Alterations in Mammary Gland Synthesis and Secretion of Fatty Acids in Response to *Trans* Isomers of Octadecenoic Acid or Conjugated Linoleic Acid Isomers

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Dissertation submitted to the Faculty of the Virginia Polytechnic Institute & State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

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April 13, 2001

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Key words: *trans*10-18:1, *trans*-vaccenic acid, *trans*10,*cis*12-18:2, rumenic acid, milk fat, grazing

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(ABSTRACT)

Experiments were conducted to investigate: 1) production of *trans*-18:1 and *cis*/*trans*-18:2 isomers due to input of forage and corn grain in continuous culture fermenters, 2) concentrations of *trans*-18:1 and *cis*/*trans*-18:2 isomers in blood and milk fat of grazing cows fed a grain supplement containing solvent- or mechanically-extracted soybean meal, 3) plasma and milk fatty acid profiles of lactating cows in response to a conjugated linoleic acid (CLA) mixture infused into the rumen, and 4) effects of *cis*9,*trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) on *de novo* synthesis and desaturation of milk fatty acids in lactating cows fed unsaturated oils. In the first study, rumen fermenters were fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g/d corn replacing equal portions of forage DM. Outflow of *trans*11,*cis*15-18:2 (11/15LA) in effluents was greater when DM input was clover or grass only. With each increment of corn, output of 9/11CLA increased until it was 205% greater compared with forage alone. *Trans*11-18:1 (TVA), an intermediate in 18:2n6 (LA) and 18:3n3 (LN) hydrogenation, output increased by 28% with corn addition. Outputs of *trans*10-18:1 and 10/12CLA nearly doubled as corn increment increased. In the second experiment, Holstein cows grazing mixed clover-grass pastures were fed a grain supplement (6.7 kg/d) containing 1.7 kg solvent-extracted soybean meal (SES, 15 mg LA/g of DM), 1.9 kg mechanically-extracted soybean meal (MES, 24 mg LA/g of DM), or 1.9 kg MES plus 30 g of liquid methionine hydroxy analog (MESM). Cows fed MES or MESM had greater concentrations of LA, TVA, 9/11CLA, and 11/15LA in blood compared

with cows fed SES. Daily yields of 18:0 (SA), LA, LN, TVA, and 9/11CLA in milk fat also were greater for cows fed MES or MESM compared with SES. In experiment 3, four Holstein cows were used in a 4×4 Latin square to determine plasma and milk fatty acid profiles during infusion of a CLA mixture at 0, 45, 90, or 180 g/d for 48 h into the rumen. Relative to the control, infusion of 180 g CLA/d decreased milk fat percentage and yield. Lower milk fat yield resulted from depressed concentrations of saturated 6:0 to 16:0 medium-chain fatty acids (MCFA). Concentrations of TVA, *trans*10-18:1, and 10/12CLA in blood plasma, and yields in milk fat increased in response to each dose of CLA. Stearic acid yield also increased as dose of CLA increased. Yield of *cis*9-18:1 (OA) in milk fat, however, was lower at 180 g CLA/d. In experiment 4, four cows were fed high-oleic (HO) or high-linoleic (HL) (2.5% of DM) oil for 11 d prior to abomasal infusion (15 g/d) of 9/11CLA or 10/12CLA for 48 h (2×2 factorial). Milk fat percentage and yield decreased 25% due to infusion of 10/12CLA compared with 9/11CLA, regardless of diet. Lower fat yields resulted from lower MCFA concentrations and yields. Regardless of diet, concentration (but not yield) of SA increased 40% when 10/12CLA was infused compared with 9/11CLA. Concentrations and yields of OA, 9/11CLA, and 20:4n6 also were reduced by infusing 10/12CLA compared with 9/11CLA regardless of diet. Thus, in addition to inhibiting de novo fatty acid synthesis, 10/12CLA appeared to inhibit desaturation via Δ^6 and Δ^9 desaturases. Significant implications from the above studies include: 1) 11/15LA and TVA are the primary intermediates flowing out of the rumen during hydrogenation of pasture lipids, 2) replacing forage DM with starch, OA, and LA increases synthesis of *trans*10-18:1 and 10/12CLA in the rumen, 3) desaturation of TVA produced in the rumen provides an alternate source for 9/11CLA in milk fat, and 4) 10/12CLA decreases *de novo* synthesis and desaturation of milk fatty acids.

ACKNOWLEDGEMENTS

I sincerely appreciate the input and participation of Dr.'s M. A. Barnes, C. E. Polan, T. W. Keenan, and D. S. Kronfeld as members of the committee during my Ph.D. program.

I also would like to thank my advisor, Dr. J. H. Herbein, for his support and advice during the past five years. He gave me the freedom to conduct a tremendous amount of work, which has certainly opened up opportunities for my future in research.

I am indebted to Dr. C. E. Polan for allowing me to participate in the studies that became Chapters 1 and 2. Also, Chapter 1 would not have been possible without the cooperation of Dr. W. H. Hoover and T. K. Miller-Webster, West Virginia University, who conducted the incubations.

I extend my gratitude to W. A. Wark. She went beyond the call of duty to provide help in the laboratory or the dairy farm. Her dedication to optimizing conditions for the separation of 18:1 and 18:2 isomers was invaluable.

DEDICATION

To my wife Carmen and my parents.

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INTRODUCTION

Diet is considered a contributing factor in the onset or progression of some cancers, coronary heart disease, diabetes, and obesity (Grundy, 1998). Epidemiological studies indicated diet composition may account for 35% of human cancer deaths (Doll, 1992). Saturated fatty acid (14:0 and 16:0, primarily) overload, exposes muscle to insulin resistance, hyperinsulinemia, or enhanced production of triglycerides and cholesterol by the liver (Grundy, 1998).

Over one half of the fatty acids in milk fat are saturated (Chilliard et al., 2000). However, milk and dairy products also contain many "forms" of 18:1 and 18:2 derived from hydrogenation of unsaturated fatty acids in the rumen. *Trans*11-18:1 (*trans*-vaccenic acid) and *cis*9,*trans*11-18:2 (CLA) in dairy products are examples of hydrogenation intermediates that may have implications in human health (Parodi, 1999). Milk fat-derived CLA resulted in reduced growth of human mammary cancer cells or chemically-induced tumors in rat mammary tissue (Ip et al., 1999; O'Shea et al., 2000). Desaturation of dietary *trans*11-18:1 in human tissues may enhance CLA concentrations in blood plasma (Adlof et al., 2000), and maintain CLA status.

The profiles of unsaturated fatty acids in diets for lactating cows vary and might affect the production of *trans*11-18:1 or *cis*9,*trans*11-18:2 in the rumen and their incorporation in milk fat. The highest concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat have been achieved during grazing (Precht and Molkentin, 1997; Kelly et al., 1998). Diets supplemented with unsaturated oils also increase *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat (Chilliard et al., 2000). Bacteria in the rumen hydrogenate dietary unsaturated fatty acids to different extents resulting in accumulation of a variety of *trans*-18:1 (Kemp et al., 1975). Under certain conditions, greater starch and linoleic acid (18:2n6) availability in the rumen might increase the production of *trans*10,*cis*12-18:2 or *trans*10-18:1, which were associated with reduced concentrations of milk fat due to reduced medium-chain fatty acid content of the fat (Piperova et al., 2000; Baumgard et al., 2000b).

