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USE OF RUMEN MODIFIERS TO MANIPULATE RUMINAL FERMENTATION
AND IMPROVE NUTRIENT UTILIZATION AND LACTATIONAL
PERFORMANCE OF DAIRY COWS

by

Christopher M. Dschaak

A dissertation submitted in partial fulfillment
of requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Animal Science

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2012

ABSTRACT

Use of Rumen Modifiers to Manipulate Ruminal Fermentation and Improve Nutrient Utilization and Lactational Performance of Dairy Cows

by

Christopher M. Dschaak, Doctor of Philosophy

Utah State University, 2012

Major Professor: Jong-Su Eun
Department: Animal, Dairy, and Veterinary Sciences

Overall hypothesis in a series of lactation studies reported in this dissertation was that supplementing different rumen modifiers would have consistent responses on ruminal fermentation and lactational performance under optimal ruminal fermentative conditions.

First experiment investigated the influence magnesium exchanged zeolite on ruminal fermentation and lactational performance. Intake of dry matter (**DM**), milk yield, milk fat concentration, and feed efficiency were not affected. Milk protein concentration tended ($P = 0.15$) to be higher for the zeolite total mixed ration (**TMR**). Ruminal pH tended to increase ($P = 0.11$) by feeding the sodium bicarbonate or the zeolite.

A second lactation experiment determined the influence of quebracho condensed tannin extract (**CTE**) on ruminal fermentation and lactational performance. Supplementing CTE decreased intakes of DM and nutrients regardless of forage level thereby increasing feed efficiency. Milk yield and components were not affected. Milk urea N (**MUN**) and total VFA concentration decreased by supplementing CTE. Cows fed

CTE had decreased ruminal ammonia-N and MUN concentrations, indicating that less ruminal N was lost as ammonia.

A third lactation trial assessed whole safflower seeds (**SS**) on ruminal fermentation, lactational performance, and milk fatty acids. Feeding the Nutrasaff SS TMR (**NSST**) decreased intake of neutral detergent fiber. Digestibilities of nutrients, milk yield and components, ruminal pH, ruminal VFA, and ammonia-N were similar. Ruminal C16:0 fatty acid (**FA**) concentration increased with the cottonseed TMR (**CST**), while C18:1 *cis*-9 and C18:2 n-6 tended ($P = 0.10$ and $P = 0.09$, respectively) to increase with SS supplementation. Supplementing SS decreased milk C16:0 concentration, whereas it increased C18:1 *cis*-9 and C18:1 *trans*-9. Milk C18:1 *trans*-11 FA and *cis*-9, *trans*-11 conjugated linoleic acid increased and tended ($P = 0.07$) to increase with feeding the NSST.

Feeding zeolite would cost-effectively replace sodium bicarbonate as a ruminal buffer, whereas CTE may change the route of N excretion, having less excretion into urine, but more into feces. Whole SS can be an effective fat supplement to lactating dairy cows without negative impacts on lactational performance and milk FA. These studies demonstrate that the three rumen modifiers can positively manipulate ruminal fermentation.

PUBLIC ABSTRACT

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A series of lactation studies reported in this dissertation hypothesized that supplementing different rumen modifiers would have consistent responses on ruminal fermentation and lactational performance under optimal rumen conditions.

The first experiment investigated the influence of magnesium exchanged zeolite on ruminal fermentation and lactational performance. Intake of dry matter (**DM**), milk yield, milk fat, and feed efficiency were not affected. Milk protein concentration tended ($P = 0.15$) to be higher for cows fed the zeolite. Ruminal pH tended to increase ($P = 0.11$) by feeding the sodium bicarbonate or the zeolite.

A second lactation experiment determined the influence of quebracho condensed tannin extract (**CTE**) on ruminal fermentation and lactational performance. Supplementing CTE decreased intakes of DM and nutrients thereby increasing feed efficiency. Milk yield and components were not affected. Milk urea N (**MUN**) and total VFA concentration decreased by supplementing CTE. Cows fed CTE had decreased ruminal ammonia-N and MUN concentrations, indicating that less ruminal N was lost as ammonia.

A third lactation trial assessed whole safflower seeds (**SS**) on ruminal fermentation, lactational performance, and milk fatty acids. Feeding Nutrasaff SS decreased intake of neutral detergent fiber. Digestibilities of nutrients, milk yield and components, ruminal pH, ruminal VFA, and ammonia-N were similar. Ruminal C16:0 fatty acid (**FA**) concentration increased when feeding cottonseed, while C18:1 *cis*-9 and C18:2 n-6 tended ($P = 0.10$ and $P = 0.09$, respectively) to increase with SS supplementation. Supplementing SS decreased milk C16:0 concentration, whereas it increased C18:1 *cis*-9 and C18:1 *trans*-9. Milk C18:1 *trans*-11 FA and *cis*-9, *trans*-11 conjugated linoleic acid increased and tended ($P = 0.07$) to increase with feeding the Nutrasaff SS.

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CONTENTS

	Page
ABSTRACT.....	ii
PUBLIC ABSTRACT	iv
ACKNOWLEDGMENTS	vi
LIST OF TABLES.....	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS.....	xiv
 CHAPTER	
1. INTRODUCTION	1
References	5
2. REVIEW OF LITERATURE	9
Ruminant Digestion and Metabolism.....	9
Relationship between Carbohydrate and Nitrogen Metabolism in the Rumen.....	11
Energy and Nitrogen Synchronization in the Rumen.....	13
Ruminal Fermentation of Starch and Its Impacts on Lactational Performance	15
Manipulation of Ruminal Fermentation	18
Manipulation of Carbohydrate Fermentation	18
Manipulation of Nitrogen Metabolism.....	22
Manipulation of Lipid Fermentation	25
Use of Feed Additives in Dairy Diets	28
Zeolite.....	28
Clinoptilolite Zeolite	29
Condensed Tannins	32

	Ruminal fermentation when feeding CT.....	36
	Animal performance when feeding CT.....	38
	Fat Supplementation to Dairy Cows	42
	Yield of milk and milk components and DMI	45
	Unsaturated fat supplementation to dairy cows	48
	Biohydrogenation of FA in the rumen and its impacts on milk fat depression	53
	References	55
3.	EFFECTS OF SUPPLEMENTATION OF NATURAL ZEOLITE ON INTAKE, DIGESTION, RUMINAL FERMENTATION, AND LACTATIONAL PERFORMANCE OF DAIRY COWS	91
	Introduction	91
	Materials and Methods	93
	Cows and Experimental Diets	93
	Sample Collections, Calculations, and Chemical Analyses	95
	Statistical Analyses.....	97
	Results and Discussion.....	98
	Chemical Composition of Diets	98
	Intake, Digestibility, Milk Production and Composition, and BW	98
	Ruminal Fermentation Characteristics	100
	Implications	104
	References	105
4.	EFFECTS OF SUPPLEMENTING CONDENSED TANNIN EXTRACT ON INTAKE, DIGESTION, RUMINAL FERMENTATION, AND MILK PRODUCTION OF LACTATING DAIRY COWS ¹	118
	Introduction	118
	Materials and Methods	120
	Cows and Experimental Design and Diets	120
	Sampling, Data Collection, and Chemical Analyses.....	122
	Ruminal Fermentation Characteristics	125
	Statistical Analyses.....	126

Results and Discussion	128
Characteristics of Experimental Diets	128
Intake and Digestibility	128
Milk Production and Its Efficiency	130
Ruminal Fermentation Characteristics	132
Milk FA Composition	136
Conclusions	137
References	137
5. RUMINAL FERMENTATION, MILK FATTY ACID PROFILES, AND PRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COWS FED TWO DIFFERENT SAFFLOWER SEEDS	157
Introduction	157
Materials and Methods	159
Cows, Experimental Design, and Diets	159
Sampling, Data Collection, and Chemical Analyses	161
Ruminal Fermentation Characteristics	164
Statistical Analyses	165
Results and Discussion	166
Characteristics of Experimental Diets	167
Intake and Digestibility	168
Milk Production and Its Efficiency	169
Ruminal Fermentation Characteristics	170
FA Profiles in Ruminal Fluid and Milk	171
Conclusions	177
References	177
6. SUMMARY AND CONCLUSIONS	196
APPENDIX	201
VITA	210

LIST OF TABLES

Table	Page
3.1. Ingredient composition of the control diet.....	111
3.2. Chemical composition of the treatment diets on a DM basis	112
3.3. Nutrient intake and total tract digestibility of lactating dairy cows fed different ruminal buffer additives.....	113
3.4. Milk production and composition, efficiencies of DM and N use, and BW of lactating dairy cows fed different ruminal buffer additives.....	114
3.5. Ruminal fermentation characteristics of lactating dairy cows fed different ruminal buffer additives	116
4.1. Ingredients and chemical composition of the TMR fed to lactating cows	146
4.2. Fatty acid composition of the TMR fed to lactating cows.....	148
4.3. Nutrient intake and total tract digestibility of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation	150
4.4. Milk production and composition and efficiencies of DM and N use for milk production of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation.....	151
4.5. Ruminal fermentation characteristics of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation	153
4.6. Fatty acid composition in the milk of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation.....	155
5.1. Ingredients of the TMR fed to lactating cows	184
5.2. Chemical composition of oilseeds and the diets (DM basis)	186
5.3. Nutrient intake and total tract digestibility of lactating cows fed different safflower seeds.....	188

5.4.	Milk production and composition and efficiencies of DM and N use for milk production of lactating cows fed different safflower seeds	189
5.5.	Ruminal fermentation characteristics of lactating cows fed different safflower seeds	191
5.6.	Fatty acid composition in the ruminal fluid of lactating cows fed different safflower seeds.....	193
5.7.	Fatty acid composition in the milk of lactating cows fed different safflower seeds	194

LIST OF FIGURES

Figure	Page
2.1. A theoretical scheme showing carbohydrate and protein utilization by ruminal bacteria.....	82
2.2. Interventions to manipulate fermentation and metabolism in the rumen. Sometimes the target organisms have several functions, in other cases the metabolic pathways are linked, for example by the availability of H ₂	83
2.3. Possible sites targeted by feed additives to improve carbohydrate fermentation in the rumen	84
2.4. Possible sites targeted by feed additives to improve nitrogen metabolism in the rumen	85
2.5. Manipulation of lipid fermentation in the rumen. Major reactions in the rumen and possible sites targeted (increase or decrease) for modifications are depicted.	86
2.6. Skeletal structure of clinoptilolite.....	87
2.7. Chemical structure of condensed tannins	88
2.8. Biohydrogenation pathways in the rumen	89
2.9. Relationship between the change in the fat content of milk and the <i>trans</i> -10 18:1 fatty acid concentration of milk fat (expressed as % of total fatty acids).....	90
3.1. Dry matter intake and milk yield of lactating dairy cows fed different ruminal buffer additives. Treatments were TMR without buffer (CD), CD and sodium bicarbonate TMR (SBD), and CD and zeolite TMR (ZD). Each point represents the mean of 10 observations (SEM = 1.19 and 1.46 for DMI and milk yield, respectively)	117

LIST OF ABBREVIATIONS

AA = amino acid

A:P = acetate-to-propionate ratio

ADF = acid detergent fiber

AIA = acid insoluble ash

BH = biohydrogenation

BW = body weight

CH₄ = methane

CLA = conjugated linoleic acid

CP = crude protein

CS = whole linted-cottonseed

CSS = conventional safflower seed

CSST = conventional safflower seed total mixed ration

CST = whole linted-cottonseed total mixed ration

CT = condensed tannins

CTE = condensed tannin extract

CTL = control diet without Nutrasaff safflower seed addition

DM = dry matter

DIM = days in milk

DMI = dry matter intake

FA = fatty acids

FCM = fat-corrected milk

HF = high forage

HF+CTE = high forage diet with condensed tannin extract

HF-CTE = high forage diet without condensed tannin extract

LF = low forage

LF+CTE = low forage diet with condensed tannin extract

LF-CTE = low forage diet without condensed tannin extract

LRCpH = Lethbridge Research Centre Ruminant pH Measurement System

MFD = milk fat depression

MP = metabolizable protein

MUN = milk urea nitrogen

N = nitrogen

NaHCO₃ = sodium bicarbonate

NDF = neutral detergent fiber

NE_L = net energy for lactation

NFC = nonfibrous carbohydrates

NH₃ = ammonia

NH₃-N = ammonia nitrogen

NPN = non-protein nitrogen

NSS = Nutrasaff safflower seed

NSST = Nutrasaff safflower seed total mixed ration

OM = organic matter

PUFA = polyunsaturated fatty acids

RDP = rumen-degradable protein

RUP = rumen-undegradable protein

SBD = sodium bicarbonate diet

SEM = standard error of least square means

SS = safflower seed

TMR = total mixed ration

VFA = volatile fatty acids

ZD = zeolite diet

CHAPTER 1

INTRODUCTION

There have been extensive research efforts to acquire better approaches focused on the area of "ruminal microbial fermentation and forage utilization by ruminants". This focus is justified by a challenge to minimize nutrient excretion and maximize use of nutrient by the ruminant production systems. We all know that we "feed the rumen" when we feed ruminants. Yet, in today's production scenario, we need to be more aware of how and why we feed the rumen because of the greater array of feedstuffs available and environmental concerns. One of most challenging research areas in ruminant nutrition is to integrate biological constraints with feeding practices to identify issues to improve our ability to reduce the variability and increase the efficiency associated with "optimizing ruminal fermentation and maximizing ruminant production". Ruminal fermentation and function influence all productive processes and, ultimately, performance in dairy cows. Providing the right nutrients creates an optimal environment that allows rumen microbes to function efficiently, giving cows the nutrients they need to convert feed into milk to enhance profitability. By maximizing rumen microbial activity and bacterial protein production, cows can make the most of their feed to efficiently maximize milk and its components. The end-products of fermentation such as VFA are absorbed across the rumen wall and used for energy and protein synthesis. The outflow of microbial biomass and VFA from the rumen influences the nutritional status of the animal as well as the efficiency of nutrient utilization. The rumen is therefore a highly efficient organ in the context of the evolution of an herbivore subsisting on forage with its attribution to maintaining rumen function. In order to minimize nutrient waste and

maximize its use by dairy cows, there is strong need to optimize ruminal fermentation with better understanding of microbial dynamics in the rumen.

Manipulation of ruminal fermentation involves improving ruminant productivity by maximizing the efficiency of feed utilization. Therefore, considerable research efforts have focused on methods to modify ruminal fermentation using rumen modifiers to optimize rumen functions for the benefit of ruminants. The ultimate goal of manipulation of ruminal fermentation is to maximize microbial fermentation and improve animal performance.

Use of dietary ruminal buffers, as a rumen modifier, has been suggested to ameliorate the occurrence of ruminal acidosis, especially when lactating diets include large amounts of readily fermentable carbohydrate. Sodium bicarbonate is commonly used as an exogenous buffer to stabilize ruminal pH in cows that can potentially suffer from ruminal acidosis (Clark et al., 2009). This chemical feed additive is characterized by an acid dissociation constant ($pK_a = 6.25$), which is close to the normal ruminal pH. Sodium bicarbonate is generally recognized as an efficient buffer because of its high acid-consuming capacity in the rumen, and its mode of action is well documented (Erdman, 1988; Russell and Chow, 1993).

Research has continued to identify cheaper mineral buffers that exhibit the same mode of action as the established buffers. The natural zeolite clinoptilolite has a high attraction for water and a large number of cations, such as K^+ , NH_4^+ , Ca^{2+} , and Mg^{2+} , which can be reversibly bound or released, depending upon the surrounding conditions (Mumpton, 1999). The high affinity of zeolites for water and osmotically active cations may facilitate ruminal fermentation, and osmotic activity may regulate pH in the rumen

by buffering against hydrogen ions of organic acids. In addition, supplementing zeolite in dairy diets may improve N utilization, because zeolite gradually releases excess ammonia in the rumen and allows rumen microorganisms to capture the ammonia into microbial protein for assimilation into the animals' digestive systems (Mumpton, 1999). Johnson et al. (1988) reported that ruminal pH increased when synthetic zeolite was added to the diet, and addition of the synthetic zeolite, with or without sodium bicarbonate, resulted in negative effects on feed intake, milk production, milk component yield, and nutrient digestibility in lactating Holstein cows. However, there is a lack of experimental results regarding the effects of long-term feeding of lactating dairy cows with clinoptilolite, a natural zeolite, on its potential as a ruminal buffering agent.

In ruminants fed high quality forage diets, most proteins are rapidly degraded releasing between 56 and 65% of the N concentration in the rumen during fermentation; consequently, large losses of N occur (25-35%) as ammonia into urine (Min et al., 2000). Natural plant compounds, such as condensed tannin extract (**CTE**), are a rumen modifier and have the ability to reduce proteolysis and improve animals' N retention. Aerts et al. (1999) found that condensed tannins (**CT**) in birdsfoot trefoil (*Lotus corniculatus*) and big trefoil (*L. pedunculatus*) markedly protected ribulose-1, 5-bisphosphate carboxylase/oxygenase from degradation. Condensed tannin reduced the growth of a range of bacterial strains from the rumen (Molan et al., 2001; Min et al., 2002). These effects of CT on retarding forage N degradation supported more milk production from cows fed birdsfoot trefoil over alfalfa silage (Hymes-Fecht et al., 2005). Tannin-rich forages are not agronomically suited in many areas. Hence, a concentrated source of CT may be a possible alternative approach to feeding tannin-rich forages to manipulate

ruminal fermentation, enhance N utilization, and improve lactational performance of dairy cows.

Fat supplements in lactation dairy diets allows for the maintenance of energy density while increasing fiber intake, resulting in stabilization of ruminal fermentation (Allen, 1997). In the western and central United States, safflower (*Carthamus tinctorius* L., Asteraceae) has been widely grown because of tolerance to hot and dry climates (Li and Mündel, 1996; Bradley et al., 1999). Alizadeh et al. (2010) reported that SS can be included up to 5% of dietary DM alongside cottonseed (CS) for early lactating cows without affecting feed intake while maintaining normal ruminal fermentation, peripheral energy supply, and milk production. Whole Nutrasaff SS (NSS), a new variety of SS (Safflower Technologies International, Sidney, MT), contain higher oil and lower fiber concentrations than traditional SS varieties (Bergman et al., 2007). Nutrasaff SS can replace CS and be fed to lactating dairy cows without negative impacts on lactational performance up to 3% DM (Dschaak et al., 2010). Feeding the NSS improved efficiency of use of feed N to milk N and decreased MUN. In addition to the benefits on nutrient utilization, feeding NSS enhanced functional quality of milk with increased *cis-9, trans-11* conjugated linoleic acid (CLA) concentration, which is an additional benefit to human health (Dschaak et al., 2010). However, effects of feeding SS on ruminal fermentation has not been assessed, and therefore further research is needed to identify other CLA isomers or 18:1 *trans* FA would affect milk fat yield when SS are fed in lactation dairy diets.

The overall hypothesis in a series of lactation studies reported in this dissertation was that supplementing different rumen modifiers would have consistent responses on

ruminal fermentation under optimal ruminal fermentative conditions with adequate supply of forage fiber in lactation dairy diets. The objective of this research was to evaluate the use of zeolite, quebracho CTE, and Nutrasaff SS as potential rumen modifiers and assess ruminal fermentation characteristics, nutrient utilization, and lactational performance. All lactation experiments were performed using typical lactation diets in Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) containing relatively high forage. Therefore, high quality of alfalfa hay (high CP but low NDF concentrations) was fed to provide 50 to 75% of the dietary forage with total forage levels averaging 45 to 55% of the dietary DM in the experiments. We have reported that due to positive nutritive contribution by the high quality of alfalfa hay, overall fermentative conditions have not been interfered when sizable dietary proportions of readily fermentable carbohydrates are included in dairy cow diets (Holt et al., 2010). These approaches may help to compare efficacy of supplementing the rumen modifiers investigated in this project and extrapolate overall results to be used in dairy ration formulation.

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CHAPTER 2

REVIEW OF LITERATURE

Ruminant Digestion and Metabolism

The rumen is a large fermentation chamber providing an anaerobic environment, constant temperature and pH, and good mixing. The rumen microbial population contains billions of complex microbiota made of anaerobic bacteria ($\sim 10^{11}$ cells/mL), anaerobic protozoa ($\sim 10^5$ cells/mL), anaerobic fungi ($\sim 10^3$ cells/mL), and methanogen archaea ($\sim 10^9$ cells/mL) as the main groups of microorganisms which anaerobically break down and ferment the ingested plant materials (Jouany and Morgavi, 2007). Ruminants evolved to consume and subsist on roughage and grasses consisting predominantly of celluloses. Ruminants typically face several challenges on feeding, because plant matter is difficult to digest, relatively low in fat and protein, and the majority of nutrients are located within strong cell wall fraction (Vaughan et al., 2011). Many microbes synthesize cellulolytic enzymes which allow them to utilize dietary celluloses and other plant cell wall materials. The bacteria in the rumen can be classified into two groups; those that utilize fiber and those that utilize nonfiber sources of carbohydrates (primarily sugars and starch). Microbial fermentation is a slow process, requiring time and space in order to occur.

Several processes are involved in ruminal function, and generally these involve: reduction in size of feed particles, degradation of substrate, utilization of substrate by microorganisms, synthesis of microbial biomass, formation of VFA, ammonia (NH_3), methane, and carbon dioxide as end-products of substrate fermentation, intra-ruminal recycling of microbial matter (death, predation), absorption of VFA and ammonia, and

finally, outflow of ruminal contents. Nutrients that are absorbed differ markedly from those consumed because of microbial fermentation in the rumen (Allen et al., 2005). Organic matter is partially fermented to VFA (primarily acetic, propionic, and butyric acids), feed protein is partially degraded to AA and NH_3 , which are incorporated into microbial protein of high biological value, and unsaturated fatty acids (**FA**) are biohydrogenated and isomerized to varying degrees.

The major fuels for dairy cows are VFA from ruminal and intestinal fermentation of OM, glucose from starch digestion in the small intestine, and nonesterified FA and AA absorbed from the gastrointestinal tract and mobilized from body reserves. Between half and two-third of total ME may originate from VFA produced from fermentative degradation by microorganisms in the rumen. More than 60% of absorbed AA are from microbial protein flowing from the rumen, with the remainder from feed protein escaping ruminal degradation (Allen et al., 2005).

Feeding value of a diet as well as feed requirement for milk synthesis are affected by animal factors such as the level of DMI, the type of diet consumed, and the physiological state of the cow. The type of nutrient absorbed affects partitioning between maintenance, milk production, body deposition, and efficiency of energy utilization for productive functions (Dijkstra et al., 2007; Friggens and Newbold, 2007). Ruminal digestibility not only depends on the types of material ingested and their intrinsic degradation characteristics, but also it is influenced by all the ingredients combined in a diet and the fermentation conditions encountered in the rumen with that particular diet. Changes in rumen function affect the interactions between individual types of nutrient with respects to their digestibility, site of digestion and availability for the cow's metabolism, and

productive functions. It is clear that the rumen plays a major role in the digestion of feed, and the fermentative processes in the rumen largely determine the site and extent of feed digestion. Variation in ruminal fermentation is a major cause of variation in the quantity and type of nutrients absorbed from the gastrointestinal tract.

Ensuring adequate flow of nutrients from the rumen to support high milk production should first take maximum advantage of ruminal fermentation. Maximum ruminal fermentation is characterized by high ruminal digestion of nutrients (DM, OM, NDF, ADF, and CP) in conjunction with an optimum level of efficiency of microbial protein synthesis. In efficient dairy cow diets, over half of the protein requirements of the animal are met with microbial protein produced during the fermentation of feed nutrients. This significant function depends largely on providing dietary energy and protein in appropriate ratios and amounts. Therefore, the importance of energy and protein interactions within the rumen has been researched with quantitative description of the event as influenced by diet composition, along with consideration of how such processes may be manipulated in order to improve the overall efficiency of ruminant livestock production (Beever, 1993).

Relationship between Carbohydrate and Nitrogen Metabolism in the Rumen

Microbial metabolism in the rumen and the amount of absorbed energy and AA available to meet requirements from ruminal fermentation depend on DMI, content of carbohydrate, protein fractions in diet ingredients, microbial growth on the fiber and non-fiber carbohydrates consumed, and the unique rates of digestion and passage of the individual feed carbohydrate and protein fractions that are being fed. The nutrient

components of diet used for microbial maintenance and growth in the rumen have been subdivided into more specific aspects of ADF, NDF, structural carbohydrate (**SC**), non-structural carbohydrate (**NSC**), RDP, and RUP. These different nutrient components have their unique physical and chemical characteristics and utilization efficiencies (Van Soest, 1994).

Microbial growth is largely dependent on availability of both N and carbohydrates in the rumen. From the degradation and fermentation of fiber and starch, microorganisms derive their energy as ATP and their N from feed protein degradation. The overall process illustrating the coordination of N and carbohydrate is shown in Figure 2.1. Plant tissues contain carbohydrates of one kind or another, and provide the primary source of energy for both the ruminal organisms primarily used for FA synthesis in adipose tissue. Therefore, production of adequate levels of acetate in the rumen is essential to maintain adequate quantities of milk fat. Propionic acid provides energy through conversion to blood glucose in the liver and is also used in milk lactose synthesis. Butyric acid provides energy to the rumen wall and is largely converted to ketones during absorption through the rumen epithelium. Starch is the primary energy component in grains, and is considered the primary driver of microbial protein synthesis in the rumen. Hexoses and pentoses produced through microbial breakdown are subsequently used by the ruminal microbial population to supply the precursors of macromolecules and energy needed for growth and maintenance (Van Soest, 1994). It is generally accepted that the energy available for ruminal microbial yield is largely dependent on the rate of carbohydrate digestion in the rumen (Hoover and Stokes, 1991).

The majority of true protein and NPN entering the rumen is broken down to NH_3 which is required for bacteria as a main source of N to synthesize microbial protein. The microbial protein is a high quality protein for the animal that is highly digestible in the small intestine and has a good balance of the essential AA. Ammonia is most efficiently incorporated into microbial protein when the diet is rich in soluble carbohydrates, particularly starch. Because proteolysis occurs rapidly in the rumen, it usually exceeds the rate of utilization by microorganisms with an accumulation of NH_3 . Ammonia, in excess of that used by the microorganisms, is absorbed across the rumen wall, converted to urea in the liver, released as blood urea N, and excreted into urine or recycled back to the rumen via saliva or diffusion through the rumen wall. This inefficient recycling of N is energetically costly to the animal. Because of the uncertain contributions to inefficiency of conversion of dietary CP to tissue or milk protein, this route needs to be further characterized to improve N retention (Firkins and Reynolds, 2005). Although protein sources and NDF are major nutrients supplying NH_3 -N and energy, respectively, NSC in ruminal fermentation plays a significant role with respect to optimization of microbial protein yield in the rumen.

Energy and Nitrogen Synchronization in the Rumen

The efficiency of microbial protein synthesis is defined as the amount of microbial N reaching the duodenum per unit of OM truly degraded in the rumen. In order to improve the efficiency of microbial protein synthesis, the rate of growth by microorganisms should match the rate of substrate availability. Otherwise, feed degradation promotes wasteful mechanisms decreasing the efficiency of microbial protein synthesis, and factors that increase energy consumption without increasing the microbial biomass must be

controlled. Synchronization is the provision of both RDP (NPN and rumen-degradable true protein) and energy (ruminally fermentable carbohydrate) to the rumen, so that microorganisms can utilize both simultaneously (Seo et al., 2010). There have been many attempts to test the 'synchrony' hypothesis. The basic assumption on the effect of synchronization of energy and N availability is that a lack of synchrony between the rates at which energy and N become available to the microbes. This will lead to a reduced efficiency of microbial capture of N. Under this condition, ATP produced from fermentation of dietary carbohydrate will be inefficiently used for microbial growth (Chamberlain and Choung, 1995). Therefore, synchronous nutrient availability should allow more efficient use of nutrients, thus enhancing microbial protein yield, increasing nutrient supply to the animal, and potentially improving performance of the animal (Hall and Huntington, 2008).

In a review, Cabrita et al. (2006) reported that synchronization of rapid fermentation with fast degradable starch and protein stimulated greater microbial protein passage than slowly degradable synchronized diets. Furthermore, starch digestibility in the rumen affected utilization of nutrients more than protein digestibility. The authors concluded that the ruminal synchronization of substrate release can enhance the amount and efficiency of microbial protein yield. It has been reported that a synchronous supply of energy and N to the rumen enhances the efficiency of microbes in capturing N and in utilizing ATP for microbial growth (Richardson et al., 2003), which may indicate that synchronized feeds increase microbial protein production in the rumen and enhance rumen fermentation efficiency, thereby improving nutrient utilization and animal performance (Seo et al., 2010).

Conversely, when uncoupled fermentation occurs i.e., when there is asynchronous carbohydrate and protein degradation in the rumen, large amounts of $\text{NH}_3\text{-N}$ are absorbed across the rumen wall into the blood, and energy use for microbial protein synthesis will decrease (Bach et al., 2005). Cabrita et al. (2006) reported that across a large number of studies in lactating dairy cows, there was no consistent effect of attempts to synchronize carbohydrate fermentation and N availability on ruminal NH_3 concentration or microbial N supply in lactating dairy cows. Kim et al. (2000) showed no significant effect of nutrient synchrony on the efficiency of microbial growth or VFA production. Ichinohe and Fujihara (2008) reported that the microbial N supply was greater for an asynchronous diet than it was for a synchronous diet. Kaswari et al. (2007) and Richardson et al. (2003) also showed that there were no differences in the efficiencies of microbial protein synthesis or N deposition between diets that were formulated to have different synchronization characteristics. These results indicate that efforts should be made to maintain a continuous ruminal energy supply pattern while supplying the appropriate quantity of rumen degradable N instead of simply pursuing synchronization of energy and N release.

Ruminal Fermentation of Starch and Its Impacts on Lactational Performance

The proportion of starch and dietary content of rapidly degradable carbohydrates in the diet determines ruminal fermentation dynamics which affects the VFA profile (Lechartier and Peyraud, 2011) and varies by species and physiological status of animal as well as physical and chemical processing methods. Few studies have attempted to evaluate in vivo the effect of mechanical processing of corn grain on starch digestion. Remond et al. (2004) reported an increase in ruminal starch digestion between whole or

processed grain. Supplementing whole and processed corn grain did not affect milk yield and composition (Remond et al., 2004). Pelleting decreases particle size, thus shifting the site of carbohydrate digestion from post-ruminal sites to the rumen (Kiran and Mutsvangwa, 2007). Therefore, it can be expected that ruminal starch digestibility would be higher for pelleted barley compared with dry-rolled barley, which could potentially alter urea-N recycling to the rumen and microbial protein synthesis in the rumen, thus having the potential to improve lactational performance of dairy cows.

The theory behind processing grain is shifting the site of starch digestion from the rumen to the small intestine. If total tract digestibility of starch is not affected, there is no direct benefit in a shift of the site of starch digestion. Improved starch digestion is partially achieved via its increased degradation in the rumen (Tagari et al., 2008). Indeed the nature of glucogenic substrates (propionate derived from starch fermentation or glucose derived from small intestinal starch digestion) has little effect on milk yield and composition (Remond et al., 2004).

