 g DM for Low NFC, Medium NFC, and High NFC, respectively (Figure 2.2). For each NFC category, the mean concentration of DCAD was above 25 mEq/100 g DM as recommended by NRC (2001). The mean DCAD concentration of the current analysis (Table 2.2) was 7.3 mEq/100 g DM greater than Iwaniuk and Erdman (2015). This was due to the exclusion of negative DCAD diets and low DCAD observations from the current analysis. Milk yield, milk fat yield, milk protein yield, and DMI were higher for this study (Table 2.2) compared to a previous analysis (Iwaniuk and Erdman, 2015). Milk fat percent was similar, but milk protein percent was lower. No data for NFC was provided by Iwaniuk and Erdman (2015), but mean NDF was 3.6% lower in the current analysis while CP was similar. This would suggest that the mean NFC for the current analysis could be higher, although no means for fat or ash were provided by Iwaniuk and Erdman (2015) to calculate NFC for comparison.

2.3.1 Milk fat yield

Regression analysis (Table 2.3) identified no difference in milk fat yield in Medium NFC compared to the model intercept (Low NFC). This is consistent with Batajoo and Shaver (1994) who reported no difference in milk fat yield when treatments of 24, 30, 36, and 42% DM NFC were fed. Contrary to our result, Valadares Filho et al. (1999) reported that a reduction in milk fat yield occurred when NFC levels reached 42.8% DM. Significant

decreases in milk fat yield have been linked to low rumen pH (Griinari et al., 1998) as an altered pathway of biohydrogenation isomerizes linoleic acid to *trans*-10 *cis*-12 CLA, a bioactive FA known to cause MFD (Baumgard et al., 2000b). Comparing rumen pH measures for Batajoo and Shaver (1994) to Valadares et al. (1999), increasing NFC reduced mean rumen pH for both. However, the reduction in milk fat yield observed in Valadares Filho et al. (1999) at 42.8% NFC compared to 36% was not dependent on mean rumen pH, as both were 6.08. The extent of the daily range or nadir of rumen pH and length of time rumen pH was below 6.0 can impact a cows risk for subacute ruminal acidosis (SARA; Dohme et al., 2008); neither were reported for comparison. The difference in forage concentration for Valadares Filho et al. (1999) 42.8% NFC treatment and Batajoo and Shaver (1994) 42% NFC treatment was 12.2%. Perhaps a greater forage concentration for Batajoo and Shaver (1999) impacted fermentation characteristics and the range and nadir rumen pH (Dohme et al., 2008; Schwab et al., 2006). The current study found reduced milk fat yield occurred at NFC concentrations >45.3% DM (Figure 2.3), a value greater than the one found by Valadares Filho et al. (1999). Differences in forage type and amount fed were apparent, as Valadares Filho et al. (1999) fed alfalfa silage as the only forage, where all studies within High NFC fed corn silage. The work of Valadares Filho et al. (1999) differs from other reports (Broderick, 1985; Onetti et al., 2002; Ruppert et al., 2003) which support our results for High NFC with observations that milk fat yield was reduced when corn silage was the main or sole forage source compared to alfalfa. Different factors such as forage amount or type can impact rumen pH and milk fat yield beyond NFC (Robinson and McQueen, 1997; Schwab et al., 2006). The 42.8% NFC treatment in Valadares et al. (1999) had only 35% forage, where the mean forage concentration for treatments within Medium NFC for this analysis was 54.5%, closer to that of Batajoo and Shaver (1994). The disagreement between results in amount of forage fed may have led to a difference in rumen pH range or nadir but neither were reported. Improved understanding of the role of biohydrogenation in MFD (Bauman and Griinari, 2003) and how to better utilize forage and dietary components to stabilize rumen pH could have changed how increasing NFC impacted milk fat yield from high producing cows for studies in this analysis.

With regard to DCAD, milk fat yield increased linearly with increasing DCAD (Figure 2.3), similar to that found in the meta-analysis of Iwaniuk and Erdman (2015). Greater milk fat yield within each level of NFC was found as DCAD increased (Table 2.3, Figure 2.3) but

the response to DCAD did not vary by NFC category. The slope for response to DCAD was not different (P = 0.125) for the per unit increase in DCAD for High NFC compared to Medium NFC. An interaction of DCAD and concentrate (a proxy for NFC) on milk fat yield was apparent in Apper-Bossard et al. (2006), but fat and ash were not reported to estimate NFC. Additions of buffer that increase DCAD reduced the daily range of rumen pH (Apper-Bossard et al., 2010), suggesting this could be one mechanism by which DCAD increases milk fat yield. Milk fat yield response to DCAD was also not different between Low NFC and High NFC. The response to increasing DCAD for Low NFC was greater than expected as diets with NFC concentration below 42% are at lower risk of MFD (Griinari et al., 1998; Valadares Filho et al., 1999). Harrison et al. (2012), however, did report greater milk fat yield (0.009 kg/d) per unit increase in DCAD from 32 to 53 mEq/100 g DM feeding diets with NFC concentrations within Low NFC. Our results suggest a response of 0.0043 kg/d per unit increase of DCAD for Low NFC, half that of Harrison et al. (2012).

Rumen and blood pH are increased when additional buffers that increase DCAD are fed (Hu and Murphy, 2004). Increasing rumen pH or reducing the daily range of rumen pH associated with greater DCAD could reduce the *trans*-10 biohydrogenation pathway of linoleic acid shown to be related to MFD (Griinari et al., 1998; Apper-Bossard et al., 2010). Diets higher in NFC (>45%) may have a greater response to DCAD if daily range of rumen pH is greater (Apper-Bossard et al., 2010). Diets lower in NFC may be at more risk for MFD than previously thought because of the increase in milk fat yield with additional DCAD. The transfer of short-chain fatty acids (SCFA) from the rumen fluid to the ruminal epithelium and finally into the bloodstream requires the exchange of bicarbonate to neutralize the proton from the dissociated SCFA to maintain neutrality within the cell (Penner, 2019). For SCFA such as lactate, the proton can be dissociated within the cell requiring either bicarbonate or sodium/hydrogen channels to neutralize or transport the proton out of the cell (Aschenbach et al., 2011; Penner, 2019). This suggests that rumen buffering is mediated not only from bicarbonate production from saliva but also from the absorption process of SCFA which requires bicarbonate from the blood (Aschenbach et al., 2011). Tucker et al. (1994) showed that the inclusion of 1% Na sesquicarbonate to diets increased blood bicarbonate. In a meta-analysis, Hu and Murphy (2004) showed that blood bicarbonate increased linearly with DCAD. The increase in rumen pH, or reduction in the daily range of rumen pH, when high

concentrations of a buffer are fed could be facilitated by direct rumen neutralization of protons, as well as the increased concentration of carbonate in the blood facilitating the transfer of SCFA (Hu and Murphy, 2004; Aschenbach et al., 2011; Iwaniuk and Erdman, 2015; Penner, 2019; Apper-Bossard et al., 2010).