In addition to reducing *de novo* synthesis of saturated medium-chain fatty acids in the mammary gland, exogenous CLA mixtures (*cis*9,*trans*11-18:2 and *trans*10,*cis*12-

18:2) apparently reduced desaturation of 18:0 to *cis*9-18:1 (Loor and Herbein, 1998; Chouinard et al., 1999a). Activity of Δ^9 desaturase in the mammary gland leads to endogenous synthesis of *cis*9-16:1, *cis*9-18:1, and *cis*9,*trans*11-18:2 and their secretion in milk fat (Enjalbert et al., 1998; Griinari et al., 2000). Arachidonic acid (20:4n6) also is synthesized endogenously in the mammary gland by desaturation and elongation of 18:2n6. Concentration of 20:4n6 in milk fat also was reduced due to abomasal infusion of a CLA mixture (Loor and Herbein, 1998). When purified preparations of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 were infused into the abomasum, the concentration of 18:0 relative to basal levels was increased in response to either isomer (Baumgard et al., 2000b). Thus, it is still unclear which CLA isomer affects the desaturation process.

The objectives of the present study were:

1. To evaluate the production of biohydrogenation intermediates when red clover or orchardgrass alone or with three levels of supplemental corn grain were incubated in continuous culture fermenters.
2. To determine the effect of pasture and two sources of supplemental soybean meal (as sources of dietary 18:2n6) on concentrations of *cis* and *trans* isomers of 18:1 and *cis*,*trans* isomers of 18:2 in blood plasma and in milk fat.
3. To determine plasma and milk fatty acid profiles upon infusion of three doses of CLA into the rumen of lactating cows.
4. To determine plasma fatty acid extractions by the mammary gland, *de novo* synthesis of medium-chain fatty acids, and desaturation of long-chain fatty acids in lactating cows fed oil-supplemented diets and infused with *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 via the abomasum.

LITERATURE REVIEW

Lipids

Classification and Fatty Acid Nomenclature

There is no widely accepted definition for the term “lipid”. Most textbooks describe lipids as a group of naturally occurring compounds, which are readily soluble in organic solvents. They include fatty acids and their derivatives, carotenoids, terpenes, steroids, and bile acids. In terms of structure and function, however, many of these compounds do not have much in common (Christie, 1998). A more specific definition is one that describes “lipids” as: fatty acids, their naturally occurring derivatives (esters or amides), and substances related biosynthetically (e.g. prostanoids, alcohols) or functionally (e.g. cholesterol) to these compounds (Christie, 1998).

The most common lipid classes in nature consist of fatty acids linked by an ester bond to glycerol or other alcohols. In addition to fatty acids, lipids may also contain alkyl moieties such as phosphoric acid or carbohydrates. Lipids are further subdivided into two broad classes. Simple lipids are those which, upon hydrolysis, yield at most two types of primary product per mole. Complex lipids yield three or more primary hydrolysis products per mole (Christie, 1998). Phospholipids (contain a polar phosphorus moiety and a glycerol backbone) and glycolipids (contain a polar carbohydrate moiety) belong to the complex lipid class. In contrast, triglycerides (contain a glycerol backbone) and cholesterols esters (contain a cholesterol backbone) are considered simple lipids (Figure 1).

Fatty acids are compounds synthesized in nature via condensation of 2-carbon units (malonyl-CoA), derived from acetyl-CoA, by a fatty acid synthase complex. They are saturated (no double bonds) and contain between 14 to 24 carbon atoms in a straight chain. Addition of a double bond, desaturation, to saturated fatty acids produces monounsaturated fatty acids. Addition of two or more double bonds results in synthesis of polyunsaturated fatty acids (PUFA). When double-bonded carbon atoms in a PUFA alternate with a single bond (-C=C-C=C-), the acid is referred to as conjugated. In contrast, when double-bonded carbon atoms in a PUFA alternate with two single

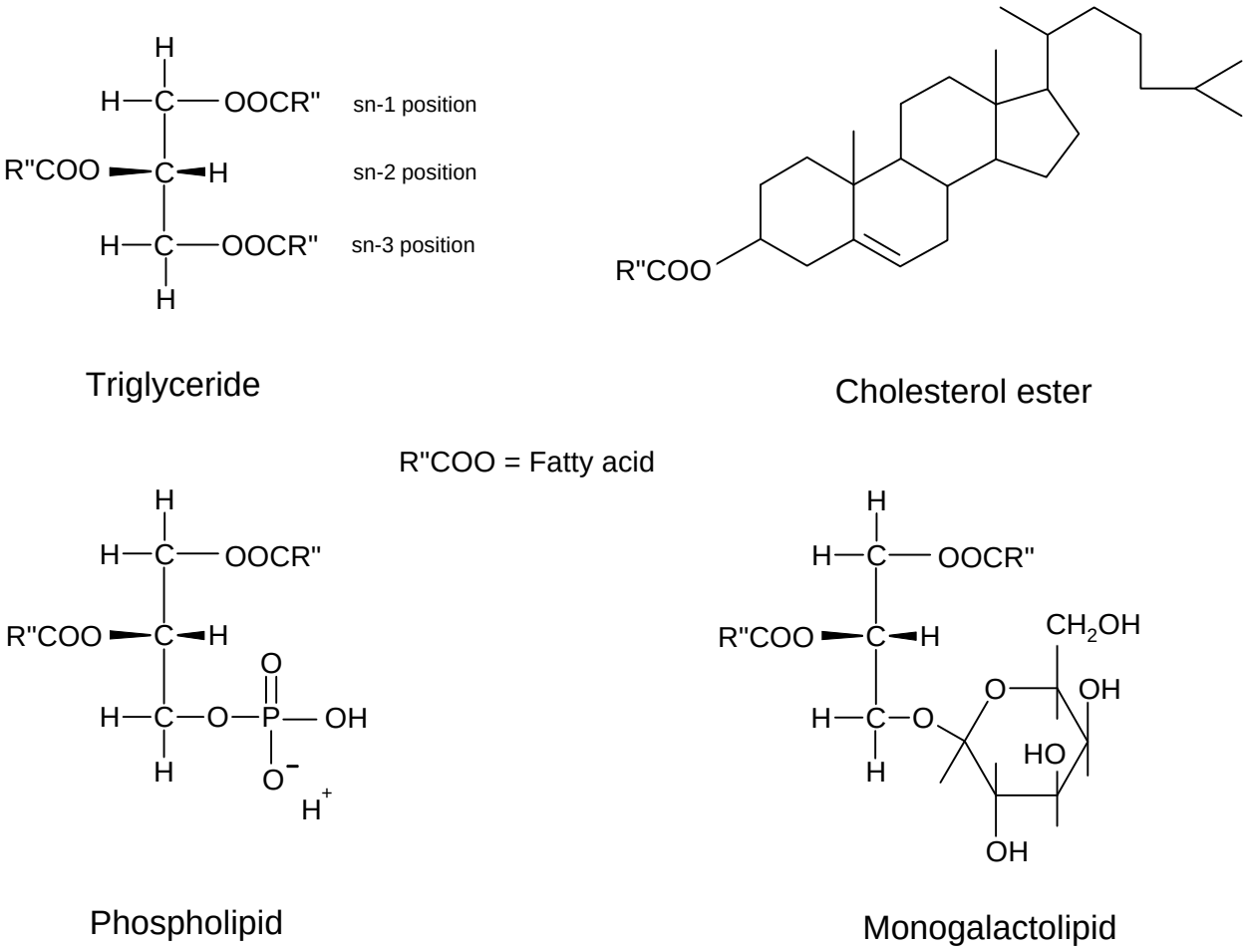


Figure 1. Major lipid classes found in nature.

bonds (-C=C-C-C=C-), the acid is referred to as isolated. The geometrical configuration of the double bond can be either *cis* or *trans*, depending on the position of the alkyl groups: *cis* (on the same side), *trans* (on opposite sides).