Increasing grain content of the diet usually decreases milk fat concentration, a change most commonly explained by changes in ruminal fermentation. High grain diets and diets higher in ruminally degraded starch typically increase propionate proportion, but decrease acetate proportion, acetate-to-propionate ratio, fiber digestibility, milk fat concentration, and DMI (Plaizier et al., 2008; Lechartier and Peyraud, 2011). The decrease in milk fat concentration with high grain diets has, therefore, been classically explained by a general shift from lipogenesis to gluconeogenesis. Recent research, however, suggests that the depression in milk fat may instead be caused by an accumulation of *trans*-FA in the rumen caused by low ruminal pH on high grain diets

(Hollmann et al., 2011). Increasing the amount of grain fed generally results in an improvement of milk protein concentration (Yang and Beauchemin, 2007). This is likely due to an increased supply of energy and microbial protein in association with a larger quantity of starch digested in the rumen and the intestine, which might have provided more propionate for glucose synthesis and more glucose for absorption in the intestine. Mackle et al. (2000) suggested that the mechanism by which an increased dietary energy level influences milk protein is related to increased microbial protein synthesis in the rumen.

Other factors that may influence ruminal starch digestion include rumen protozoa (Kiran and Mutsvangwa, 2010), level of DM and starch intake, and grain variety (Lechartier and Peyraud, 2010). Protozoa reduce the rate of starch digestion in the rumen through ingestion of starch digesting bacteria as well as ingestion of starch granules and sugars. Protozoa engulf starch rapidly and consume lactate, thereby keeping the ruminal pH from becoming more acidic (Williams and Coleman, 1997). Kiran and Mutsvangwa (2010) reported that partial defaunation of sheep has been shown to increase ruminal starch digestibility (Kiran and Mutsvangwa, 2010). Starch disappearance was less in faunated (35.0%) than defaunated (40.7%) rumen fluid, but corn variety did not affect starch disappearance (van Zwieten et al., 2008). When no protozoa were present, the disappearance of starch was higher after 6 and 12 h incubation compared with presence of protozoa. However, early in vivo studies with sheep or cattle reported variable results, indicating decrease (Veira and Ivan, 1983) or increase (Mendoza et al., 1993) of ruminal starch degradation upon defaunation.

Manipulation of Ruminal Fermentation

Manipulating ruminal fermentation involves maximizing the efficiency of feed utilization and increasing ruminant productivity (i.e., increase in milk, meat, and wool production). Therefore, considerable research efforts have focused on methods to modify ruminal fermentation (Figure 2.2). The main objectives of manipulating ruminal fermentation are to enhance beneficial processes, minimize, alter, or delete inefficient processes, and minimize, alter, or delete processes harmful to the host (Nagaraja et al., 1997). Manipulation of ruminal fermentation can be thought of as a procedure, whereby optimal conditions are sought by maximization and/or minimization of fermentation. One approach to achieve nutrient synchrony that has received considerable attention is the manipulation of dietary carbohydrate and protein sources (NRC, 2001; Cabrita et al., 2006). Other main targets have also been identified for feed additives to optimize some rumen functions for the benefit of ruminants: 1) decreased methane production in favor of propionate to improve the energy balance of animals; 2) reduced feed protein degradation to increase bioavailability of AA in the small intestine; 3) reduced degradation rate of rapidly fermentable carbohydrates (starch, sucrose) and control lactic acid concentration; and 4) improved fiber digestion (Jouany and Morgavi, 2007).

Manipulation of Carbohydrate Fermentation

The major sites that are targeted for modification of carbohydrate fermentation in the rumen are depicted in Figure 2.3 (Jouany and Morgavi, 2007). Cellulose and hemicellulose constitutes more than 17% of most ruminant diets. Since they are structurally complex and not completely digested, the extent of their fermentation in the

rumen is less than desirable. Increasing fiber or starch fermentation will result in increased VFA production in the rumen. Increased production of propionate is beneficial to the animal by affecting the capture of fermentation energy (i.e., decreased methane production in the rumen).

Ingestion of large quantities of rapidly fermentable carbohydrate can result in production of large quantities of VFA, leading to high ruminal lactate concentrations and subsequently ruminal acidosis. Feeding more concentrate also decreases ruminal pH because grains are generally more rumen digestible than forages. The decrease in ruminal pH favors growth of *Streptococcus bovis* causing changes in fermentation favoring lactate production and its accumulation, and the lactate is 10 times stronger than either acetic, propionic, or butyric acid (Bramley et al., 2008). Therefore, lactate accumulation should be prevented by either inhibiting lactic acid production or enhancing its fermentation to propionate. Lactic acid accumulation in the rumen is aided principally by a low pH, which favors increased production because of growth of *Lactobacillus spp.*, and decreased fermentation due to inhibition of lactate-utilizing bacteria (Bramley et al., 2008).

The dietary profile of NFC has the potential to alter the supply of metabolizable nutrients to the animal because NFC differs in digestion and fermentation characteristics (Hall et al., 2010). Although NFC has been represented as a single value for feeds or diets, the types of carbohydrates in this fraction can vary greatly. For example, the NFC in corn grain is mostly starch (65 to 70% of DM), citrus pulp provides sugars (12 to 40% of DM), and neutral detergent-soluble fiber (NDSF; largely pectic substances; 25 to 44% of DM) and sugars (mono- and disaccharides) are predominant in molasses (Hall, 2002).

Cows consuming ground corn (starch) had the greatest MUN and milk protein concentration and yield and tended ($P = 0.09$) to have the greatest DMI, but had a lesser milk fat concentration compared to cows consuming dried citrus pulp (sugar and pectin) and sucrose + molasses (sugar) (Hall et al., 2010). They also reported the sucrose + molasses diets supported greater DMI, milk protein yield, and 3.5% fat- and protein-corrected milk yield than did dried citrus pulp diets. Ammonia concentration tended ($P = 0.09$) to be greater with ground corn than with dried citrus pulp and sucrose + molasses.

Carbohydrate supply profoundly influences the amount of ruminal $\text{NH}_3\text{-N}$ assimilated into microbial protein. Hristov et al. (2005) fed diets high in alfalfa hay that were supplemented with 20.6% purified glucose, starch, or oat fiber. Feeding glucose decreased ammonia production and decreased the amount of microbial N synthesized from ammonia on a gram/d basis. Less $\text{NH}_3\text{-N}$ was converted into microbial N for the fiber treatment, although one might expect the fiber to stimulate the abundance of ammonia-using cellulolytic bacteria.

Carbohydrate source and availability appear to influence the amount of microbial protein synthesis and efficiency of microbial protein synthesis in potentially different ways. Increasing the dietary proportion of grain in a ration increased microbial N flow to the duodenum by about 30%, but efficiency of microbial protein synthesis was not affected by level of grain; instead, efficiency of microbial protein synthesis was increased when high-moisture corn was replaced with dry ground corn (Firkins et al., 2007). In another study reported by Voelker and Allen (2003), high-moisture corn was reduced from 36 to 11% of DM by substitution with beet pulp, and microbial N flow was linearly decreased without affecting efficiency of microbial protein synthesis. In this case, the

amount of rumen-degradable starch was probably decreased below the levels needed to support optimum microbial protein synthesis. Cereal grains differ in their starch content, with wheat containing (DM basis) 77% starch, corn 72%, and barley and oats 57 to 58% (Gozho and Mutsvangwa, 2008). The authors reported that differences also exist among these cereal grains in their rates and extents of ruminal starch degradation, with 55 to 70% of corn starch, 80 to 90% of barley and wheat starch, and 92 to 94% of oats starch being digested in the rumen. In principle, the rate and extent of fermentation of dietary carbohydrates in the rumen are important parameters that determine nutrient supply to the animal (Hall, 2004).

Increasing the dietary content of rapidly degradable carbohydrates sharply decreased DMI (-2.1 kg/d) by replacing corn with wheat (Lechartier and Peyraud 2010). Gozho and Mutsvangwa (2008) reported DMI tended ($P = 0.10$) to be lower for cows fed the wheat-based TMR than those on the barley-based TMR. Cows fed barley-, corn-, or oats-based TMR consumed 2.2, 2.0, or 1.2 kg/d of DM more, respectively, compared with those fed the wheat-based TMR (Gozho and Mutsvangwa, 2008). Increasing the amount of concentrate in the diet linearly increased milk yield, protein yield, and milk protein concentration, but it decreased milk fat content, and tended ($P = 0.10$) to decrease milk fat yield (Lechartier and Peyraud, 2010). Feeding wheat as a rapidly degradable starch source did not affect milk yield, protein yield, or milk protein concentration, but reduced milk fat yield and milk fat concentration (Lechartier and Peyraud, 2010). Milk yield tended to be lower ($P = 0.06$) in cows fed the wheat-based diet compared with those fed the barley-based TMR, and on average, cows fed barley-, corn-, and oats-based TMR produced 3.4, 2.1, and 1.6 kg/d more milk, respectively, compared with those fed the

wheat-based TMR (Gozho and Mutsvangwa, 2008). The authors reported that feeding the corn-based TMR resulted in greater fat concentration compared with feeding the barley-based or wheat-based TMR, whereas fat yield was only higher in cows fed corn-compared with wheat-based TMR diets. Milk protein concentration was higher in cows fed corn based-TMR compared with those fed oats-based TMR, whereas protein yield tended ($P = 0.05$ and $P = 0.10$, respectively) to be lower in cows fed wheat-based TMR compared with those fed barley-based TMR or corn-based TMR (Gozho and Mutsvangwa, 2008). Nitrogen balance was similar across diets, and urine excretion of purine derivatives indicated higher microbial protein availability at the small intestine in cows fed barley- and corn-based diets compared with those fed oats- and wheat-based diets (Gozho and Mutsvangwa, 2008).

Replacing corn with wheat and feeding a rapidly fermentable carbohydrate source in diets increased the yield of *trans*-10 C18:1 and reduced the yield of *trans*-11 C18:1 in milk. These findings suggest that increasing the amount of rapidly degradable carbohydrates induces a microbial change resulting in a profound modification of ruminal biohydrogenation (BH) and isomerization of C18:2 (Lechartier and Peyraud, 2010). The linear increase of the yield of *trans*-10 C18:1 in milk as forage level decreased also suggests a shift in the BH pathways of C18:2 that became oriented to *trans*-10 C18:1 instead of *trans*-11 C18:1 (Bauman and Griinari, 2003).

Manipulation of Nitrogen Metabolism

Nitrogen utilization is affected by many factors, such as dietary N concentration, degradability, microbial community, and their interaction with other nutrients (Firkins and Reynolds, 2005). The fermentation scheme of nitrogenous substances and sites that

are targeted for modification (increase or decrease) is depicted in Figure 2.4 (Jouany and Morgavi, 2007). The strategy for manipulating N metabolism in the rumen is to enhance ruminal escape of dietary protein by minimizing its degradation and optimizing microbial protein production from NPN. Minimization of protein degradation can be achieved by intervening at the proteolysis, peptidolysis, or AA deamination stages. This will reduce losses incurred in the conversion of dietary protein to microbial cell protein. Most efforts to improve urea utilization have been directed toward minimizing NH_3 absorption by aiming to reduce the rate of urea hydrolysis in the rumen and/or to increase the ability of rumen microorganisms to assimilate NH_3 , thus reducing N loss to the animal. Urea also can be recycled from the blood to the rumen, either via saliva or by direct transfer across the ruminal wall. Therefore, NH_3 is a prime intermediate in the conversion of dietary N to microbial N.

Besides N (NH_3) and carbon skeletons (organic acids and branched-chain FA), microbial protein synthesis in the rumen requires other nutrients, such as sulfur and vitamins. However, such nutrients usually are not limiting. These nutrients or cofactors are supplied directly from the feed, synthesized *de novo* in the rumen, or provided by microbial cross-feeding. The goal in manipulating microbial protein synthesis is to increase efficient production by improving NH_3 assimilation and urea recycling, thereby minimizing N excretion. Urea recycling provides a great advantage when ruminants are fed low-protein diets (Reynolds and Kristensen, 2007).

Feed processing, such as treatments related to heat, can be used to manipulate site of digestion as well as degradation characteristics in the rumen and is therefore a helpful tool in optimizing ruminant production under certain circumstance (Yu et al., 2002).

Historically heat and chemical treatments are probably the most studied and widely used methods for reducing ruminal degradation of feed proteins. Yu et al. (2002) reported that heat treatment (pressure toasting, dry roasting, extrusion, etc.), except pelleting, can reduce the rate and extent of ruminal degradation of protein in legume seeds, thus resulting in a potential increase in the supply of protein (as AA source) to the small intestine.

Decreasing the amount of MP in the diet decreased milk yield by about 3 kg compared with the adequate protein diet (Lee et al., 2011). Lowering the amount of dietary CP was reported by Cyriac et al. (2008) to have a similar effect. Increasing dietary MP linearly increased yields of milk and ECM, but a quadratic relationship was observed for yields of protein and lactose (Weiss et al., 2009). Those yields increased as MP increased and reached their maximums when diets contained approximately 11% MP. Lowering dietary level of protein below requirements of the animal tended ($P = 0.06$) to decrease total VFA concentration resulting in increased ruminal pH of protein deficient diets and tended to decrease ($P = 0.09$) $\text{NH}_3\text{-N}$ concentration in ruminal fluid (Lee et al., 2011).

Hall et al. (2010) reported that changing the proportion of protein degradability in the diet did not affect DMI or milk yield. Cows consuming diets that contained a greater proportion of protein as RDP had greater milk protein concentration and tended to have greater protein production ($P = 0.13$) and MUN ($P = 0.07$), respectively (Hall et al., 2010). Milk fat concentration tended ($P = 0.15$) to be less with increased RDP (Hall et al., 2010). Likewise, NH_3 concentration tended ($P = 0.09$) to be greater by increased RDP, while total VFA and molar proportions of major VFA (acetate, propionate, and butyrate)

were not affected by increased RDP (Hall et al., 2010). Increased ruminal protein degradation, decreased microbial capture of ammonia, or increased catabolism of protein by the animal including protein in excess of requirements could result in the increased $\text{NH}_3\text{-N}$ concentration.

Manipulation of Lipid Fermentation

Manipulation of ruminal lipid metabolism depicted in Figure 2.5 is aimed at controlling antimicrobial effects of FA to minimize disruption of ruminal fermentation, so that higher levels of fat can be included in the diet. In addition, control of ruminal BH has been investigated to alter the absorption of selected FA that may improve nutritional qualities of animal food products or even enhance animal performance (Nagaraja et al., 1997). Increasing dietary polyunsaturated FA intake enhances the polyunsaturated FA content of ruminant meat and milk (Dewhurst et al., 2006; Scollan et al., 2006). Improvement of the FA profile of ruminant products can be achieved by two distinct approaches: 1) Modification of the FA profile during meat or milk processing or 2) Modification through the changes in animal diet (Lourenco et al., 2010). The latter might simply result in greater bypass of dietary FA from the rumen, or might be a consequence of altered microbial metabolic activity.

Commonly used fat supplements in dairy diets are natural fats which include plant oils, oilseeds and animal or animal/vegetable blends, and fats that have been modified with the intent of making them more ruminally "inert" (rumen protected fats). Oils rich in unsaturated FA are relatively more digestible in the small intestine than saturated fats, but when fed unprotected and at high concentrations can interfere with ruminal fermentation and metabolic processes such as milk fat synthesis in the mammary gland.

Oilseeds are commonly extruded (mechanically squeezing the oil from the seed) to enhance their handling, intake, or digestibility, which can significantly reduce their resistance to BH (Jenkins and Lundy, 2001). Extrusion of the oilseeds appears to consistently depress milk fat concentration across a number of oilseed sources. The extrusion process of oilseeds likely results in a faster and greater availability of oil in the rumen than when whole oilseeds are fed (Staples, 2006).

Safflower is an oilseed rich in unsaturated FA, mainly in the form of linoleic acid. Wu et al. (1994) reported that adding 2.2% safflower oil to the diet resulted in increased milk yield as well as C18:1, C18:2, and C18:3 FA in the milk. Rindsig and Schultz (1974) showed that adding 250 mL of safflower oil daily to the ration decreased milk fat concentration. The milk had higher concentrations of C18:1 and C18:2. Bell et al. (2006) reported a decrease in yield and concentration of fat when diets were supplemented with safflower oil. Conjugated linoleic acid (CLA) concentrations were significantly higher in the milk of cows supplemented with the safflower oil (Bell et al., 2006). Feeding free oil depressed milk fat yield, but feeding oil as part of whole oilseeds did not alter milk fat yield (Grummer, 1991).

The potentially negative impact of unsaturated fat from oilseeds as safflower could be minimized if the oilseeds are fed either whole or coarsely cracked rather than extruded (Faldet and Satter, 1991). Jenkins and Lundy (2001) concluded that whole seeds were broken by the cow both in the chewing and by microbial action during the rumination process, so processing was considered unnecessary before feeding. They found that whole seeds provide some protection from BH because of the nature of their hard outer seed coat. This would allow the oil to be released at a slower rate in the rumen, or some

of the oil may escape rumen BH and be absorbed in the small intestine. Disruption of the seed coat exposes the oil to the microbial population, which may result in the potential for fermentation problems and BH. Whole oil seeds release FA slowly and have minimal effects; extruded or ground oilseeds expose more of the FA to the rumen microorganisms and thus have greater impacts (NRC, 2001).

Little research has been conducted on feeding whole, unprocessed safflower seed (SS) to dairy cows. Godfrey (2006) showed that feeding unprocessed SS resulted in 50% of the seeds being excreted in the manure. Feeding coarsely ground SS at 2% of diet DM to dairy cows improved feed efficiency by 11% (Godfrey, 2006). Dschaak et al. (2010) using a new variety of safflower seed (Nutrasaff SS) reported that it can be fed to lactating dairy cows without negative impacts on lactational performance up to 3% DM. Feeding the Nutrasaff SS diet improved dietary N use for milk production as indicated by increase in efficiency of use of feed N to milk N and a decrease in MUN concentration. The enhanced functional quality of milk with increased *cis-9, trans-11* CLA concentration due to increasing the addition of Nutrasaff SS was an additional benefit to human health. The authors stressed further research was needed on the effects of Nutrasaff SS on ruminal fermentation with an emphasis on microbial protein synthesis (Dschaak et al., 2010).

However, Lammoglia et al. (1999) suggested that the whole SS needed to be processed to improve digestibility. The recommendations were to process (roll) the SS with enough pressure to crack about 90% of the seed hulls without extracting the oil (Lammoglia et al., 1999). Feeding coarsely ground SS at 2% of diet DM to dairy cows improved feed efficiency by 11% (Godfrey, 2006). Milk fat concentration was unchanged

when cows were fed rolled SS at 10% of dietary DM in diets containing at least 50% of the forage as alfalfa (Stegeman et al., 1992).

Use of Feed Additives in Dairy Diets

As rumen modifiers, many feed additives have been developed and extensively used in dairy diets to manipulate ruminal fermentation, improve nutrient utilization, and enhance lactational and environmental performance. Feed additives are a group of feed ingredients that can cause an animal response in a non-nutrient role, such as pH shift, growth, or metabolic modifier. Several feed additives contain nutrients, such as sodium in sodium bicarbonate or protein in yeast culture, substances such as probiotics, prebiotics, some organic acids involved in metabolic pathways, herbs, and plant extracts. Feed additives are not a requirement, nor are they a guarantee for high productivity or profitability. There has been continued interest in use of feed additives on dairy diets due to potentially positive results and improved profit margins, but securing consistent results on the use of feed additives has been challenged on-farm.

Zeolite

Zeolites are crystalline, hydrates of aluminosilicates of alkali and alkaline earth cations that have an infinite three-dimensional structure with interconnecting channels and large pores, capable of trapping molecules of proper dimensions. Zeolites are unique with respect to other minerals that possess cation adsorption and exchange capacity. Zeolites have the ability to selectively adsorb specific cations while reject others. This property is known as “molecular sieving.” Flanigen (1984) describes the silica and alumina tetrahedra as primary building units. The large structural cavities and the entry

channels leading into them contain water molecules, which form hydration spheres around exchangeable cations. Due to the presence of alumina, zeolites exhibit a negatively charged framework counter-balanced by positive cations, resulting in a strong electrostatic field on the internal surface. Each zeolite has its own unique chemical composition, crystalline structure (similar to honeycomb), and therefore, possesses its own set of adsorption properties. Water moves freely in and out of the pores; however, the zeolite framework remains rigid. Exchangeable cations maintain electrical neutrality within the structure.

Clinoptilolite Zeolite

Clinoptilolite (**CLN**) is a member of the natural zeolite family and is the most widely used natural zeolite in animal studies due to its structural stability under high temperatures and acidic conditions. Clinoptilolite has widespread applications in agriculture due to its high affinity for ammonium (NH_4^+) and K^+ ions and its stable behavior at high temperatures and low pH. The high cation exchange capability, open cage-like structures with large internal and external surface areas (Figure 2.6) and high permeability make CLN a versatile additive with many uses. Clinoptilolite's high affinity for NH_4^+ (Barbarick and Pirella, 1984; Blanchard et al., 1984) and other ions enable it to be used to remove NH_4^+ from the rumen of cattle. The high cation exchange capacity of the inner matrix and large surface area of dehydrated channels and cavities are the properties that allow CLN to adsorb NH_4^+ . Therefore, the NH_4^+ will not be lost to the atmosphere. Physical elements such as pH, NH_4^+ concentration, and temperature all affect the ability of CLN to absorb the NH_4^+ ions.

The ion exchange capabilities of zeolites influence microbial and animal metabolism through the preferential trapping and release of cations (McCollum and Galyean, 1983). In an attempt to reduce the toxic effects of high NH_4^+ content of ruminal fluids when high N compounds are added to the diets of cattle, natural zeolites were introduced into the rumen of animals (White and Ohlroggi 1974). White and Ohlroggi (1974) found that NH_4^+ ions were immediately ion exchanged into the zeolite structure and held there for several hours until released by the regenerative action of Na^+ entering the rumen in saliva during after-feeding fermentation. Data from White and Ohlroggi (1974) using both in vivo and in vitro showed that up to 15% of the NH_4^+ in the rumen could be taken up by the zeolite, allowing gradual release of excess N into the rumen which contributes to capturing the N into microbial protein for assimilation into the animals' digestive systems. The zeolite's ability to act as a reservoir for NH_4^+ permits the addition of supplemental N to the animal feed while protecting the animal against the production of toxic levels of NH_3 in the rumen (White and Ohlroggi 1974). Pond et al. (1981) postulated that the zeolite bound free NH_3 in the gastrointestinal tract, thereby preventing its accumulation to toxic levels in the system. Peterson (1980) also stated that zeolite, when introduced into an acidic environment, will exchange constituent ions for hydrogen ions that could allow zeolite to act as a buffering agent.

In contrast, Sweeney et al. (1980) observed no effect of a synthetic zeolite on NH_3 concentrations. Peterson (1980) stated that CLN, when introduced into an acidic environment, exchanged constituent ions for hydrogen ions that could allow CLN to act as a buffering agent. Sweeney et al. (1980) reported a higher acetate-to-propionate ratio and reduced DM digestibility and blood K levels in dairy cattle consuming zeolites.

Increased DMI has been observed in beef and dairy cattle consuming zeolites, but growth response has not been consistent (Mumpton and Fishman, 1977; Sweeney et al., 1980). Galyean and Chabot (1981) found no increase in intake when CLN was supplemented to beef steers. Eng et al. (2003) conducted four feedlot studies feeding CLN at 1.2% of the diet. The steers were housed in concrete pens for a more complete manure collection. Improved steer performance was only observed in one of the four trials. Average daily gain was increased by 4.7% from 1.75 to 1.83 kg/day by feeding zeolite. Therefore, feed conversion was also improved by 3% in one trial. However, addition of 1.2% zeolite to the diet did not affect manure-N levels.

Natural zeolites have proven to be effective in protecting animals against mycotoxins and aflatoxins, in feed and digestive system (Dvorak, 1989; Shell et al., 1992) resulting in measurable improvements in animal health (Galabov et al., 1991; Kovac et al., 1995; Lon-wo et al., 1993). Thilsing-Hansen and Jorgensen (2001) showed that the addition of zeolite to the daily ration during the last month of pregnancy prevented parturient paresis as well as subclinical hypocalcemia of Jersey cows.

Zeolite has the potential to reduce N and P in manure and minimize the negative effects of odor and other gaseous emissions such as NH_3 and hydrogen sulfide. The loss of NH_3 from manure is a function of total NH_3 content, pH of the manure, and NH_3 concentration of the ambient air. Ammonia loss can be decreased by reducing manure pH or NH_4^+ ion concentration. As a manure treatment additive, zeolite is efficient in controlling NH_3 and effective in absorbing volatile organic compounds and odor. According to Emfema (2005), zeolite allowed better performance of intestinal microflora, eliminated NH_3 odor, and contributed to a healthier environment for animals and humans.

Kithome et al. (1999) performed a study to determine the effect of pH on NH_4^+ adsorption by natural CLN zeolite. The authors reported a linear effect of pH; as pH increased, NH_4^+ adsorption capacity of zeolite increased. The amount of NH_4^+ ion adsorbed also increased with increasing initial NH_4^+ ion concentration.

Condensed Tannins

Tannins are polyphenols compounds that are widely distributed in legumes, trees, fruit, and shrubs (Min et al., 2003). Although tannins are chemically a diverse and ill-defined group, it is usual to divide them into two types: the hydrolysable and the condensed tannins. Hydrolysable tannins contain a carbohydrate as a central core (Haslam, 1989) and in most plants occur mainly in fruit pods and plant galls as protection against insect herbivory (McLeod, 1974). Condensed tannins (CT) are secondary plant compounds found in some plant species. These plant products are polymers of flavan-3-ol (catechin) or flavan-3, 4-diol (proanthocyanidins) units linked by C-C bonds (Figure 2.7) (Waghorn et al., 1997; McMahan et al., 2000) and are not susceptible to anaerobic enzyme degradation (McSweeney et al., 2001). The CT are typically found in plant cell vacuoles (Min et al., 2003), but location can vary among plant species. Condensed tannins are probably the most extensively studied plant secondary metabolites with reference to their physiological and nutritional consequences, and are the most common type of tannin present in cell walls, stems, bark, leaves, flowers, and seeds of trees, shrubs, and browse (McMahan et al., 2000; McSweeney et al., 2001; Min et al., 2003). Condensed tannin concentration of plants can also be influenced by maturity, temperature, and soil fertility as well as grazing (McMahan et al., 2000). Tannin concentration in birdsfoot trefoil (*Lotus corniculatus*) was reported to increase with

increasing plant maturity and was higher in regrowth than in spring growth (Cassida et al., 2000).

The structure of CT can vary greatly. They have various molecular weights and variable complexities, both of which affect their ability to bind proteins in aqueous solutions (Makkar, 2003). Monomers can be linked by C-4 and C-8 or C-4 and C-6 interflavan bonds, which alter the shape of the compound (Haslam, 1989; Barry and McNabb, 1999). Condensed tannins are termed proanthocyanidins because they release bright red anthocyanad chloride when treated with HCL/butanol (Waghorn, 2008).

Condensed tannins form complexes with many compounds, including protein and carbohydrates (cellulose, hemicellulose, and pectin) to form stable complexes (Barry and McNabb, 1999). Condensed tannins can bind protein by hydrogen bonding at near neutral pH (pH 6.0 to 7.0) in the rumen to form CT-protein complexes, and then dissociate and release bound protein at pH less than 3.5 as they enter the abomasum and small intestine (Barry et al., 2001; Mueller-Harvey, 2006). Thus, these CT-containing plants can protect dietary protein against degradation in the rumen and increase N utilization, resulting in reduction in MUN concentration and nitrogenous waste excretion and improved nutritional status of the animal. Therefore, feeding CT-containing forages in lactating dairy diets will be a promising means to improve N utilization and reduce excretion of nitrogenous waste by dairy cows.

The size and chemical structure of CT can have an effect on their reactivity, impact of digestion, and nutritional value (Molan et al., 2002, Waghorn, 2008). Some evidence indicates that CT have stronger binding affinity for proteins with a high molecular weight, flexible tertiary structure, and high AA content (Asquith and Butler, 1986,

Hagerman and Butler, 1991). Feeding forages with high concentrations of CT (10% DM or greater) has been shown to reduce feed intake, N utilization, and carbohydrate digestion (Waghorn et al., 1997; Barry and McNabb, 1999). Tannins may reduce intake of forage legumes by decreasing palatability or by negatively affecting digestion because of the astringency caused by the formation of complexes between tannins and salivary glycoproteins (Landau et al., 2000). High concentrations of CT depressed ruminal carbohydrate digestion in sheep fed big trefoil (*Lotus pedunculatus*), while no suppression in carbohydrate digestion was observed in sheep fed birdsfoot trefoil (Barry and McNabb, 1999). Alternatively, feeding forages containing moderate levels of CT (2-5% DM) have proven to be beneficial to animal production and nutrient utilization.

Condensed tannins have positive and negative effects on ruminants depending on the level of concentration as well as molecular weight (McSweeney et al., 2001). Moderate levels of CT at 5.5% DM can be beneficial to ruminants by improving N retention in the rumen where they decrease protein metabolism and increase absorption of plant AA in the small intestine (Waghorn, 1990; Barry and McNabb, 1999; Min et al., 2003). Also, CT have been known to decrease frothy bloat and reduce internal parasites in ruminant animals (Min et al., 2004; Min et al., 2006; Terrill et al., 2007). Concentrations above 5.0 to 6.0% DM have the potential to decrease voluntary intake and impair digestion of protein, P, cellulose, and hemicellulose by ruminant microorganisms (Barry and McNabb, 1999; McSweeney et al, 2001; Pagan-Riestra et al., 2009). However, the effect of CT also depends on other factors such as animal species, physiological state of the animal, and composition of the diet (McSweeney et al., 2001).

The interaction of tannins with protein alters the partitioning of N within the cow, shifting the route of excretion away from urine toward feces (Powell et al., 1994; Beauchemin et al., 2008; Waghorn, 2008). This reduction in urinary N reduces volatile N losses after land application of dairy manure (Misselbrook et al., 2005), which would in turn reduce environmental losses through nitrate leaching, NH₃ volatilization, and nitrous oxide emissions. Confirming these effects of CT-containing forages, Powell et al. (2009) reported that the ratio of N excreted in feces and urine was highest for low-tannin and high-tannin birdsfoot trefoil treatments and lowest for the alfalfa treatment.

Recently the effects of hydrolysable tannins and quebracho tannins (CT, 5% on DM basis) on the in vitro fermentation of ground wheat and corn grains by mixed ruminal bacteria was examined (Martínez et al., 2006). Regardless of the source of tannin, microbial fermentation was inhibited in both grains, as demonstrated by a decline in gas production, DM disappearance, and VFA and NH₃ production. However, these effects were more pronounced for wheat than corn grain, mostly during the initial stages of the incubation (Martínez et al., 2006). The authors found that tannins did not prevent bacterial attachment to starch granules, but starch hydrolysis was slowed indirectly as a result of a tannin-mediated reduction in the degradation of the surrounding protein matrix. Therefore, tannins are likely to be more effective at modulating the rate of starch digestion in grains that possess a readily degradable protein matrix such as wheat and barley. Wang et al. (1999) investigated varieties of barley with low amounts of tannins, but concentrations of CT on DM basis were low (0.2%), and far from those concentrations of CT that were shown to have some positive effects on protein utilization (1 to 4% on DM basis).