2.3.2 Milk fat percent

Regression analysis found no difference in milk fat percent for Medium NFC compared to the model intercept (Low NFC; Table 2.3). This result was surprising as others (Batajoo and Shaver 1994; Valadares Filho et al., 1999) have reported a reduction in milk fat percent with increasing NFC. Both of these studies fed alfalfa silage as the sole forage, where corn silage was the main forage source in all the studies in the current meta-analysis. Batajoo and Shaver (1994) linked the reduction in milk fat percent to a reduction in rumen pH, similar to previous research (Sievert and Shaver, 1993). Batajoo and Shaver (1994) did not however, report a reduction in milk fat yield, suggesting milk fat synthesis was not reduced. Milk fat percent was reduced ($P < 0.01$) 0.67 percentage units between High NFC and Low NFC (Table 2.3, Figure 2.4). High levels of NFC (>46% DM) have been connected to a reduction in milk fat percent (Iwaniuk et al. 2015), possibly because of an increase in the range or nadir of rumen pH when cows are fed diets rich in NFC (Dohme et al., 2008; Apper-Bossard et al., 2010). Griinari et al. (1998) showed a reduction in milk fat percent of 0.22 percentage unit when NFC was increased from 31 to 47.5 % DM. This response was magnified when SFA was substituted with PUFA, as milk fat percent was reduced from 3.33 to 2.49%.

Milk fat percent had a positive linear relationship with DCAD (Figure 2.4), consistent with Iwaniuk and Erdman (2015). Milk fat percent within each NFC level was positively related to DCAD (Table 2.3), although differences were not detected in the slopes for the response to DCAD within a level of NFC. Apper-Bossard et al. (2010) also reported an increase in milk fat percent with increasing DCAD for diets within our categories of low and medium NFC. More recently, research confirmed that milk fat percent in cows fed diets low in NFC can be increased through additional DCAD and that even cows producing high fat percent (>4.0%) benefit (Harrison et al., 2012; Guiling et al., 2017). Our results demonstrated milk fat percent improved ($P < 0.001$) 0.011% and 0.014% percentage units per unit of DCAD for Low NFC and Medium NFC, respectively (Table 2.3). These results are similar to

that of Guiling et al. (2017), who reported that increasing DCAD 16.6 mEq/100 g DM increased milk fat percent by 0.014% per unit of DCAD. Apper-Bossard et al. (2006) showed greater milk fat percent by elevating DCAD in higher concentrate diets with milk fat percentages of 3.41, 3.55, and 3.80 when cows were fed a DCAD of 4.0, 15.6, 30.6 mEq/100 g DM, respectively. This is consistent with our results for High NFC, where milk fat percentage increased ($P < 0.001$) 0.022% per unit increase in DCAD.

Jenkins and Harvatine (2014) used continuous cultures to evaluate the extent to which K sesquicarbonate impacts biohydrogenation of linoleic acid from soybean oil. They reported that additions of K sesquicarbonate increased in culture pH and reduced the total *trans*-10 *cis*-12 CLA and increased *cis*-9 *trans*-11 CLA, the normal pathway of biohydrogenation. Rumen pH is increased with diets greater in DCAD (Iwaniuk and Erdman, 2015) which could mitigate the production of MFD bioactive intermediates by promoting the *trans*-11 biohydrogenation pathway of linoleic acid (Griinari and Bauman, 1999; Guiling et al., 2017). The positive milk fat percent response to DCAD was evident in all NFC levels (Table 2.3). This suggests that diets lower in NFC may still require higher DCAD to buffer the rumen and blood that supports transport of SCFA across the rumen epithelium, which may indirectly reduce bioactive intermediates that reduce milk fat synthesis.

2.3.3 Milk yield

Milk yield increased as NFC increased (Figure 2.5). An additional 2.66 kg/d ($P<0.01$) of milk was attributed to Medium NFC and 5.33 kg/d for High NFC compared to the model intercept (Low NFC; Table 2.3). Valadares Filho et al. (1999) detected 3.6 kg/d greater milk yield when NFC was increased from 36.2 to 42.8% DM. High levels of NFC have not consistently resulted in increased milk yield. For example, Griinari et al. (1998) increased NFC from 31.0 to 47.5% DM and reported a reduction in milk yield of 2.8 kg/d. High NFC diets can reduce rumen pH and feed intake while increasing the risk of ruminal acidosis (Allen, 1997). Greater milk yield for High NFC suggests that a change in microbial populations or fermentation led to greater propionate production aiding lactose synthesis but producing biohydrogenation intermediates that impact milk fat synthesis as milk fat percent and milk fat yield were significantly reduced (Bauman et al., 2011a).

Milk yield was increased ($P < 0.01$) by DCAD for Low NFC, but DCAD had no effect on milk yield in Medium NFC (Table 2.3, Figure 2.5). A tendency ($P < 0.1$) for DCAD to reduce milk yield was detected for High NFC (Table 2.3); caution may be appropriate given the limited number of DCAD values above 40 mEq/100 g DM in High NFC (Figure 2.2). Previous meta-analyses examining the effects of DCAD on milk yield found a quadratic relationship or nonlinear increase (Iwaniuk and Erdman, 2015; Hu and Murphy, 2004). These studies included diets with a range of DCAD levels from -6.77 to 81.09 mEq/100 g DM for Iwaniuk and Erdman (2015) and -19.10 to 63.57 mEq/100 g DM for Hu and Murphy. (2004). Diets balanced for negative DCAD have resulted in a reduction in milk yield (Escobosa et al., 1984; Tucker et al., 1988; Apper-Bossard and Peyraud, 2004) and thus are not appropriate when considering how to increase milk yield. Exclusion of negative DCAD may explain the lack of consistent response to DCAD with that of Iwaniuk and Erdman (2015). The difference in slope for a unit increase in DCAD was 0.145 kg/d greater milk yield for Low NFC compared to High NFC (Table 2.3). No difference in slope between Low NFC and Medium NFC was detected. The response of milk yield to increasing DCAD for Low NFC is positive, which is consistent with Iwaniuk and Erdman (2015). The lack of response for Medium NFC was consistent with Iwaniuk et al. (2015), who reported no increase in milk yield in cows fed that range of NFC with increasing DCAD. The tendency for reduced milk yield with increasing DCAD for High NFC possibly follows a biological pattern. Iwaniuk and Erdman (2015) reported that increasing DCAD from 0 to 50 mEq/100 g DM increased rumen pH from 6.31 to 6.46 and the acetate to propionate ratio from 2.01 to 2.71. Their work did not find a reduction in propionate production, but Fraley et al. (2015) showed a reduction in the molar production of propionate and an increase in the ratio of acetate to propionate with greater K carbonate supplementation which increased DCAD from 16.0 to 53.5 mEq/100 g DM. If the production of propionate is reduced, a reduction in milk yield would be expected given the relationship to gluconeogenesis and lactose synthesis (Aschenbach et al., 2010; Lin et al., 2016).

2.3.4 Milk protein yield and percent

Milk protein yield increased with increasing NFC (Figure 2.6). An additional ($P < 0.05$) 0.153 and 0.176 kg/d of milk protein were detected for Medium NFC and High NFC, respectively, compared to the model intercept (Low NFC, Table 2.3). Apper-Bossard et al.

(2010) reported an improvement in milk protein from 0.99 kg/d to 1.21 kg/d when NFC was increased from 39.98 to 43.73%. Valadares Filho et al. (1999) reported that milk protein percent or yield was positively linearly related to NFC, consistent with what was detected in the current study. The increase in milk protein yield was a combination of greater milk protein percent ($P < 0.05$) and milk yield for Medium NFC compared to Low NFC (Table 2.3, Figure 2.7). Only greater milk yield contributed to greater milk protein yield for High NFC. There was no response in milk protein yield or milk protein percent to DCAD at any NFC level (Table 2.3, Figure 2.6 and 2.7), consistent with Iwaniuk and Erdman (2015). The positive linear relationship with DCAD and milk protein yield found previously (Hu and Murphy, 2004) may have most likely been related to an increase in milk yield and not protein percent.