The trivial names of most fatty acids arose from the botanical or zoological species from which they were isolated. Such names may cause confusion because they do not provide information regarding the structure of the acid. Systematic nomenclature identifies a fatty acid and describes its structure on the basis of number of carbon atoms and number and position of unsaturated bonds relative to the carboxyl group(s). The substituted groups and their positions are identified and the optical activity and geometric configuration at double bonds also are designated (Lobb and Chow, 2000). Modifications to this naming system have evolved, however, and resulted

in different naming systems. For example, vaccenic acid from the Latin *vacca* is commonly used to refer to 18-carbon monounsaturated fatty acids with a double bond in position 11 of the carbon chain. Shorthand designations, which give the number of carbons and double bonds in the molecule separated by a semicolon, are the most commonly used in the literature. One example is oleic acid, which is designated *cis*9-18:1 and indicates the geometry of the double bond as well as the number of carbons in the molecule. In the following sections of this chapter and subsequent chapters, fatty acids will be described by their shorthand designations as outlined in Table 1.

Metabolism of Unsaturated Fatty Acids in the Rumen

Lipolysis

Overview

Prior to hydrogenation, fatty acids in triglycerides, phospholipids, and galactolipids in the diet are rapidly hydrolyzed in the rumen (Kepler et al., 1970). The overall lipolytic activity during fermentation results from the combination of bacterial as well as plant lipases. Most of the knowledge regarding lipolytic enzymes, however, has come from *in vitro* studies with pure cultures of rumen microorganisms or mixed rumen fluid. *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* strain S2 produce extracellular lipases capable of hydrolyzing triglycerides, galactolipids, and phospholipids (Mackie et al., 1991). However, the former accounts for ~1% of the viable population compared with ~25% for *B. fibrisolvens* (Latham et al., 1972). While rumen protozoal suspensions hydrolyzed small amounts of triglyceride or galactolipid (Wright, 1961; Singh and Hawke, 1979), cultures of anaerobic fungi did not hydrolyze exogenous lipid (Mackie et al., 1991). Leaves from a wide variety of pasture grasses
also appeared to have substantial lipase and phospholipase activity. Free fatty acids accounted for up to 60% of total radioactivity from ^{14}C-triolein and ^{14}C-ryegrass monogalactolipid (53% 18:3n3) after they were incubated with extracts from grass leaves in autoclaved rumen fluid (Faruque et al., 1974a).

Table 1. Systematic name, trivial name, and shorthand designation of fatty acids identified in feedstuffs, rumen fermenters, blood plasma, or milk fat [1].

Systematic name	Trivial name	Short-hand designation
Saturated fatty acids		
Butanoic	Butyric	4:0
Hexanoic	Caproic	6:0
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
Monounsaturated fatty acids		
Cis isomers		
*cis*9-tetradecanoic	Myristoleic	*cis*9-14:1
*cis*9-hexadecenoic	Palmitoleic	*cis*9-16:1
*cis*9-octadecenoic	Oleic	*cis*9-18:1
*cis*11-octadecenoic	*Cis*-vaccenic	*cis*11-18:1
*cis*12-octadecenoic		*cis*12-18:1
*cis*13-octadecenoic		*cis*13-18:1
*cis*15-octadecenoic		*cis*15-18:1
Trans isomers		
*trans*9-hexadecenoic	Palmitelaidic	*trans*9-16:1
*trans*6/7/8-octadecenoic [1]		*trans*6/7/8-18:1
*trans*9-octadecenoic	Elaidic	*trans*9-18:1
*trans*10-octadecenoic		*trans*10-18:1
*trans*11-octadecenoic	*Trans*-vaccenic	*trans*11-18:1
*trans*12-octadecenoic		*trans*12-18:1
*trans*13/14-octadecenoic [1]		*trans*13/14-18:1
*trans*15-octadecenoic		*trans*15-18:1
*trans*16-octadecenoic		*trans*16-18:1
Polyunsaturated fatty acids		
Cis configuration		
9,12-octadecadienoic	Linoleic	18:2n6
9,12,15-octadecadienoic	α-linolenic	18:3n3
11,14,17-octadecatrienoic	Eicosatrienoic	20:3n3
5,8,11,14-octadecatrienoic	Arachidonic	20:4n6
5,8,11,14,17-eicosapentaenoic	Eicosapentaenoic	20:5n3
Isolated configuration		
c9,t12-octadecadienoic		*cis*9,*trans*12-18:2
t9,t12-octadecadienoic		*trans*9,*trans*12-18:2
t9,c12-octadecadienoic	Linolelaidic	*trans*9,*cis*12-18:2
c9,t11,c15-octadecatrienoic		*cis*9,*trans*11,*cis*15-18:2
Conjugated configuration		
c9,t11-octadecadienoic	Rumenic acid	*cis*9,*trans*11-18:2
c9,c11-octadecadienoic		*cis*9,*cis*11-18:2
t10,c12-octadecadienoic		*trans*10,*cis*12-18:2
c10,c12-octadecadienoic		*cis*10,*cis*12-18:2

[1] Isomers that could not be separated by gas chromatography are presented as single fatty acids.

According to the reaction specificity, bacterial lipases found in nature can be classified as nonspecific, 1,3-specific, and fatty acid specific (Mackie et al., 1991). Lipases from *A. lipolytica* hydrolyzed triglycerides from olive oil (70% *cis*9-18:1) more rapidly than those containing saturated fatty acids with 14 carbons or less (Henderson, 1971). On average, 67 and 80% of 18:2n6 and 18:3n3 were released from soybean oil triglycerides during incubation with rumen contents (Van Nevel and Demeyer, 1996a,b). In addition, lipolysis of soybean oil increased in proportion with substrate availability (Immig et al., 1993).

Factors Affecting Lipolysis

pH

The hydrolytic activity of lipase isolated from *A. lipolytica* during exponential growth peaked when the pH of the medium was 7.4 (Henderson, 1971). As the pH decreased to 7.1 and 6.6, lipase activity also decreased. Based on similar findings with other bacterial lipases, it was concluded that ruminal lipases are most active at or close to neutral pH (Henderson, 1971). Lower rumen pH due to dietary manipulation could be detrimental to microbial lipases or species of lipolytic bacteria. The total counts of viable lipolytic bacteria in rumen fluid, decreased 96% due to feeding lactating cows a high-grain/low-forage (80:20) diet compared with a diet similar in amounts of forage plus grain (45:55) (Latham et al., 1972). The proportion of *B. fibrisolvens* strains, as a percentage of total isolated bacteria, also decreased from 30 to 3% by feeding the high-grain/low-forage diet. Hence, *in vitro* lipolysis of tripalmitin, triolein, or linseed oil in suspensions of mixed rumen bacteria isolated from cows on the high-concentrate diet, was 3-fold lower. These results were subsequently confirmed in sheep fed high-grain diets, or when the pH was reduced from 6.8 to 5.6 during incubations of soybean oil with rumen fluid (Gerson et al., 1983; Gerson et al., 1985).

Diet

Independent of the level of rumen pH high-grain diets appear to reduce the numbers of *B. fibrisolvens* and lipolytic vibrios, and might account for the overall reduction in hydrolysis of unsaturated fatty acids. Stepwise adaptation of sheep to a

high-grain diet, however, increased the proportion of *A. lipolytica* while reducing pH to 5.4 (Mackie et al., 1991). A similar effect could explain the lack of differences in the rates of lipolysis (11%/h) of soybean oil, when rumen fluid was obtained from cows fed a high-grain or low-grain diet supplemented with soybean oil for 3 wk (Beam et al., 2001). These results raise the question of whether bacterial counts are a true reflection of lipase activity. Besides changes in the composition of the rumen microflora, direct effects of diet ingredients might affect lipase activity. Greater levels of glucose in the culture medium reduced the production of lipase by pure strains of aerobic bacteria (Jaeger et al., 1994).

Other evidence supports the view that the extent of lipolysis in the rumen could be affected by diet. Hydrolytic activity of rumen fluid from cows fed fresh pasture compared with hay was greater (Faruque et al., 1974a,b; Singh and Hawke, 1979). In addition, lipolysis of triolein in rumen fluid was greater when sheep were fed immature grass compared with mature grass (Gerson et al., 1986). As indicated earlier, the contribution of plant lipases to the overall process of lipolysis might be higher when actively growing pasture is fed. More recently, it was found that the primary factor affecting the rate of lipolysis of 18:2n6 in soybean oil was the concentration of soybean oil in the culture medium (Beam et al., 2000). Lipolysis declined from 44%/h to 22%/h as the concentration of soybean oil increased from 2 to 10%.