Ruminal fermentation when feeding CT

While the potential of CT to reduce fiber digestion has been documented, little research has been published on the effects of CT on VFA production. Although changes in VFA production have been reported when feeding CT, there are conflicting results between studies. Dahlberg et al. (1988) reported an increase in total VFA concentration and a shift from acetate production to propionate when feeding birdsfoot trefoil in continuous cultures. The shift from acetate to propionate observed by Dahlberg et al. (1988) may have been due to a slower rate of fiber degradation or suppression of cellulose digestion by CT present in birdsfoot trefoil. Khiaosa-Ard et al. (2009) also reported a shift in VFA production; total VFA concentrations remained the same between the treatments, but the molar proportion of propionate was increased, reducing the acetate-to-propionate ratio which may have been due to a reduction in NDF degradation caused by the CT present in sainfoin. de Oliveira et al. (2007) reported no effects on VFA production or proportions when feeding high and low tannin-containing diets. Carulla et al. (2005) and Bhatta et al. (2009) reported no change in total VFA; however, there were decreases in the molar proportion of acetate and increases in propionate when feeding CT to sheep and in vitro batch cultures. This may be due to a decreased rate of fiber digestion or a reduction in fiber degradability when feeding tannins (Waghorn et al., 1994; Carulla et al., 2005).

Moderate levels of CT (0.5-4.7% DM) have been found to have beneficial effects on ruminal fermentation. When 0.25 and 1.45% CT of DM, respectively, were fed to sheep (John and Lancashire, 1981), higher CT decreased protein solubility and ruminal $\text{NH}_3\text{-N}$ concentration, while it increased the amount of N retained when compared to the less CT.

Molan et al. (2001) suggests that CT not only reduce protein degradation in the rumen by binding with protein, but also altered the microbial population, particularly those strains most involved in proteolysis. Condensed tannins have also been shown to decrease digestion of cellulose by ruminal fungi by inhibiting endoglucanase activity (McAllister et al., 1994). Proteolysis by *Streptococcus bovis* and *Butyrivibrio fibrisolvens* was reduced when cultures were exposed to CT (Jones et al., 1994). This reduction in proteolysis is further confirmed by Dahlberg et al. (1988), when continuous cultures offered forage with CT exhibited a 90% reduction in ruminal NH₃-N concentration when compared to alfalfa hay. Scharenberg et al. (2007) also noted a decrease in ruminal NH₃-N concentration when comparing sheep fed sainfoin hay to those fed sainfoin hay with added polyethylene glycol (PEG), further documenting the N binding effects of the CT found in sainfoin. Polyethylene glycol forms an insoluble complex with tannins in the rumen, therefore preventing CT from binding to protein (Mantz et al., 2009). Reduced NH₃-N production was also observed in continuous cultures when comparing sainfoin hay to grass-clover hay (Khiaosa-Ard et al., 2009).

Feeding CT has also been shown to affect BH in the rumen. Some studies (Jones et al., 1994; Molan et al., 2001) have shown that CT from different legume forages inhibit cell growth and division of ruminal microorganisms, particularly *B. fibrisolvens*, that are responsible for ruminal BH (Jenkins et al., 2008). Grazing birdsfoot trefoil vs. perennial ryegrass led to increased concentrations of C12:0, C14:0, C16:0, C18:2 n-6, and C18:3 n-3, but reduced concentrations of *cis*-9 C18:1, *cis*-9, *trans*-11 CLA, and *trans*-11 C18:1 FA in milk (Turner et al., 2005). Addition of PEG, which blocks the action of tannins in the digestive tract, showed that the effect on C18:3 n-3 (i.e., reduced BH) was almost

certainly related to the effects of CT in birdsfoot trefoil, while effects on BH intermediates, notably a reduced *trans*-11 C18:1 concentration, were probably related to some other component in birdsfoot trefoil. Fermentors receiving tannins from carob, acacia leaves, or quebracho exhibited an increase in C18:1 and a reduction in C18:0, suggesting an alteration in the activity of microorganisms (Vasta et al., 2009). Piredda et al. (2002) showed increased C18:3 n-3 in milk when comparing white ginger (*Hedychium coronarium*) pasture with perennial ryegrass at either vegetative or reproductive stages. There were no consistent effects on *trans*-11 C18:1 or *cis*-9, *trans*-11 CLA. Vasta et al. (2007) reported that the intramuscular fat of lambs fed a carob-based diet (2.7% CT on DM basis) contained lower percentages of the *cis*-9, *trans*-11 CLA and *trans*-11 C18:1 FA than that of lambs fed the same diet supplemented with polyethylene glycol. The authors suggested that when CT from carob were not deactivated by polyethylene glycol, CT might have reduced the activity of ruminal bacteria, resulting in lower ruminal BH. However, a converse result was reported by Priolo et al. (2005) that the intramuscular FA of the lambs fed white ginger (1.8% CT on DM basis) contained higher levels of *cis*-9, *trans*-11 CLA than that of lambs fed a concentrate diet (0.91 vs. 0.46 g/100 g FA). Based on the results reported in the literature, the effects of tannins on FA composition of milk and meat may depend on source of CT, concentration of CT, and diet composition.

Animal performance when feeding CT

Shifts in ruminal fermentation patterns, as observed above, may ultimately result in changes in animal performance. Altering ruminal fermentation can have beneficial effects on energy status and milk composition. Decreased enteric methane (**CH₄**) emissions due to feeding CT may increase energy absorption by the animal. Furthermore, modifications

in VFA production and ruminal BH may affect energy status and milk production, as well as the FA composition of the milk. In addition, reducing protein degradation in the rumen, as is consistently reported when feeding CT, can increase the protein available in the small intestine, resulting in improved N utilization and animal production.

Waghorn et al. (1987) reported decreased ruminal N digestion and $\text{NH}_3\text{-N}$ concentration when feeding sheep birdsfoot trefoil compared to those receiving the same forage with added PEG. This study also found that the abomasal digesta in sheep fed birdsfoot trefoil contained 50% more essential AA and 14% more non-essential AA. Protein that bypasses the rumen improves N efficiency and is used more efficiently in the small intestine, as long as it contains essential AA (Van Soest, 1994). A 50% increase of essential AA entering the small intestine may increase N utilization by the animal (Waghorn et al., 1987). Woodward et al. (2000) reported increased milk protein in cows fed birdsfoot trefoil compared to those fed birdsfoot trefoil with PEG. In addition, an increase in milk yield was observed in cows grazing birdsfoot trefoil compared to those grazing birdsfoot trefoil with PEG, ryegrass, or ryegrass with PEG. The authors reported that CT in birdsfoot trefoil contributed to 46% of the difference in milk production between cows on birdsfoot trefoil or ryegrass. These results suggest that decreased protein degradation in the rumen, and the subsequently increased availability of protein in the small intestine when feeding birdsfoot trefoil may be responsible for improving milk production and milk protein in this study (Woodward et al., 2000). In a different experiment, N partitioning was measured in dairy cows fed increasing proportions of fresh birdsfoot trefoil, compared to those on ryegrass pasture (Woodward et al., 2009). Total N intake and N output did not vary with increasing birdsfoot trefoil. However, N

partitioned to milk increased with increasing birdsfoot trefoil because of an increased milk yield and similar milk protein concentration when increasing proportions of birdsfoot trefoil. Furthermore, while total N excretion remained unchanged, a shift in N from the urine to the feces was observed (Woodward et al., 2009). This reduction in urinary N excretion could potentially lead to reductions in NH₃ emissions from manure sources.

Another beneficial effect associated with tannins is the reduction in ruminal CH₄ emission (Woodward et al., 2001; Makkar, 2003). Although the exact mechanism is not clear, CT are effective in lowering CH₄ emissions, because they appear to have a negative effect on methanogenic bacterial activity as well as altering protozoal activity (Animut et al., 2008). A depression in CH₄ production was observed when feeding goats quebracho tannin, supplementing CT at 5.0% DM (Animut et al., 2008). The authors attributed this to a reduction in protozoa numbers and the methanogens associated with them or a reduced fiber digestion, which ultimately reduced H₂ substrate methanogens use for CH₄ production (Animut et al., 2008). Diets containing CT of birdsfoot trefoil at levels of 2.6% (DM basis) resulted in decreased CH₄ emission per kilogram of digested DM in dairy cattle (Woodward et al., 2001). Although no conclusive explanations were given, the authors suggest that tannins might have had a deleterious effect on methanogenic bacteria, corroborating the hypothesis that the oxidized catechin, a CT, decreases the growth of methanogenic bacteria (Scalbert, 1991). Adding Acacia tannin extract powder to the diet of sheep at a rate of 2.5% of DM intake decreased enteric CH₄ emission by about 12% with only a marginal decrease in fiber digestion (Carulla et al., 2005). Beauchemin et al. (2007) found feeding up to 1.8% DM of quebracho tannin to steers did

not reduce enteric CH₄ emissions or digestibility of the dietary DM. Furthermore, de Oliveira et al. (2007) reported that there was no depression in CH₄ production when feeding low levels of CT (1.0% DM). These studies show that tannins hold some promise in terms of CH₄ abatement, but the CT structure and concentration of tannin need considerable refinement to ensure CH₄ emission is lowered without negatively affecting fiber digestion and milk production.

Cows grazing birdsfoot trefoil produced 17% less CH₄ per unit of DMI than their counterparts grazing ryegrass (Woodward et al., 2004). They calculated that 66% of this reduction was due to the action of tannins in the birdsfoot trefoil, and also estimated that if the energy saved from this decrease of CH₄ were absorbed, it could result in an increase of 0.6 kg of daily milk production. Cows grazing birdsfoot trefoil increased milk production, and similar concentrations of milk fat and protein when compared to those grazing ryegrass (Woodward et al., 2004). Cows offered birdsfoot trefoil silage also had decreased CH₄ emissions per unit of DMI and per kg of milk solids produced when compared to those fed ryegrass silage (Woodward et al., 2001). The cows fed birdsfoot trefoil silage also increased milk production and DMI. Increased milk production was also observed in lactating ewes grazing birdsfoot trefoil when compared to those grazing birdsfoot trefoil with PEG (Wang et al., 1996). Turner et al. (2005) grazed lactating cows on either birdsfoot trefoil or ryegrass pasture, with half of the cows in each treatment receiving PEG. An increase in milk production was noted when cows grazed birdsfoot trefoil when compared with ryegrass, as well as in cows that grazed birdsfoot trefoil when compared to those on birdsfoot trefoil and PEG. These results further confirm the feeding value of birdsfoot trefoil and beneficial effects of CT on animal production. A

decrease in C18:0 and increase in C18:2, C18:3, and n-3 FA were found in the milk from cows fed birdsfoot trefoil when compared to those fed ryegrass, suggesting CT altered BH in the rumen, and subsequently the FA composition of the milk (Turner et al., 2005). This decrease in saturated FA and increase in n-3 FA in milk when feeding birdsfoot trefoil could have beneficial effects on human health.

Condensed tannins have also been reported to decrease parasite loads. Marley et al. (2003) found that lambs grazing birdsfoot trefoil had reduced helminth numbers when compared to those grazing ryegrass and white clover. Niezen et al. (1995) found that sheep fed the tannin-containing legume sulla (*Hedysarum coronarium*) had increased ADG and decreased numbers of parasites when compared to their counterparts fed alfalfa. Although the exact mechanism is unknown, Athanasiadou and Kyriazakis (2004) suggested that CT may reduce worm numbers or mitigate the effects of parasitism. However, more research is needed to understand the anthelmintic properties of CT, as responses vary with the source of CT.

Fat Supplementation to Dairy Cows

Fat supplements are simply considered energy sources, hence their economic value derives largely from their ability to increase NE_L intake by cows and have been used for years to increase diet energy density and increase milk yield (Jenkins and McGuire, 2006). Factors that account for essentially all the differences among fat supplements as energy sources are GE concentration of the supplement, digestibility of the supplement, effects of the supplement on digestibility of other dietary components, and effects on DMI (Weiss et al., 2011). Dietary fat usually constitutes approximately 3 to 5% of dairy lactation diets, and is a component of almost every feedstuff including forages and grains.

Commonly used fat supplements can be grouped into natural fats which include plant oils, oilseeds, animal or animal/vegetable blends, and fats that have been modified with the intent of making them more ruminally "inert" (rumen protected fats). The latter group includes calcium salts of FA and hydrogenated fat. Generally, oleic acid (C18:1) and linoleic acid (C18:2) predominate in most seed lipids such as canola, corn, and soybean in the form of triglyceride, although linseed (flaxseed) is rich in linolenic acid (C18:3). Forages are characterized by high content of galactolipid and phospholipid, and contain substantial quantities of the monounsaturated FA, oleic acid (C18:1), and the two most abundant polyunsaturated FA, linoleic acid (C18:2) and linolenic acid (C18:3) (Mir et al., 2006).

Replacing digestible carbohydrate with digestible fat increases the energy concentration in the diet because fat has much higher GE and the efficiency of converting DE from fat to NE_L is higher (Weiss and Pinos-Rodríguez, 2009). Thus, fat can be used to overcome the limitation in energy supplies in diets for high production cows, while maintaining NDF and forage content. NRC (2001) states that fat supplements increase the absorption of fat-soluble nutrients, such as fat-soluble vitamins and tend to increase reproductive efficiency. Staples (2006) reported that supplementing cows with fat can have several beneficial effects: usually increasing the energy density of the diet resulting in increased milk production and feed efficiency, which usually translates into more profit. Less heat may be produced in the rumen during digestion of fat supplemented diets, because dietary long-chain FA are not fermented in the rumen, thus reducing heat increment (NRC, 2001). Less heat produced during digestion would help cows during heat stress conditions. Because of its energy density and no contribution to heat

increment, feeding fat is common during the summer months when DM intake will likely be depressed. Reduced dustiness and particle separation of some finely ground feedstuffs during diet mixing if liquid fat is supplemented can be observed. As a result, fat inclusion can be a good choice for diet formulation.

During early lactation, high-producing cows cannot consume enough feed to meet their energy needs. Fats contain 2.25 times more energy than the starches and digestible fiber found in grains and forages. In early lactation, cows are usually in negative energy balance and intake of energy is the primary limitation on milk yield (Allen, 2000), and limited by DMI capacity suggesting that rumen fill, not energy demand, regulates intake (Weiss and Pinos-Rodríguez, 2009). At the same time, adequate amounts of forage and fiber can be fed to maintain a healthy rumen. Harvatine and Allen (2005) explained that adding fat to the diet increases energy density without increasing ruminal acid production, thus stabilizing ruminal pH relative to addition of grain thereby reducing the severity of the negative energy balance the animal experiences. The increase in energy density achieved through the feeding of supplemental fat may enhance lactational performance (Weiss et al., 2011) as well as metabolic efficiency of dairy cattle (Kronfeld et al., 1980). Without sufficient dietary energy, cows will produce milk less than their potential milk production (Harvatine and Allen, 2006b). Evidence suggests that feeding of certain fats also seems to improve reproductive performance (Butler, 2005).

The NRC (2001) recommended diets contain up to 4% DM from supplemental fat and total dietary fat not exceeding 7% DM, considering about 3% DM fat from cereal grains and forages. It is recommended that diets for lactating cows not exceed 5% total fat from natural fat sources which include forages, cereal grains, oilseeds, and tallow.

Two to 3% fat could be supplied by the forages and normal cereal grains found in the diet. Other 2 to 3% could be supplied from oilseeds or tallow. An additional 2 to 3% fat (to make a total of 8% fat in the total ration) can be added by using specialty or ruminally inert fats. Exceeding this much fat may negatively affect dairy cattle.

Results from feeding fat supplements, as a whole, may differ between studies and by sources of fat supplements and rate of supplementation (Allen, 2000). Possible explanations for the variation in response to supplementation of individual fat supplements include management practices, stage of lactation, and nutritional effects. Differences between fat supplements may be levels of saturation, ratios of individual FA, and the level to which the fat is protected from processing in the rumen (Allen, 2000). Results from individual fat supplements cannot be generalized to all supplements, and inconsistent results, both within supplement and overall supplements, are found in DMI and milk yield.

Yield of milk and milk components and DMI

Fat supplementation to high forage diets allows the ration to be high in energy density without the negative side effects of a starchy high-grain diet. Adding fat supplements to high forage diets may supply the animals with enough energy to maintain high levels of milk production and milk component yield with minimal use of cereal grains. High grain diets had lower milk fat content than high forage diets (Yang and Beauchemin, 2009). This could be attributed to milk fat depression caused by elevated levels of propionate production in the rumen causing a shift of lipid synthesis precursors away from the mammary gland and toward insulin-sensitive tissues of the body. A recent study by Harvatine and Allen (2006a) showed that the addition of fats to the diet allows for the

maintenance of energy density while increasing fiber intake, which stabilizes rumen fermentation. A fat supplement that maximizes DMI and ruminal fiber digestion increases milk production and milk component yield, and improves health and reproduction of dairy cows.

Several studies have reported no significant effect of lipid supplementation on milk production and milk protein concentration of lactating cows (Selberg et al., 2004; Rodríguez-Sallaberry et al., 2007; Do Amaral, 2008). Mattos et al. (2004) also reported no effect of unprotected lipid supplementation (fish oil or olive oil) on milk production and milk fat and milk protein concentration. Donovan et al. (2000) reported an increase in milk yield corresponding to an increase in dietary fish oil concentration from 0 to 1% (DM basis), followed by a linear decrease as fish oil concentration increased from 1 to 3%. Whitlock et al. (2002) found that milk production and milk fat concentration were lower for lactating cows that consumed either a diet containing 2% fat from fish oil or a combination of 1% fat from fish oil and 1% fat from extruded soybeans, compared to cows fed a control diet or a diet containing 2% fat from extruded soybeans. The significant reduction in milk yield reported by Donovan et al. (2000) and Whitlock et al. (2002) might be attributed to the reduction in intake associated with dietary fish oil or related to differences in parity.

Generally, milk fat concentration has increased when the recommended amount of supplemental fat is fed to dairy cows. Zheng et al. (2005) tested the effects of feeding vegetable oil high in total C18 on performance of dairy cows and found that using oils derived from cottonseed, soybean, and corn had no effect on milk production or milk protein concentration, but the oil supplementation affected milk fat concentration; when

the supplement was derived from cottonseed, there was no difference in milk fat concentration, but when fed with oils derived from soybean or corn the milk fat concentration was significantly decreased.

The effect of fat supplementation on the DMI of dairy cows has varied and is dependent on the type and amount of fat fed. Factors that determine if a reduction in DMI will occur due to fat supplementation include the degree of ruminal protection of the fat supplement and the amount of fiber fed in the diet. The reduction in fiber digestibility observed with diets containing a high level of polyunsaturated FA is a result of the change in microbial population and ruminal environment that is attributed to a reduction of DMI (Doreau and Chilliard, 1997). Feeding of protected fats seems to have a less pronounced effect on DMI, because the slow release of the unsaturated FA from the calcium salt complex prevents rapid modifications of the ruminal environment, therefore minimizing the effect of these fats on fiber digestion. The depression in DMI from fat feeding is due, in part, to a reduction of fiber digestion leading to prolonged rumen fill and decreased palatability attributed to fat supplements (Allen, 2000). In addition, dietary fat supplementation increases plasma concentration of cholecystokinin (Bradford et al., 2008), which can inhibit reticuloruminal motility (Matzinger et al., 2000).

Harvatine and Allen (2006b) investigated Holstein cows fed different fat supplements at varying levels of saturation. Four diets were fed, three of which were supplemented at a rate of 2.5% of diet DM with a saturated, intermediate, or unsaturated fat supplement. In the study, all fat supplemented diets showed a decreased DMI compared with control. Furthermore, there was a linear decrease in DMI with increasing levels of unsaturated fat. This suggests that there may be specific characteristics to each fat supplement that

decreases DMI (Harvatine and Allen, 2006b). In a study by Mattos et al. (2004), addition of fish oil to the diet reduced DMI in the prepartum and postpartum periods by 30.3 and 18.1%, respectively, compared to cows fed an olive oil rich diet. In this study, prepartum rations contained 2% oil (DM basis) and postpartum diets contained 1.8% oil. These data agree with the reduction in DMI observed in dairy cows fed a diet containing 4.5% sunflower and fish oils (Shingfield et al., 2006), as well as for primiparous cows fed diets containing fish oil at 1 to 2% (DM basis) (AbuGhazaleh et al., 2002). Ruminant infusion of fish oil also resulted in a decrease in intake (Castañeda-Gutiérrez et al., 2007). Feeding of protected fats or abomasal infusion of fats seem to have a less pronounced effect on DMI, which could be related to ruminal inertness and minimizing the effects of these fats on fiber digestion. Other studies agree that abomasal infusion of long-chain FA or *trans* FA causes a reduction in DMI (Romo et al., 2000). On the other hand, no effect of lipid supplementation was observed in cows fed a mixture of protected palm and fish oil at 2.7% of dietary DM compared to cows receiving a no fat diet (Allred et al., 2006). Feeding of protected fats up to 5% of dietary DM also had no significant effect on DMI (Moallem et al., 2000; Schroeder et al., 2003). Discrepancies between studies suggest that the effects of feeding protected fats on DMI of dairy cows may depend on animal parity, stage of lactation of the animals, source of the fat, protection of the fat, degree of saturation of the FA, and proportion of fat in the diet.

Unsaturated fat supplementation to dairy cows

As the unsaturation of a rumen available fat increases, so does the negative impact it will have on rumen fermentation (NRC, 2001). Unsaturated free FA have relatively short half-lives in ruminal fermentation, because they are rapidly hydrogenated by microbes to

more saturated end products. If the ruminal microorganisms' capacity for BH is exceeded, unsaturated FA can accumulate in the rumen and potentially interfere with fermentation, especially fiber fermentation. Diets containing more than 6% rumen-available fat (especially unsaturated forms) have long been known to inhibit fiber digestion by ruminal microbes and in some cases, decrease feed intake (Bradford et al., 2008). Harvatine and Allen, (2006b) reported that unsaturated FA had no negative effects on ruminal digestibility of OM, starch, or NDF compared with no FA supplementation. When fat supplements inhibit ruminal fermentation, limited hindgut fermentation may lessen the fiber digestibility depression in the whole digestive tract (Jenkins, 1988), but increased fiber excretion in feces often still occurs (Palmquist and Jenkins, 1980). The amount of unsaturated FA appearing in the small intestine is likely influenced not only by the source of supplemental fat but also by DMI influencing passage rate of digesta, the amount of fat fed influencing ruminal fermentation, and the fiber content of diet influencing mastication of oilseeds and release rate of oil (Staples et al., 1998).

Several mechanisms have been proposed to explain how lipids interfere with ruminal fermentation. The lipid "coating" theory and the theory of direct antimicrobial effects have received the most attention (Zheng et al., 2005). The coating theory attempts to explain reduced fermentation by a lipid layer over feed particles that inhibit digestion of cellulose. This lipid covering is proposed to cause detrimental effects by inhibiting close contact of microbial cells or their hydrolytic enzymes with feed particles.

Unsaturated FA act as antimicrobial agents by interfering with normal function of the ruminal microbes. The antimicrobial effect of dietary lipids is associated with the degree of unsaturation of the FA present (Zhang et al., 2008; Yang et al., 2009). Polyunsaturated

FA are more toxic for biohydrogenating bacteria than di- or monoenoic FA (Maia et al., 2007, 2010), and thus oils containing PUFA such as linolenic acid would be expected to have a greater effect on ruminal BH and fermentation processes than those rich in linoleic acid or oleic acid. Staples (2006) speculated that the act of BH by bacteria is an attempt to protect themselves, as unsaturated fats can be toxic to bacteria, primarily cellulolytic bacteria. During the process of BH of unsaturated fats in the rumen, the conversion to the saturated state may be incomplete. Bauman and Lock (2006) also stated that unsaturated FA are toxic to many rumen bacteria, so the major transformation that dietary lipids undergo in the rumen is BH of polyunsaturated FA. If feeding unsaturated fats reduces the numbers or activity of cellulolytic bacteria in the rumen, then DMI, milk production, and milk fat concentration can decrease.

Excessive concentrations of unsaturated fat will interfere with fiber digestion in the rumen, and high concentrations of total fat may decrease DMI (Eastridge, 2006). Sanchez et al. (2004) stated that although DMI may be reduced slightly, unsaturated FA increase milk production and feed efficiency of high producing dairy cows. According to Avila et al. (2000), limited evidence indicates that fiber digestibility is not affected, nor is changes in ruminal fermentation patterns substantially when diets include whole oilseeds.

Jenkins and McGuire (2006) stated that untreated vegetable oils high in unsaturated FA have only limited ability to alter milk FA composition. The delivery of unsaturated FA to mammary tissue is limited even when their dietary concentration is high because of the transformation of dietary unsaturated FA during BH. Fat supplementation has been shown to differentially change the FA profile of milk, depending on the fat supplement being ingested by the cow. Therefore, ruminant milk fat concentration and composition

can be extensively modified by nutritional factors, in particular fat supplementation of the diet (Shingfield et al., 2008). Milk FA composition may be improved due to an increased proportion of total unsaturated FA in the milk, including CLA, *trans*-vaccenic acid (C18:1 *trans*-11), and n-3 FA. Research has shown that the *cis*-9, *trans*-11 CLA isomer has anticarcinogenic and antiatherosclerotic properties (Huth et al., 2006). Increasing the proportion of these FA in milk may improve its consumer appeal, enhancing its salability and therefore benefiting the dairy industry.

Milk fat yield was not significantly affected by feeding 4.6% high-C18:1 sunflower seeds vs. 4.34% high-C18:2 sunflower seeds providing similar dietary FA (AbuGhazaleh et al., 2003), or by feeding 0.5 kg/d rapeseed oil vs. sunflower oil in concentrate supplemented to grazing cows (Rego et al., 2009). However, feeding a diet containing 2% fish oil increased the concentrations of CLA and C18:1 *trans*-11 in milk fat to 356% and 502%, respectively, of basal amounts when no fish oil was fed (Donovan et al., 2000). Increasing the amount of fish oil in the diet to 3% resulted in no further increase in the concentration of these FA, but did increase the profile of n-3 FA in milk when no fish oil was added to the diet. Most of the increase in n-3 FA was due to EPA and DHA, because there was no change in C18:3 concentration. Similar results were observed when feeding a diet with 2% fat from fish oil, 2% fat from extruded soybeans, or a combination of both. All dietary treatments increased the proportion of n-3 FA in milk, with no significant difference among fat supplements (Whitlock et al., 2002).

Concentrations of C18:1 *trans*-11 and *cis*-9, *trans*-11 CLA were higher in milk fat from cows fed fish oil than milk fat from cows receiving the extruded soybean-supplemented diet. This is in contrast to the data obtained by AbuGhazaleh et al. (2002),

who reported that concentrations of *cis*-9, *trans*-11 CLA and C18:1 *trans*-11 were increased in milk fat by all fat supplements, with no differences in milk CLA and C18:1 *trans*-11 observed among fat supplements. Feeding fish oil also increased the proportion of individual and total n-3 FA as well as of total CLA in milk when compared to cows fed olive oil (Mattos et al., 2004). Selberg et al. (2004) also reported an increase in the milk FA concentration of CLA (namely the *trans*-10, *cis*-12 isomer) and *trans*-C18:1 FA due to supplementation with calcium salt of CLA and calcium salt of *trans*-FA. In addition, supplementation of CLA decreased short- to medium-chain FA concentrations and increased both C18:2 and C18:3 amounts in milk fat.

The higher concentration of CLA observed when feeding fish oil can be explained by the modification of ruminal or systemic functions caused by fish oil feeding, which stimulates increased conversion of C18:2 and C18:3 present from other feeds, to C18:1 *trans*-11 and CLA (Whitlock et al., 2002). Cows supplemented with n-3, n-6, or a mixture of both FA, had higher C18:1 *trans*-11 to CLA ratio in ruminal digesta compared with no fat supplementation, indicating that the fat supplements increased milk CLA concentration mainly by increasing ruminal production of C18:1 *trans*-11. This observation indicates a significant role for mammary Δ^9 -desaturase in milk CLA production (AbuGhazaleh et al., 2002). The decrease in short- and medium-chain FA observed with CLA supplementation can be explained by the increased proportion of long-chain FA in milk as a result of feeding increasing amounts of these FA, and by their inhibition of de novo synthesis of short- and medium-chain FA in the mammary gland (Selberg et al., 2004).

Biohydrogenation of FA in the rumen and its impacts on milk fat depression

Lipids are extensively altered in the rumen, resulting in marked differences between the FA profiles of lipids in the diet (mostly unsaturated FA) and lipids leaving the rumen (mostly saturated FA). When dietary material enters the rumen, it enters a large fermentation vat, where it undergoes a wide range of chemical changes performed by the microbial population. Ruminant microbes transform lipids entering the rumen via two major processes, lipolysis and BH (Jenkins et al., 2008). Lipids entering the rumen are first transformed by microbial lipases in a process called lipolysis. After lipolysis, unsaturated FA undergo BH by ruminal microbes. This process depicted in Figure 2.8 converts the unsaturated FA to saturated FA via isomerization to *trans* FA intermediates, followed by hydrogenation of the double bonds (Chilliard et al. 2007). Several previous theories have been proposed to explain diet-induced milk fat depression (MFD) including acetate deficiency (Tyznik and Allen, 1951), P-hydroxybutyrate deficiency (Van Soest and Allen, 1959), glucogenic-insulin theory (McClymont and Vallance, 1962), and *trans* FA theory (Davis and Brown, 1970). However, subsequent work either provided little support or indicated that those theories could not adequately explain the MFD under different dietary conditions.

Under certain dietary situations the rumen environment is altered, and a portion of BH occurs via a pathway that produces *trans*-10, *cis*-12 CLA and *trans*-10 18:1 FA (Figure 2.8). Therefore, dietary situations causing MFD alter the pathways of ruminal BH resulting in changes in the specific *trans*-18:1 FA and CLA isomers. As shown in Figure 2.6, this '*trans*-10 FA shift' in BH pathways and the associated increase in the *trans*-10 18:1 FA concentration of milk fat are indicative of the complex changes in ruminal BH

pathways, which is a characteristic of MFD. Although *trans*-10 18:1 FA does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007), it is relatively easy to analyze compared with *trans*-10, *cis*-12 CLA and other CLA isomers. Therefore, in general, this FA can serve as a surrogate marker for the type of alterations in ruminal BH that characterize diet-induced MFD (Lock et al., 2007). This is highlighted in Figure 2.9, which shows the relationship between the content of *trans*-10 18:1 FA concentration in milk fat and milk fat concentration (Bauman and Griinari, 2003). Also shown in Figure 2.8 are the three predominant ways in which dietary components can impact the risk of MFD: 1) through increasing substrate supply of C18 unsaturated FA, 2) by altering the rumen environment and BH pathways, and 3) via changes in the rate of ruminal BH at various steps in the BH process.