2.3.5 Dry matter intake and feed efficiency

Compared to Low NFC, DMI was greater (2.04 kg/d; $P < 0.05$) for Medium NFC but not different for High NFC (Table 2.3). This contradicts with results from Valadares Filho et al. (1999) who detected a maximum at 36.2% DM, and a reduction in DMI when NFC was increased to 42.8% DM. More consistent with our result, Apper-Bossard et al. (2010) reported an additional 3.3 kg/d DMI as NFC increased from 40.0 to 43.7% DM. No reduction in DMI for High NFC differed from previous research (Griinari et al., 1998). Treatments within High NFC in this analysis also increased milk yield but milk fat percent and yield were significantly reduced. This suggests that the negative effects of elevated NFC concerning DMI and acidosis were not apparent, but sub-acute rumen acidosis may have been apparent in the studies contributing to the meta-analysis for High NFC. Feed efficiency was not different among NFC levels (Table 2.3, Figure 2.9). This is consistent with Batajoo and Shaver (1994) who determined no difference in FE in cows fed 36 and 42% NFC, and Apper-Bossard et al. (2010) for FE in cows fed diets containing 39.9 to 43.9% NFC.

No interaction of DCAD within a level of NFC impacted DMI (Table 2.3, Figure 2.8). This is consistent with Guiling et al. (2017) who reported no change in DMI or milk yield when DCAD was increased from 37.7 to 54.3 mEq/100 g DM when cows were fed 38% NFC. Harrison et al. (2012) did report an increase in DMI when DCAD was elevated from 32 to 53 mEq/100 g DM, but feed efficiency was improved by 0.08 unit. This increase in FE related to DCAD was evident for Medium NFC in our analysis as well (Table 2.3, Figure 2.9). Greater

milk fat percent and yield without additional DMI means more energy supplied by either the diet or body reserves would need to be partitioned to the mammary gland for milk fat synthesis (Baumgard et al., 2017). Increasing NFC had no impact on FE, but increasing DCAD within Medium NFC elevated ($P<0.05$) FE 0.003 units per unit increase in DCAD. This suggests that the additional energy supplied in the diet for Medium NFC compared to Low NFC, or greater mobilization of body reserves was partitioned to the mammary gland for milk and milk fat synthesis.

2.3.6 Mechanism of action

Milk fat yield was not different for Low NFC and Medium NFC but significantly reduced for High NFC. Studies have shown that increasing NFC can significantly reduce milk fat yield (Griinari et al., 1998; Iwaniuk et al., 2015). The production of bioactive FA intermediates such as *trans*-10 *cis*-12 CLA tied to reduced rumen pH is a potential mechanism for this significant reduction in milk fat yield (Baumgard et al., 2000a; Bauman et al., 2011). Increasing DCAD had a positive linear effect on milk fat yield for all NFC levels. Some studies (Harrison et al., 2012; Guiling et al., 2017) have shown greater DCAD increased the concentration of *trans*-11 18:1 FA while reduced *trans*-10 18:1 FA in milk, likely with a reduction of *trans*-10 *cis*-12 production in the rumen. Increased rumen pH or reduction in the variability of rumen pH with DCAD could reduce the biohydrogenation of linoleic acid to *trans*-10 *cis*-12 CLA. To evaluate this mechanism further, more studies feeding varying levels of NFC and DCAD with measures of *trans*-10 18:1 FA, *trans*-10 *cis*-12 CLA, measures of milk fat synthesis, and measures of daily rumen pH variation are needed. Few studies reported the concentration of linoleic acid in diets to include its role as a significant risk factor for production of *trans*-10 *cis*-12 CLA in the rumen. The mean fat concentration across all treatment diets included in our study was 3.7% with the highest treatment at 5.9% DM. This may be considered relatively low as recommendations suggest feeding higher levels of dietary fat (Palmquist and Jenkins, 2017). The benefits of increasing DCAD on milk fat yield suggest a greater ability to increase rumen pH or reduce the variability in rumen pH, enhancing SCFA absorption by the rumen epithelium, and an indirect reduction of bioactive FA intermediates that reduce milk fat synthesis.

2.4 Conclusions

The objective of this study was met as the response of milk fat yield to DCAD and NFC was determined. No difference in milk fat yield for Low NFC compared to Medium NFC was found, but a reduction in milk fat yield with High NFC was observed due to a dramatic decrease in milk fat percent. This effect is consistent with previous research, although, the decline in milk fat yield occurred at higher NFC levels than previously reported. There was a positive relationship of milk fat yield to DCAD for all NFC levels. A tendency for a greater increase in milk fat yield to DCAD was found for High NFC compared to Medium NFC, which supports our hypothesis that DCAD modifies the response of milk fat yield to NFC. Dietary formulations intended to increase the yield of milk fat should consider both NFC and DCAD. This analysis suggest that treatments within Medium NFC (40.97 to 44.82% diet DM) had the greatest total milk fat and protein yield (2.34 kg/d), and that increasing DCAD within this NFC level improved milk fat yield.

Table 2.1: Brief description of studies used in the analysis

Reference	Diets	Breed (number of cows)	Range of NFC (%DM)	Range of DCAD[1] (mEq/100 g DM)
Alfonso-Avila et al. (2017)	5	Holstein (35)	47.0	10.7 to 32.6
Apper-Bossard et al. (2010)	6	Holstein (6)	39.5 to 44.8	11.0 to 32.7
Borucki Castro et al. (2004)	4	Holstein (4)	33.3 to 35.9	14.0 to 45.0
Chan et al. (2005)	3	Holstein (33)	39.8 to 40.9	22.7 to 54.6
Erdman et al. (2011)	3	Holstein (45)	38.9 to 42.6	28.1 to 33.6
Guiling et al. (2017)	2	Holstein (10)	37.4 to 38.3	37.7 to 54.3
Harrison et al. (2012)	2	Holstein (26)	37.5 to 38.3	32.0 to 53.0
Hu et al. (2007a)	4	Holstein (6)	46.3 to 49.1	20.5 to 47.5
Hu et al. (2007b)	2	Holstein (16) Jersey (8)	46.3 to 49.5	21.8 to 51.1
Iwaniuk et al. (2015)	12	Holstein (60)	40.6 to 47.9	16.4 to 54.4
Lamar et al. (2013)	2	Holstein (16)	38.7 to 39.7	29.0 to 31.0
Martins et al. (2015)	3	Holstein (16)	40.9 to 41.7	9.8 to 29.0
Mooney and Allen (2007)	5	Holstein (40)	43.1 to 43.4	16.1 to 27.6
Wildman et al. (2007)	4	Holstein (32)	40.4 to 44.2	25.0 to 50.0
Zali et al. (2019)	3	Holstein (9)	45.3 to 45.6	32.7 to 36.9

[1]DCAD was calculated as (K + Na) - (Cl + S)

Table 2.2: Mean, standard deviation, and range of nutrient composition, milk production responses, feed intake and feed efficiency across fifteen studies used in the meta-analysis[1]

Variable	Mean	SD	Minimum	Maximum
Diet composition				
NFC (% of DM)	42.9	3.86	33.3	49.5
DCAD (mEq/100 g DM)	29.9	12.13	9.8	54.6
K (% of DM)	1.52	0.42	0.91	2.64
Na (% of DM)	0.36	0.17	0.17	0.79
Cl (% of DM)	0.50	0.18	0.29	1.11
S (% of DM)	0.22	0.07	0.11	0.37
CP (% of DM)	16.3	1.36	14.2	19.9
NDF (% of DM)	30.9	3.39	26.3	38.8
Fat (% of DM)	3.7	0.86	2.6	5.9
Ash (% of DM)	6.8	1.23	3.5	9.0
Milk				
Yield (kg/d)	34.7	5.37	22.0	47.3
Fat percent (%)	3.59	0.45	2.39	4.32
Fat yield (kg/d)	1.23	0.24	0.72	2.01
Protein percent (%)	3.07	0.15	2.78	3.56
Protein yield (kg/d)	1.05	0.15	0.69	1.22
Dry matter intake (kg/d)	22.7	2.93	15.3	27.8
Feed efficiency	1.53	0.20	1.0	1.94

[1] Studies represent 62 observations, 60 diet treatment diets, 362 cows.