The data indicate that lower fiber availability leads to reduced numbers of *B. fibrisolvens,* which seem to be the primary lipolytic bacterium in the rumen under "normal" feeding conditions. Rumen bacteria synthesize lipases that readily hydrolyze 18-carbon unsaturated fatty acids found in lipids from forages and oilseeds, and makes them available for hydrogenation.

Biohydrogenation

Overview

Reiser (1951) provided the first evidence of biohydrogenation in the rumen. Upon incubation of 18:3n3 with rumen fluid, he observed greater amounts of 18:2 isomers accumulating. *In vitro* studies indicated that 18:0 accounted for 20% of the disappearance of *cis*9-18:1, 18:2n6, or 18:3n3 when incubated with rumen contents

from sheep (Shorland et al., 1957). *Trans*-18:1 isomers accounted for 17, 48, and 67% of the original *cis*9-18:1, 18:2n6, or 18:3n3 substrate. Linoleic acid, in particular, resulted in the formation of conjugated 18:2 isomers. Subsequently, it was confirmed *in vivo* that 18:0 and *trans*-18:1 (45 and 34% of total rumen fatty acids) were the major products of hydrogenation (Wood et al., 1963).

Kinetics of Biohydrogenation

The first detailed studies of the biohydrogenation process in the rumen were conducted by Polan et al. (1964). Among twenty pure cultures of rumen bacteria, only certain strains of *B. fibrisolvens* could hydrogenate ^{14}C-18:2n6 to 18:1 isomers, but not to 18:0. Anaerobic conditions and a hydrogen atmosphere were required for complete hydrogenation. Hydrogenation of 18:2n6 to 18:0 decreased as the amount of substrate increased. Accumulation of 18:1 isomers was favored by high concentrations of 18:2n6 in the medium. Mixed rumen bacteria obtained during grazing in the summer had more hydrogenating activity compared with rumen bacteria in rumen fluid from grazing in the winter. Results led to three major conclusions. Unsaturated fatty acids in the rumen could function as terminal acceptors for metabolic hydrogen, high levels of 18:2n6 compete for hydrogenation of 18:1 isomers to 18:0, changes in microbial populations and(or) activity could affect the extent of hydrogenation.

The initial step during hydrogenation of 18:2n6 or 18:3n3, involved an isomerization to *cis*9,*trans*11-18:2 or *cis*9,*trans*11,*cis*15-18:3 via Δ^{12}*cis*, Δ^{11}*trans* isomerase (Kepler and Tove, 1967). The K_m for the isomerization of 18:2n6 was lower (1.2×10^{-5} M) compared with 18:3n3 (1.2×10^{-5} M). This enzyme (isolated from *B. fibrisolvens*) had a pH optimum between 7.0 and 7.2, it was found in the cytoplasm and particulate fraction of the cell extract, it did not require cofactors (ATP, CoA, Mg^{++}, or NAD^{+}) for activity, and could carry out isomerization in the presence of oxygen. Isomers with *cis* and *trans* double bonds at positions 9 or 11 were competitive inhibitors of enzymatic activity (Kepler et al., 1970). All isolated *cis*/*trans* isomers of 18:2n6 also were inhibitory.

Following isomerization of 18:2n6 or 18:3n3 in pure cultures of *B. fibrisolvens* or mixed rumen fluid, reductive hydrogenation (via Δ^{9}*cis*, Δ^{11}*trans* reductase; Hughes et

al., 1980) of the double bond at carbon 9 resulted in accumulation of *trans*11-18:1 or *trans*11,*cis*15-18:3, primarily. A further reduction of the double bond at carbon 15 of *trans*11,*cis*15-18:3 yielded *trans*11-18:1 as the major 18:1 isomer (Wilde and Dawson, 1966; Kepler et al., 1966; Kepler and Tove, 1967). The reductase (isolated from *B. fibrisolvens*) had a pH optimum between 7.2 and 8.2, required iron for activity, it was found in the particulate fraction of the cell extract, and required strict anaerobic conditions.

Factors Affecting Biohydrogenation

pH

Similar to observations with bacterial lipases, activity of Δ^{12}*cis*, Δ^{11}*trans* isomerase and Δ^{9}*cis*, Δ^{11}*trans* reductase could be reduced at pH below neutrality. The reduction in activity for both enzymes was linear between pH 7.2 to 5.3 (Kepler and Tove, 1967; Hughes et al., 1980). Diet-induced low rumen pH was shown to affect the extent of lipolysis and hydrogenation. Thus, due to lower rates of lipolysis, hydrogenation of 18:2n6 and 18:3n3 from soybean oil decreased by 59 and 63% in rumen fluid from cows fed a high-grain/low-forage diet (Latham et al., 1972). *Trans*-18:1 isomers in rumen fluid also accumulated when high-concentrate diets were fed (Tove and Matrone, 1962), indicating that reduction to 18:0 also is affected by lower pH and(or) changes in bacterial populations. Despite substantial inhibition (65%) of lipolysis in rumen fluid during stepwise (6.8 to 5.2) reductions in pH (*in vitro*), hydrogenation of 18:2n6 and 18:3n3 from soybean oil was not markedly altered until the pH was 5.2 (Van Nevel and Demeyer, 1996a). At this pH, hydrogenation of 18:2n6 and 18:3n3 averaged 65% compared with 96% when the pH was 6.8.

It appears that lipolytic activity in the rumen is more sensitive to pH changes, and could be responsible for most of the decreases in apparent hydrogenation observed when high-grain diets are fed. From a review of the literature with fattening beef cattle, it was reported that hydrogenation of 18:2n6 or 18:3n3 was between 35 to 60% or 50 to 80% when the level of concentrate in the diet was above 70% (DM basis) (Jouany et al., 2000). These values represented a 25 to 35% decrease in hydrogenation of 18:2n6, and a 20 to 38% decrease in the hydrogenation of 18:3n3.

Diet

Pasture

Few *in vivo* data on the biohydrogenation of fatty acids from pasture have been published. Based on the changes in fatty acid profiles of rumen fluid after feeding fresh pasture, it was initially deduced that 18:2n6 and 18:3n3 in grasses and legumes were hydrogenated substantially. Feeding fresh grass-clover pasture for 2 h tripled the concentration of 18:1 isomers, whereas concentration of 18:3n3 accounted for less than 1% of total fatty acids in rumen fluid samples taken from 0 to 6 h after feeding (Hawke and Robertson, 1964). Infusion of linseed oil into the rumen during the 2-h feeding period, further increased concentrations of 18:1 and 18:2 isomers, but reduced 18:0 concentration.

In subsequent studies, using ryegrass monogalactolipids (the primary lipid class in pastures) as substrates, it was shown that *trans*-18:1 isomers and 18:0 were the primary fatty acids produced during hydrogenation of mixed pasture in the rumen of grazing cows (Singh and Hawke, 1979). The maturity of the plants in mixed pastures also seems to affect the extent of hydrogenation, because hydrogenation of triolein was 2-fold greater in rumen fluid from sheep fed immature ryegrass compared with mature ryegrass (Gerson et al., 1986). Immature grass was characterized by a higher leaf to stem ratio, crude protein content, and total fatty acid content compared with mature grass.

In sheep fed fresh lucerne (18% 18:2n6 and 40% 18:3n3; pre-bloom), apparent hydrogenation (100 - 100 $\times$ [C18 unsaturated flow to duodenum/C18 unsaturated intake]) of 18:2n6 and 18:3n3 averaged 61 and 91% (Doreau and Poncet, 2000). When the same forage was fed as hay, effectively reducing total fatty acid and 18:2n6 and 18:3n3 intake, the degree of hydrogenation of 18:3n3, but not 18:2n6, decreased. Hydrogenation of pasture 18:3n3 in grazing cows averaged 96% (Chilliard et al., 2000).