Work of Bauman and Griinari (2001) identified that specific *trans* FA isomers rather than total *trans* FA are more crucial to diet-induced MFD. Previous studies confirmed that abomasal infusion of *cis*-9, *trans*-11 CLA (Baumgard et al., 2000) or *trans*-11 and *trans*-12 mixture (Griinari et al., 2000) had no effect on milk fat yield. An increase of milk fat proportion of *trans*-10 C18:1 rather than total *trans*-C18:1 was associated with MFD (Griinari et al., 1998), and this was confirmed by others (Piperova et al., 2000). In addition, altered ruminal condition by MFD diets also increased rumen production of *trans*-10, *cis*-12 CLA and its subsequent incorporation into milk fat (Bauman and Griinari, 2001). Thus, a portion of the dietary C18:2 undergoes BH via a unique pathway that produces *trans*-10, *cis*-12 CLA which is further biohydrogenated into *trans*-10 C18:1. Based on the preceding results, Bauman and Griinari proposed the "BH theory"

that under certain dietary conditions, the pathways of ruminal BH are altered to produce unique FA intermediates which depress milk fat synthesis.

Several FA intermediates have been identified to be inhibitory of milk fat synthesis, and they have different potencies on MFD. Baumgard et al. (2002) found that milk fat yield was decreased by 48% by abomasal infusion of *trans*-10, *cis*-12 CLA. Saebo et al. (2005) abomasally infused *trans*-10, *cis*-12 CLA and *trans*-10, *trans*-12 CLA. *Trans*-10, *trans*-12 CLA infusion had no effect on milk fat yield, whereas *trans*-10, *cis*-12 CLA decreased milk fat (Saebo et al., 2005). Comparable reductions in milk fat yield by different amounts of *trans*-10, *cis*-12 CLA supplied indicated anti-lipogenic effect from other 10, 12 isomers in the CLA mixture. Evidence from this experiment showed that *cis*-10, *trans*-12 CLA exerts at least as potent MFD effects as *trans*-10, *cis*-12 CLA (Saebo et al., 2005). Similarly, Perfield et al. (2007) reported milk fat yield was reduced by 15% for *cis*-9, *trans*-11 CLA mixture and by 27% for *trans*-10, *cis*-12 CLA infusion, whereas *trans*-9, *trans*-11 CLA had no effect on milk fat yield which ruled out the effect of *trans*-9, *trans*-11 CLA in 9,11 CLA mixture on MFD. Perfield et al. (2007) proposed that *trans*-9, *cis*-11 CLA in the CLA mixture infused was responsible for MFD, although its potency was less than *trans*-10, *cis*-12 CLA.

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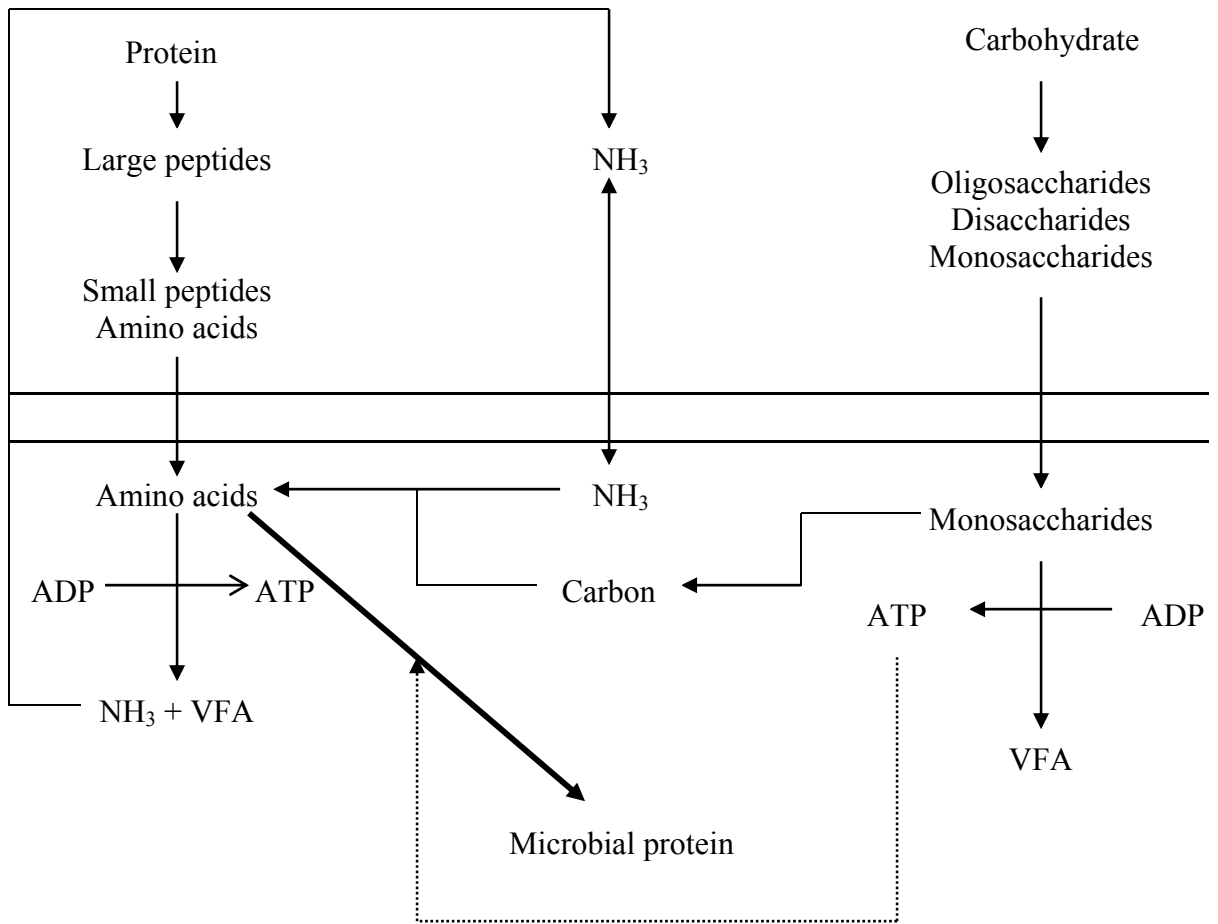


Figure 2.1. A theoretical scheme showing carbohydrate and protein utilization by ruminal bacteria. (Cotta and Russell, 1996)

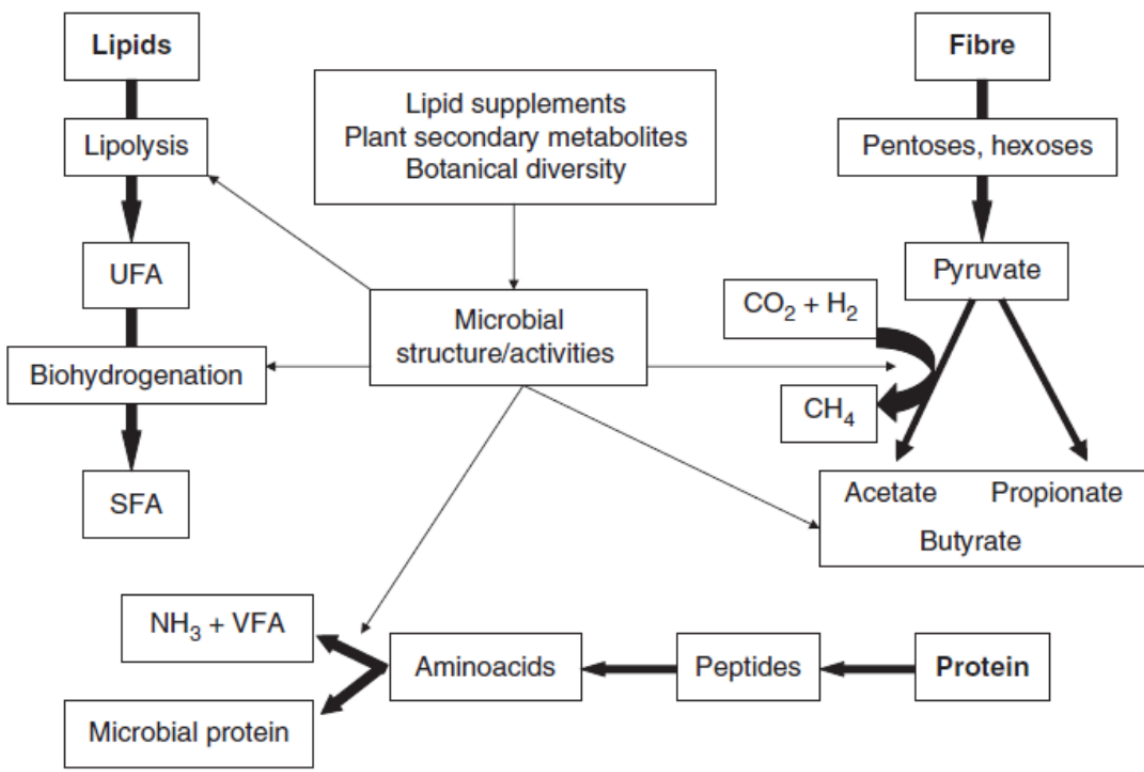


Figure 2.2. Interventions to manipulate fermentation and metabolism in the rumen. Sometimes the target organisms have several functions, in other cases the metabolic pathways are linked, for example by the availability of H₂. (Lourenco et al., 2010)

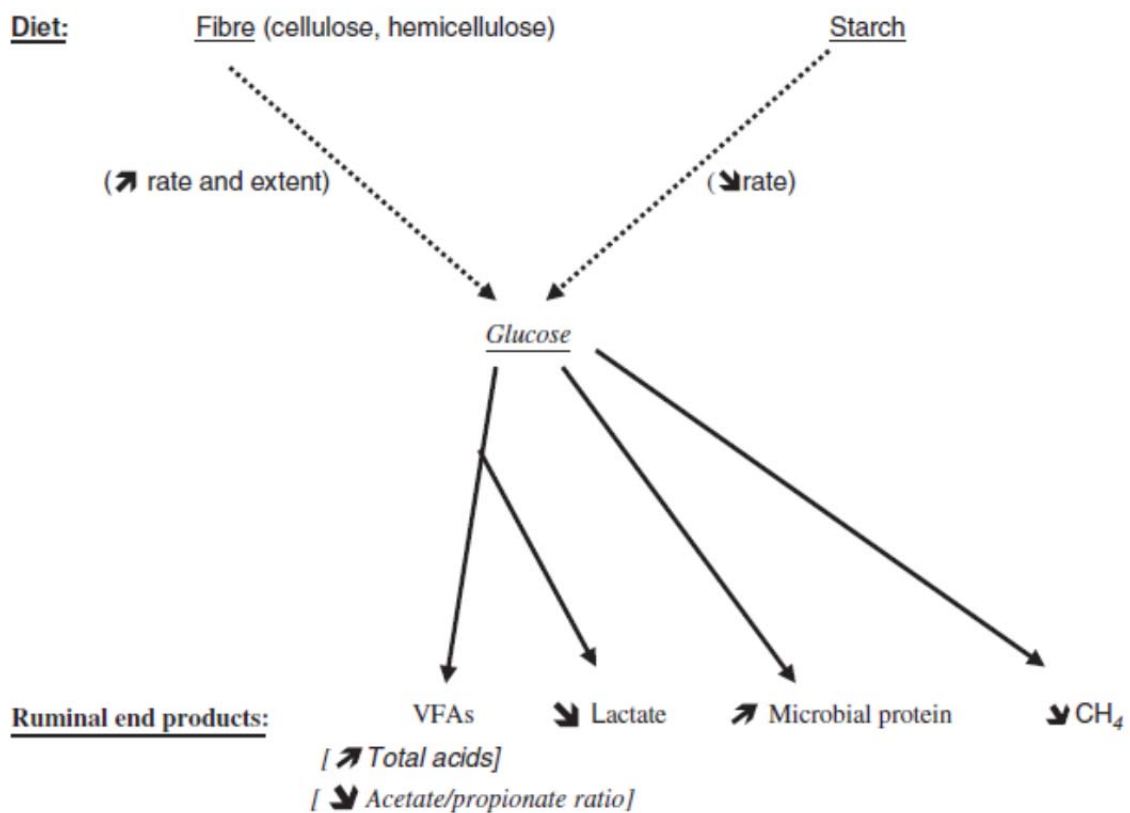


Figure 2.3. Possible sites targeted by feed additives to improve carbohydrate fermentation in the rumen. (↗ : increase of the function; ↘: decrease of the function; Jouany and Morgavi, 2007)

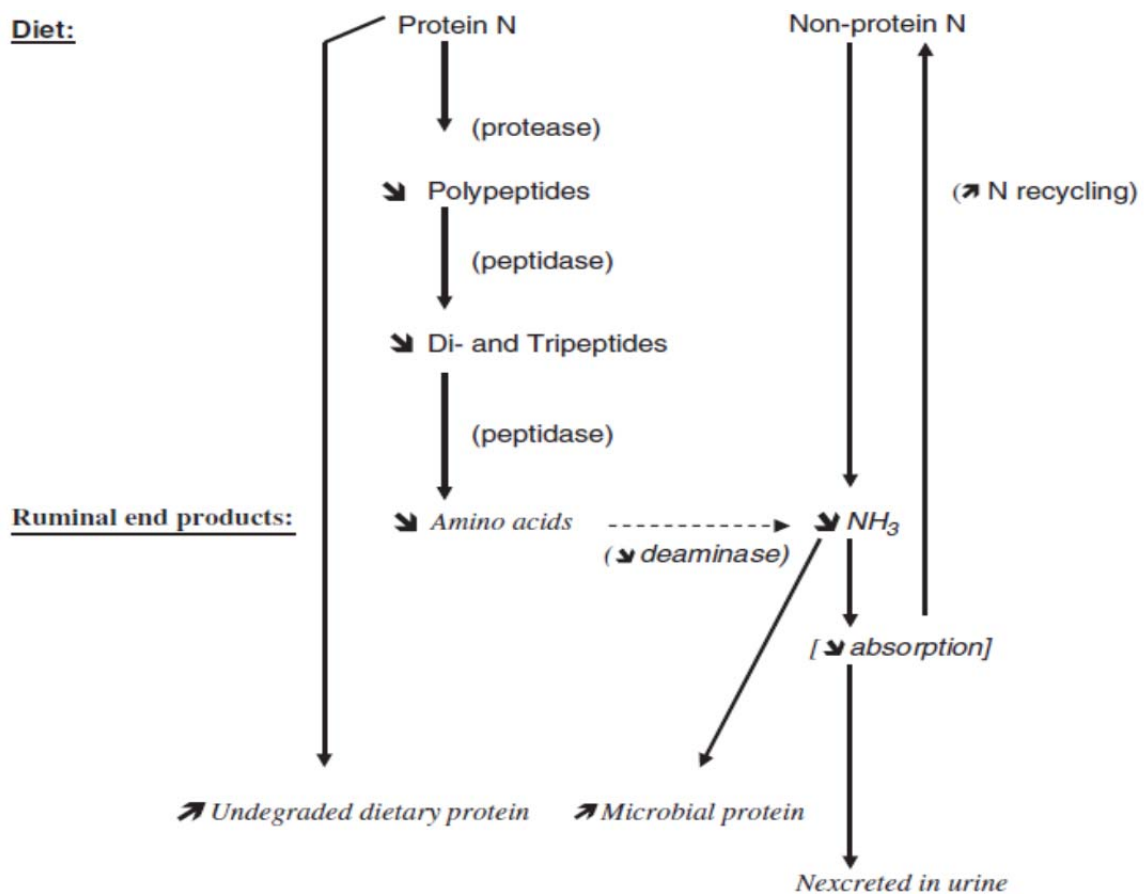


Figure 2.4. Possible sites targeted by feed additives to improve nitrogen metabolism in the rumen. (↗ : increase of the function; ↘ : decrease of the function; Jouany and Morgavi, 2007)

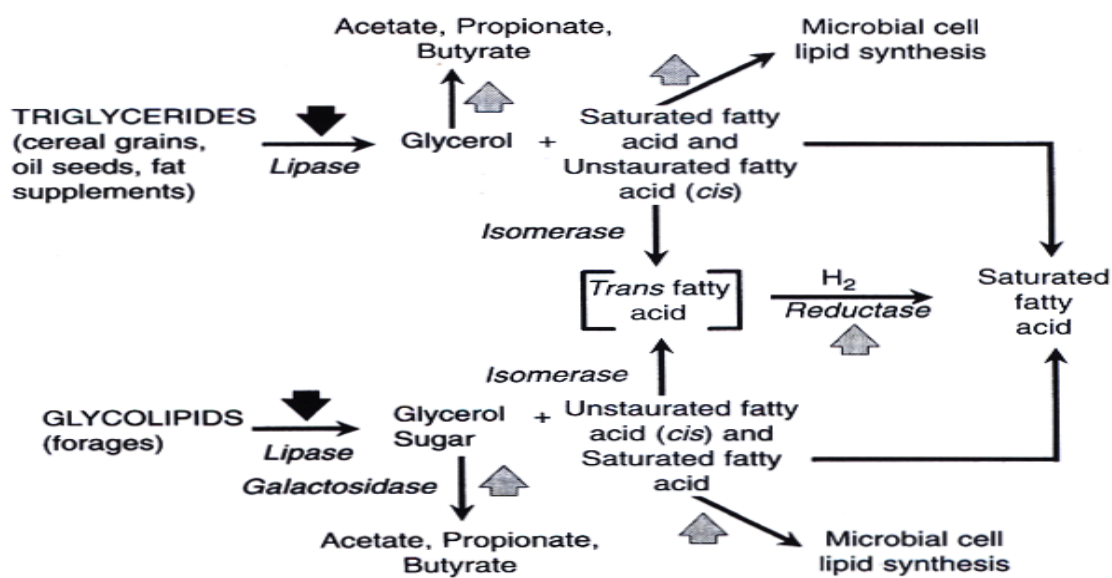


Figure 2.5. Manipulation of lipid fermentation in the rumen. Major reactions in the rumen and possible sites targeted (increase or decrease) for modifications are depicted. (Nagaraja et al., 1997)

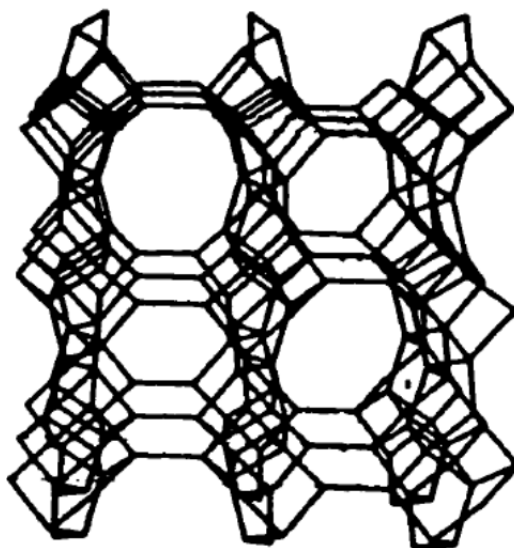
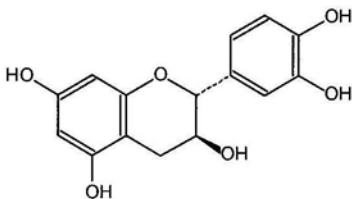
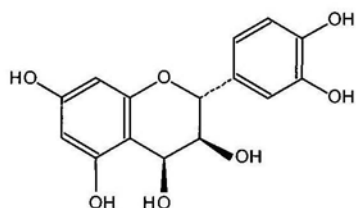


Figure 2.6. Skeletal structure of clinoptilolite. (Flanigen, 1984)

Flavan-3-ol (catechin)



Flavan-3,4-diol (leucoanthocyanidin)



Condensed tannin

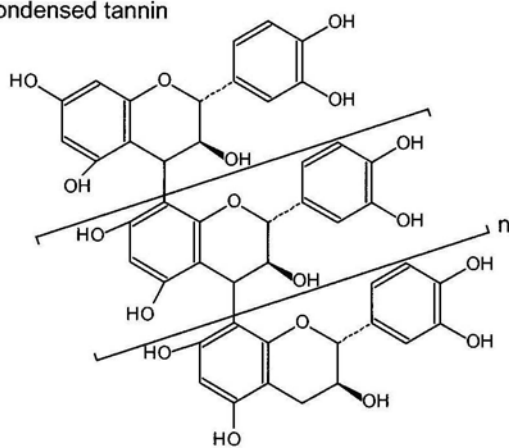


Figure 2.7. Chemical structure of condensed tannins. (McMahon et al., 2000)

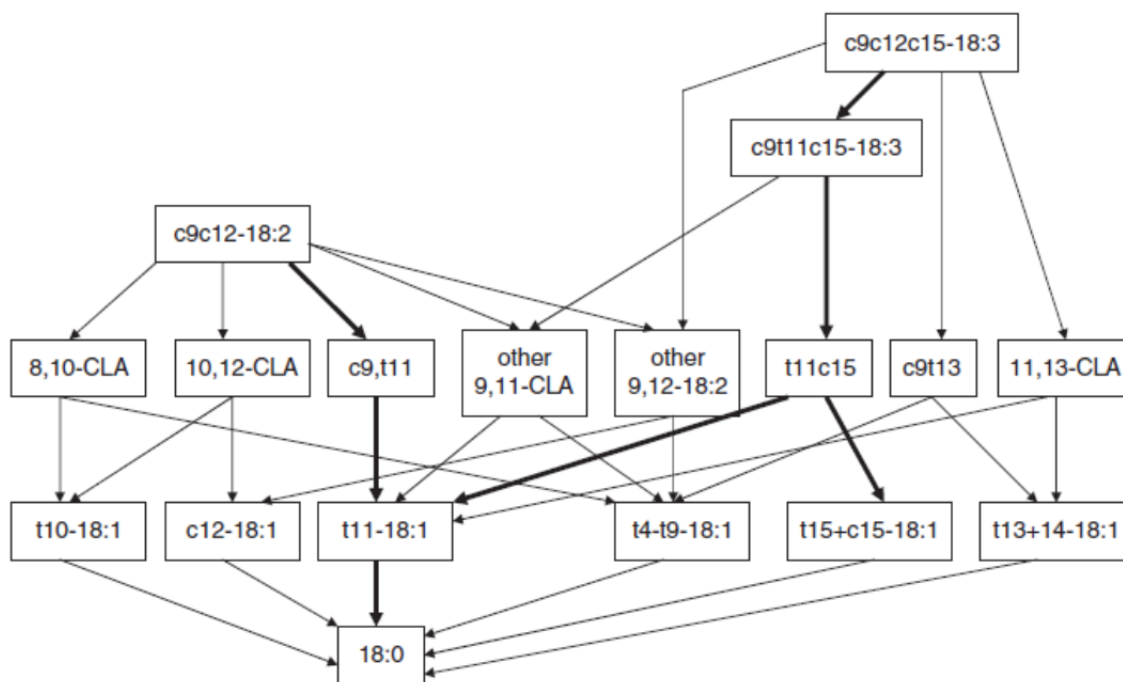


Figure 2.8. Biohydrogenation pathways in the rumen. (Chilliard et al., 2007)

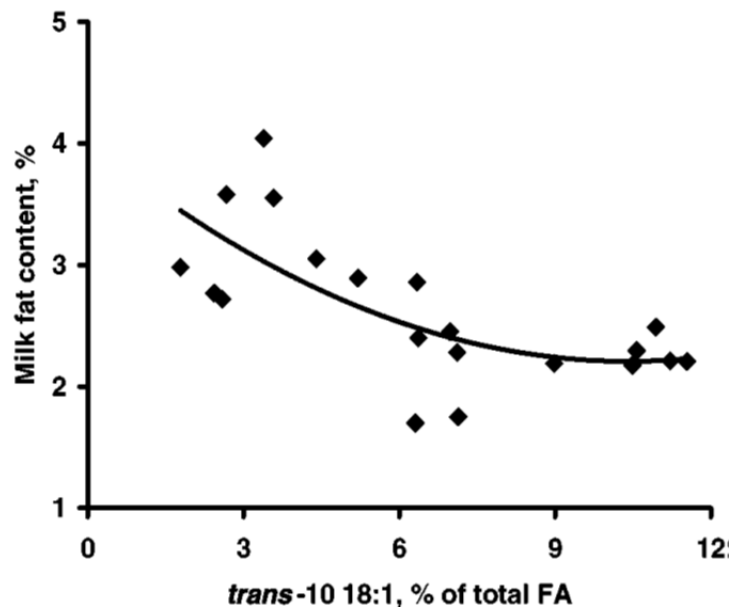


Figure 2.9. Relationship between the change in the fat content of milk and the *trans*-10 18:1 fatty acid concentration of milk fat (expressed as % of total fatty acids). (Bauman and Griinari (2003))

CHAPTER 3
EFFECTS OF SUPPLEMENTATION OF NATURAL ZEOLITE ON INTAKE,
DIGESTION, RUMINAL FERMENTATION, AND LACTATIONAL
PERFORMANCE OF DAIRY COWS¹

INTRODUCTION

Sizable inclusion of readily fermentable carbohydrate (**RFC**) feedstuffs in dairy rations causes appearance of digestive disorders such as subacute ruminal acidosis in dairy cattle if appropriate precautions are not taken. Strategic use of dietary ruminal buffers has been suggested as a sound approach to ameliorate the occurrence of ruminal acidosis, especially when lactating diets include large amounts of RFC. Commonly used as an exogenous buffer, sodium bicarbonate (**NaHCO₃**) is involved in the stabilization of ruminal pH in cows that can potentially suffer from ruminal acidosis (Clark et al., 2009). This chemical feed additive is characterized by an acid dissociation constant ($pK_a = 6.25$), which is close to the normal ruminal pH. Therefore, NaHCO₃ is generally recognized as an efficient buffer because of its high acid-consuming capacity in the rumen, and its mode of action is well documented (Erdman, 1988; Russell and Chow, 1993).

Any mineral additive to a diet is costly for the producer, whereas significant improvements in performance are not always achieved (Rogers et al., 1985; Harrison et al., 1986). Therefore, research is continuing to identify cheaper mineral buffers that

¹Dschaak, C. M., J.-S. Eun, A. J. Young, R. D. Stott, and S. Peterson. 2010. Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. *Prof. Anim. Sci.* 26:647–654.

exhibit the same mode of action as the established buffers. The natural zeolite clinoptilolite has a high attraction for water and a large number of cations, such as K^+ , NH_4^+ , Ca^{2+} , and Mg^{2+} , which can be reversibly bound or released, depending upon the surrounding conditions (Mumpton, 1999). The high affinity of zeolites for water and osmotically active cations may facilitate ruminal fermentation, and osmotic activity may regulate pH in the rumen by buffering against hydrogen ions of organic acids. In addition, supplementing zeolite in dairy diets may improve nitrogen (N) utilization, because zeolite gradually releases excess ammonia (NH_3) in the rumen and allows rumen microorganisms to capture the NH_3 into microbial protein for assimilation into the animals' digestive systems (Mumpton, 1999).

Johnson et al. (1988) reported that ruminal pH increased when synthetic zeolite was added to the diet; however, the change in pH was only 0.2 units, and addition of the synthetic zeolite, with or without $NaHCO_3$, resulted in negative effects on feed intake, milk production, milk component yield, and nutrient digestibility in lactating Holstein cows. To our knowledge, there is a lack of experimental results regarding the effects of long-term feeding of lactating dairy cows with clinoptilolite, a natural zeolite, on its potential as a ruminal buffering agent.

The objectives of this study were 1) to investigate whether natural zeolite could replace $NaHCO_3$ as a buffer in dairy cattle diet, and 2) to assess the effects of $NaHCO_3$ and natural zeolite additions on feed intake, milk production and composition, digestibility, and ruminal fermentation characteristics when added to a lactating dairy diet.

MATERIALS AND METHODS

Cows and Experimental Diets

The experiment was carried out using 30 Holstein cows consisting of 7 primiparous and 23 multiparous cows. At the start of the experiment, DIM averaged 52 ± 23.0 . For 1 wk prior to feeding experimental diets, all cows were fed a diet without ruminal buffer. This 1-wk phase was used as the covariate period, thus milk yield and DMI were determined. At the end of the covariate period, 10 cows were assigned to one of 3 dietary treatments; control diet without ruminal buffer (**CD**), 1.4% SB diet (**SBD**), and 1.4% clinoptilolite zeolite diet (**ZD**) on DM basis. The cows were assigned to the dietary treatments based on previous milk yield, DIM, and parity. The experiment was conducted in a completely randomized design over 12 wk. Cows were weighed at approximately 0830 h at the beginning of the trial and end of wk 4, 8, and 12, and these weights were used to calculate the mean BW of cows for each month. Average BW was 676 ± 71.8 kg at the beginning of the experiment and 726 ± 70.2 kg at the end of the experiment. The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of Institutional Animal Care and Use Committee at Utah State University.

The diets contained 57% forage (67% alfalfa hay and 33% corn silage) and 43% concentrate mix on average (Table 3.1). The diets are typical for high-producing dairy cows in northern Utah containing more alfalfa hay than corn silage, and baled alfalfa hay is commonly fed to provide 50 to 75% of the dietary forage with total forage levels averaging 45 to 55% of the dietary DM. Diets were formulated based on NRC (2001)

recommendations to provide sufficient NE_1 and protein, vitamins, and minerals to produce 38 kg/d of milk with 3.5% fat and 3.0% true protein.

The clinoptilolite zeolite used in this study (RuMagTM; ZeoTech Corporation, Fort Worth, TX) is a complex rumen buffer containing Mg and Ca exchanged zeolite and Mg and calcium hydroxide. Hydrothermal process used to chemically bond hydrate of Mg lime to high, cation-exchangeable and absorptive clinoptilolite zeolite results in a high-quality, prilled rumen buffer with bioavailable Mg and Ca conditioning properties of zeolite. Supplementation rate of clinoptilolite zeolite used in this study (1.4% DM) was based on the manufacturer's recommendation for an adult lactating dairy cow.

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, and were fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0530 and 1630 h with approximately 60% and 40% of total daily feed allocation at each feeding, respectively. Feed offered and refused was recorded daily, and daily samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0500 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry-lot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the Wednesday p.m. and Thursday a.m. milkings of each week throughout experiment. Milk samples were preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA), and stored at 4°C. Individual milk samples were analyzed for fat, true protein, lactose, and milk urea N (**MUN**) by the Rocky Mountain DHIA Laboratory (Logan, UT), with mid-infrared wave-bands (2 to 15 μ m) procedures using an infrared

instrument (Bentley 2000, Bentley Instruments, Chaska, MN) calibrated weekly using raw milk standards provided by Eastern Laboratory Services (Fairlawn, OH). An enzymatic procedure was used to determine MUN concentration using a Chemspec 150 instrument (Bentley Instruments, Chaska, MN). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and protein content of the milk of an individual cow.

Sample Collections, Calculations, and Chemical Analyses

Samples of the TMR fed and orts for individual cows were collected for 7 d at wk 4, 8, and 12, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Analytical DM content of samples was determined by oven drying at 135°C for 3 h. Organic matter was calculated as the difference between DM and ash contents, with ash content determined by combustion at 550°C for 5 h. Measurement of CP ($N \times 6.25$) was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000; method 990.03). The NDF and ADF concentrations were sequentially determined using an ANKOM^{200/220} Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite and heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO) were included in the analysis of NDF. Another set of dried, ground samples was sent to Cumberland Valley Analytical Service (Hagerstown, MD) to determine Ca, P, Mg, K, and Na (AOAC, 2000; method 985.01).