[2] Energy corrected milk [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)] (NRC, 2001) divided by dry matter intake.

Table 2.3: Response of milk yield, milk composition, feed intake and feed efficiency to dietary level of NFC and DCAD[1]

	Model Intercept[2]	Medium NFC	High NFC	Low NFC : DCAD	Medium NFC: DCAD	High NFC : DCAD
Milk (kg/d)	31.61***	2.66**	5.33**	0.067*[a]	0.026[ab]	-0.078†[b]
	±1.85	±0.85	±1.80	±0.025	±0.022	±0.044
Fat (kg/d)	1.149***	0.094	-0.242**	0.004*	0.003**	0.007***
	±0.103	±0.072	±0.082	±0.0018	±0.001	±0.0017
Fat (%)	3.46***	-0.002	-0.667**	0.011***	0.014***	0.022***
	±0.153	±0.139	±0.221	±0.003	±0.003	±0.006
Protein (kg/d)	0.949***	0.153**	0.176*	0.002	-0.001	-0.002
	±0.067	±0.047	±0.066	±0.001	±0.001	±0.002
Protein (%)	2.99***	0.162*	0.141	0.004	-0.0014	-0.0002
	±0.065	±0.068	±0.091	±0.001	±0.001	±0.002
DMI (kg/d)	21.01***	2.04*	1.66	0.038	0.013	-0.007
	±1.13	±0.92	±1.39	±0.027	±0.021	±0.033
Feed efficiency	1.52***	-0.044	-0.032	0.001	0.003*	0.001
	±0.069	±0.059	±0.064	±0.001	±0.001	±0.001

[1]Regressions were performed with Low NFC as the intercept. The range of NFC for NFC Low NFC, Medium NFC, and High NFC was (33.3-40.90, 40.97-44.82, and 45.3-49.5% DM), respectively. The range for DCAD within Low NFC, Medium NFC, and High NFC was (14.0-54.6, 9.8-54.4, and 10.7-54.4 mEq/100 g DM), respectively.

[2]Values in each cell are means of the regression above ± SEM. Differences from zero for each regression term are denoted by ***P<0.001, ** P<0.01, * P<0.05, and † P<0.10. Interaction values within a response variable that do not share a letter differ (P<0.05)

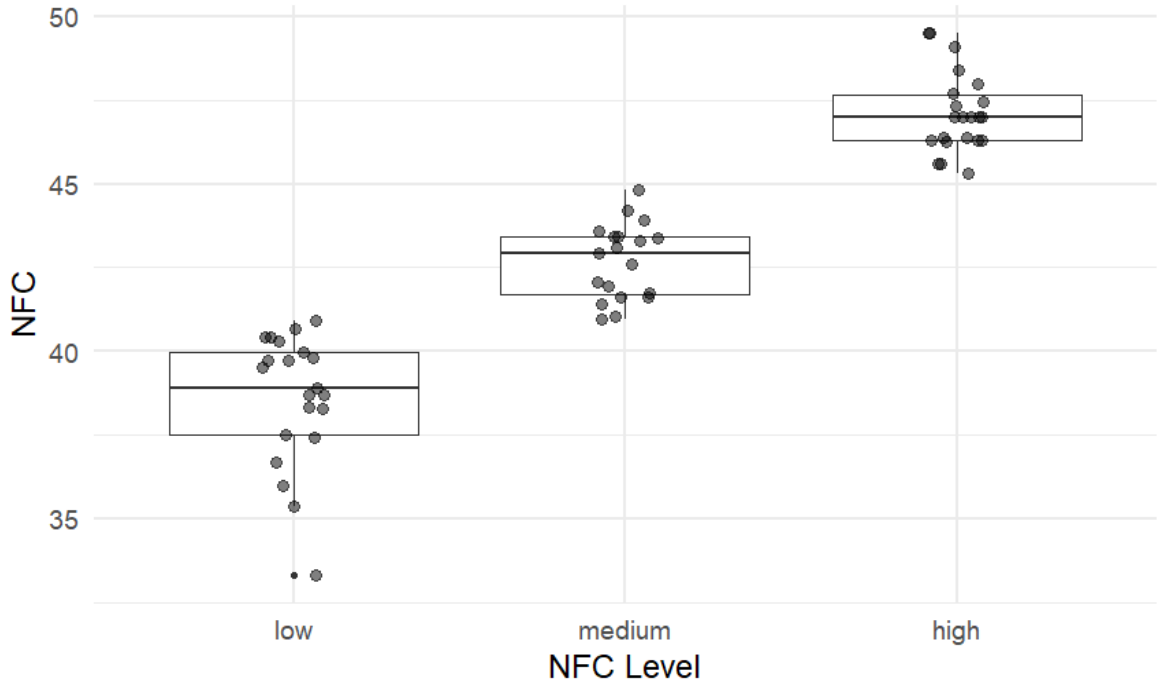

Figure 2.1: Distribution of treatment observations for NFC within each NFC level. The dots represent individual dietary treatments (n=60) from studies (n=15) with 362 cows.