Leaves from pasture grasses have substantial lipase and phospholipase activity (Faruque et al., 1974a). In addition, numbers of cellulolytic bacteria (primarily *B. fibrisolvens*) are substantially higher in pasture-fed or grazing animals compared with animals fed conserved forages (Wolstrup et al., 1974). Thus, greater lipolytic and

hydrogenating activity during grazing could be a combination of plant and microbial factors.

Preserved Forages and Supplemental Oils

A review of the literature indicated that hydrogenation of *cis*9-18:1, 18:2n6, and 18:3n3 from diets composed of ~50% ensiled forage (3.3 to 4.5% lipid) is remarkably constant, and averages 55, 76, and 81%, respectively, (Doreau and Ferlay, 1994). However, the extent of hydrogenation of unsaturated fatty acids increases in proportion with dietary intake. Greater hydrogenation of *cis*9-18:1 (38 to 73%), 18:2n6 (70 to 95%), and 18:3n3 (89 to 98%) was observed when supplemental oils (2.5 to 10% of DM) containing high concentrations of these fatty acids were fed (Murphy et al., 1987; Doreau and Chilliard, 1997; Kalscheur et al., 1997a; Wachira et al., 2000). Hydrogenation of 20:5n3 (EPA) and 22:6n3 (DHA), averaged 75% in sheep fed supplemental fish oil at 3% of DM (Wachira et al., 2000).

The extent of hydrogenation also varies due to method of processing fat supplements. It is typically believed that supplementing free oils or unprotected fat above 3.5% of DM might depress fiber digestibility (Palmquist, 1991). There are four main processes commonly used to protect fats (triglyceride or free fatty acids) from ruminal hydrogenation and potential negative effects on microbes. These include encapsulation of triglycerides in a matrix of aldehyde-treated protein, formation of calcium salts of saturated or unsaturated fatty acids, pelleting of hydrogenated oils with small amounts of starch to form “prilled” fat, and extrusion of oilseeds (Gulati et al., 1997). Compared with feeding free oils, hydrogenation of *cis*9-18:1 and 18:2n6 from extruded oilseeds or prilled fat was 30% lower. However, less than 20% of formaldehyde-treated cottonseed oil was hydrogenated (Gulati et al., 1997). The extent of hydrogenation of formaldehyde-treated CLA (60% CLA; *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 primarily) *in vitro* averaged 30% compared with 90% for the unprotected mixture (Gulati et al., 2000).

Fatty Acid Intermediates During Biohydrogenation

Trans-18:1 isomers produced during hydrogenation with *B. fibrisolvens* reflected the double bond positions of the substrates. Thus, hydrogenation of 18:2n6 led to production of *trans*11-18:1, primarily, but *trans*9-18:1 also accumulated. Incubating a mixture of *cis*9,*trans*11-18:2 (39% of total fatty acids), *trans*10,*cis*12-18:2 (3%), and *cis*8,*trans*10-18:2 (54%) resulted in accumulation of *trans*8-18:1 (27%), *trans*9-18:1 (7%), *trans*10-18:1 (10%), *trans*11-18:1 (46%), and *trans*12-18:1 (9%) (Kepler et al., 1966). Although *B. fibrisolvens*, accounts for a large number of total rumen bacteria, numerous isomers are produced during hydrogenation of unsaturated fatty acids by the mixed rumen microflora (Table 2).

Human colonic bacteria also have hydrogenating activity. Incubation of 18:2n6 with fecal homogenates led to detectable concentrations of *trans*11-18:1 (4% of total fatty acids), *cis*9,*trans*11-18:2 (11%), *trans*,*trans*-18:2 (4%), and greater 18:0 (15%) concentration compared with unsupplemented controls (Howard and Henderson, 1999). Addition of 18:3n3 to the culture medium, however, increased the concentrations of an unidentified *cis*,*trans*18:3 isomer only. In the terms of tissue lipid metabolism, the significance of hydrogenation in the large intestine of human is questionable because fatty acid absorption from this compartment into blood has not been demonstrated.

Catalytic Hydrogenation

Overview

Industrial hydrogenation, or hardening, of vegetable oils improves flavor stability and melting behavior, and has been carried out since the beginning of the century (Veldsink et al., 1997). The major aim of the process is to hydrogenate 18:3n3, maintain 18:2n6, and minimize 18:0 formation. Consequently, the selectivity of the hydrogenation of double bonds in *cis*9-18:1, 18:2n6, or 18:3n3 is important. For industrial purposes, the hydrogenation of 18:1 to 18:0 and the accumulation of *cis*-18:1 versus *trans*-18:1 are the most important parameters (Veldsink et al., 1997). Selective hydrogenation leads to lower levels of conjugated 18:2 isomers. Hydrogenated vegetable or animal fat supplements are commonly fed to dairy cattle, because the

greater degree of saturation makes them unavailable ("inert") to rumen microbes, which has been associated with lower fiber digestibility (Palmquist et al., 1993).

Besides saturation, migration and *cis*/*trans* isomerization of double bonds occur during hydrogenation leading to a broad product distribution. The major *trans*-18:1 isomers produced during catalytic hydrogenation are: *trans*9-, *trans*-10, *trans*11-,and *trans*12-18:1 which account for 15, 23, 19, or 13% of total *trans*-18:1 (Veldsink et al., 1997). The overall process involves three steps. Hydrogenation is typically carried out in a batch autoclave over a nickel-based catalyst at the following conditions: 110-215 °C, 0.5-2.5 kg/cm^2 H_2 pressure, and 0.01-0.15 wt% nickel (Veldsink et al., 1997; Jung and Ha, 1999). Compared with selective hydrogenation, non-selective hydrogenation requires lower amounts of nickel catalyst, higher hydrogen pressure, but similar temperature (Jung and Ha, 1999). The degree of unsaturation of the hydrogenated product during non-selective hydrogenation is reduced by 40% after only 1 h of reaction, whereas it requires up to 6 h of reaction under selective hydrogenation conditions to reach similar degrees of unsaturation (Jung and Ha, 1999).

The formation of conjugated linoleic acid (CLA) isomers during hydrogenation of soybean oil is dependent on the type and duration of the process. Selective hydrogenation was more favorable for the formation of CLA compared with non-selective hydrogenation (Jung and Ha, 1999). Concentrations of CLA isomers (mixture of *cis*9,*trans*11-18:2, *trans*10,*cis*12-18:2, and *trans*,*trans*-18:2) peaked (98 mg total CLA/g oil) at 210 min during selective hydrogenation. During non-selective hydrogenation, CLA concentration peaked (9 mg/g of oil) after 35 min then decreased through 56 min when the reaction stopped.

Ruminal and industrial hydrogenation result in saturation of double bonds from unsaturated fatty acids in dietary oils. However, it is apparent that the mechanisms involved differ greatly. *Trans*11-18:1 is the major intermediate of rumen hydrogenation compared with *trans*10-18:1 during catalytic hydrogenation. Enzymatic isomerization of dietary 18:2n6 in the rumen, results in formation of *cis*9,*trans*11-18:2 primarily. However, this isomer only accounts for 27% of CLA formed as a result of selective or non-selective industrial hydrogenation of soybean oil.

Table 2. Ability of ruminal bacteria to hydrogenate *cis*9-18:1, 18:2n6, and 18:3n3 [1].