Digestibilities of feed DM and nutrients were measured at wk 4, 8, and 12 using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 200 g wet weight) were collected for each cow from the rectum twice daily (a.m. and p.m.) every 12 h, moving ahead 2 h each day for the 6 d of fecal sampling. This schedule provided 12 representative samples of feces for each cow. Samples were immediately subsampled (about 50 g), composited across sampling times for each cow and each period, dried at 55°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total tract nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, orts, and feces using the following equation: apparent digestibility = $100 - [100 \times (AIA_d/AIA_f) \times (N_f/N_d)]$, where AIA_d = AIA concentration in the diet actually consumed, AIA_f = AIA concentration in the feces, N_f = concentration of the nutrient in the feces, and N_d = concentration of the nutrient in the diet actually consumed.

Ruminal fluid was taken using Geishauser probe 4 h after the morning feeding on wk 4, 8, and 12. The fluid was collected with a solid, tube-like probe with rows of small holes on the end (Geishauser, 1993). Rumenocentesis is reported to be superior to the use of an oral stomach tube for determining ruminal pH as the latter technique is susceptible to saliva contamination (Nordlund and Garrett, 1994). However, rumenocentesis is a more invasive technique involving surgical preparation of the centesis site, as well as chemical and physical restraint, and suffers from a risk of localized abscesses or peritonitis. An alternative technique developed by Geishauser (1993) utilizes a weighted oro-ruminal probe and suction pump, requires minimal time to perform, and is less invasive than rumenocentesis. The pH of the ruminal fluid was measured within 5 min of

collecting the samples using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid were added to 1 mL of 25% of meta-phosphoric acid, and the samples were retained for VFA determination. Another 5 mL of the ruminal fluid were mixed with 1 mL of 1% sulfuric acid for $\text{NH}_3\text{-N}$ analysis. All samples were stored frozen (-40°C) until analysis.

Ruminal VFA were quantified using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) with a capillary column ($30\text{ m} \times 0.32\text{ mm i.d.}$, $1\text{ }\mu\text{m}$ phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by $5^\circ\text{C}/\text{min}$ to 185°C , and then by $3^\circ\text{C}/\text{min}$ to 220°C , and held at this temperature for 1 min. The injector temperature was 225°C , the detector temperature was 250°C , and the carrier gas was helium. Concentration of $\text{NH}_3\text{-N}$ in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRX^o, Dynex Technologies, Chantilly, VA).

Statistical Analyses

Daily intake and milk yield were reduced to weekly means before data analysis. Data for DMI, BW, and milk yield obtained during the covariate period were used as covariates for the corresponding measurements during the treatment period. Analysis of variance was conducted using the MIXED procedure (Littell et al., 1998) of SAS (SAS Institute, 2001) for a completely randomized design with repeated measures for all the statistical analyses in this study. The model included the effects of treatment, week, and the interaction between treatment and week, with the random variable being the cow within treatment. Simple, autoregressive one, and compound symmetry covariance

structures were used in the analysis depending on low values for the Akaike's information criteria and Schwartz's Bayesian criterion. For all models used, degrees of freedom were estimated with the Kenward-Roger specification in the models. Means were compared using a protected ($P < 0.05$) LSD test. Least square means are reported throughout. Treatment effects were declared significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.15$.

RESULTS AND DISCUSSION

Chemical Composition of Diets

The CP, NDF, and ADF concentrations of alfalfa hay and corn silage were 18.6 ± 0.78 and $6.21 \pm 0.401\%$, 40.0 ± 0.03 and $40.9 \pm 0.28\%$, and 30.2 ± 0.28 and $22.8 \pm 0.62\%$, respectively, indicating that the alfalfa hay was of good quality. Concentrations of CP, ADF, and NDF were similar among all dietary treatments (Table 3.2). Mineral concentrations did not differ across dietary treatments except that the SBD contained higher concentration of Na compared to the CD and ZD. All diets used in this study contained sufficient total NDF according to NRC (2001) recommendations. Generally, diets that are low in fiber are associated with ruminal acidosis; reduced rumination, saliva secretion, and fiber digestion (Yang and Beauchemin, 2006).

Intake, Digestibility, Milk Production and Composition, and BW

Intake of DM averaged 26.5 kg/d across treatments, and did not differ due to inclusion of SB or zeolite (Table 3.3). This lack of effect across treatments on DMI was consistent throughout the experiment (Figure 1). Sherwood et al. (2006), using zeolite at 1.2% of DM, and Cole et al. (2007), using zeolite at 2.0% of DM, similarly reported no

effect on DMI when supplementing zeolite to beef steer finishing diets. Previous work by Johnson et al. (1988) using lactating dairy cows, reported a decrease in DMI when synthetic zeolite was added at 2.0% of dietary DM. Similar to our results, Johnson et al. (1988) found no effect on DMI with the addition of NaHCO_3 in dairy cow diets. Kennelly et al. (1999) reported that addition of NaHCO_3 did not affect intake of DM, CP, and NDF when cows were fed a high or low forage diet. Addition of either NaHCO_3 or zeolite in the diets assessed in this study did not influence intake of OM, CP, NDF, and ADF.

Digestibilities of DM and nutrients (OM, CP, NDF, and ADF) did not differ by the addition of NaHCO_3 or zeolite (Table 3.3). Supplementing finishing diets of beef steers with zeolite did not affect DM digestibility (Cole et al., 2007). Johnson et al. (1988) reported lower digestibilities of DM and OM with added synthetic zeolite, but suggested that part of this reduction could be attributed to consumption of the indigestible synthetic zeolite. In addition, the authors observed that CP digestibility decreased, but ADF digestibility did not differ with added synthetic zeolite (Johnson et al., 1988). However, Cole et al. (2007) reported that digestibility of CP was not affected by addition of 1.0 or 2.0% zeolite supplemented to the diets of finishing steers. Similar to our result, Johnson et al. (1988) showed that addition of SB did not affect apparent digestibilities of DM and OM.

Yield of milk and 4% FCM averaged 40.7 and 40.0 kg/d, respectively (Table 3.4), and were similar in response to adding NaHCO_3 or zeolite. Lack of effect of supplementing the ruminal buffers in milk yield was consistent throughout the experiment (Figure 3.1). It seems that the zeolite at 1.4% DM used in this study was too low to affect milk yield. Similar to our result, Katsoulos et al. (2006) and Bosi et al.

(2002) observed no difference in milk yield of dairy cows supplemented with zeolite at 1.25 % and 1.0% on DM basis, respectively. However, dairy cows fed 2.5% (Katsoulos et al., 2006) and 2.0% DM zeolite (Garcia Lopez et al., 1992) increased milk yield. Katsoulos et al. (2006) speculated that the higher milk production by cows fed 2.5% zeolite could be due to increased production of propionate in the rumen and/or increased postruminal digestion of starch. On the other hand, Johnson et al. (1988) reported that supplementing synthetic zeolite at 2.0% decreased milk yield as well as 4% FCM yield, and the reduction in milk yield was likely associated with decreased DMI and digestibility.

Milk composition and yield were not influenced by supplementing ruminal buffers except that feeding the ZD tended to increase milk true protein concentration ($P = 0.15$; Table 3.4). In general, it has been accepted that dietary buffers do not consistently alter protein percentage of milk (Cassida et al., 1988; Harrison et al., 1989; Xu et al., 1994). Despite the tendency to increase milk protein concentration by zeolite, MUN and efficiency of N use for milk N were not affected by dietary treatments. Dairy efficiency, calculated as 4% FCM divided by DMI, was not influenced by dietary treatments. In addition, mean BW and BW change were similar among dietary treatments.

Ruminal Fermentation Characteristics

Ruminal pH tended to increase ($P = 0.11$) by supplementing NaHCO_3 or zeolite (Table 3.5). Johnson et al. (1988) reported an increase in ruminal pH when synthetic zeolite was added to the diet; however, like in our case, the change was only 0.2 units. Bosi et al. (2002) reported no effect of supplementing zeolite at 1.0% DM on ruminal pH when dairy cows were fed a typical lactation diet with a forage to concentrate ratio of

45:55. In beef finishing feedlot diets, the addition of zeolite at 1.2% DM increased ruminal pH (Eng et al., 2006). Survival rates of cellulolytic bacteria decrease when pH drops to less than 6.2 (Calsamiglia et al., 1999), thus reducing fiber digestion and causing various negative effects on ruminal fermentation. Because the ruminal pH in the CD was 6.42, which is over 6.2, the increase in ruminal pH of 0.12 units and 0.19 units by the SBD and the ZD, respectively, would have no physiological significance, and would not affect overall ruminal fermentation.

High concentrate diets are often associated with lower ruminal pH and decreased fiber digestibility (Yang et al., 2002; Eun and Beauchemin, 2005). Ruminal buffers have been shown to prevent milk fat depression associated with feeding corn silage or low fiber diets (Harrison et al., 1989; Xu et al., 1994; Kennelly et al., 1999), by helping to stabilize rumen pH and thus providing a more favorable environment for microbial growth. Marden et al. (2008) reported that stabilization of ruminal pH with NaHCO_3 was not associated with a lower lactate concentration, and consequently suggested that NaHCO_3 may have stabilized the pH through its strong capacity to neutralize protons (Le Ruyet and Tucker, 1992). Erdman et al. (1982) reported an increase in rumen pH, from 6.13 to 6.43, in early lactating dairy cows receiving 1.0% NaHCO_3 . Therefore, to offset the potential negative effect of high concentrate diets on the rumen environment, supplementing a buffer in lactating diets is recommended. However, such benefits have not been observed from the addition of buffer to diets that contained alfalfa as the primary forage (Bath et al., 1985). The experimental diets assessed in this study contained 38% alfalfa hay of high quality being clean, bright green, and fine stemmed. Feeding a high forage diet would have reduced the rate of fermentation acid production in

the rumen, because less starch is fermented in the rumen compared with feeding a high concentrate diet (Yang and Beauchemin, 2006). Therefore, it is likely that a high forage NDF concentration with high quality alfalfa hay provided a normal, fermentative environment, eliminating potentially positive effects of supplementing NaHCO_3 or zeolite. Further research is needed to determine if supplementing zeolite in a high concentrate, lactating diet would prove effective by increasing ruminal pH, as feeding the high concentrate diet will lower ruminal pH with more fermentable carbohydrate in the diet.

Total VFA concentration tended to decrease ($P = 0.14$) when cows were fed the ZD (Table 3.5), whereas molar proportions of major VFA (acetate, propionate, and butyrate) and acetate to propionate and acetate + butyrate to propionate ratios were not affected by dietary treatment. Decreased total VFA concentration by the ZD would not have resulted in a lower fiber digestion, because digestibilities of NDF and ADF were not influenced by supplementing buffers. Bosi et al. (2002) observed that the inclusion of zeolite in the diet of lactating dairy cows had no effect on concentration and molar proportion of VFA. Johnson et al. (1988) reported no effect on ruminal VFA concentration with inclusion of NaHCO_3 ; however, the authors reported that propionate decreased with added synthetic zeolite, while other VFA were unaffected (Johnson et al., 1988). The effects of supplementing zeolite on ruminal VFA composition have been variable among studies. For instance, McCollum and Galyean (1983) observed that when steers were fed a high concentrate diets, molar proportion of propionate increased by the addition of 2.5% DM zeolite in their ration, but not when 1.5% DM was added. Katsoulos et al. (2006) reported that supplementation of a concentrate diet for dairy cows with 2.5% DM of zeolite

reduced the incidence of clinical ketosis and increased milk yield. The authors suggested that the positive impacts could have resulted from possible enhancement of propionate production in the rumen (Katsoulos et al., 2006). In contrast, Sweeney et al. (1984) observed a decrease in propionate and an increase in acetate, resulting in increased acetate to propionate ratio when Holstein steers and heifers were fed 5% clinoptilolite zeolite. Similarly, Johnson et al. (1988) reported an increase in the acetate to propionate ratio with synthetic zeolite, but because acetate concentration was unchanged, the higher ratio was due to decreased propionate.

Concentration of ruminal $\text{NH}_3\text{-N}$ was not affected by dietary treatment. Similar to our result, Bosi et al. (2002) reported ammonia level in ruminal fluid was not affected by feeding zeolite to lactating dairy cows at 1.0% of dietary DM. Johnson et al. (1988) reported ruminal $\text{NH}_3\text{-N}$ was not affected by addition of synthetic zeolite or NaHCO_3 in dairy cattle diets. In contrast, Hemken et al. (1984) reported a decrease in the concentration of $\text{NH}_3\text{-N}$ when feeding natural zeolite to dairy cows, but the positive effect of supplementing zeolite was obtained when cows were fed a diet containing urea as a source of protein. Mumpton and Fishman (1977) reported that the zeolite's ability to act as a reservoir can result in protecting the animal against ammonia overload in the rumen. It is possible that, after the release of ammonia consequent to each meal, zeolite absorbs high levels of NH_3 concentration in the rumen and then releases NH_3 when its concentration is reduced (Bosi et al., 2002), which may explain no effects of supplementing zeolite on $\text{NH}_3\text{-N}$ concentration in this study. Although adsorption sites on zeolite may be tied up by ammonia in the rumen and thus limit the capacity of excreted zeolite to bind ammonia on the pen surface, some studies suggest that the feeding of

zeolite may reduce N losses from manure (Eng et al., 2003; Cole et al., 2007). Cole et al. (2007) reported that zeolite addition to the feedlot pen surface using an in vitro ammonia emission system (Cole et al., 2005) decreased ammonia losses by 51 to 86%; however, apparent CP digestibility and N retention and excretion were not affected by addition of zeolite in beef finishing diet. The slow rate of NH₃ emission could render zeolite more effective at adsorbing ammonium because of the longer time for contact between the ammonium and zeolite in the manure.

The most significant findings in this study were that supplementing natural zeolite in lactation dairy diet had minor impacts on ruminal fermentation and lactational performance of dairy cows. The lack of effects of supplementing the ruminal buffer was consistent throughout the long-term feeding experiment during early to midlactation. High NDF concentration together with high dietary proportion of high quality alfalfa hay may dilute potential effects of supplementing natural zeolite in the experimental diet assessed in this study. Further research is needed for the zeolite used in this study to determine if the product influences ruminal fermentation characteristics when added to high concentrate, lactation dairy diets with focus on its potential to reduce subacute ruminal acidosis.

IMPLICATIONS

Supplementing zeolite had no negative impacts on productive performance and ruminal fermentation except for a tendency to reduce VFA production, which indicates that the zeolite product used in this study would replace NaHCO₃ as a ruminal buffer additive cost-effectively in lactation dairy diet. In addition to zeolite maintaining the

rumen environment similar to NaHCO_3 , an additional finding of a trend toward increased milk protein and the estimated cost of zeolite projected to be lower than NaHCO_3 suggests that the net income of the farmer will increase when using this product. The real test will be when this product is used in a low ruminal pH fermentative environment. With its increased exchange rate for ions, the difference may be greater than in the current study.

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Table 3.1. Ingredient composition of the control diet

Ingredient	% DM
Alfalfa hay	37.9
Corn silage	19.3
Corn grain, steam flaked	13.7
Whole linted-cottonseed	4.41
Cottonseed extender	2.82
Dried sugar beet pulp	5.69
Soybean meal, expeller	1.66
Canola meal	2.09
Molasses, sugar beet	1.20
Corn dried distillers grains with solubles	2.79
Corn hominy	5.47
Blood meal	1.10
Mineral and vitamin mix ¹	1.87

¹Contained (per kilogram of DM) a minimum 250,000 IU of vitamin A; 65,000 IU of vitamin D; 2,100 IU of vitamin E; Fe 400 mg; Cu 540 mg; Zn 2,100 mg; Mn 560 mg; Se 15 mg; I 35 mg; Co 68 mg; and 19.6 g of Rumensin (Elanco Animal Health, Greenfield, IN).

Table 3.2. Chemical composition of the treatment diets on a DM basis (%)

Item	Dietary treatment ¹		
	CD	SBD	ZD
DM, %	64.5	64.4	63.9
CP	17.8	17.7	17.7
NDF	33.8	33.9	33.9
ADF	22.3	22.2	22.5
Ca	1.10	1.06	1.11
P	0.38	0.37	0.36
Mg	0.41	0.38	0.43
K	2.22	1.92	2.11
Na	0.233	0.395	0.255
NE _l ² , Mcal/kg	1.58	1.56	1.58

¹CD = control diet without buffer; SBD = sodium bicarbonate diet composed of CD and sodium bicarbonate (1.4% DM); and ZD = zeolite diet composed of CD and clinoptilolite zeolite (1.4% DM).

²Based on tabular value (NRC, 2001).

Table 3.3. Nutrient intake and total tract digestibility of lactating dairy cows fed different ruminal buffer additives

Item	Dietary treatment ¹			SEM	<i>P</i>
	CD	SBD	ZD		
Intake, kg/d					
DM	26.5	26.4	26.7	1.19	0.98
OM	23.7	23.8	23.9	1.07	0.99
CP	4.72	4.71	4.63	0.204	0.94
NDF	8.57	8.76	8.84	0.387	0.88
ADF	5.76	5.75	5.76	0.255	0.99
Digestibility, %					
DM	72.9	72.5	73.0	0.47	0.72
OM	74.6	74.1	75.0	0.48	0.43
CP	77.2	76.8	76.9	0.46	0.79
NDF	47.9	48.0	48.7	1.03	0.83
ADF	45.9	44.7	44.0	1.20	0.57

¹CD = control diet without buffer; SBD = sodium bicarbonate diet composed of CD and sodium bicarbonate (1.4% DM); and ZD = zeolite diet composed of CD and clinoptilolite zeolite (1.4% DM).

Table 3.4. Milk production and composition, efficiencies of DM and N use, and BW of lactating dairy cows fed different ruminal buffer additives

Item	Dietary treatment ¹			SEM	<i>P</i>
	CD	SBD	ZD		
Milk production, kg/d					
Actual	41.5	41.0	39.6	1.46	0.62
4% FCM	40.1	40.2	39.5	1.54	0.94
Milk composition, %					
Fat	3.77	3.94	3.84	0.100	0.48
True protein	2.94	2.93	3.09	0.063	0.15
Milk urea nitrogen, mg/dL	14.7	14.2	13.4	0.48	0.18
Milk component yield, kg/d					
Fat	1.57	1.62	1.52	0.079	0.70
True protein	1.21	1.20	1.22	0.056	0.98
Efficiency					
4% FCM/DMI	1.54	1.56	1.43	0.077	0.49
Milk N/N intake ²	0.27	0.26	0.27	0.008	0.56
BW					
kg	709	704	707	5.2	0.74
Change in BW, kg/d	0.34	0.30	0.32	0.049	0.82

¹CD = control diet without buffer; SBD = sodium bicarbonate diet composed of CD and sodium bicarbonate (1.4% DM); and ZD = zeolite diet composed of CD and clinoptilolite zeolite (1.4% DM).

²Efficiency of use of feed nitrogen to milk nitrogen = (total milk protein, kg/d ÷ 6.38) ÷ nitrogen intake, kg/d.

Table 3.5. Ruminant fermentation characteristics of lactating dairy cows fed different ruminal buffer additives

Item	Dietary treatment ¹			SEM	<i>P</i>
	CD	SBD	ZD		
Ruminal pH	6.42	6.54	6.61	0.061	0.11
Total VFA, mM	114.4	113.8	103.8	4.44	0.14
Individual VFA, mol/100 mol					
Acetate (A)	62.8	62.5	63.9	0.74	0.37
Propionate (P)	22.4	22.0	21.6	0.70	0.74
Butyrate (B)	10.8	11.0	10.5	0.21	0.17
Valerate	1.68	1.81	1.69	0.633	0.28
Isobutyrate	0.82 ^b	0.97 ^a	0.81 ^b	0.027	< 0.01
Isovalerate	1.17 ^b	1.39 ^a	1.18 ^b	0.058	0.02
A:P	2.85	2.90	3.01	0.124	0.65
(A + B):P	3.33	3.41	3.50	0.140	0.70
NH ₃ -N, mg/dL	10.7	11.6	11.7	0.68	0.58

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹CD = control diet without buffer; SBD = sodium bicarbonate diet composed of CD and sodium bicarbonate (1.4% DM); and ZD = zeolite diet composed of CD and clinoptilolite zeolite (1.4% DM).

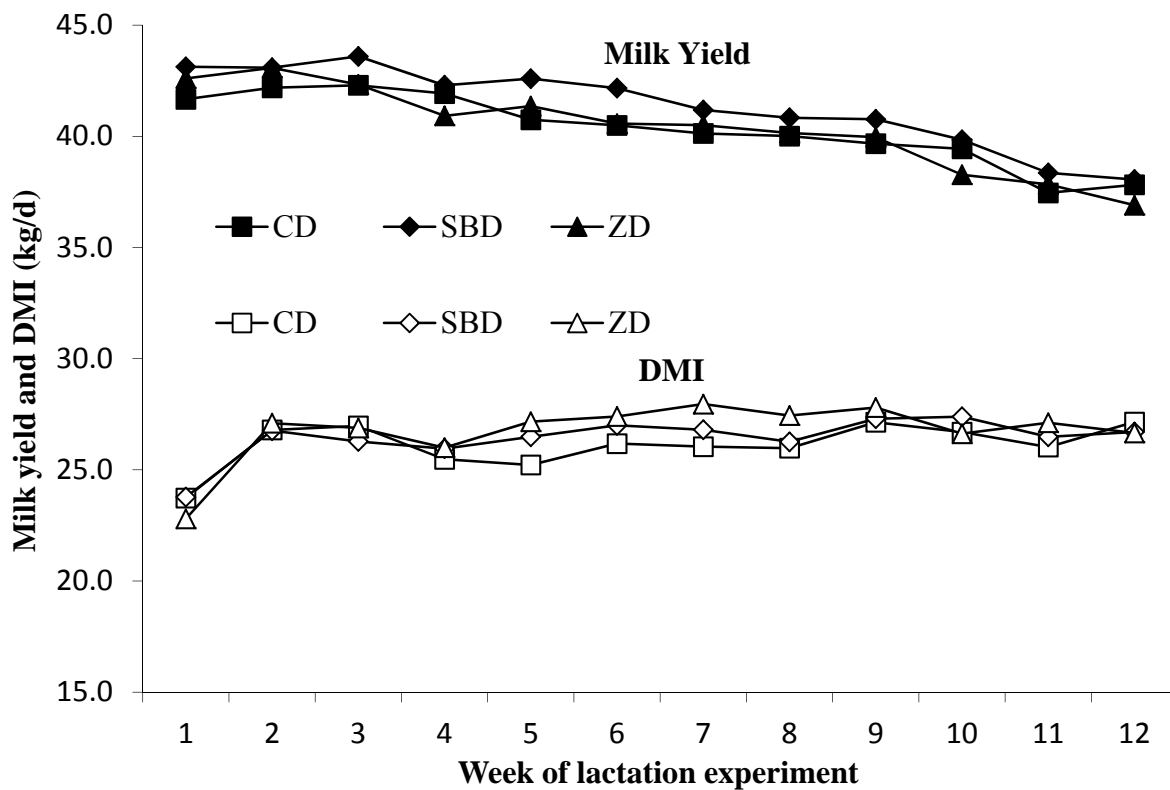


Figure 3.1. Dry matter intake and milk yield of lactating dairy cows fed different ruminal buffer additives. Treatments were TMR without buffer (CD), CD and sodium bicarbonate TMR (SBD), and CD and zeolite TMR (ZD). Each point represents the mean of 10 observations (SEM = 1.19 and 1.46 for DMI and milk yield, respectively).

CHAPTER 4
EFFECTS OF SUPPLEMENTING CONDENSED TANNIN EXTRACT ON
INTAKE, DIGESTION, RUMINAL FERMENTATION, AND MILK
PRODUCTION OF LACTATING DAIRY COWS¹

INTRODUCTION

In ruminants fed high quality forage diets, most proteins are rapidly solubilized releasing between 56 and 65% of the N concentration in the rumen during fermentation; consequently, large losses of N occur (25-35%) as ammonia (NH_3) into urine (Min et al., 2000). Research is needed to improve animals' N retention. Natural plant compounds with known ability to reduce proteolysis such as condensed tannin extract (CTE), offer a promising means of achieving this goal. Aerts et al. (1999a) found that condensed tannins (CT) in birdsfoot trefoil (*Lotus corniculatus*) and big trefoil (*L. pedunculatus*) markedly protected ribulose-1,5-bisphosphate carboxylase/oxygenase from degradation by mixed rumen microorganisms. Molan et al. (2001) demonstrated that CT concentrations of 400 μg CT/mL or greater reduced the growth of a range of bacterial strains from the rumen. Furthermore, Min et al. (2002) reported that when the diet was changed from perennial ryegrass/white clover pasture (which does not contain CT) to birdsfoot trefoil (3.2% CT on DM basis) in sheep, populations of the proteolytic rumen bacteria decreased, confirming that the CT in the forages greatly reduces rumen proteolytic bacterial growth.

¹Dschaak, C. M., C. M. Williams, M. S. Holt, J.-S. Eun, A. J. Young, and B. R. Min. 2010. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *J. Dairy Sci.* 94:2508–2519.

These effects of CT on retarding forage N degradation supported more milk production from cows fed birdsfoot trefoil over alfalfa silage (Hymes-Fecht et al., 2005). Alfalfa does not produce tannins except in the seed coats. Thus, feeding CT-containing forages in the diets containing alfalfa as a main forage source may increase N utilization and improve animal performance. However, tannin-rich forages are not agronomically suited in many areas, thus, may not be readily available. Hence, a concentrated source of CT may be a possible alternative approach to feeding tannin-rich forages to enhance N utilization and improve lactational performance of dairy cows if similar dietary concentrations of tannins are provided from CTE as is seen in tannin-rich forage diets.

Although supplementation of CTE in lactating dairy diets has been extensively investigated, there is lack of information in literature on how ruminal fermentation characteristics are altered depending upon dietary composition, particularly forage-to-concentrate-ratio which is considered one of the main driving forces directly affecting ruminal fermentation and production performance of lactating dairy cows. In addition, some studies (Jones et al., 1994; Molan et al., 2001) have shown that CT from different legume forages inhibit cell growth and division of ruminal microorganisms, including *Butyrivibrio fibrisolvens*, that is among the ones responsible for ruminal biohydrogenation (Jenkins et al., 2008). Grazing birdsfoot trefoil vs. perennial ryegrass led to increased concentrations of C12:0, C14:0, C16:0, C18:2 n-6, and C18:3 n-3 fatty acids (FA), and reduced concentrations of *cis*-9 C18:1, *cis*-9, *trans*-11 conjugated linoleic acids (CLA), and *trans*-11 C18:1 FA in milk (Turner et al., 2005). The ruminal microbial population is an integral system with numerous interrelationships. Thus, it is likely that the inhibitory effects of CT influence ruminal biohydrogenation of unsaturated FA,

leading to an altered biohydrogenation pathway (Vasta et al., 2008) and consequently, changes in FA composition of milk.

We hypothesized that supplementation of CTE would decrease ruminal NH_3 concentration and improve utilization of N for milk production, but its impacts would differ between high (**HF**) and low forage (**LF**) diets. The objective of this study was to assess ruminal fermentation characteristics, digestibility, and lactational performance of early to midlactating dairy cows fed HF or LF diet without or with CTE supplementation. Additionally, we were interested in a possible link between supplementation of CTE and milk FA composition.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University.

Cows and Experimental Design and Diets

Eight multiparous lactating Holstein cows were used; 4 cows were surgically fitted with ruminal cannula. Days in milk ranged from 52 to 68 and from 49 to 73 for noncannulated and cannulated cows, respectively, at the start of the experiment. Average BW was 692 ± 69.7 kg at the beginning of the experiment and 710 ± 75.9 kg at the end of the experiment.

The design of the experiment was a double 4×4 Latin square with each period lasting 21 d (14 d of treatment adaptation and 7 d of data collection and sampling). The cows were allocated to squares by whether they were surgically cannulated, and the 2

squares were conducted simultaneously. Within square, cows were randomly assigned to a sequence of 4 diets. A 2×2 factorial arrangement was used; HF or LF diet with a forage-to-concentrate ratio of 59:41 or 41:59 (DM basis; Table 4.1), respectively, was combined without or with CTE to form 4 treatments: HF diet without CTE (**HF-CTE**), HF diet with CTE (**HF+CTE**), LF diet without CTE (**LF-CTE**), and LF diet with CTE (**LF+CTE**; Table 4.1). Water-soluble quebracho CTE in powder form (99% solubility; Chemtan Company Inc., Exeter, NH) was a crude extract of the bark of *Shinopsis* spp., and the appropriate quantity of quebracho CTE was applied into the mixer wagon to be mixed with other ingredients added to the HF+CTE and the LF+CTE at a rate of 3% DM. The same quebracho CTE product was reported to contain 75% CT concentration with a small amount of simple phenolics (Min et al., 2006).

The forages used were alfalfa hay and corn silage. Table 4.1 shows diet composition. The concentrate contained steam-flaked barley and a pelleted supplement, and the formulation of the concentrate differed for the LF and the HF diets. The diets are typical of high producing cow diets in northern Utah with the inclusion of Rumensin (approximately, 300 mg/head/d; Elanco Animal Health, Greenfield, IN), and were formulated based on NRC (2001) recommendations to provide sufficient NE_L , metabolizable protein, vitamins, and minerals to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein.

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, and were fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0830 and 1500 h with approximately 70% and 30% of total daily feed allocation at each feeding, respectively. Feed offered and

refused was recorded daily, and daily samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a drylot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the a.m. and p.m. milkings on 2 consecutive days (d 16 and d 17) in each period. Individual milk samples were analyzed for fat, true protein, lactose, and MUN by the Rocky Mountain DHIA Laboratory (Logan, UT). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Yields of milk fat, true protein, and lactose were calculated by multiplying milk yield from the respective day by fat, true protein, and lactose concentrations of the milk from an individual cow.

Sampling, Data Collection, and Chemical Analyses

Chopped alfalfa hay, corn silage, and concentrates were sampled weekly to determine DM concentration. Diets were adjusted weekly to account for changes in DM concentration. Diets of TMR samples were collected on d 20 and 21 for particle size analysis by using the Penn State Particle Separator as described by Kononoff et al. (2003) equipped with 3 sieves (19, 8, and 1.18 mm) and a pan. The recommended proportions for TMR are 2 to 8% on the 19-mm sieve, 30 to 50% on the 8-mm sieve, and 30 to 50% on the 1.18-mm sieve (Kononoff and Heinrichs, 2007). All diets were within the recommended range except the top screen for the HF diet which was greater than the recommendation.

Samples of the TMR fed and orts for individual cows were collected daily during the data collection period, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard

model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Contents of DM of the samples were used to calculate intakes and digestibilities of DM and nutrients.

Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N content was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM^{200/220} Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). Starch concentration of feed was determined by a two-step enzymatic method (Rode et al., 1999) with a microtiter plate reader (Dynatech Laboratories, Chantilly, VA) to read glucose release colorimetrically at 490 nm.

Weekly samples of dietary ingredients were analyzed for total FA concentration and FA profile according to the procedure of Sukhija and Palmquist (1988) and Kleinschmit et al. (2007) using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) fitted with a flame ionization detector. The injector port temperature was 230°C with a split ratio of 20:1. The column was 100 m, and its inside diameter was 0.25 mm (CP-Sil 88, Varian, Lake Forest, CA). The carrier gas was helium at a rate of 2.0 mL/min. Initial oven temperature was 50°C held for 1 min, and then increased to 145°C at a rate of 5°C per min and held for 30 min. The temperature was then increased at 10°C/min to 190°C

and held for 30 min. Finally, the temperature was raised at 5°C/min to 210°C and held for 35 min. The total run per sample lasted 123.5 min.