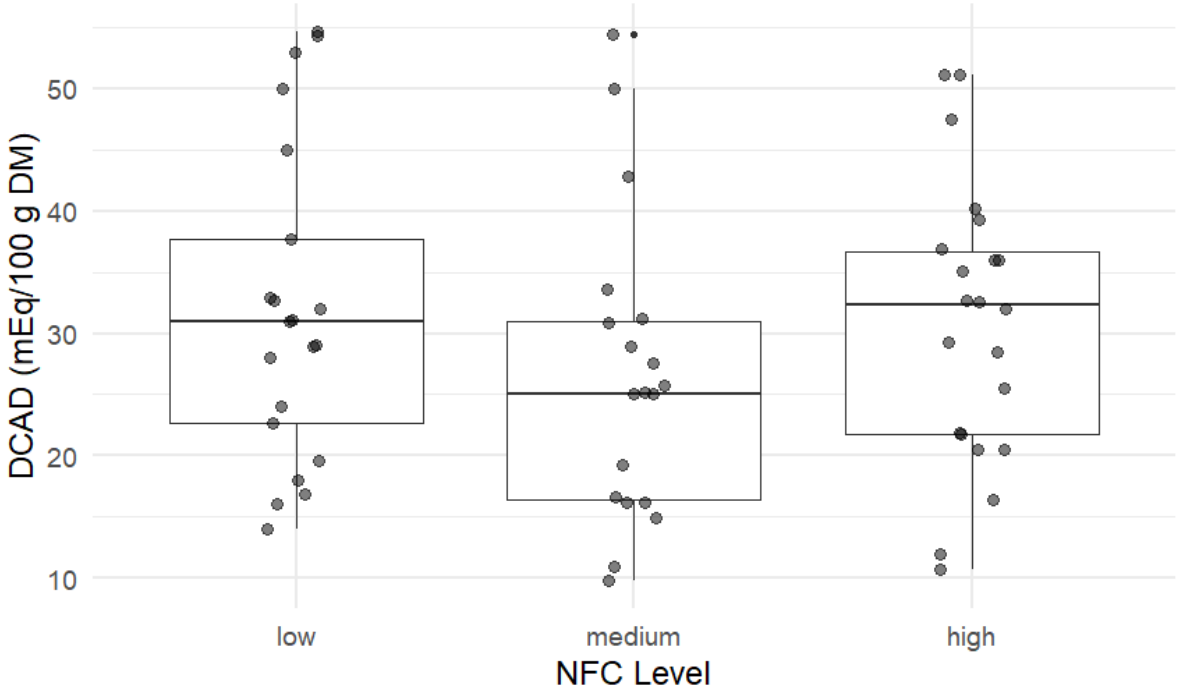

Figure 2.2: Distribution of treatment observations for DCAD within each NFC level. The dots represent individual dietary treatments (n=62) from studies (n=15) with 362 cows.

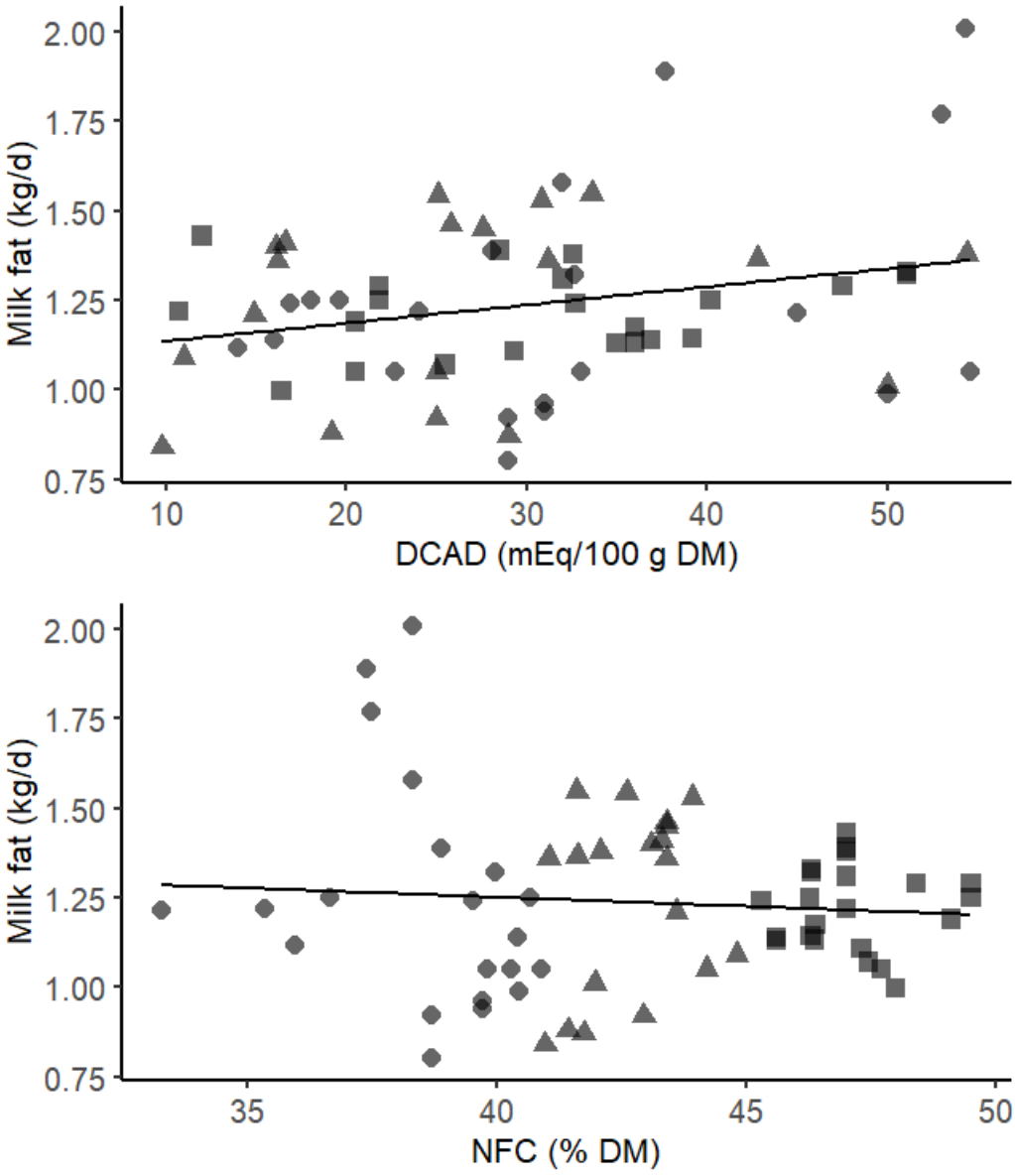

Figure 2.3 Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on milk fat yield