	***cis*9-18:1**	**18:2n6**	**18:3n3**
Organism	**Products of hydrogenation (% of total fatty acids recovered)**		
Butirivibrio fibrisolvens A38		cis9,trans11-18:2 = 100%	cis9,trans11,cis15-18:3 = 100%
Ruminococcus albus	Not hydrogenated	cis9,trans11-18:2 *	cis9,trans11,cis15-18:3 *
		trans18:1 = 95%	trans11,cis15-18:2 *
		cis18:1 = 5%	trans18:1= 95%
			cis18:1 = 5%
Fusocillus T344	trans11-18:1 = 5%	cis9,trans11-18:2 *	cis9,trans11,cis15-18:3 *
	cis18:1 = 5%	trans18:1 = 65%	cis9,trans11-18:2 *
	18:0 = 90%	18:0 = 35%	cis15-18:1 = 85%
Fusocillus babrahamensis	18:0 = 80%	cis9,trans11-18:2 *	cis9,trans11,cis15-18:3 *
	18:0-OH = 20%	trans18:1 = 70%	trans11,cis15-18:2 = 15%
		18:0 = 30%	cis15-18:1 = 85%
R8/5 Gram-negative rod	cis9-18:1 = 60%	trans18:1 = 50%	trans11,cis15-18:2 = 25%
	18:0-OH = 40%	18:0 = 40%	cis15-18:1 = 50%
			trans15-18:1 = 25%
EC7/2 Gram-negative rod	not hydrogenated	trans11-18:1 = 100%	trans11,cis15-18:2 = 70%
			trans11-18:1 = 30%
LM8/1B, Gram negative rod	Not hydrogenated	trans11-18:1 = 100%	trans11,cis15-18:2 = 100%
Eubacterium W461		cis9,trans11-18:2 *	cis9,trans11,cis15-18:3 *
		trans18:1 = 50%	trans11,cis15-18:2 *
		trans11 = 65%	trans18:1 = 50%
		trans10 = 18%	trans11 = 65%
		trans12 = 12%	trans10 = 20%
		trans9 = 2%	trans12 = 15%
		cis18:1 = 50%	trans9 = 1%
		cis11 = 85%	cis18:1 = 50%
		cis10 = 10%	cis11 = 95%
		cis9 = 1%	cis10 = 5%
Coccus F2/6		cis9,trans11-18:2 *	cis9,trans11,cis15-18:2 *
		trans18:1 = 95%	trans11,cis15-18:2 *
		trans10 = 30-70%	trans18:1 = 95%
		trans11 = 70-30%	trans10 = 30-70%
		cis18:1 = 5%	trans11 = 70-30%
			cis18:1 = 5%
Propionibacterium sp.	Not hydrogenated	trans10,cis12-18:2 = 50%	Not hydrogenated
		trans10-18:1 = 10%	
Pseudomonad	cis9-18:1 = 8%		
	18:0-OH = 67%		
	trans10-18:1 = 25%		

[1] Compiled from: Kemp et al., 1975; Hazlewood et al., 1976; Mortimer and Niehaus, 1972; Verhulst et al., 1987.

* Transient intermediate.

Significance of Biohydrogenation

There is no known physiological advantage for microbial biohydrogenation in the rumen. The current trend of thought is that unsaturated fatty acids are more toxic to anaerobic bacteria than the products of hydrogenation (Keweloh and Heipieper, 1996). Therefore, one function of biohydrogenation may be to destroy growth-inhibiting substances. Another suggestion, widely accepted among ruminant nutritionists, is that biohydrogenation allows for disposal of excess reducing power (metabolic hydrogen) in the rumen (Polan et al., 1964). Because bacterial lipids are largely saturated, biohydrogenation might serve to convert dietary fatty acids into a suitable form for incorporation into the bacterial cell without the large energy expenditure required for *de novo* synthesis of long-chain saturated fatty acids (Jenkins, 1994).

Duodenal Flow, Digestibility, and Absorption of Fatty Acids

Microbial Lipids

In adult ruminants, lipid digestion begins in the reticulo-rumen. As noted in the preceding sections, intense lipolysis and hydrogenation characterize initial stages of ruminant digestion by microorganisms. Microbes also synthesize fatty acids *de novo*. Palmitic and stearic acid are the primary components of phospholipids and free fatty acid fractions, which account for 30 or 35% of the total bacterial lipid (Jenkins, 1994). Uptake of preformed fatty acids, due to supplemental fat, increases the proportion of free fatty acids in the bacterial cytoplasm (Bauchart et al., 1990). Fatty acids with 15 and 17 carbons also can be synthesized. Synthesis of fatty acids occurs primarily from volatile fatty acids. Odd-numbered fatty acids are produced through α-oxidation of existing long chain fatty acids or from propionyl-CoA (Emmanuel, 1978). Branched-chain fatty acids are formed from isobutyrate or branched amino acids (Tweedie et al., 1966). *Trans*16:1 or *trans*14:1 isomers arise from desaturation of 14:0 and 16:0 via an anaerobic pathway (Emmanuel, 1978). Although considerably variable, the mixture of liquid and particle-adherent bacteria flowing out of the rumen contains between 100 and 200 mg lipid/g DM (Demeyer and Doreau, 1999).

Rumen Outflow of Fatty Acids

Total Fatty Acids

Duodenal flow of total fatty acids exceeds intake when basal diets are fed, but is often decreased when fat-supplemented diets are fed (Doreau and Ferlay, 1994). Results using radioactive fatty acids indicated that absorption of fatty acids across the rumen wall is low (Bickerstaffe et al., 1972). However, other data indicated that absorption of fatty acids across the rumen wall could increase in proportion with fatty acid availability (Goosen, 1975). In reviewing the literature, it was concluded that negative balances were obtained when supplemental fat was >5% of DM (Doreau and Ferlay, 1994). Fresh pasture, whether fed or grazed, most often induce negative duodenal flows (Outen et al., 1975; Bauchart et al., 1984; Doreau and Ferlay, 1994).

Isomers of 18:1 and 18:0

Effects of Supplemental Oil

The proportions of 18:1 isomers flowing to the small intestine reflect the extent of hydrogenation in the rumen. As indicated earlier, a number of *cis* and *trans*-18:1 isomers can arise during hydrogenation of *cis*9-18:1, 18:2n6, and 18:3n3. Separation of individual *cis* and *trans* isomers, however, is labor intensive. Thus, very few *in vivo* data describing the individual flows of 18:1 isomers derived from hydrogenation are available.

Bickerstaffe et al. (1972) quantified the flow of *cis* and *trans* isomers of 18:1 with double bonds at positions 7 through 16, when goats were fed supplemental soybean oil (60% 18:2n6). Total flow of *cis*-18:1 isomers averaged 5 g/d. Oleic acid accounted for 84% of total *cis*-18:1 isomers in the feed, but only 43% of total *cis*-18:1 isomers in duodenal digesta. *Cis*11- and *cis*12-18:1 were 12 and 1% of total fatty acids in feed, but increased to 13 and 17% of total *cis*-18:1 isomers at the duodenum. The proportions of *cis*7-, *cis*8-, *cis*10-, and *cis*13- through *cis*16-18:1 each were less than 1% of total fatty acids in feed, but each averaged 4% of total *cis*-18:1 in duodenal digesta.

Trans-18:1 isomers were not detected in the diet. However, duodenal flow averaged 13 g/d compared with 5 g/d for *cis*-18:1 isomers when supplemental oil was fed. The concentration of *trans*11-18:1 in duodenal chime represented 57% of total

trans-18:1 isomers. *Trans*7-, *trans*8-, *trans*9-, and *trans*10-18:1 averaged 1, 1, 2, and 4% of total *trans*-18:1. Whereas, *trans*12-, *trans*13-, *trans*14-, *trans*15-, and *trans*16-18:1 accounted for 6, 6, 8, 7, and 9% of total *trans* isomers of 18:1. The flow of 18:0 averaged 56 g/d.