Weighted composite milk samples from individual cows were analyzed for FA composition. Milk fat was extracted by boiling milk in a detergent solution (Hurley et al., 1987). Extracted fat was derivatized to methyl esters using an alkaline methylation procedure by mixing 40 mg of fat with a sodium methoxide methylation reagent (NaOCH₃/MeOH) as described by Chouinard et al. (1999). After FA methyl esters were formed, anhydrous calcium chloride pellets were added and allowed to stand for 1 h to remove water in the sample. Samples were then centrifuged at 1016 × g at 4°C for 20 min.

Separation of FA was achieved by using a GLC (model 6890 series II) fitted with a flame ionization detector. Samples containing methyl esters in hexane (1 µL) were injected through the split injection port (100:1) onto the column (CP-Sil 88). Oven temperature was set at 80°C and held for 10 min, then increased to 190°C at 12°C/min for 39 min. The temperature was then increased again to 218°C at 20°C/min and held for 21 min. Injector and detector were set at 250°C. Total run time was 71 min.

Heptadecadenoic acid was used as a qualitative internal standard. Individual FA concentrations were obtained by taking the specific FA area as a percentage of total FA, and were reported as g/100 g FA methyl esters.

Feed DM and nutrient digestibility was measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 200 g wet weight) were collected for each cow from the rectum twice daily (a.m. and p.m.) every 12 h, moving ahead 2 h each day for the 6 d of

fecal sampling beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were immediately subsampled (about 50 g), composited across sampling times for each cow and each period, dried at 55°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total tract nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, orts, and feces using the following equation: apparent digestibility = $100 - [100 \times (AIA_d/AIA_f) \times (N_f/N_d)]$, where AIA_d = AIA concentration in the diet actually consumed, AIA_f = AIA concentration in the feces, N_f = concentration of the nutrient in the feces, and N_d = concentration of the nutrient in the diet actually consumed (Eun and Beauchemin, 2005).

Ruminal Fermentation Characteristics

Ruminal pH was continuously measured for 2 consecutive days starting on d 18 using the Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as described by Penner et al. (2006). Readings in pH buffers 4 and 7 were recorded prior to placing the LRCpH system in the rumen. Ruminal pH readings were recorded every 30 s and stored by the data logger. After about 48 h of continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C water, and millivolt readings were recorded in pH buffers 4 and 7. The daily ruminal pH data was averaged for each minute and summarized as minimum pH, mean pH, and maximum pH. In addition, daily episodes, duration (h/d), and area (pH × min) when ruminal pH was less than 5.5 were calculated. The threshold 5.5 was assigned because it has been defined by others (Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal contents were sampled from cannulated cows 0, 3, and 6 h after the a.m. feeding on d 20 and 21. Approximately 1 L of ruminal contents was obtained from the anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral locations within the rumen, composited by cow, and strained through a polyester screen (pore size 355 μm ; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five milliliters of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid and samples were retained for $\text{NH}_3\text{-N}$ determination. Concentration of $\text{NH}_3\text{-N}$ in the ruminal contents was determined as described by Rhine et al. (1998). Another 5 mL of the filtered ruminal fluid was taken at 3 h after the a.m. feeding and added to 1 mL of 25% of meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a GLC (model 6890 series II) with a capillary column (30 m \times 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA), and flame-ionization detection. Crotonic acid was used as an internal standard. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium.

Statistical Analyses

Data were summarized for each cow by measurement period. All data were statistically analyzed using the mixed model procedure in SAS (SAS Institute, 2001). Data for intake, BW, digestibility, energy balance, and milk production were analyzed with a model that included the effects of forage level in the diet (HF vs. LF), square (noncannulated vs. cannulated cows), CTE supplementation (without vs. with CTE), and

the interaction between forage level in the diet and CTE. Cow, period, and cow by period by square were the terms of the random statement.

Data for VFA profiles and $\text{NH}_3\text{-N}$ concentration were analyzed with a model that included the effects of forage level (HF vs. LF), CTE supplementation (without vs. with CTE), and the interaction between forage level and CTE. In addition, the fixed effect of time after feeding was included using the repeated option. Cow and period were the terms of the random statement. The covariance structure that resulted in the lowest values for the Akaike's information criteria and Schwartz's Bayesian criterion was used (Littell et al., 1998). Data for DMI were reported using the heterogeneous compound symmetry structure, whereas milk yield were analyzed by the autoregressive structure. Data for milk components and efficiency of feed and N utilization were analyzed using the unstructured covariance structure. In addition, data for VFA and $\text{NH}_3\text{-N}$ were analyzed by the unstructured and unstructured variance-covariance structure.

Residual errors were used to test main effects and interactions. Differences were considered significant at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$. When the interaction between forage level in the diet and CTE was $P < 0.10$, contrasts were used to examine the effects of CTE within forage level using single degree of freedom contrasts. Contrasts were considered significant at $P < 0.05$. Results are reported as least square means.

RESULTS AND DISCUSSION

Characteristics of Experimental Diets

Ingredients and chemical composition of experimental diets are listed in Table 4.1. Alfalfa hay was used as main forage in the HF and the LF diets, and the alfalfa hay was high quality being clean, bright green, and fine stemmed with a chemical composition of 21.7 ± 1.68 , 40.6 ± 0.09 , and $30.7 \pm 0.27\%$ DM for CP, NDF, and ADF, respectively. Therefore, digestive and nutritive contribution by the alfalfa hay in the current study would be relatively high due to its large dietary proportion (31.9 and 24.4% DM for the HF and the LF diet, respectively) as well as high nutritive quality. Corn silage used in this study contained 7.2 ± 0.70 , 41.7 ± 1.67 , and $23.4 \pm 1.12\%$ DM for CP, NDF, and ADF, respectively. Concentrations of CP were similar between the HF and the LF diet, and RDP to RUP ratio was maintained at 70:30 on average across diets. Concentrations of NDF and ADF were higher in the HF diet compared with the LF diet, whereas starch concentration was higher in the LF than the HF diet (29.4 vs. 22.9% on average).

Fatty acid profiles of experimental diets are presented in Table 4.2. *Cis*-9 C18:1 concentration was higher in the LF diet, while C18:3 n-3 was present at higher concentration in the HF diet. Diets were similar in other FA and total saturated and unsaturated FA.

Intake and Digestibility

The effects of increasing the forage proportion of the diet observed in this study were as expected based on previous literature (Yang et al., 2001; Eun and Beauchemin, 2005). Yang et al. (2001) reported no difference on DM intake in response to forage-to-

concentrate ratio of 35:65 or 55:45 (DM basis) in lactating cows, whereas Eun and Beauchemin (2005) reported that total tract digestibilities of DM, OM, and NDF were not affected by forage-to-concentrate ratio of 60:40 vs. 34:66 (DM basis) in lactating cows. The discussion of these effects is limited as the focus of this study was to understand the interaction between level of forage in the diet and CT supplementation, rather than the effects of forage-to-concentrate ratio, per se.

Supplementing CTE in the diet decreased intake of DM, OM, CP, NDF, and ADF regardless of level of forage (Table 4.3). Previous studies on the effects of CT on feed intake in ruminants have yielded inconsistent results. Decreases of DMI with CT have been already reported either in cows (McNabb et al., 1996 with 5.5% of CT; Priolo et al., 2000 with 2.5% of CT) or in sheep (Barry and McNabb, 1999 with 7.5 to 10.0% of CT). This depressing effect would be attributed to degraded palatability (Cooper and Owen-Smith, 1985) or to short-term effect of astringency (Landau et al., 2000). Others found no effect of CT on DMI either on Jersey heifers (0.60% of quebracho CTE; Baah et al., 2007) and lactating dairy cows (0.45% of CT, Benchaar et al., 2008). Conversely, DMI increases were also observed with CT as in Woodward et al. (2001) and Carulla et al. (2005) with 2.59% and 2.50% of CT, respectively. These results suggest that CT fed at relatively high concentrations has negative effects on feed intake in ruminants, and the effects may vary with the source of CT.

Although supplementing CTE in the diet decreased feed intake, total tract digestibilities of DM and nutrients were not affected regardless of level of forage in the diet. Similar to our result, Benchaar et al. (2008) observed no effects on digestibilities of DM and nutrients (OM, CP, and ADF) with the addition of quebracho CTE at 0.64% of

DM. In contrast, Beauchemin et al. (2007) reported that digestibility of CP decreased in beef cattle with the addition of 1 to 2% of quebracho CTE. Carulla et al. (2005) observed that supplementing the diet of sheep with 2.5% DM of CTE from black wattle tree decreased digestibilities of OM, CP, NDF, and ADF. Although binding effect of tannins to protein improves efficiency of N utilization in ruminants (Aerts et al., 1999b), it may influence CP digestibility depending upon concentration and source of tannins. In fact, a diet containing 1.8% CT from big trefoil resulted in a reduced CP digestibility, whereas the same concentration of CT from birdsfoot trefoil had less effect on CP digestibility (Waghorn and Shelton, 1997). Similar to our findings, Baah et al. (2007) reported digestibilities of DM, OM, and NDF were not altered in LF diets supplemented with quebracho CTE at 0.6% DM when fed to Jersey heifers. Based on CTE supplementation having no effect on digestibility in this study, it is likely that the quebracho CTE supplementation at 3% DM in the diets tested in our study simply depressed feed intake due to palatability without affecting feed digestion.

Milk Production and Its Efficiency

Milk yield averaged 34.6 and 36.1 kg/d for the HF and the LF diet, respectively, and was not influenced by CTE supplementation (Table 4.4) which agreed with Benchaar et al. (2008) and Aguerre et al. (2010) with lower dietary inclusions of CTE (0.64 and 1.8%, DM basis, respectively). Concentrations of milk fat, true protein, and lactose were not affected by CTE supplementation. Yield of milk components was not altered by CTE supplementation. Milk fat yield and composition were not affected when supplementing CTE in lactation dairy diets up to 1.8% DM (Aguerre et al., 2010). Benchaar et al. (2008) reported that milk composition was not changed when supplementing quebracho CTE to

lactating dairy cows. When supplementing CTE at different levels, Aguerre et al. (2010) reported that milk true protein concentration increased at 0.45% CTE supplementation, while supplementing CTE at 1.8% DM decreased milk true protein concentration. It seems that milk composition depends on concentration of CT in the diet tested, and in our case, CTE supplementation at 3% DM would result in minor impacts on milk composition. However, it is not clear how a smaller dose of CTE at 1.8% DM tested by Aguerre et al. (2010) decreased milk true protein concentration.

Concentration of MUN decreased by 16.9% and 16.2% in the HF and the LF diet, respectively in this study. Milk urea N decreased by 7.2% in cows supplemented with CTE at 1.8% DM, but not at lower concentrations of 0.45 to 0.9% DM (Aguerre et al., 2010). Likewise, Benchaar et al. (2008) observed no effects of CTE supplemented at 0.64% of DM on MUN. Although CP concentration in our diets was relatively high compared with typical lactation diets, the CP concentration and RDP to RUP ratio were similar across diets. Therefore, effect of CTE supplementation at 3% of DM used in this study would not be influenced by diets to elicit positive impact on MUN concentration. Milk urea N is used as a management tool to improve dairy herd nutrition and monitor the nutritional status of lactating dairy cows. Urinary N excretion has been shown to have a positive linear relationship with MUN (Ciszuk and Gebregziabher, 1994; Jonker et al., 1998). Elevated MUN indicates excess CP has been fed to the dairy cow for her level of production (Broderick and Clayton, 1997; Jonker et al., 1998). Broderick (1995b) reported that MUN more clearly reflected dietary CP intake than ruminal ammonia concentration. In our case, however, it is clear that forming CT-protein complexes decreased protein degradation and $\text{NH}_3\text{-N}$ production in the rumen as discussed later,

resulting in decreased MUN concentration. However, CTE supplementation did not affect milk protein concentration and yield in spite of reduced MUN in this study. Nelson (1996) suggested that when total milk protein was 3.0 to 3.2% and MUN concentration was 12 to 16 mg/dL, protein degradability fractions and NE_L were likely in balance. The MUN concentration and the milk protein concentration in our study were within this range, and CT supplementation clearly decreased MUN concentration. Lower MUN by CTE supplementation suggests that supplementing CTE in lactating dairy diets may reduce N excretion into urine due to the fact that urinary N excretion has been shown to have a positive linear relationship with MUN (Jonker et al., 1998).

Contrary to the effect of CTE on MUN, efficiency of N use for milk N was not affected by CTE supplementation (Table 4.4) probably due to no effect of CTE on milk protein yield. Feed efficiency (milk yield/DMI) increased by CTE supplementation in the HF diet, but not in the LF diet, leading to a tendency for an interaction between forage level and CTE supplementation ($P = 0.07$). Aguerre et al. (2010) reported that feed efficiency was not influenced by supplementing CTE up to 1.8% DM. Makkar (2003) indicated that the greater animal performance observed in some studies when animals were fed CT has been related to the protection of dietary protein from rumen microbial degradation, resulting in increased supply of AA to the intestine and greater absorption into blood. However, in our case it is likely that the negative impacts of CTE supplementation on feed intake resulted in an increased feed efficiency in the HF diet.

Ruminal Fermentation Characteristics

Feeding the LF diet decreased ruminal pH with mean ruminal pH of 6.47 and 6.33 in the HF and the LF diet, respectively (Table 4.5). Due to the higher starch concentration in

the LF diet (Table 4.1), we expected that the LF diet would reduce ruminal pH. However, there was no remarkable depression on ruminal pH as was shown in minimum pH and $\text{pH} < 5.5$. Yang and Beauchemin (2007) fed LF diets (35:65 of forage-to-concentrate ratio) to lactating dairy cows and observed mean ruminal pH of 6.02 with area under $\text{pH} 5.5 \times \text{min}$ of 75, whereas feeding the LF diet in this study resulted in area under $\text{pH} 5.5 \times \text{min}$ of 1.73. Although we used barley grain as a main ingredient of the concentrate mixture, dietary concentration of the barley grain was 35.5% DM in this study which is notably lower than that of LF diet (56.1% DM) tested by Yang and Beauchemin (2007). Thus, the relatively low dietary concentration of barley grain in the LF diet and proper particle size distributions of the diet prevented the decrease in ruminal pH.

Supplementation of CTE did not influence ruminal pH in the HF or the LF diet (Table 5). Similar to our results, Benchaar et al. (2008) and Aguerre et al. (2010) reported no effects on ruminal pH by feeding CTE to dairy cows. We could not test effects of CTE supplementation on its potential to prevent ruminal acidosis by reducing rapid starch hydrolysis, because feeding the LF diet did not create a severely acidotic fermentative environment in the rumen.

Supplementation of CTE decreased total VFA concentration regardless of level of forage in the diet (Table 4.5). Decreased VFA production corresponds to the decreased DMI in this study. Molar proportions of acetate, propionate, and butyrate increased in the HF diet, but not in the LF diet due to CTE supplementation, resulting in interactions between forage level and CT supplementation. Supplementing CTE in the HF diet decreased the acetate-to-propionate (**A:P**) ratio, but in the LF diet, CT supplementation increased A:P ratio, resulting in an interaction between forage level and CT

supplementation. Effects of CTE on total VFA concentration and VFA pattern have been variable among studies depending on the dosage rate and the source of CT. For example, Beauchemin et al. (2007) reported that despite a lack of effect of quebracho CTE supplementation on digestibility of DM, increasing supplementation levels of quebracho CTE (up to 2% of DMI) tended ($P = 0.08$) to decrease total VFA concentration, and decreased acetate molar proportion and A:P ratio. In contrast, despite a reduction in digestibility of OM, Carulla et al. (2005) observed no change in total VFA concentration in sheep supplemented with black wattle tree CTE, but molar proportion of acetate decreased, and that of propionate increased in sheep fed diets supplemented with CTE. Benchaar et al. (2008) and Aguerre et al. (2010) reported total concentration of VFA and molar proportions of individual VFA were not affected by feeding quebracho CTE.

An interesting finding of this experiment is that molar proportions of VFA were affected by CTE in the HF diet, but not in the LF diet. In addition, CTE supplementation decreased A:P ratio in the HF diet, whereas CTE supplementation increased A:P ratio in the LF diet. The mechanism whereby CTE supplementation resulted in an opposite effect on A:P ratio between the HF and the LF diets is difficult to explain. Supplementing CTE would beneficially manipulate ruminal fermentation in the HF diet that contained higher dietary proportion of alfalfa hay compared with the LF diet containing higher level of steam flaked barley. A favorable change in acetate to propionate ratio in the HF diet due to CTE supplementation may contribute to increasing feed efficiency discussed earlier. The differences in the A:P ratio between the HF and the LF diets detected in this study may be due to several factors, such as ruminal bacterial growth rates due to different starch concentrations between the HF and the LF diet, substrate affinities with CT (alfalfa

hay protein vs. barley protein), or microbial inhibition. Further research needs to elucidate how CTE supplementation would beneficially affect ruminal fermentation in HF diets, particularly diets containing high dietary proportion of alfalfa hay.

Concentration of $\text{NH}_3\text{-N}$ tended to decrease ($P = 0.09$) by supplementation of CTE (Table 4.5). Decrease in $\text{NH}_3\text{-N}$ concentration was reported when CTE was supplemented at 1.8% DM, while at lower concentration (0.45 and 0.9% DM) no effects were observed (Aguerre et al., 2010). As another point of view, diet containing 1.8% of CT from big trefoil resulted in decreased ruminal $\text{NH}_3\text{-N}$ concentration, whereas the same concentration of CT from birdsfoot trefoil had lesser effects (Waghorn and Shelton, 1997). The N binding effects of CT have been well documented (Waghorn et al., 1987; Barry and McNabb, 1999; Beauchemin et al., 2007). The CT present in birdsfoot trefoil have been found to inhibit the growth of proteolytic bacteria and may also precipitate plant protein, making it less available for proteolysis (Min et al., 2000; Molan et al., 2001), thereby inhibiting NH_3 production. When protein is rapidly degraded in the rumen, NH_3 is produced more quickly than the microbes can utilize it for protein synthesis, resulting in more protein being degraded than synthesized (Broderick, 1995a). Powell et al. (2009) recently reported a shift of N excretion routes by feeding CT-containing forages, because the ratio of N excreted in feces and urine was greatest for low-tannin and high-tannin birdsfoot treatments and lowest for alfalfa treatment. Reduced urinary N excretion would result in reduced environmental losses through nitrate leaching, NH_3 volatilization, and nitrous oxide emissions (Place and Mitloehner, 2010). Because supplementation of CTE had no effect on N utilization efficiency for milk production in this study, it may have no effect on N retention, suggesting that

supplementation of CTE in lactation dairy diets may change the route of N excretion, having less excretion into urine but more into feces. It is also possible that the tendency on lower ruminal $\text{NH}_3\text{-N}$ with CTE supplementation was due in part to the lower CP intake as reported in Table 4.3.

Milk FA Composition

In general, supplementation of CTE had minor impacts on milk FA profiles regardless of forage level in the diet (Table 4.6). Concentration of total C18:1 *trans* FA increased with CTE supplementation. Cows fed CTE-supplemented diets tended to increase ($P = 0.06$) *trans*-10, *cis*-12 CLA. Supplementing CTE increased the proportion of C18:3 n-3 FA regardless of forage level. In addition, supplementing CT tended to increase ($P = 0.07$) C20:1 FA. There is limited information on the use of CTE to alter the ruminal biohydrogenation process and manipulate the FA profile of bovine milk and meat. Benchaar and Chouinard (2009) reported that milk FA composition was not affected by supplementing quebracho CTE. Jones et al. (1994) showed inhibition of the growth of *B. fibrisolvens* which is involved in ruminal biohydrogenation of FA. Using 24-h in vitro batch culture, Kronberg et al. (2007) reported that quebracho CTE reduced biohydrogenation of C18:3 FA. Using continuous cultures, Khiaosa-Ard et al. (2009) reported an inhibition of the last step of biohydrogenation by CTE supplied at a rate of 7.89% DM, leading to the accumulation of *trans*-11 C18:1 FA. It is likely that the CTE supplementation at 3% DM tested in this study would not interfere in the biohydrogenation process in the rumen and consequently, no major impacts on FA profiles in the milk.

CONCLUSIONS

Experimental cows used in this study maintained overall productive performance without any negative response on digestibility, milk production, and ruminal fermentation when cows were fed CTE-supplemented diets. Condensed tannins decreased intakes of DM and nutrients, but CT improved feed efficiency in the HF diet. The greater response to CTE supplementation in the HF diet may have resulted from a higher dietary proportion of alfalfa hay in the HF diet, which highlights that CTE supplementation needs to be focused on diets containing high forage N degradability in the rumen. The most significant findings in this study were that cows receiving CTE-supplemented diets decreased ruminal $\text{NH}_3\text{-N}$ and MUN concentrations without loss of milk protein yield. This indicates less ruminal N was lost as NH_3 due to decreased CP degradation by rumen microorganisms in response to CTE supplementation. Therefore, dietary manipulation with the use of CTE in dairy diets may alter ruminal metabolism and N excretion into urine. Due to lack of its effect on N utilization efficiency, however, the beneficial effect may be an increase of N excretion into feces, a more stable form of N, influencing ratio of fecal N to urinary N, but not total N excretion.

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Table 4.1. Ingredients and chemical composition of the TMR fed to lactating cows (n = 4)

Item	Diet ¹			
	HF		LF	
	-CTE	+CTE	-CTE	+CTE
Ingredient, % of DM				
Alfalfa hay	31.9	30.9	24.4	23.6
Corn silage	27.6	26.7	16.3	15.8
Barley, steam flaked	16.6	16.1	35.5	34.4
Beet pulp, pellets	4.00	3.89	3.98	3.89
Corn DDGS ²	9.30	9.01	9.26	9.00
Canola meal	3.71	3.61	3.70	3.61
Wheat middlings	2.28	2.22	2.28	2.21
Calcium carbonate	1.08	1.05	1.07	1.05
Sodium sesquicarbonate	1.03	1.01	1.03	1.01
Blood meal, flash dried	0.86	0.83	0.85	0.83
Salt	0.66	0.64	0.65	0.64
Urea	0.37	0.36	0.37	0.36
Magnesium oxide	0.04	0.04	0.04	0.04
Vitamins and minerals ³	0.57	0.55	0.57	0.55
Condensed tannin extract	–	3.0	–	3.0
Chemical composition, % of DM				

DM, %	54.1 ± 1.73	54.7 ± 1.23	60.1 ± 0.54	60.1 ± 0.53
OM	90.0 ± 0.15	90.6 ± 0.63	90.5 ± 0.71	90.5 ± 0.77
CP	18.5 ± 0.24	18.6 ± 0.27	18.4 ± 0.79	18.6 ± 0.68
RDP, % of CP ⁴	69.1	69.4	69.6	69.8
RUP, % of CP ⁴	30.9	30.6	30.4	30.2
NDF	37.3 ± 0.67	37.6 ± 1.35	34.1 ± 0.23	35.4 ± 0.38
ADF	21.9 ± 0.01	21.4 ± 1.09	18.3 ± 0.40	18.7 ± 0.33
Starch	23.0 ± 0.07	22.4 ± 0.71	29.3 ± 0.92	29.5 ± 0.92
Ether extract	3.64 ± 0.102	3.58 ± 0.013	3.59 ± 0.157	3.62 ± 0.262
NE _L , Mcal/kg ⁴	1.64	1.62	1.73	1.70

¹HF-CTE = high forage (HF) without condensed tannin extract (CTE), HF+CTE = HF with CTE, LF-CTE = low forage (LF) without CTE, and LF+CTE = LF with CTE.

²Dried distillers grains with solubles.

³Formulated to contain (per kg DM): 71.3 g of P, 68.9 g of K, 94.6 mg of Se (from sodium selenate), 6.56 g of Cu (from copper sulfate), 25.8 g of Zn (from zinc sulfate), 4131.3 KIU of Vitamin A, 515.4 KIU of Vitamin D, 5728.8 IU of vitamin E, and 19.6 mg of Rumensin[®] (Elanco Animal Health, Greenfield, IN).

⁴Based on tabular value (NRC, 2001).

Table 4.2. Fatty acid composition of the TMR fed to lactating cows (n = 4)

Fatty acids ¹	Diet ²			
	HF		LF	
	-CTE	+CTE	-CTE	+CTE
C14:0	0.38 ± 0.035	0.36 ± 0.029	0.35 ± 0.027	0.38 ± 0.029
C16:0	20.9 ± 0.28	21.3 ± 0.66	20.8 ± 0.65	21.8 ± 0.24
C17:0	0.17 ± 0.023	0.17 ± 0.009	0.17 ± 0.025	0.17 ± 0.007
C18:0	2.60 ± 0.189	2.60 ± 0.141	2.60 ± 0.109	2.80 ± 0.087
C18:1 <i>trans</i> -9	0.08 ± 0.004	0.07 ± 0.008	0.07 ± 0.019	0.07 ± 0.022
C18:1 <i>cis</i> -9	20.0 ± 0.29	19.6 ± 0.17	21.2 ± 0.60	21.2 ± 0.52
C18:2	45.0 ± 0.56	44.9 ± 0.26	45.5 ± 0.44	44.8 ± 0.75
C18:3 n-6	0.55 ± 0.159	0.50 ± 0.137	0.49 ± 0.128	0.45 ± 0.141
C18:3 n-3	8.31 ± 0.299	8.16 ± 0.212	6.51 ± 0.438	6.14 ± 0.387
C20:0	0.53 ± 0.043	0.52 ± 0.109	0.48 ± 0.055	0.49 ± 0.011
C20:2	0.04 ± 0.006	0.03 ± 0.013	0.03 ± 0.015	0.05 ± 0.033
C20:4	0.18 ± 0.015	0.20 ± 0.017	0.20 ± 0.038	0.19 ± 0.009
C22:0	0.41 ± 0.042	0.48 ± 0.040	0.43 ± 0.012	0.49 ± 0.012
C24:0	0.73 ± 0.073	0.72 ± 0.021	0.59 ± 0.053	0.61 ± 0.087
Saturated fatty acids	26.1 ± 0.59	26.7 ± 0.37	26.2 ± 0.71	27.2 ± 0.27
Unsaturated fatty acids	73.9 ± 0.59	73.3 ± 0.37	73.8 ± 0.71	72.8 ± 0.27

¹Fatty acids composition expressed as g/100 g fatty acid methyl esters.

²HF-CTE = high forage (HF) without condensed tannin extract (CTE), HF+CTE = HF with CTE, LF-CTE = low forage (LF) without CTE, and LF+CTE = LF with CTE.

Table 4.3. Nutrient intake and total tract digestibility of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet ¹				SEM	Significance of effect ²		
	HF		LF			FL	CTE	FL × CTE
	-CTE	+CTE	-CTE	+CTE				
Intake, kg/d								
DM	27.7	25.4	27.5	26.3	1.25	0.53	< 0.01	0.32
OM	24.0	22.8	24.6	23.8	1.17	0.10	0.04	0.63
CP	4.94	4.68	5.08	4.96	0.224	0.02	0.03	0.41
NDF	10.0	9.37	9.67	9.19	0.495	0.14	< 0.01	0.59
ADF	5.75	5.39	5.12	4.89	0.287	< 0.01	< 0.01	0.54
Digestibility, %								
DM	68.8	65.9	71.1	70.0	2.28	0.17	0.39	0.70
OM	70.2	67.6	72.4	71.2	2.30	0.23	0.42	0.76
CP	70.8	67.1	73.4	70.5	3.01	0.29	0.24	0.87
NDF	53.1	51.1	55.0	54.9	3.55	0.43	0.76	0.79
ADF	49.8	47.5	48.1	48.7	3.89	0.95	0.82	0.72

¹HF-CTE = HF without CTE, HF+CTE = HF with CTE, LF-CT = LF without CTE, and LF+CTE = LF with CTE.

²FL = forage level in the diet (high vs. low forage), CTE = condensed tannin extract (without vs. with CTE supplementation), and FL × CTE = interaction between FL and CTE.

Table 4.4. Milk production and composition and efficiencies of DM and N use for milk production of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet ¹				SEM	Significance of effect ²		
	HF		LF			FL	CTE	FL × CTE
	-CTE	+CTE	-CTE	+CTE				
Milk yield, kg/d	34.1	35.0	36.2	36.0	1.82	< 0.01	0.42	0.21
Milk composition, %								
Fat	3.75	3.69	3.59	3.61	0.111	0.02	0.75	0.42
True protein	3.07	3.05	3.11	3.09	0.059	0.05	0.28	0.76
Lactose	4.83	4.81	4.89	4.87	0.049	< 0.01	0.15	0.95
MUN, mg/dL	14.8	12.3	14.8	12.4	0.80	0.91	< 0.01	0.96
Milk component yield, kg/d								
Fat	1.29	1.29	1.30	1.30	0.091	0.84	0.92	0.93
True protein	1.05	1.05	1.12	1.10	0.050	< 0.01	0.55	0.43
Lactose	1.66	1.69	1.76	1.75	0.099	0.04	0.84	0.61
Efficiency								
Milk yield/DMI	1.23 ^b	1.37 ^a	1.33	1.37	0.071	0.05	< 0.01	0.07
Milk N/N intake ³	0.229	0.231	0.236	0.236	0.0116	0.13	0.75	0.80

^{a,b}Means in the same row within HF and LF subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

¹HF-CTE = HF without CTE, HF+CTE = HF with CTE, LF-CTE = LF without CTE, and LF+CTE = LF with CTE.

²FL = forage level in the diet (high vs. low forage), CTE = condensed tannin extract (without vs. with CTE supplementation), and FL × CTE = interaction between FL and CTE.

³Efficiency of use of feed N to milk N = ((milk true protein, kg/d ÷ 0.93) ÷ 6.38) ÷ N intake, kg/d (Dschaaka et al., 2010).

Table 4.5. Ruminal fermentation characteristics of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet ¹				SEM	Significance of effect ²		
	HF		LF			FL	CT	FL × CTE
	-CTE	+CTE	-CTE	+CTE				
Minimum pH	5.63	5.45	5.39	5.30	0.152	0.02	0.73	0.71
Mean pH	6.43	6.50	6.33	6.33	0.080	< 0.01	0.38	0.35
Maximum pH	7.01	7.07	6.93	6.89	0.074	0.02	0.84	0.27
pH < 5.5								
Daily episodes	0.00	0.25	1.00	0.50	0.401	0.04	0.62	0.17
Duration, h/d	0.00	0.06	0.19	0.29	0.161	0.12	0.54	0.85
Area, pH × min	0.00	0.17	1.17	2.28	1.193	0.14	0.53	0.64
Total VFA, mM	129.5	121.8	137.0	131.9	3.44	< 0.01	0.03	0.64
Individual VFA ³								
Acetate (A)	64.3 ^b	65.9 ^a	57.9	58.9	0.96	< 0.01	0.09	< 0.01
Propionate (P)	18.7 ^b	20.3 ^a	25.7	24.4	0.69	< 0.01	0.65	0.01
Butyrate (B)	10.5 ^b	11.5 ^a	13.0	13.6	0.49	< 0.01	0.16	< 0.01
A:P	3.48 ^a	3.29 ^b	2.29 ^b	2.47 ^a	0.114	< 0.01	0.83	< 0.01
NH ₃ -N, mg/dL	10.4	8.49	8.52	7.60	0.902	0.10	0.09	0.52

^{a,b}Means in the same row within HF and LF subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

¹HF-CTE = HF without CTE, HF+CTE = HF with CTE, LF-CTE = LF without CTE, and LF+CTE = LF with CTE.