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

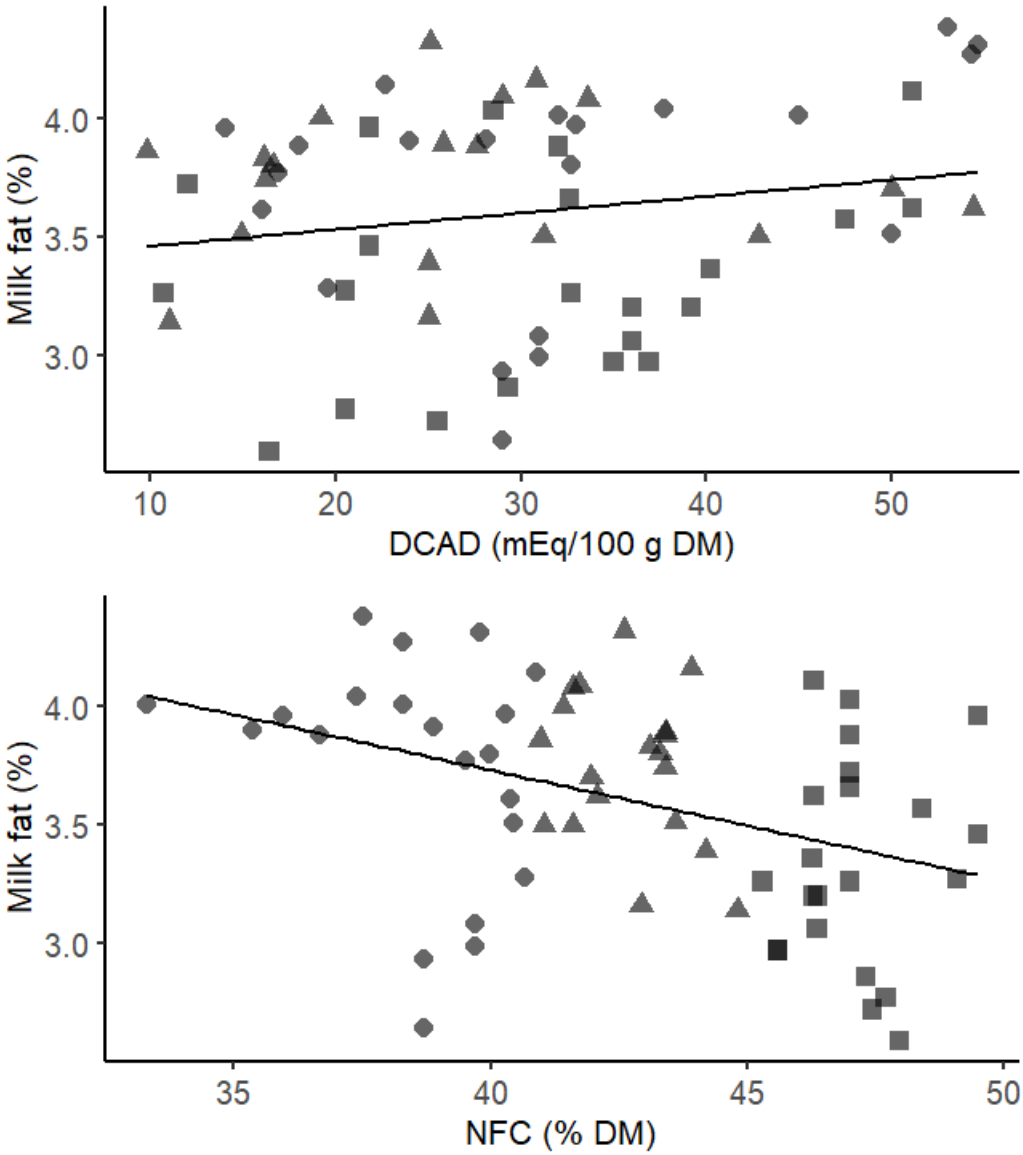

Figure 2.4: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on milk fat percent.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

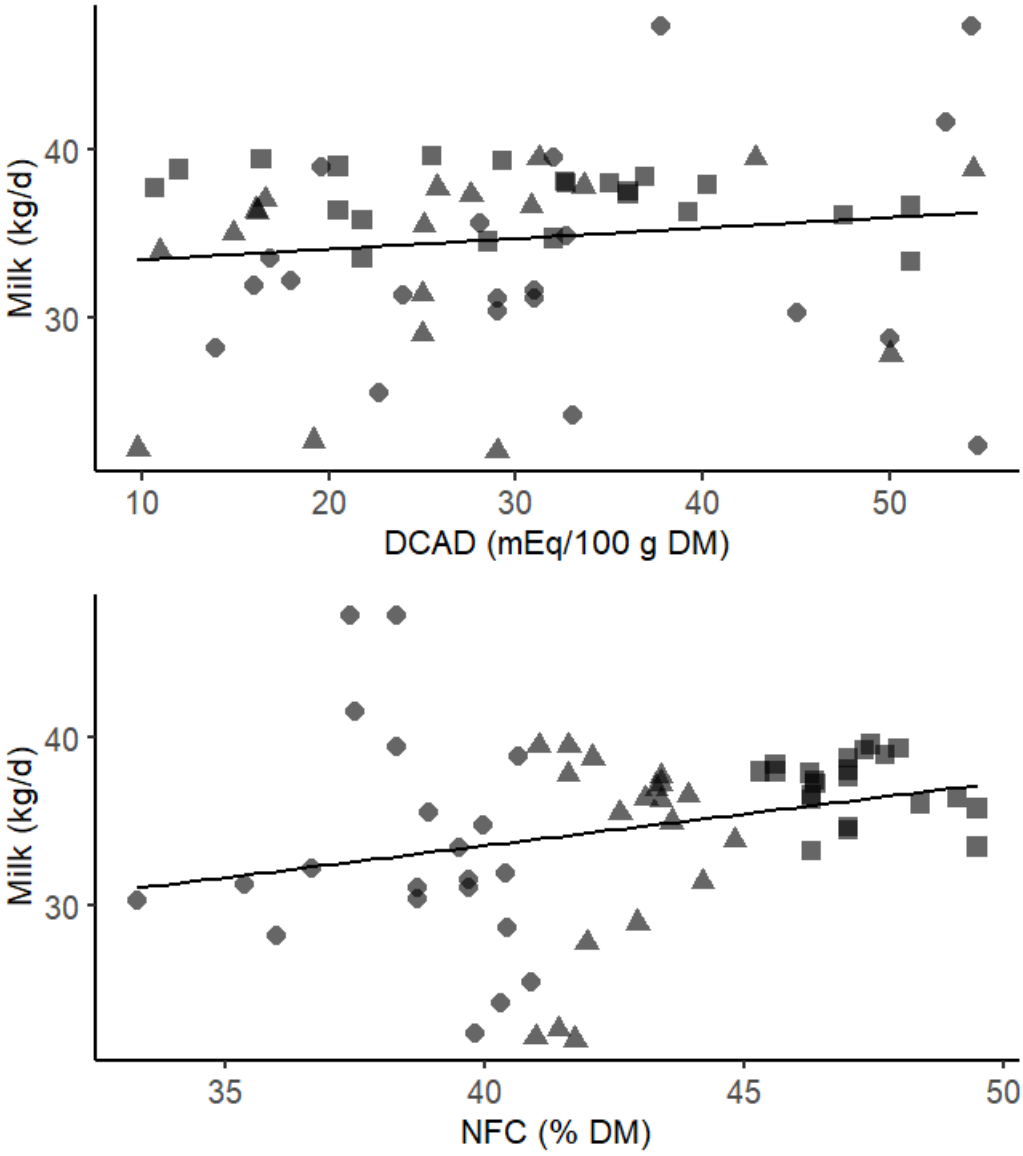

Figure 2.5: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on milk yield.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