More recent studies with sheep and lactating cows have quantified total *cis*-18:1 and *trans*-18:1 isomer flows in response to various supplemental oils, oilseeds, and ratio of forage to concentrate in the diet. In lactating cows fed diets supplemented with 0, 6, or 12% of DM with rapeseeds (50% *cis*9-18:1), the flow of *cis*-18:1 isomers to the duodenum increased from 48 g/d for controls to 118 and 142 g/d due to supplemental oilseeds (Murphy et al., 1987). Duodenal flow of stearic acid also increased from 253 g/d for controls to 504 and 740 g/d when diets containing 6 or 12% rapeseeds were fed. The greater flow of 18:0, was directly proportional to the linear increase in hydrogenation of supplemental *cis*9-18:1 (Murphy et al., 1987). Similar responses were observed when high-oleic sunflower oil (78% *cis*9-18:1) was supplemented at 3.7% of DM compared with a basal diet (Kalscheur et al., 1997a). Total *trans*-18:1 flow also increased by 223 g/d due to feeding high-oleic oil. Results suggest that supplemental *cis*9-18:1 is hydrogenated to 18:0 primarily, but might also be isomerized to *trans*-18:1 isomers.

In cows fed (3% of DM) a blend of soybean oil (54% 18:2n6) and hydrogenated soybean oil (38% *trans*-18:1) in equal proportions, *cis*-18:1 flow to the duodenum was not different compared with a basal diet, but total *trans*-18:1 flow was 115 g/d greater (Wonsil et al., 1994). Stearic acid flow was 148 g/d greater due to feeding the oil blend compared with controls. Despite the availability of *trans*-18:1 in the diet, hydrogenation of *cis*9-18:1 and 18:2n6 increased from 62 and 69% for controls to 82 and 79% when the oil blend was fed. Supplemental (3.7% of DM) high-linoleic sunflower oil (65% 18:2n6) also increased duodenal flow of *trans*-18:1 isomers and 18:0 flow, without affecting total *cis*-18:1 flow (Kalcheur et al., 1997a). Greater hydrogenation was responsible for the enhanced flow of *trans*-18:1 and 18:0 due to oil supplementation.

In the above studies, it was apparent that *trans*-18:1 and 18:0 flow to the duodenum was proportional to the extent of hydrogenation of *cis*9-18:1 and 18:2n6, as their level of supplementation increased. Linolenic acid hydrogenation averaged 85%

regardless of diet across all experiments (Murphy et al., 1987; Wonsil et al., 1994; Kalscheur et al., 1997a). Inclusion of whole linseeds in the diet of sheep, however, increased the flow of *trans*-18:1 isomers and 18:0 by 6 and 16 g/d compared with controls (Wachira et al., 2000). Hydrogenation of supplemental 18:3n3 was 92% compared with 80% for basal 18:3n3.

Effect of Amount of Concentrate

The amount of dietary concentrate and buffer in diets of lactating cows also affects the outflow of *cis*-18:1, *trans*-18:1, and 18:0 from the rumen. When a low-forage/high-concentrate (25:75) diet without buffer was fed, the flow of *cis*-18:1, *trans*-18:1, and 18:0 was 67, 97, and 22% higher compared with feeding a high-forage/low-concentrate (60:40) diet (Kalscheur et al., 1997b). Hydrogenation of 18:2n6 and 18:3n3 also were lower when the low-forage diet was fed. Addition of buffer (MgO plus $NaHCO_3$) to the low-forage diet increased ruminal pH and probably altered the growth rate and the profiles of ruminal bacteria (Kalscheur et al., 1997b). As a result, hydrogenation of 18:2n6 and 18:3n3 and *trans*-18:1 flow to the duodenum in cows fed the low-forage diet plus buffer was comparable with that from cows fed the high-fiber diet. These results confirmed early evidence (Tove and Matrone, 1962) indicating that high-concentrate diets inhibit the growth of bacteria which carry out the reduction of *trans*-18:1 to 18:0 in the rumen.

Taken together, results are consistent with original *in vitro* observations demonstrating that hydrogenation of unsaturated fatty acids in the rumen leads to accumulation of *trans*-18:1 isomers. Despite the paucity of data, it is likely that *trans*11-18:1 is the major isomer flowing into the small intestine when supplemental 18:2n6 and 18:3n3 are fed. Early studies with pure cultures of *B. fibrisolvens* showed that it could only hydrogenate 18:2n6 to *trans*-18:1, but not to 18:0 (Polan et al., 1964). Because rumen outflow of 18:0 is greater due to oil supplementation, it is evident that there are other groups of hydrogenating bacteria capable of completing this final step of the pathway.

Intestinal Absorption of Fatty Acids

Overview

Due to extensive lipolysis and hydrogenation in the rumen, lipids in post-ruminal digesta are mainly saturated non-esterified fatty acids (from dietary and microbial origin; 70%). Small variable amounts of microbial phospholipids (10 to 20%) are also present, absorbed on particulate matter (Leat and Harrison, 1975). In the acidic conditions of the abomasum and duodenum (pH 2.0 to 2.5), non-esterified fatty acids are fully protonated which enhances absorption onto the surface of particulate matter (Leat and Harrison, 1975).

Lipid digestion occurs in a biphasic medium that consists of an insoluble particulate phase to which non-esterified fatty acids and phospholipids are attached, and a soluble micellar phase containing dissolved fatty acids. Transfer of non-esterified fatty acids to the micellar phase occurs gradually, as digesta goes through the intestinal tract; 5% of the total transfer occurs in the duodenum, 20% in the upper jejunum, 25% in the mid and lower jejunum, and 50% in the ileum (Leat and Harrison, 1975). Bile secretion in the duodenum favors the interaction of fatty acids with bile phospholipids and water, leading to the formation of a liquid crystalline phase. With increasing pH, this phase is dispersed in the presence of bile salts to form the micellar solution.

With conventional diets, 15 to 25% of total fatty acids are readily absorbed in the upper jejunum (pH 2.8 to 4.2); whereas, 55 to 65% of digesta fatty acids are absorbed in the middle and the lower jejunum (pH 4.2 to 7.6). Pancreatic lipase and colipase systems convert triglycerides into free fatty acids and 2-monoacylglycerols, which constitute an important factor in the micellar solubilization of free fatty acids (Moore and Christie, 1984). Under these dietary conditions, and because of the optimal pH (pH 7.5) for lipase activity, triglyceride hydrolysis and fatty acid absorption do not take place prior to the mid jejunum (Leat and Harrison, 1975).

Digestibility of Fatty Acids

Generally, the intestinal absorption coefficient of individual fatty acids ranges from 80% (for saturated fatty acids) to 92% (for polyunsaturated fatty acids) in conventional diets with a fat content of 2 to 3% of DM (Bauchart, 1993; Doreau and

Ferlay, 1994). Moore and Christie (1984) suggested that the ability of the ruminant animal to absorb fatty acids with 16 and 18 carbon chains might be due to the greater degree of dispersion of long chain fatty acids in intestinal contents and also to the greater solubilization of these unesterified fatty acids by bile-salt/lysophosphatidylcholine micelles.

Increasing dietary lipid generally results in higher apparent intestinal fatty acid digestibility because of dilution of bile fatty acids and bacterial fatty acid production in the large intestine. However, fatty acid digestibility in dairy cows seems to decrease progressively at higher intakes of supplemental fat. Palmquist (1991) found that true digestibility of fatty acids decreased from 95 to 78% when Ca-salts were supplemented at 500 g/d (2% of DM) and 1,000 g/d (6% of DM) to dairy cows. These decreases suggested limited secretion and activity of pancreatic lipase and biliary lipids (bile salts), which may affect lipid absorption in ruminants at high fat intakes.