²FL = forage level in the diet (high vs. low forage), CTE = condensed tannin extract (without vs. with CTE supplementation), and FL × CTE = interaction between FL and CTE.

³Expressed as mol/100 mol.

Table 4.6. Fatty acid composition in the milk of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Fatty acids ¹	Diet ²				SEM	Significance of effect ³		
	HF		LF			FL	CTE	FL × CTE
	-CTE	+CTE	-CTE	+CTE				
C16:0	34.4	33.7	34.6	34.2	0.94	0.57	0.38	0.71
C16:1 <i>trans</i> -9	0.46	0.55	0.52	0.50	0.034	0.81	0.08	0.01
C16:1 <i>cis</i> -9	1.28	1.31	1.52	1.34	0.124	0.05	0.31	0.13
C17:0	0.57	0.62	0.60	0.61	0.039	0.85	0.16	0.43
C17:1 <i>cis</i> -10	0.16	0.17	0.18	0.16	0.014	0.71	0.81	0.43
C18:0	8.82	9.03	8.25	8.36	0.508	0.03	0.57	0.85
C18:1 <i>cis</i> -9	17.8	18.4	17.2	17.5	0.76	0.21	0.49	0.83
C18:1 <i>trans</i> -10	0.27	0.29	0.28	0.28	0.020	0.94	0.61	0.37
C18:1 <i>cis</i> -11	0.87	0.91	0.87	0.95	0.077	0.77	0.32	0.68
C18:1 <i>trans</i> -11	1.23	1.38	1.23	1.32	0.085	0.70	0.12	0.71
C18:1 <i>trans</i> , total	2.50	2.76	2.50	2.73	0.146	0.91	0.04	0.87
<i>Cis</i> -9, <i>trans</i> -11	0.32	0.37	0.38	0.42	0.032	0.09	0.15	0.82
CLA								
<i>Trans</i> -10, <i>cis</i> -12	0.06	0.08	0.07	0.08	0.008	0.77	0.06	0.81
CLA								
C18:2 n-6	2.68	2.65	2.74	2.89	0.119	0.21	0.59	0.41
C18:3 n-3	0.35	0.39	0.34	0.39	0.024	0.74	0.02	0.79

C18:3 n-6	0.10	0.11	0.11	0.11	0.009	1.00	0.64	0.60
C20:0	0.12	0.14	0.13	0.12	0.007	0.45	0.12	0.03
C20:1	0.11	0.13	0.13	0.17	0.015	0.12	0.07	0.57
C20:2	0.10	0.12	0.10	0.11	0.011	0.56	0.11	0.76

¹Fatty acid composition was expressed as g/100 g fatty acid methyl esters. C18:1 *trans*, total = *trans*-4,5 C18:1 + *trans*-6,8 C18:1 + *trans*-9 C18:1 + *trans*-10 C18:1 + *trans*-11 C18:1 + *trans*-12 C18:1 + *trans*-13,14 C18:1; CLA = conjugated linoleic acid.

²HF-CTE = HF without CTE, HF+CTE = HF with CTE, LF-CTE = LF without CTE, and LF+CTE = LF with CTE.

³FL = forage level in the diet (high vs. low forage), CTE = condensed tannin extract (without vs. with CTE supplementation), and FL × CTE = interaction between FL and CTE.

CHAPTER 5
RUMINAL FERMENTATION, MILK FATTY ACID PROFILES, AND
PRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COWS FED TWO
DIFFERENT SAFFLOWER SEEDS¹

INTRODUCTION

Addition of fats in lactation dairy diets allows for the maintenance of energy density while increasing fiber intake, resulting in stabilization of ruminal fermentation (Allen, 1997). In addition, a fat supplement that maximizes DMI and ruminal fiber digestion increases milk production and milk component yield, and improves health and reproduction of dairy cows (Overton and Waldron, 2004). The need for various fat sources that are digestible in the small intestine, easy to use, and cost-effective has drawn a lot of attention with the increasing costs of ration ingredients. In the western and central United States, safflower (*Carthamus tinctorius* L., Asteraceae) has been widely grown because of tolerance to hot and dry climates (Li and Mündel, 1996; Bradley et al., 1999). Safflower seed (SS) is usually 106% higher in fat and 21% lower in CP than is whole linted-cottonseed (CS; Dschaak et al., 2010). The high oil concentration of SS makes it an attractive energy-dense feed for animals with high energy requirements, such as lactating dairy cattle. Alizadeh et al. (2010) reported that SS can be included up to 5% of dietary DM alongside CS for early lactating cows without affecting feed intake while

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maintaining normal ruminal fermentation, peripheral energy supply, and milk production. We recently conducted a lactation study to assess productive performance of dairy cows fed varying levels of whole NutrasaffTM SS (**NSS**), a new variety of SS (Safflower Technologies International, Sidney, MT) containing higher oil and lower fiber concentrations than traditional SS varieties (Bergman et al., 2007). The study demonstrated that NSS can replace CS and be fed to lactating dairy cows without negative impacts on lactational performance up to 3% DM (Dschaak et al., 2010). Feeding the NSS improved efficiency of use of feed N to milk N and decreased MUN; however, how feeding the SS affects ruminal fermentation and FA profiles have not been assessed.

In addition to the benefits on nutrient utilization, feeding NSS enhanced functional quality of milk with increased *cis*-9, *trans*-11 conjugated linoleic acid (**CLA**) concentration, which is an additional benefit to human health (Dschaak et al., 2010). However, the beneficial effect of NSS was counterbalanced by an unfavorable increase of *trans*-10 18:1 fatty acid (**FA**). Many dietary treatments producing high levels of CLA also induce a shift in the major biohydrogenation (**BH**) pathways characterized by increased accumulation of *trans*-10 and *trans*-11 18:1 FA. The increase in the *trans*-10 18:1 content of milk fat is indicative of complex changes in ruminal BH pathways (Lock et al., 2007). Therefore, further research is needed to identify if other CLA isomers or 18:1 *trans* FA would be involved to affect milk fat yield when SS are fed in lactation dairy diets.

We hypothesized that supplementation of SS in lactation dairy diet would improve nutrient utilization and milk FA profiles, but a conventional SS (**CSS**) and NSS elicit

different milk FA profiles due to their unique FA compositions. Our objective was to assess lactational performance, ruminal fermentation, and milk FA profiles and their impacts on milk fat yield when dairy cows were fed CSS or NSS.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University.

Cows, Experimental Design, and Diets

Nine multiparous lactating Holstein cows were used; 3 cows were surgically fitted with ruminal cannula, and they consisted of one of 3 squares. Days in milk ranged from 70 to 108 and from 100 to 144 for noncannulated and cannulated cows, respectively, at the start of the experiment. Average BW was 656 ± 130.9 kg at the beginning of the experiment and 705 ± 123.3 kg at the end of the experiment.

The design of the experiment was a replicated 3×3 Latin square with each period lasting 21 d (14 d of treatment adaptation and 7 d of sampling and data collection). The 3 squares were conducted simultaneously. Within square, cows were randomly assigned to a sequence of 3 dietary treatments consisting of: CS TMR without whole SS (**CST**), CSS TMR (**CSST**), and NSS TMR (**NSST**) (Table 5.1). The CSS (variety S-208) is a normal white hull seed cultivar and a linoleic oil variety, containing 37.6% ether extract and 42.2% NDF, whereas the NSS contains higher oil (45.8% ether extract) and lower fiber concentration (23.7% NDF) than CSS (Table 5.2). The diets had approximately 63.0% forage and 37.0% concentrate. The CSS and NSS added to the CSST and NSST diets

replaced whole-linted CS in the CST diet, and Table 5.1 shows diet composition. In our previous study (Dschaak et al., 2010), efficiency of use of feed N to milk N increased by feeding NSS, and we speculated that N solubility of NSS may be lower than that of CS, thereby influencing ruminal ammonia production and consequently MUN concentration. Although N utilization was not our primary interest in the current study, we would assess N fermentation in the rumen when SS was supplemented. In addition, Dschaak et al. (2010) reported that milk fat concentration was greatly affected when NSS was included at 4% DM with a 15% reduction, and at the inclusion rate increase of 18:1 *trans*-10 milk FA was much more pronounced compared with lower inclusion rates of NSS. These findings indicate that inclusion of SS more than 3% DM may induce complex changes in ruminal BH pathways and cause diet-induced milk fat depression. Consequently, the CSS and the NSS were added at 3.0 and 3.1% DM, respectively, to formulate isonitrogenous diets and avoid possible negative impacts of feeding SS on milk fat yield. Diets were formulated based on NRC (2001) recommendations to provide sufficient NE_L , metabolizable protein, vitamins, and minerals to produce 35 kg/d of milk with 3.5% fat and 3.0% true protein.

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, and were fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0830 and 1500 h with approximately 70% and 30% of total daily feed allocation at each feeding, respectively. Feed offered and refused was recorded daily, and daily samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry-lot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the a.m. and p.m. milkings on 2 consecutive days (d 16 and d 17) in each period. Individual milk samples were analyzed for fat, true protein, lactose, and MUN by the Rocky Mountain DHIA Laboratory (Logan, UT). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Yields of milk fat, true protein, and lactose were calculated by multiplying milk yield from the respective day by fat, true protein, and lactose concentrations of the milk from an individual cow.

Sampling, Data Collection, and Chemical Analyses

Corn silage, chopped alfalfa hay, grass hay, and concentrates were sampled weekly to determine DM concentration. Diets were adjusted weekly to account for changes in DM concentration. Diets of TMR samples were collected on d 20 and 21 for particle size analysis by using the Penn State Particle Separator as described by Kononoff et al. (2003) equipped with 3 sieves (19, 8, and 1.18 mm) and a pan. The recommended proportions for TMR are 2 to 8% on the 19-mm sieve, 30 to 50% on the 8-mm sieve, and 30 to 50% on the 1.18-mm sieve (Kononoff and Heinrichs, 2007), and all diets were within the recommended ranges.

Samples of the TMR fed and Orts for individual cows were collected daily during the data collection period, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Contents of DM of the samples were used to calculate intakes and digestibilities of DM and nutrients.

Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N content was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM^{200/220} Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Weekly samples of dietary ingredients were analyzed for total FA concentration and FA profile according to the procedure of Sukhija and Palmquist (1988) and Kleinschmit et al. (2007) using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) fitted with a flame ionization detector. The injector port temperature was 230°C with a split ratio of 20:1. The column was 100 m, with an inside diameter of 0.25mm (CP-Sil 88, Varian, Lake Forest, CA). The carrier gas was helium at a rate of 2.0 mL/min. Initial oven temperature was 50°C held for 1min, and then increased to 145°C at a rate of 5°C per min and held for 30 min. The temperature was then increased at 10°C/min to 190°C and held for 30 min. Finally, the temperature was raised at 5°C/min to 210°C and held for 35 min. The total run per sample lasted 123.5 min.

Weighted composite milk samples from individual cows were analyzed for FA composition. Milk fat was extracted by boiling milk in a detergent solution (Hurley et al., 1987). Extracted fat was derivatized to methyl esters using an alkaline methylation procedure by mixing 40 mg of fat with a sodium methoxide methylation reagent

($\text{NaOCH}_3/\text{MeOH}$) as described by Chouinard et al. (1999). After FA methyl esters were formed, anhydrous calcium chloride pellets were added and allowed to stand for 1 h to remove water in the sample. Samples were then centrifuged at $1,016 \times g$ at 4°C for 20 min.

Separation of FA was achieved by using a GLC (model 6890 series II) fitted with a flame ionization detector. Samples containing methyl esters in hexane (1 μL) were injected through the split injection port (100:1) onto the column (CP-Sil 88). Oven temperature was set at 80°C and held for 10 min, then increased to 190°C at $12^\circ\text{C}/\text{min}$ for 39 min. The temperature was then increased again to 218°C at $20^\circ\text{C}/\text{min}$ and held for 21 min. Injector and detector were set at 250°C . Total run time was 71 min. Heptadecanoic acid was used as a qualitative internal standard. Individual FA concentrations were obtained by taking the specific FA area as a percentage of total FA, and were reported as g/100 g FA methyl esters.

Feed DM and nutrient digestibility was measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 200 g wet weight) were collected for each cow from the rectum twice daily (a.m. and p.m.) every 12 h, moving ahead 2 h each day for the 6 d of fecal sampling beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were immediately subsampled (about 50 g), composited across sampling times for each cow and each period, dried at 55°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total tract nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, orts, and feces using the following equation: apparent digestibility = $100 - [100$

$\times (AIA_d/AIA_f) \times (N_f/N_d)]$, where AIA_d = AIA concentration in the diet actually consumed, AIA_f = AIA concentration in the feces, N_f = concentration of the nutrient in the feces, and N_d = concentration of the nutrient in the diet actually consumed (Eun and Beauchemin, 2005).

Ruminal Fermentation Characteristics

Ruminal pH was continuously measured for 2 consecutive days starting on d 18 using the Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as described by Penner et al. (2006). Readings in pH buffers 4 and 7 were recorded prior to placing the LRCpH system in the rumen. Ruminal pH readings were taken every 30 s and stored by the data logger. After about 48 h of continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C water, and millivolt readings were recorded in pH buffers 4 and 7. The daily ruminal pH data was averaged for each minute and summarized as minimum pH, mean pH, and maximum pH. In addition, daily episodes, duration (h/d), and area (pH \times min) when ruminal pH was less than 5.5 were calculated. The threshold 5.5 was assigned because it has been defined by others (Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal contents were sampled from cannulated cows at 0, 3, and 6 h after the a.m. feeding on d 20 and 21. Approximately 1 L of ruminal contents were obtained from the anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral locations within the rumen, composited by cow, and strained through a polyester screen (pore size 355 μ m; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five milliliters of the filtered ruminal fluid were added to 1 mL of 1% sulfuric acid and samples were retained for ammonia-N (**NH₃-N**) determination. Concentration of NH₃-N in the ruminal

contents was determined as described by Rhine et al. (1998). Another 5 mL of the filtered ruminal fluid were taken at 3 h after the a.m. feeding and added to 1 mL of 25% of metaphosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a GLC (model 6890 series II) with a capillary column (30 m × 0.32 mm i.d., 1 µm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA), and flame-ionization detection. Crotonic acid was used as an internal standard. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium. In addition, 15 mL of blended ruminal fluid were collected and freeze-dried for ruminal FA analysis. The samples were methylated (Kramer et al., 1997) and analyzed for long-chain FA by GLC (model CP-3380; Varian, Walnut Creek, CA). Fatty acid methyl esters were separated on a 100 m × 0.25 mm × 0.2 µm film thickness fused-silica capillary column (SP-2560, Supelco, Bellefonte, PA). Verification of peak identity was established by comparison of peak retention times to known standards.

Statistical Analyses

Data were summarized for each cow by measurement period. All data were statistically analyzed using the mixed model procedure in SAS (SAS Institute, 2001). Data for intake, digestibility, and milk production were analyzed with a model that included the effect of dietary treatment. Cow, period, and cow by period by square were the terms of the random statement.

Data for VFA profiles and NH₃-N concentration were analyzed with the same model except that the fixed effect of time after feeding was included using the repeated option.

Cow and period were the terms of the random statement. The covariance structure that resulted in the lowest values for the Akaike's information criteria and Schwartz's Bayesian criterion was used (Littell et al., 1998). Data for DMI and milk yield were reported using the heterogeneous compound symmetry structure, whereas efficiency of feed was analyzed by the autoregressive structure. Data for milk components, efficiency of N utilization and VFA were analyzed using the unstructured covariance structure. In addition, data for $\text{NH}_3\text{-N}$ was analyzed by the unstructured and compound symmetry variance-covariance structure.

Residual errors were used to test dietary treatments. Least squares means are reported throughout, and differences were considered significant at $P < 0.05$, and trends were discussed at $P < 0.10$. Treatment means were compared using a protected ($P < 0.05$) LSD test.

RESULTS AND DISCUSSION

In recent years the number of safflower varieties available has increased dramatically, and they have been popularly grown in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) because of reasonable economic returns, a great option in crop rotations, and a potential as a fat supplement in dairy diets. The main findings show that SS supplementation did not affect nutrient digestibility and lactational performance principally through minimal effects on ruminal fermentation. Collectively, this suggests that SS supplementation is effective for maintain normal productive performance of lactating dairy cows without deleterious effects on milk and milk fat yields at a dietary inclusion rate investigated in this study.

Characteristics of Experimental Diets

Chemical composition of experimental diets is listed in Table 5.2. Concentrations of CP as well as fractions of RDP and RUP were similar across dietary treatments. Replacing CS with NSS decreased NDF and ADF concentrations in the NSST due to lower NDF (23.7% DM) and ADF (18.4% DM) concentrations of NSS compared with those of CS (58.8 and 40.8% DM of NDF and ADF, respectively). As expected, CSS contained higher NDF (42.2%) and ADF (32.0%) concentrations compared with the NSS, resulting in medium range of fiber concentrations among diets. Ether extract concentration increased by adding SS in diets due to higher ether extract concentration of CSS (37.6%) and NSS (45.8%) compared with that of CS (17.2%), resulting in increased NE_L in the diets.

Fatty acid profiles of experimental diets are presented in Table 5.2. Due to higher C16:0 concentration of CS (26.1 g/100 g FA methyl esters) compared with that of CSS and NSS (7.3 and 7.8 g/100 g FA methyl esters, respectively), the CST contained higher C16:0 than either the NSST or the CSST. Dschaak et al. (2010) reported when substituting CS with NSS, C16:0 decreased in diets regardless of NSS level. Addition of SS increased C18:2 concentration due to higher C18:2 concentration in CSS and NSS (76.1 and 79.7 g/100 g FA methyl esters, respectively) compared with that of CS (49.8 g/100 g FA methyl esters). Increased concentration of C18:2 was also reported by Dschaak et al. (2010) when CS was substituted with NSS. In addition, Beauchemin et al. (2009) observed increased C18:2 concentration in lactation diet when feeding crushed sunflower seed at 10.6% DM. Sunflower seed is another dietary oilseed that is comparable to SS due to relatively high C18:2 concentration, but the sunflower seed

contains lower C18:2 than NSS (70.1 vs. 79.7 g/100 g FA methyl esters). Adding CSS or NSS in diets decreased saturated FA, but increased unsaturated FA compared with the CST. Composition of FA was similar between the CSST and the NSST.

Intake and Digestibility

Intake of DM averaged 21.8 kg/d across all treatments and was not affected by addition of SS in the diets (Table 5.3). Alizadeh et al. (2010) and Dschaak et al. (2010) reported no effects on DMI when SS were supplemented up to 5% DM. Contrary to these findings, supplementing whole sunflower seed at a relatively high concentration (15.0% DM) resulted in decreased DMI in lactating dairy cows (Mansoori et al., 2011), while an increase in DMI was observed by Beauchemin et al. (2009) when feeding crushed sunflower seed at 10.6% DM. Intake of OM and CP were not affected by SS inclusion in this study. However, feeding the NSST decreased and tended ($P = 0.06$) to decrease intake of NDF and ADF compared with the CST and the CSST, respectively, due to lower NDF and ADF concentrations of NSS reported earlier. Substituting NSS for CS also decreased intake of NDF and ADF (Dschaak et al., 2010). Mansoori et al. (2011) observed decreased intake of NDF when feeding whole sunflower seed at high (15.0% DM) and low (7.5% DM) levels.

Total tract digestibilities of DM, OM, CP, NDF, and ADF were not influenced by SS inclusion (Table 5.3). Total tract digestibilities of DM and OM increased when cows were fed NSS up to 3.0% DM compared with those fed CS diet, but digestibilities of CP, NDF, and ADF did not differ (Dschaak et al., 2010). Similarly, total tract digestibilities of NDF and OM were not affected by feeding SS up to 5% DM (Alizadeh et al., 2010), whereas feeding crushed sunflower seed at 10.6% DM dramatically decreased DM and

OM digestibilities at 19.7 and 15.4%, respectively (Beauchemin et al., 2009).

Polyunsaturated FA exert a toxic effect on cellulolytic bacteria (Nagaraja et al., 1997) and protozoa (Doreau and Ferlay, 1995). This effect is probably through an action on the cell membrane, particularly of gram-positive bacteria (Martin et al., 2008). However, relatively small amounts of polyunsaturated FA in SS used in this study would not affect cellulolytic activities in the rumen, resulting in no effects of adding CSS or NSS on fiber digestion in the current study.

Milk Production and Its Efficiency

Addition of SS in the diets of lactating cows did not influence milk and FCM yield (Table 5.4). In reviewing the literature where SS or sunflower seed have been fed to lactating dairy cows, milk yield response has been variable: no effects (Beauchemin et al., 2009; Alizadeh et al., 2010; Dschaak et al., 2010) or negative effects (Mansoori et al., 2011). These various responses may have resulted from inclusion rate, physiological condition of experimental animals, and diet composition. Milk composition (fat, true protein, and lactose) and MUN concentration were not affected by inclusion of SS. In addition, milk component yields (fat, true protein, and lactose) did not differ due to SS inclusion. Addition of increasing amounts of NSS linearly decreased milk fat concentration and yield, but milk true protein and lactose did not differ with NSS inclusion up to 4% DM (Dschaak et al., 2010). Similarly, milk composition (fat, true protein, and lactose) was not affected by SS inclusion in early lactation diets (Alizadeh et al., 2010) or by crushed (Beauchemin et al., 2009) or whole sunflower seed (Mansoori et al., 2011). Low forage diets with rich C18:2 n-6 FA typically induce milk fat depression (Piperova et al., 2000; Loor et al., 2005; Chilliard et al., 2007), while supplementing

oilseeds in forage-based diets with lipids containing high concentration of C18:2 n-6, like in our case, does not affect milk fat synthesis (Roy et al., 2006; Chilliard et al., 2007).

Feed efficiency (milk yield/DMI and 3.5% FCM yield/DMI) and N efficiency (milk N yield/N intake) were similar among treatments (Table 5.4). Efficiency of use of feed N to milk N was improved in cows fed the 1% NSS diet, but it tended to decrease ($P = 0.08$) when NSS inclusion rate increased (Dschaak et al., 2010). Similarly, feed efficiency (3.5% FCM yield/DMI) did not differ by adding crushed sunflower seed in lactation dairy diets (Beauchemin et al., 2009).

Ruminal Fermentation Characteristics

Substitution of CS with SS did not influence ruminal pH profiles (Table 5.5). Likewise, ruminal pH was not affected by SS inclusion in early lactation diets (Alizadeh et al., 2010) and crushed sunflower seed (Beauchemin et al., 2009). In our study, mean pH was 6.2 across all diets, and minimum ruminal pH was maintained above 5.7 for all diets. Ruminal pH less than 5.8 hardly (< 2.1 h/d) occurred, signifying that all treatments did not interfere with ruminal fermentation due to adequate supply of forage NDF and its particle size which in turn would provide sufficient buffering capacity in the rumen.

Total concentration of VFA was not affected by addition of SS in diets (Table 5.5). Likewise, molar proportions of major VFA (acetate, propionate, and butyrate) did not differ across dietary treatments, and acetate-to-propionate ratio was similar between treatments. However, branched-chain VFA, isobutyrate increased with SS addition in diets. Alizadeh et al. (2010) reported that molar proportions of VFA were not changed by SS inclusion in early lactation diets, and there were no effects of supplementing crushed sunflower seed on total VFA concentration; however, butyrate proportion decreased

(Beauchemin et al., 2009). Because feed intake as well as ruminal fermentation characteristics were similar, it is apparent that ruminal fermentation would not interfere with properties associated with SS in the diets tested in this study. Concentration of $\text{NH}_3\text{-N}$ was not affected by addition of SS in the diets. Similarly, $\text{NH}_3\text{-N}$ concentration did not differ among treatments with addition of SS (Alizadeh et al., 2010). Ruminal $\text{NH}_3\text{-N}$ concentration increased by crushed sunflower seed due possibly to decrease in N uptake by ruminal microbes (Beauchemin et al., 2009). In our previous study (Dschaak et al., 2010), we reported that MUN concentration decreased in cows supplemented with 1% NSS diet compared to those fed CS diet, whereas increasing NSS inclusion level up to 4% DM tended ($P = 0.08$) to improve efficiency of use of feed N to milk N. We speculated that it was likely that the N solubility of NSS might be lower than that of CS, thereby influencing ruminal $\text{NH}_3\text{-N}$ concentration and consequently MUN concentration (Dschaak et al., 2010). However, the $\text{NH}_3\text{-N}$ concentration did not differ among treatments in this study. The discrepancy between the previous and current studies may have been resulted from differences in diet composition; the CST diet contained 34.6% non-fibrous carbohydrate, whereas a control diet having CS in the previous study (Dschaak et al., 2010) had 25.3% non-fibrous carbohydrate, suggesting that the CST diet may provide sufficient soluble carbohydrate to capture ammonia during ruminal fermentation.

FA Profiles in Ruminal Fluid and Milk

The proportion of C16:0 in ruminal fluid was decreased by addition of SS in the diets, having a similar proportion between the CSST and the NSST (Table 5.6). Greater intake of dietary C16:0 (565, 499, and 480 g/d for the CST, the CSST, and the NSST,

respectively) contributed to the higher proportion of the C16:0 in ruminal content of cows fed the CST. Although the concentration of C18:0 in the diets was low, and concentrations of unsaturated C18 FA, such as C18:2 *cis*-9, *cis*-12 and C18:3 *cis*-9, *cis*-12, *cis*-15 in the diets, were high (Table 5.2), C18:0 was the major long-chain FA in ruminal fluid on all diets. Concentrations of C18:0 and C18:1 *trans*-11 were not affected by addition of SS in the diets. However, concentrations of C18:1 *cis*-9 ($P = 0.10$) and C18:2 n-6 ($P = 0.09$) tended to increase by cows fed SS diets compared with those fed the CST. Concentrations of C18:1 *trans*-11 and C18:1 *cis*-9 in ruminal fluid of dairy cows were not affected by feeding flaxseed, but concentration of C18:3 *cis*-9, *cis*-12, *cis*-15 increased (Cortes et al., 2010). The process of ruminal BH reduces the ruminal outflow of polyunsaturated FA and contributes to accumulation of *cis* and *trans* isomers in ruminant products, including *trans* monoenes. Mosley et al. (2002) demonstrated that C18:1 *cis*-9 could be a precursor for several *trans*-FA isomers including C18:1 *trans*-11 FA. The extent to which C18:1 *trans*-11 is hydrogenated to C18:0 via group B microorganisms depends on conditions in the rumen (Jenkins and McGuire, 2006). Hence, the extent and type of the ruminal BH process will determine both the amounts and structures of FA leaving the rumen (Fievez et al., 2007). The increased proportions of C18:1 *cis*-9 and C18:2 n-6 FA in ruminal content of cows fed the CSST and the NSST indicate that CSS and NSS were partially protected from microbial BH, and these SS did not proceed to completion of ruminal BH. Feeding lipids in the form of seeds rather than oils has often been suggested to limit ruminal BH, because seed hulls would restrict bacterial access to lipids. Jenkins and Bridges (2007) found that whole seeds provide some protection from ruminal BH and help lessen the severity of digestion problems by encapsulation of

antimicrobial FA within the hard outer seed coat. The oil would be released at a slower rate in the rumen, or some of the oil may escape ruminal BH (Jenkins and Bridges, 2007). Therefore, feeding whole oilseeds represents a means by which favorable changes in milk FA profile can be obtained (Grummer, 1991).

Physiological conditions of ruminal BH of unsaturated FA have been reported to be sizably affected by some factors such as ruminal pH, amount of added fat, number of double bonds in FA, and ruminal turnover rate (Kalscheur et al., 1997a; Beam et al., 2000; Jenkins and Adams, 2002). Because we did not detect any negative impacts on ruminal fermentation, and added amounts of dietary fat were relatively small in this study, it is likely that feeding SS may not greatly interfere with the ruminal BH, exerting its impacts on proportions of only C18:1 *cis*-9 and C18:2 n-6 FA in ruminal content.

Adding SS in diets of dairy cows decreased the proportion of C16:0 FA in milk with the concentration in the NSST diet being the lowest (Table 5.7). The higher proportion of C16:0 in milk by feeding the CST was associated with higher C16:0 in the CST diet as well as ruminal fluid. Similarly, Dschaak et al. (2010) reported a linear decrease of C16:0 in milk with increasing levels of NSS up to 4% DM. Inclusion of sunflower oil at 5.2% DM (Roy et al., 2006) and flaxseed at 4.2 (Cortes et al., 2010) or 6.5% DM (Caroprese et al., 2010) decreased C16:0 FA in milk, while supplementing fish oil at 1.1% DM (Caroprese et al., 2010) had no effect when compared with cows fed a control diet with no added fat supplement. Glasser et al. (2008) reported that C18:2 in lipid supplements (like in safflower and sunflower seeds) was more inhibitory than C18:3 (like in flaxseed) on the percentage of C16:0 in milk probably through the inhibition of *de novo* synthesis of C16:0.

Proportion of C18:0 FA in milk tended ($P = 0.06$) to increase when cows were fed the NSST diet (Table 5.7). Dschaak et al. (2010) reported that cows supplemented with NSS at 1% DM decreased C18:0 concentration in milk, but when supplemented at 3 or 4% DM no difference was observed when compared with CS supplementation. Addition of sunflower oil in a corn silage-based diet (Roy et al., 2006) or grazing dairy cows (Rego et al., 2009) increased the proportion of C18:0 in milk of dairy cows. In addition, the C18:0 FA concentration was higher in the milk of cows fed flaxseed than that of cows fed fish oil, which could be ascribed to a reduced BH of C18:1 to C18:0 in the rumen when cows were fed fish oil (Caroprese et al., 2010). Supplementation with flaxseed tended ($P = 0.07$) to increase the proportion of C18:0 in milk compared with a control diet (Cortes et al., 2010). Secretion of C18:0 in milk can be increased either by dietary C18:0 intake or by supplementation of C18 unsaturated FA, because they are in large part hydrogenated to C18:0 in the rumen (Chilliard et al., 2007). Lipid supplementation induces a general increase in C18:0 at the expense of the short- and medium-chain FA, resulting from an increase in mammary uptake of long-chain FA absorbed in the intestine and a decrease in mammary de novo FA synthesis (Palmquist et al., 1993; Glasser et al., 2008).