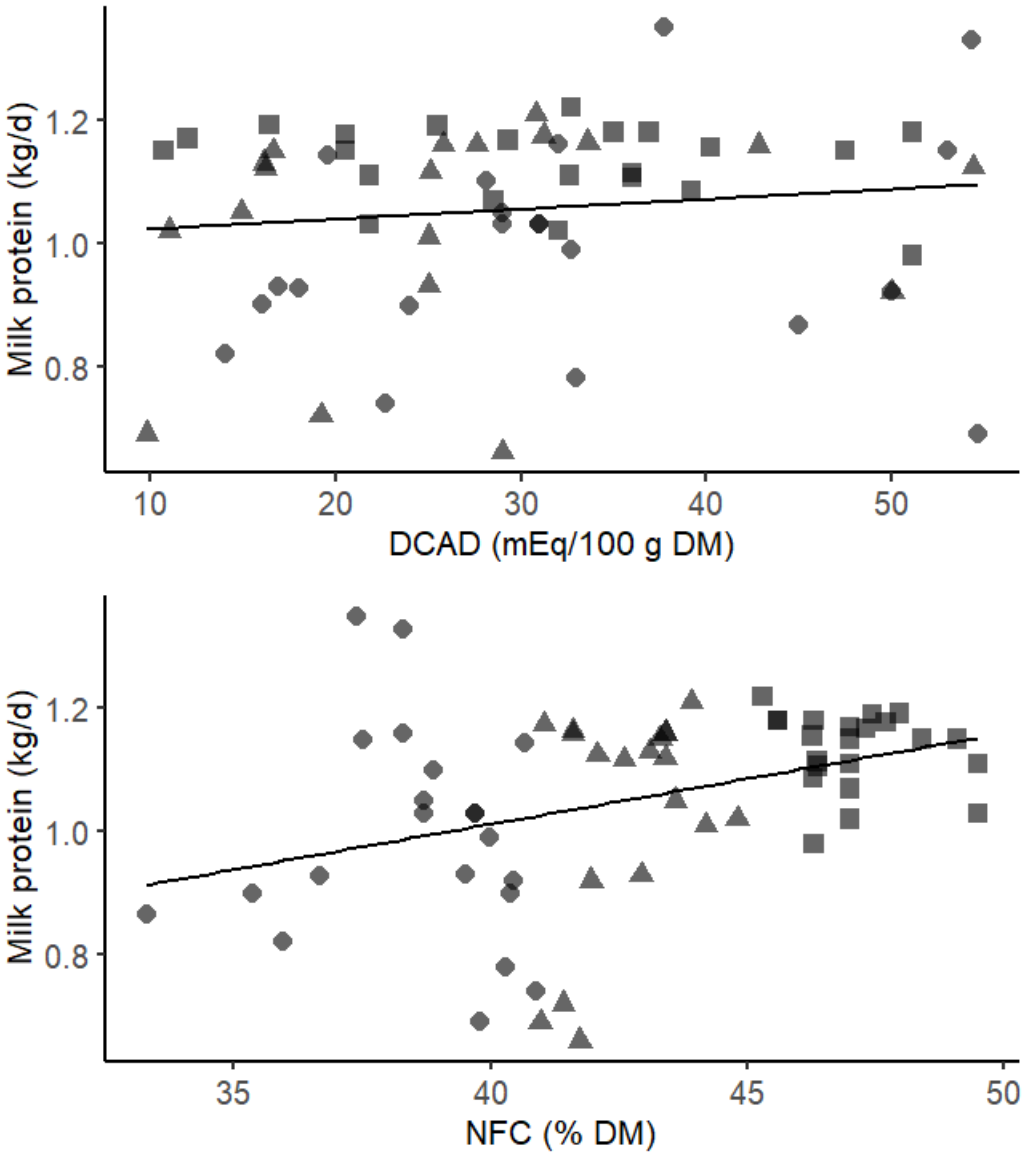

Figure 2.6: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on milk protein yield.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

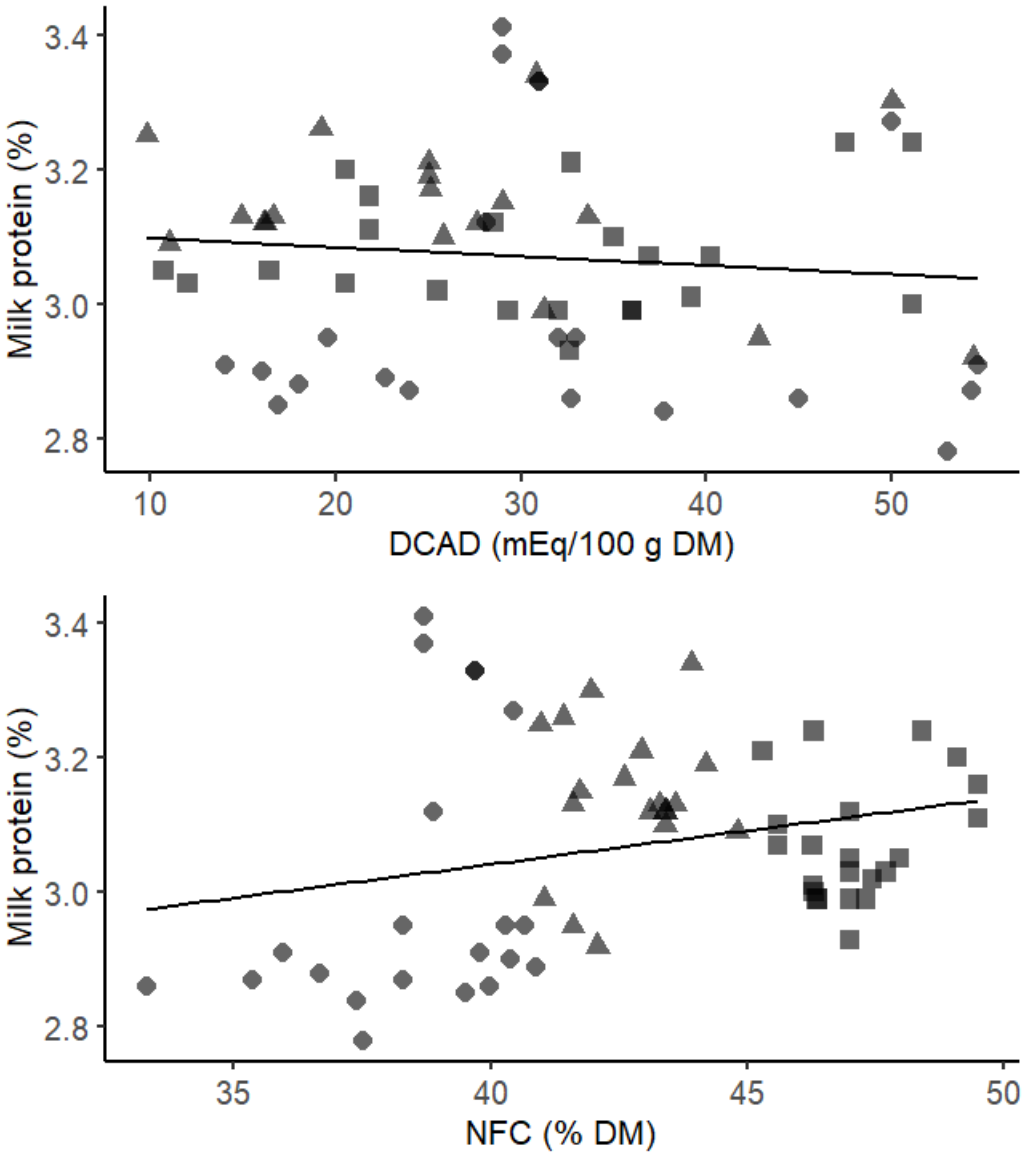

Figure 2.7: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on milk protein percent.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