Concentration of C18:1 *cis*-9 in milk increased with SS addition in diets with the NSST being the highest (Table 5.7). Dschaak et al. (2010) reported that addition of NSS showed a linear increase in the C18:1 *cis*-9 in milk with increasing amounts of NSS. Supplementation with flaxseed increased the proportion of C18:1 *cis*-9 in milk compared with a control diet (Caroprese et al., 2010; Cortes et al., 2010); however, supplementation with fish oil did not affect C18:1 *cis*-9 in milk of dairy cows (Caroprese et al., 2010). Secretion of C18:1 *cis*-9 can be increased either through direct gut absorption and

mammary secretion or mainly (ca. 80%) from ruminal BH followed by mammary desaturation of C18:0 (Chilliard et al., 2007). In our case, the increased C18:1 *cis*-9 FA in milk of cows fed SS would be a direct effect of ruminal BH, as it increased in rumen content. Concentration of C18:1 *trans*-10 increased with feeding the NSST, but not with the CSST compared with the CST. While *trans*-10, *cis*-12 CLA has been identified as a potent inhibitor of milk fat synthesis (Bauman and Griinari, 2001), C18:1 *trans*-10 FA does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007), although it responds to dietary factors, and its concentration is negatively correlated with milk fat yield response in cows (Bernard et al., 2008). In the current study, *trans*-10, *cis*-12 CLA did not differ among treatment diets, whereas C18:1 *trans*-10 FA was increased with feeding the NSST, but not with the CSST compared with the CST. The increase in the C18:1 *trans*-10 concentration of milk fat is indicative of complex changes in ruminal BH pathways, so the C18:1 *trans*-10 FA has been suggested as an alternative marker for the type of alterations in rumen BH that characterize diet-induced milk fat depression (Lock et al., 2007). As we did not find any effect of feeding the NSST on milk fat yield, the increased C18:1 *trans*-10 FA with feeding the NSST should not exert significant effects on de novo FA synthesis in mammary gland.

Concentration of *cis*-9, *trans*-11 CLA in milk tended ($P = 0.07$) to increase when feeding the NSST compared with the CST and the CSST, whereas concentration of *trans*-10, *cis*-12-CLA was not affected by dietary treatments (Table 5.7). The increase in *cis*-9, *trans*-11 CLA was linked to the increase in C18:1 *trans*-11 FA, a main precursor of *cis*-9, *trans*-11 CLA in milk. Dschaak et al. (2010) reported that supplementation of NSS linearly increased concentration of *cis*-9, *trans*-11 CLA with increasing NSS inclusion

level. Despite its ruminal origin, the majority of *cis*-9, *trans*-11 CLA in milk is synthesized within the mammary gland from C18:1 *trans*-11 via Δ^9 desaturase (Lor and Herbein, 2003). In addition to the amount and type of oil, there is increasing evidence that milk FA composition responses to lipid supplements depend on the composition of the basal diet (Chilliard and Ferlay, 2004). For example, fat supplementation in high forage diets such as the one used in our study typically result in relatively low increase in the *cis*-9, *trans*-11 CLA concentration with small increase in the C18:1 *trans*-11 isomer (Chilliard et al., 2007). On the other hand, Kalscheur et al. (1997b) reported that BH of polyunsaturated FA in the rumen was reduced with high concentrate diets causing accumulation of C18:1 *trans* isomers and increase of milk *cis*-9, *trans*-11 CLA.

It has been well established that the inclusion of unsaturated fat in dairy cow diets inhibits the de novo synthesis of short- and medium-chain FA and increases the concentration of C18 FA (Chilliard et al., 2007). In our study, proportion of short-chain FA (4:0 to 10:0) in milk was not affected by dietary treatments, while medium-chain FA (11:0 to 17:0) decreased when CS was substituted with CSS, and further decreased by NSS. Rego et al. (2009) reported that short- and medium-chain FA decreased with sunflower oil supplementation, whereas Cortes et al. (2010) reported that proportions of short- and medium-chain FA in milk fat were not affected by feeding flaxseed or fish oil. Long-chain FA ($\geq 18:0$) increased when CS was replaced with SS in the current study. Dschaak et al. (2010) reported a linear increase in long-chain FA with increasing levels of NSS in the diet. Concentration of long-chain FA in milk fat were increases by addition of flaxseed to the diet (Cortes et al., 2010).

CONCLUSIONS

Supplementing SS on 3% DM in lactation diets tested in this study did not have any negative impacts on ruminal fermentation, lactational performance, and milk fat yield. Therefore, supplementing diets with whole SS at 3% of dietary DM can be an effective strategy of fat supplementation to lactating dairy cows without negative impacts on lactational performance and milk FA profiles. Although milk FA C18:1 *trans*-11 and *cis*-9, *trans*-11 CLA increased with feeding the NSST, but not with the CSST diet, there were no differences in milk fat concentration as well as milk fat yield, suggesting that supplementing different whole SS in dairy diets would have limited impacts on mammary FA synthesis.

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Table 5.1. Ingredients of the TMR fed to lactating cows

Ingredient, % of DM	Diet ¹		
	CST	CSST	NSST
Alfalfa hay	35.9	35.8	35.8
Grass hay	4.28	4.27	4.24
Corn silage	23.0	22.9	22.9
Cottonseed, whole	2.35	-	-
CSS ² , whole	-	3.01	-
NSS ³ , whole	-	-	3.10
Corn, steam flaked	18.6	18.4	18.4
Beet pulp, pellets	6.5	6.5	6.4
Corn hominy	5.20	5.10	5.10
Corn DDGS ⁴	1.40	1.30	1.30
Soybean meal	0.73	0.72	0.73
Blood meal, flash dried	0.32	0.31	0.31
Urea	0.21	0.20	0.21
Salt	0.22	0.21	0.22
Sodium bicarbonate	0.19	0.18	0.19
Mineral and vitamin mix ⁵	1.10	1.10	1.10

¹CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

²CSS = conventional safflower seed (variety S-208).

³NSS = Nutrasaff™ safflower seed (Safflower Technologies International, Sidney, MT).

⁴Dried distillers grains with solubles.

⁵Formulated to contain (per kg DM): 71.3 g of P (from monosodium phosphate), 68.9 g of K (from potassium sulfate), 94.6 mg of Se (from sodium selenate), 6.56 g of Cu (from copper sulfate), 25.8 g of Zn (from zinc sulfate), 4,131.3 kIU of Vitamin A, 515.4 kIU of Vitamin D, 5,728.8 IU of vitamin E, and 19.6 mg of Rumensin (Elanco Animal Health, Greenfield, IN).

Table 5.2. Chemical composition of oilseeds and the diets (DM basis)

Item	Oilseed ¹			Diet ²		
	CS	CSS	NSS	CST	CSST	NSST
DM, %	91.9	92.5	95.3	63.5 ± 0.94	64.3 ± 0.96	63.3 ± 1.77
OM	94.4	97.4	96.6	93.7 ± 1.07	93.9 ± 1.12	93.9 ± 0.55
CP	19.2	15.3	17.3	15.8 ± 0.66	15.8 ± 0.63	15.8 ± 0.42
RDP, % of CP ³	-	-	-	67.1	66.9	67.1
RUP, % of CP ³	-	-	-	32.9	33.1	32.9
NDF	54.8	42.2	24.7	40.6 ± 0.63	39.7 ± 0.92	38.3 ± 0.46
ADF	40.8	32.0	18.4	26.5 ± 0.53	25.4 ± 0.71	24.7 ± 0.61
NFC ⁴	2.20	2.30	7.80	33.9 ± 0.63	34.5 ± 0.53	35.4 ± 0.80
Ether extract	18.2	37.6	46.8	3.44 ± 0.39	3.95 ± 0.33	4.42 ± 0.26
NE _L , Mcal/kg ³	-	-	-	1.51	1.53	1.55
Fatty acid ⁵						
C16:0	26.1	7.26	7.76	26.4 ± 0.87	22.7 ± 0.92	21.9 ± 0.67
C18:0	0.04	2.18	0.17	10.3 ± 0.44	10.9 ± 0.36	11.0 ± 0.38
C18:1 <i>trans</i> -9	2.86	2.79	2.51	0.34 ± 0.09	0.44 ± 0.03	0.29 ± 0.01
C18:1 <i>cis</i> -9	17.2	11.1	8.73	13.4 ± 0.32	12.8 ± 0.29	12.5 ± 0.20
C18:2	49.8	76.1	79.7	33.2 ± 1.02	38.8 ± 1.46	40.1 ± 0.92
C18:3 n-3	0.21	0.26	0.25	9.87 ± 0.37	8.54 ± 0.73	8.58 ± 0.69
SFA	31.2	9.50	9.40	40.9 ± 0.54	37.6 ± 0.87	36.8 ± 0.46
UFA	68.8	90.5	90.6	59.1 ± 0.54	62.4 ± 0.87	63.2 ± 0.46

¹Analysis performed on one composite sample for the study. CS = cottonseed, whole; CSS = conventional safflower seed (variety S-208); NSS = NutrasaffTM safflower seed (Safflower Technologies International, Sidney, MT).

²Analysis performed on 3 period samples; CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

³Based on tabular value (NRC, 2001).

⁴Non-fibrous carbohydrate = $100 - \text{CP} - \text{NDF} - \text{ether extract} - \text{ash}$.

⁵Fatty acid composition was expressed as g/100 g fatty acid methyl esters. SFA = saturated fatty acids; UFA = unsaturated fatty acids.

Table 5.3. Nutrient intake and total tract digestibility of lactating cows fed different safflower seeds

Item	Dietary treatment ¹			SEM	<i>P</i>
	CST	CSST	NSST		
Intake, kg/d					
DM	21.4	22.0	21.9	0.63	0.48
OM	19.1	19.4	19.1	0.99	0.55
CP	3.35	3.42	3.38	0.184	0.49
NDF	8.42 ^a	8.28 ^a	7.77 ^b	0.481	< 0.01
ADF	5.45	5.07	5.04	0.328	0.06
Digestibility, %					
DM	70.1	71.2	72.4	1.62	0.58
OM	72.6	73.7	74.8	1.65	0.58
CP	68.9	70.3	71.3	1.65	0.52
NDF	61.3	60.7	62.6	1.45	0.55
ADF	58.5	57.8	61.3	2.63	0.56

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

Table 5.4. Milk production and composition and efficiencies of DM and N use for milk production of lactating cows fed different safflower seeds

Item	Dietary treatment ¹			SEM	<i>P</i>
	CST	CSST	NSST		
Milk yield, kg/d	30.4	30.7	31.7	1.13	0.72
3.5% FCM yield, kg/d	29.4	28.6	28.9	1.47	0.92
Milk composition, %					
Fat	3.50	3.34	3.26	0.194	0.45
True protein	3.01	3.03	3.05	0.071	0.94
Lactose	4.62	4.66	4.65	0.058	0.88
MUN, mg/dL	11.0	11.2	10.6	0.47	0.66
Milk component yield, kg/d					
Fat	1.05	1.00	1.01	0.078	0.81
True protein	0.93	0.93	0.97	0.046	0.75
Lactose	1.40	1.43	1.47	0.052	0.66
Efficiency					
Milk/DMI	1.41	1.39	1.45	0.057	0.69
3.5% FCM/DMI	1.37	1.29	1.32	0.055	0.41
Milk N/N intake ²	0.294	0.286	0.300	0.0137	0.47

¹CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

²Efficiency of use of feed N to milk N = $((\text{milk true protein, kg/d} \div 0.93) \div 6.38) \div \text{N intake, kg/d}$ (Dschaak et al., 2010).

Table 5.5. Ruminal fermentation characteristics of lactating cows fed different safflower seeds

Item	Dietary treatment ¹			SEM	<i>P</i>
	CST	CSST	NSST		
Minimum pH	5.70	5.71	5.76	0.059	0.74
Maximum pH	6.87	6.83	6.78	0.058	0.56
Mean pH	6.23	6.21	6.26	0.068	0.90
pH < 5.8					
Daily episodes	25.7	27.3	11.7	14.67	0.72
Duration, h/d	2.12	1.24	0.18	1.068	0.48
Area, pH × min	8.42	3.00	0.60	4.102	0.43
Total VFA, mM	151.7	157.1	155.3	8.36	0.61
Individual VFA, mol/100 mol					
Acetate (A)	57.0	56.2	56.1	1.68	0.85
Propionate (P)	27.5	27.3	27.9	1.48	0.88
Butyrate	10.3	10.9	10.7	0.57	0.53
Valerate	3.17	3.36	3.08	0.573	0.74
Isobutyrate	0.65 ^b	0.74 ^a	0.72 ^a	0.015	0.04
Isovalerate	0.93	1.09	1.09	0.093	0.16
A:P	2.07	2.06	2.01	0.180	0.88
NH ₃ -N, mg/100 mL	8.47	7.20	8.00	1.381	0.83

^{a,b}Means within a row that do not have a common superscript differ at *P* < 0.05.

¹CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

Table 5.6. Fatty acid composition in the ruminal fluid of lactating cows fed different safflower seeds

Fatty acids ¹	Dietary treatment ²			SEM	<i>P</i>
	CST	CSST	NSST		
C12:0	0.41	0.41	0.47	0.065	0.80
C14:0	2.05	2.06	2.24	0.272	0.86
<i>iso</i> -15:0	1.95	2.23	2.35	0.306	0.65
<i>anteiso</i> -15:0	1.65	1.19	1.28	0.233	0.40
C15:0	0.46	0.33	0.39	0.043	0.21
<i>iso</i> -16:0	5.02	4.98	4.88	0.595	0.98
C16:0	23.5 ^a	21.6 ^b	21.8 ^b	1.10	0.02
C18:0	37.5	36.6	38.2	1.28	0.70
C18:1 <i>trans</i> -11	7.77	9.17	7.09	1.599	0.66
C18:1 <i>cis</i> -9	9.70	11.1	10.8	0.464	0.10
C18:2 n-6	5.63	6.01	6.69	0.283	0.09
C18:3 n-3	1.10	0.92	0.99	0.067	0.25
C22:6 n-3	0.74	0.63	0.84	0.133	0.59

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹Fatty acid composition was expressed as g/100 g fatty acid methyl esters.

²CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

Table 5.7. Fatty acid composition in the milk of lactating cows fed different safflower seeds

FA ¹	Dietary treatment ²			SEM	P
	CST	CSST	NSST		
16:0	37.3 ^a	34.7 ^b	32.1 ^c	1.02	< 0.01
16:1 <i>trans</i> -9	0.37	0.38	0.39	0.023	0.59
16:1 <i>cis</i> -9	1.86	1.82	1.74	0.156	0.72
17:0	0.70	0.67	0.63	0.042	0.25
17:1 <i>cis</i> -10	0.28	0.28	0.26	0.024	0.54
18:0	8.12	8.15	9.14	0.694	0.06
18:1 <i>cis</i> -9	16.2 ^c	18.1 ^b	19.8 ^a	0.82	< 0.01
18:1 <i>trans</i> -9	0.096	0.149	0.155	0.0195	< 0.01
18:1 <i>trans</i> -10	0.21 ^b	0.27 ^{ab}	0.36 ^a	0.038	0.03
18:1 <i>cis</i> -11	1.24 ^b	1.35 ^{ab}	1.43 ^a	0.073	0.02
18:1 <i>trans</i> -11	1.02 ^b	0.89 ^b	1.33 ^a	0.101	< 0.01
18:1 <i>trans</i> , total	2.34 ^b	2.63 ^b	3.37 ^a	0.169	< 0.01
<i>cis</i> -9, <i>trans</i> -11 CLA	0.33	0.34	0.45	0.044	0.07
<i>trans</i> -10, <i>cis</i> -12 CLA	0.027	0.028	0.027	0.0017	0.81
18:2 n-6	2.31	2.49	2.52	0.186	0.21
18:3 n-3	0.45	0.49	0.45	0.043	0.37
18:3 n-6	0.036	0.042	0.036	0.0042	0.49
20:0	0.10	0.11	0.10	0.008	0.65
20:1	0.080	0.097	0.088	0.0058	0.08

20:2	0.049	0.048	0.057	0.0043	0.24
MUFA	23.5 ^c	25.9 ^b	28.5 ^a	0.87	< 0.01
PUFA	3.62	3.89	3.92	0.231	0.20
SFA	71.9 ^a	70.1 ^a	67.4 ^b	0.95	< 0.01
SCFA	6.91	7.09	6.85	0.261	0.80
MCFA	60.2 ^a	58.4 ^b	54.9 ^c	1.28	< 0.01
LCFA	31.9 ^c	34.4 ^b	38.1 ^a	1.36	< 0.01

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹FA = fatty acids expressed as g/100 g fatty acid methyl esters. 18:1 *trans*, total = 18:1 *trans*-4,5 + 18:1 *trans*-6,8 + 18:1 *trans*-9 + 18:1 *trans*-10 + 18:1 *trans*-11 + 18:1 *trans*-12 + 18:1 *trans*-13,14; CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain fatty acids (C4:0 to C10:0); MCFA = medium-chain fatty acids (C11:0 to C17:1 *cis*-10); LCFA = long-chain fatty acids (C18:0 to C24:0); n-3 = 18:3 n-3 + 20:5 n-3 + 22:5 n-3; n-6 = 18:2 n-6 + 18:3 n-6 + 20:3 n-6 + 20:4 n-6 + 22:4 n-6.

²CST = whole linted-cottonseed TMR without whole safflower seed; CSST = whole conventional safflower seed TMR; NSST = whole NutrasaffTM safflower seed TMR.

CHAPTER 6

SUMMARY AND CONCLUSIONS

In dairy nutrition, the goal of manipulation of the ruminal microbial ecosystem is to improve the efficiency of converting feed to products consumable by humans. A better understanding of microbial dynamics in the rumen is needed to maximize animal production and animal welfare and secure sustainable ruminant production system. The outflow of microbial biomass and VFA from the rumen affects the nutritional status of the animal as well as the efficiency of nutrient utilization. The research presented here has addressed manipulation of ruminal fermentation using rumen modifiers (zeolite, quebracho CTE, and Nutrasaff SS) and their contribution to lactational performance of dairy cows.

Ruminal buffers have been used to stabilize ruminal pH in dairy cows because of acid-production in the rumen when lactation diets include large amounts of readily fermentable carbohydrates. Sodium bicarbonate is recognized as an efficient exogenous buffer, but research has continued to identify cheaper mineral buffers that exhibit similar responses on animal performance as the established buffers. Natural zeolite has high affinity for cations and has been used to regulate pH in the rumen by buffering against hydrogen ions of organic acids. In the first lactation study, supplementation of natural zeolite in lactation dairy diet had minor impacts on productive performance and ruminal fermentation. Dairy cows consuming zeolite maintained similar rumen environment to cows consuming sodium bicarbonate diets as indicated by no effects on ruminal pH and a tendency to reduce VFA production. The lack of effects of supplementing the ruminal buffer was consistent throughout the long-term feeding experiment during early to

midlactation. A trend toward increased milk protein and the estimated cost of zeolite projected to be lower than sodium bicarbonate suggests that the net income of the farmer would increase with effective use of zeolite in lactation dairy diets. Overall results in the study indicate that the zeolite product used in this study would cost-effectively replace sodium bicarbonate as a ruminal buffer additive in lactation dairy diet. It is likely that the zeolite at 1.4% DM used in this study would be too low to affect milk yield and other productive performance parameters. Because ruminal buffering agents are most effective when added in high concentrate diets with low ruminal pH, the real challenge in regard to the efficacy of the zeolite as a ruminal buffer additive would be the aspect that if this zeolite product is effectively used in a low ruminal pH fermentative environment.

Therefore, further research is necessary to determine if supplementing zeolite in a high concentrate, lactation diet would prove effective by increasing ruminal pH, as feeding the high concentrate diet will lower ruminal pH with more fermentable carbohydrate in the diet. With its increased exchange rate for ions, it is expected that the efficacy of the zeolite in the high concentrate diet may be greater than that reported in the current study.

Pressure from governmental and consumer agencies have necessitated minimizing nutrient waste and maximizing its use for animal production. Ruminants fed high quality forage diets have large losses of N as ammonia into urine. Tannin containing compounds such as quebracho CTE have the ability to reduce proteolysis and improve animals' N retention. Dairy cows supplemented with the CTE in the second study maintained overall productive performance without any negative response on nutrient digestibility, milk production, and ruminal fermentation. Supplementation of CTE decreased intake of nutrients which caused an improvement in feed efficiency in the high forage diet. The

negative effect of the CTE on feed intake suggests that the CTE supplemented at relatively high concentrations (3% DM) may have been resulted from lower palatability or short-term effect of astringency caused by the CTE. The greater response on N utilization by CTE supplementation in the high forage diet is likely due to a higher dietary proportion of alfalfa hay in the high forage diet, which highlights that the CTE supplementation needs to be focused on diets containing high forage N degradability in the rumen. Total VFA concentration decreased with supplementation of CTE regardless of level of forage in the diet which corresponds to the decreased DMI. However, molar proportions of VFA were affected by CTE in the high forage diet, but not in the low forage diet. In addition, CTE supplementation decreased acetate-to-propionate ratio in the high forage diet, whereas CTE supplementation increased acetate-to-propionate ratio in the low forage diet. Supplementing CTE would beneficially manipulate ruminal fermentation in the high forage diet that contained higher dietary proportion of alfalfa hay compared with the low forage diet containing higher level of steam flaked barley. Cows receiving CTE-supplemented diets decreased ruminal ammonia-N and MUN concentrations without loss of milk protein yield which would indicate less ruminal N was lost as ammonia due to decreased CP degradation by rumen microorganisms in response to CTE supplementation. Dietary manipulation with the use of CTE in dairy diets may alter ruminal metabolism and N excretion into urine. Due to lack of its effect on N utilization efficiency, however, the beneficial effect may be an increase of N excretion into feces, a more stable form of N, influencing ratio of fecal N to urinary N, but not total N excretion reducing environmental losses through nitrate leaching, ammonia volatilization, and nitrous oxide emissions. Supplementation of CTE had minor

impacts on milk FA profiles regardless of forage level in the diet. It is likely that the CTE supplementation at 3% DM tested in this study would not interfere in the biohydrogenation process in the rumen and consequently, no major impacts on fatty acid profiles in the milk.

Fats are supplemented to increase energy density of dairy diets, which ideally will lead to increased intake of energy if DM intake is not decreased. Increased energy intake should improve energy balance and benefit milk production. Safflower seed has high oil which makes it an attractive energy dense feed for lactating dairy cows that have a high energy requirement. Supplementing SS on 3% DM in lactation diets assessed in the third study did not have any negative impacts on ruminal fermentation, lactational performance, and milk fat yield. Because feed intake and ruminal fermentation characteristics did not differ compared with control diet, it is apparent that ruminal fermentation would not interfere with properties associated with SS in the diets tested in this study. Because there was no negative impacts on ruminal fermentation, and the amount of added dietary fat was relatively small in this study, it seems that feeding SS may not greatly interfere with the ruminal BH, exerting its impacts on proportions of only C18:1 *cis*-9 and C18:2 n-6 FA in ruminal content. Feeding lipids in the form of seeds would allow the oil to be released at a slower rate in the rumen, or some of the oil may escape ruminal biohydrogenation, because the seed hull would restrict bacterial access to lipids. Since there were no differences in milk fat concentration as well as milk fat yield, supplementing different whole SS (conventional and Nutrasaff) in dairy diets would have limited impacts on mammary fatty acid synthesis. Milk C18:1 *trans*-11 and *cis*-9, *trans*-11 CLA increased with feeding the Nutrasaff SS, but not with the conventional SS.

Therefore, supplementing diets with whole SS at 3% of dietary DM can be an effective strategy of fat supplementation to lactating dairy cows without negative impacts on lactational performance and milk fatty acid profiles.

In conclusion, these studies demonstrate that the 3 rumen modifiers (zeolite, quebracho CTE, and Nutrasaff SS) can positively manipulate ruminal fermentation, but supplementing the 3 rumen modifiers in typical lactation dairy diets in the Intermountain West would have limited impacts on lactational performance because of consistent ruminal fermentative conditions contributed by feeding high nutritive quality forage. Therefore, their efficacy may be influenced by the type of feed and physiological status of animals supplemented with the modifiers. The interactions that occur between rumen modifiers, rumen microbial organisms, feeds, and host are complex, and more research is needed to improve our understanding of these processes, thus securing consistent efficacy of using rumen modifiers observed in the field. Natural zeolite, quebracho CTE, and Nutrasaff SS have characteristics that make them potential rumen modifiers for dairy enterprise.

APPENDIX

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I am requesting your permission to include the paper titled: Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows, of which you are a coauthor. Your contribution will be acknowledged in a footnote to the chapter title.

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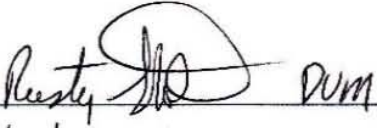
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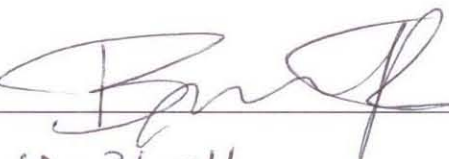
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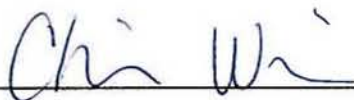
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CURRENT FIELDS OF INTEREST:

- Manipulation of ruminal fermentation and its contribution to animal production.
- Enhancement of forage utilization by ruminants.
- Improvement of nutritive value of low-quality forage for ruminants.
- Implementing a nutritional management plan to reduce environmental pollution.
- Analyzing dairy records to implement a nutritional management plan.
- Application of current strategies to improve performance of dairy cattle.

TEACHING INTEREST:

- Principles of Animal Nutrition
- Applied Ruminant Nutrition
- Dairy Cattle Production and Management
- Lactation, Milk, and Nutrition

EDUCATION:

Ph.D., Animal Science with emphasis in Dairy Nutrition, Utah State University. Logan, UT, 2008 - Present; Planned Ph.D. Dissertation Title: **Use of Rumen Modifiers to Manipulate Ruminal Fermentation and Improve Nutrient Utilization and Lactational Performance of Dairy Cows**; Advisor: Jong-Su Eun, Ph.D. Expected Date of Completion: May, 2012.

M.S., Dairy Science, Utah State University, Logan, UT, May 2009; Thesis Title: **Production Performance and Profiles of Milk Fatty Acids of Lactating Dairy Cows Fed Whole Safflower Seed Containing High Fat and Low Fiber**; Advisor: Allen J. Young, Ph.D; Research Advisor: : Jong-Su Eun, Ph.D.

B.S., Biology/Zoology with minors in agriculture and chemistry, Southern Utah University, Cedar City, UT, May, 2002.

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Research Assistant: Aug. 2006 – Mar. 2007, Dept. of Animal, Dairy, and Veterinary Science, Utah State University.

Teaching Experience:

Instructor of Applied Animal Nutrition (ADVS 3510; 3 credits) at USU, Spring, 2009 and 2010: taught *categorization of farm animal feeds into energy feeds, protein feeds, dry forages, silages and haylages, pasture and range plants, and vitamin-mineral supplements (emphasis placed on practical diet formulation, including computerization and aspects of feed delivery and nutritional management)*

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Dairy Manager: 1996 – 2005, Theron Mills and Sons Dairy Joseph, UT.

ACADEMIC HONORS:

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- **Animal, Dairy, and Veterinary Science Scholarship:** Given by Utah State University, Logan, UT; Spring, 2007.

SPECIAL SKILLS AND TECHNIQUES:

- Fermenter operation for continuous culture system.
- In vitro and in vivo techniques for evaluation of feedstuff.
- GC analyses of long chain fatty acids, volatile fatty acids, and methane.
- Basic techniques for nutrient analysis.
- Handling experimental animals (cattle and sheep).
- Statistical analysis using the Statistical Analysis System (SAS).
- Computer skill: proficient in using Microsoft Office and feed formulation software (NRC).

PROFESSIONAL MEMBERSHIP:

- American Dairy Science Association

PUBLICATIONS:

Refereed Journal Articles

Dschaak, C. M., C. T. Noviandi, J.-S. Eun, V. Fellner, A. J. Young, D. R. ZoBell, and C. E. Israelsen. 2011. Ruminant fermentation, milk fatty acid profiles, and productive performance of Holstein dairy cows fed two different safflower seeds. *J. Dairy Sci.* 94:5138–5150.

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Eun, J.-S., D. R. ZoBell, **C. M. Dschaak**, D. E. Diaz, and J. M. Tricarico. 2009. Case Study: Effects of supplementing a fibrolytic feed enzyme on the growth performance and carcass characteristics of beef steers. *Prof. Anim. Sci.* 25:382–387.

Papers in Proceedings (peer referred)

Williams, C. M., **C. M. Dschaak**, J.-S. Eun, A. J. Young, and J. W. MacAdam. 2009. Ruminant metabolism during continuous culture fermentation when replacing alfalfa (*Medicago sativa* L.) hay with birdsfoot trefoil (*Lotus corniculatus* L.) hay. Pages 148-151 in Proceedings, Western Section, American Society of Animal Science, Colorado State University, Fort Collins, CO.

Eun, J.-S., D. R. ZoBell, **C. M. Dschaak**, and D. E. Diaz. 2008. Effect of a fibrolytic enzyme supplementation on growing beef steers. Pages 418-420 in Proceedings, Western Section, American Society of Animal Science, University of Wyoming, Laramie, WY.

Abstracts in Refereed Conference Proceedings

- Dschaak C. M.**, C. M. Williams, M. S. Holt, J.-S. Eun, and A. J. Young. 2010. Effects of condensed tannins supplementation on ruminal fermentation and lactational performance of dairy cows when fed high or low forage diet. *J. Dairy Sci.* 93 (E-Suppl. 1): 196. (Abstr.)
- Dschaak C. M.**, C. M. Williams, J.-S. Eun, and A. J. Young. 2010. Effects of condensed tannins supplementation in a lactating dairy TMR diet on ruminal fermentation in continuous culture, maintained at high and low pH. *J. Dairy Sci.* 93 (E-Suppl. 1): 422. (Abstr.)
- Williams C. M., **C. M. Dschaak**, J.-S. Eun, J. W. MacAdam, and A. J. Young. 2010. Assessment of tannin-free and tanniferous legumes in lactating dairy diets using continuous culture. *J. Dairy Sci.* 92 (E-Suppl. 1): 191. (Abstr.)
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- Dschaak, C. M.**, J.-S. Eun, A. J. Young, and J. W. Bergman. 2009. Assessment of whole Nutrasaff safflower seed as a fat supplement to lactating Holstein dairy cows. *J. Dairy Sci.* 92 (E-Suppl. 1): 463. (Abstr.)
- J.-S. Eun, **C. M. Dschaak**, F. H. Bhushan, Y.-M. Kim, and A. J. Young. 2009. Effect of saponin extract supplementation on ruminal fermentation in continuous culture. *J. Dairy Sci.* 92 (E-Suppl. 1): 292. (Abstr.)
- Dschaak, C. M.**, J.-S. Eun, Y.-M. Kim, F. H. Bhushan, and A. J. Young. 2009. Shift in in vitro microbial fermentation in response to condensed tannin supplementation in mixed ruminal cultures. *J. Dairy Sci.* 92 (E-Suppl. 1): 468. (Abstr.)
- Williams, C. M., **C. M. Dschaak**, J.-S. Eun, A. J. Young, and J. W. MacAdam. 2009. Ruminal metabolism during continuous culture fermentation when replacing alfalfa (*Medicago sativa* L.) hay with birdsfoot trefoil (*Lotus corniculatus* L.) hay. *J. Anim. Sci.* 87, (E-Suppl. 3): 145. (Abstr.)
- Eun, J.-S., D. R. ZoBell, **C. M. Dschaak**, and D. E. Diaz. 2008. Effect of a fibrolytic enzyme supplementation on growing beef steers. *J. Anim. Sci.* 86, (E-Suppl. 3): 161. (Abstr.)