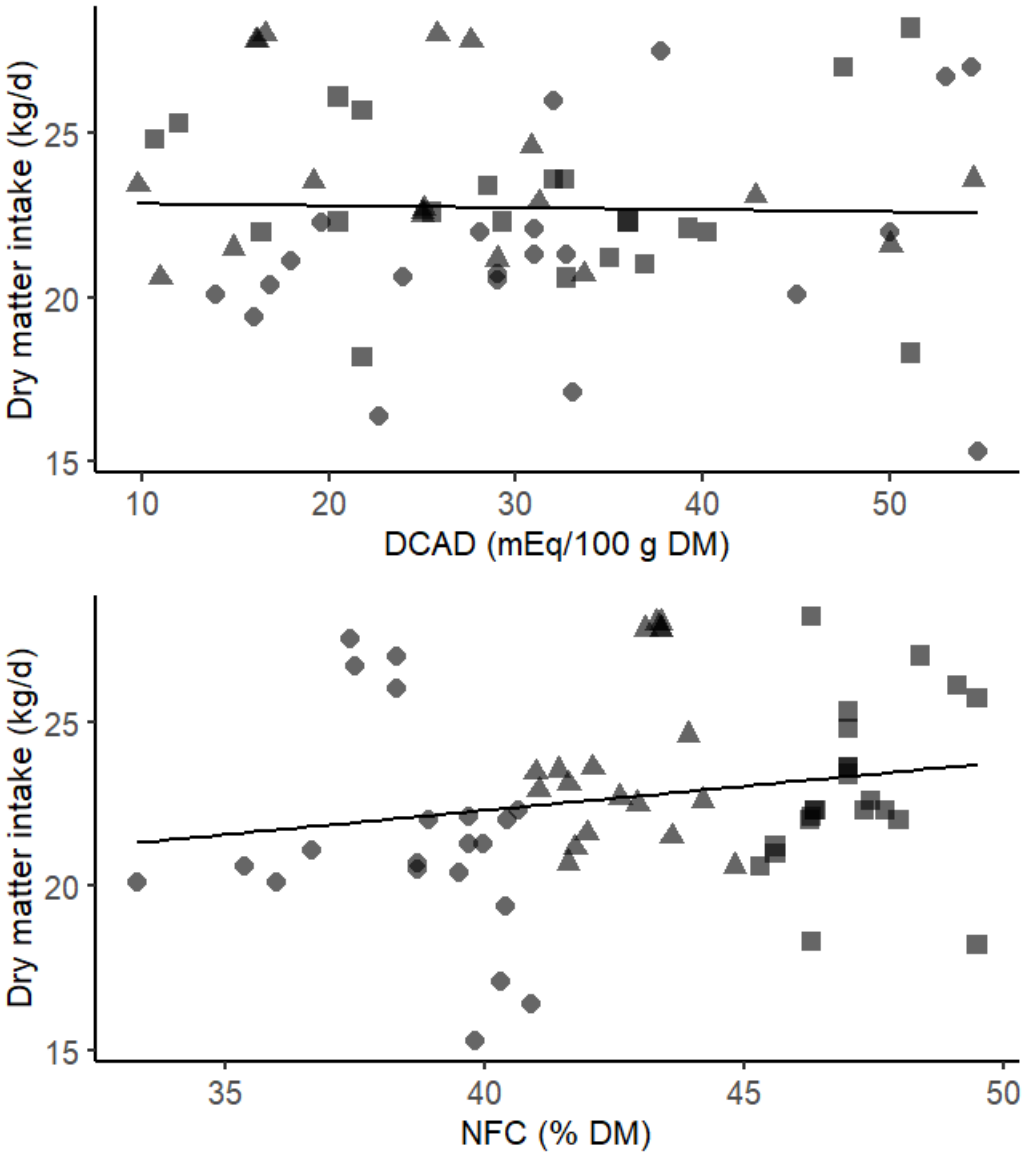

Figure 2.8: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on dry matter intake.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

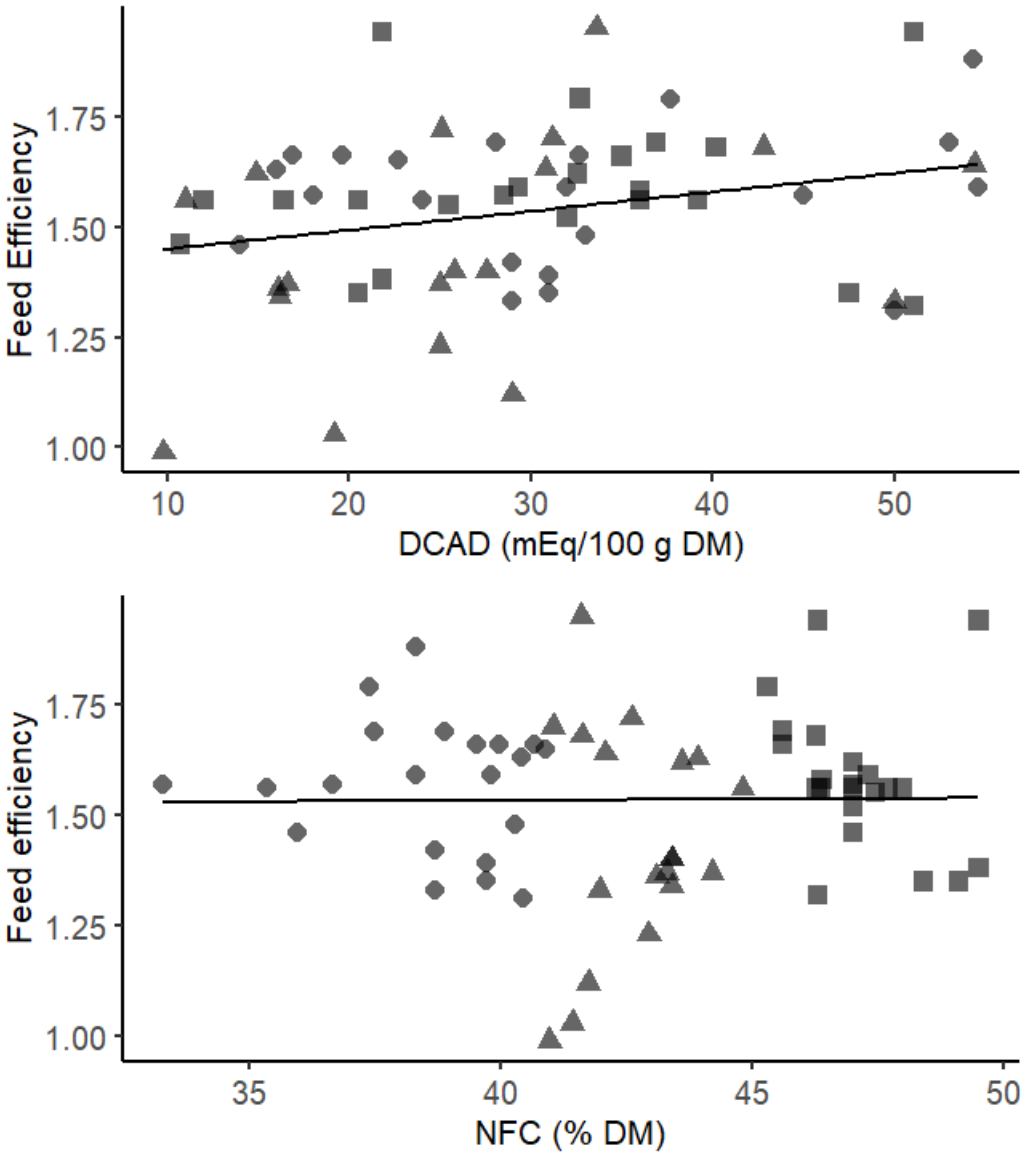

Figure 2.9: Dietary treatment observations from fifteen studies in lactating cows (n=362) to examine by meta-analysis the relationship of DCAD and NFC on feed efficiency.

Each point represents a dietary treatment with diamonds, triangles, and squares identifying Low NFC, Medium NFC, and High categories, respectively. The line indicates a fitted regression line calculated using linear regression across all observations.

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Appendix

NRC 2001 Feed codes	
Alfalfa - 1	Corn silage - 35
Barley ground - 8	Cottonseed - 37
Blood meal - 14	Fish meal - 47
Corn distillers - 23	Grass hay - 52
Corn cracked - 26	Molasses beat - 88
Corn ground - 27	Soybean meal - 88
Corn flaked - 28	