Klusmeyer and Clark (1991) reported that apparent digestibility of fatty acids with 12 to 16 and 18 (unsaturated) carbons as a percentage of flow to the duodenum was increased, whereas apparent digestibility of 18:0 was decreased by feeding diets that contained Ca-salts at 4% of the DM. However, the average apparent total digestibility of fatty acids flowing to the duodenum was 78% and was not altered by feeding Ca-salts. Steele and Moore (1968) observed a lower net digestibility for 18:0 (53%) than for 16:0 (82%) and 14:0 (100%) when sheep were fed myristic, palmitic, and stearic acids and suggested that saturated fatty acids with a higher melting point would decrease formation of micelles in the small intestine for absorption.

Fatty Acid Transport in Blood Plasma

Lymphatic Lipid Composition

In ruminants, lipid absorption and lymphatic transport to plasma are essentially continuous processes that are subject to increases when large amounts of dietary lipids are supplemented. As previously discussed, unprotected dietary lipids are subject to biohydrogenation in the rumen, yielding large amounts of *trans*-18:1 and saturated fatty acids flowing to the small intestine.

In intestinal lymph, 80% of the total lipid is associated with the very low-density lipoprotein (VLDL) fraction. The concentration of lipid in the low-density lipoprotein (LDL) and high-density lipoproteins (HDL) averages 11 and 5% (Noble et al., 1984). Among lipid fractions, triglycerides account for 71% of the VLDL fraction, with phospholipids (12%), cholesterol esters (9%), and free fatty acids (7%) making up the remainder of the VLDL. As indicated by positive arterio-venous differences, the lactating mammary gland utilizes fatty acids derived from triglycerides in VLDL of intestinal origin to a major extent (Uchide et al., 1999).

There is an appreciable selectivity in the incorporation of different fatty acids into the various lipid classes synthesized in the mucosal cells of the ruminant small intestine, and this selectivity is reflected in the fatty acid composition of the lymph lipids. On average, triglycerides of VLDL contain 16:0 (26%), 18:0 (22%), and 18:1 isomers (29%) primarily (Noble et al., 1984). Phosphatidylcholine (70% of total phospholipids) contains primarily 18:2n6 (35%), and 16:0, 18:0, or 18:1 isomers each accounts for 18% of total fatty acids. Stearic acid, 18:1 isomers, and 18:2n6 account for 23, 35, and 15% of total fatty acids in cholesterol esters.

The amount of *cis*-18:1, *trans*-18:1, and 18:0 flowing through the thoracic lymph of goats fed a diet supplemented with 5% soybean oil averaged 13, 5, and 25 g/d (Bickerstaffe et al., 1972). The higher flow of *cis*-18:1 compared with *trans*-18:1 was ascribed to partial desaturation of 18:0 in the enterocyte. Among individual isomers, *cis*9-18:1 accounted for 86% of total *cis*18:1 isomers. *Cis*6- through *cis*9-, and *cis*10- through *cis*16-18:1 each averaged 1 to 2% of total *cis*18:1 flowing through the thoracic lymph. *Trans*11-18:1 accounted for 61% of total *trans*18:1 flowing through lymph. The proportions of *trans*6- through *trans*10-, and *trans*12- through *trans*16- averaged 1,1, 8, 3, 7, 4, 6, 4, and 5% of total *trans*-18:1 isomers. Based on these data, *cis* and *trans*-18:1 isomers produced during hydrogenation of dietary lipid would be readily available for incorporation into triglycerides and synthesis of VLDL in the enterocyte.

Effects of Diet on Plasma Fatty Acids

Moore et al. (1968) found that feeding dried grass (57% 18:3n3) to sheep increased 18:3n3 concentration as a percentage of total fatty acids in plasma

cholesterol esters and phospholipids. Feeding hay (18% 18:3n3) induced a more modest response. Noble et al. (1969) infused 40 g of maize oil triglycerides (64.4% 18:2n6, and 23% *cis*9-18:1) into the rumen of sheep for one hour and detected an increase in the proportion of 18:2n6 and *cis*9-18:1 in the triglyceride fraction from 3.9 and 30.4% at 0 h to 15.1 and 35.7% at 6 h post infusion, respectively. Similarly, the proportion of 18:2n6 in the free fatty acid fraction at 6 h (11%) was increased from that at 0 h (2.5%). Concentration of 18:2n6 in the cholesterol ester and phospholipid fractions were 22.4 and 11.9% at 0 h, however, it did not attain peak values until after 24 h post infusion (29.1% and 25.8%). In the triglyceride fraction, a linear increase in 18:0 concentration from 0 h (28.2%) to 24 h (45%) also was observed.

La Count et al. (1994) infused free long chain fatty acids from canola (62.5% *cis*9-18:1) or high-oleic sunflower (86% *cis*9-18:1) for 3 d at 0, 133, 267, 400, 267, 133, and 0 g/d for a total of 21 d and reported increased *cis*9-18:1 and decreased 16:0 and 18:0 in plasma triglycerides. Abomasal infusions of 40 g linseed oil (21.3% 18:2n6 and 71.1% 18:3n3) and maize oil (22.9% *cis*9-18:1 and 64.4% 18:2n6) in sheep for 1 h resulted in rapid incorporation of 18:3n3 and 18:2n6 into plasma triglycerides (Moore et al., 1969). Concentration of 18:2n6 and 18:3n3 began to increase at 1.5 h (5% and 4.1%) and peaked at 4 h (12.4% and 34%), but remained elevated up to 10 h after infusion of linseed oil stopped. Similarly, infusion of maize oil caused 18:2n6 to increase in the triglyceride fraction from 5.6% at 1 h to 41.4% at 6 h. Concentrations of 16:0 and 18:0 varied inversely with concentrations of 18:2n6 and 18:3 while *cis*9-18:1 remained unchanged.

Feeding supplemental unsaturated oils to lactating cows also affected the fatty acid profiles in all major blood plasma lipid fractions. Lactating Jersey cows fed (3.5% of DM) supplemental canola (55% *cis*9-18:1), soybean (62% 18:2n6), or an equal mixture of both had greater concentrations of total fatty acids in plasma phospholipids, cholesterol esters, and triglycerides compared with controls (Loor et al., 1998). Oleic acid increased from 153 to 195, 100 to 151, 35 to 53, and 103 to 161 mg/g of total fatty acids in the free fatty acid, phospholipid, cholesterol ester, and triglyceride fractions, respectively, when canola oil-fed cows were compared with controls. In contrast, soybean oil intake increased linoleic acid in the free fatty acid, phospholipid, cholesterol

ester, and triglyceride fractions from 37 to 55, 327 to 366, 684 to 744, and 42 to 72 mg/g, respectively. Feeding soybean oil also increased *trans*11-18:1 from 12 to 105 mg/g in the phospholipid and triglyceride fractions, and *cis*9,*trans*11-18:2 from 0.3 to 21 mg/g in phospholipid and free fatty acid fractions.

Fatty Acid Extraction and Uptake by the Mammary Gland

Effects of Supplemental Fat

Fatty acids could influence lipoprotein lipase activity by altering lipase secretion from adipocytes, by decreasing lipase attachment to heparin sulfate proteoglycans, and by end-product inhibition of the enzyme (Neville and Picciano, 1997). It was postulated that fatty acids could regulate mammary gland lipoprotein lipase by reducing its activity during fasting and involution (Neville and Picciano, 1997). During established lactation in the cow, however, it was shown that lipoprotein lipase was not rate-limiting for uptake of plasma triglycerides at high arterial concentrations (Chilliard et al., 1986).

Prior to uptake by the mammary gland, plasma triglycerides in VLDL and chylomicrons are extensively hydrolyzed by capillary lipoprotein lipase (LPL) (West et al., 1972). Arterio-venous differences of non-esterified fatty acids, triglycerides, or the combination of both (TGFA) have shown that uptake of fatty acids by the mammary gland is largely dependent on arterial concentration (Miller et al., 1991a,b; Cant et al., 1993; Enjalbert et al., 1998). In turn, arterial concentration of fatty acids is increased due to supplemental fat. Concentrations of triglycerides and non-esterified fatty acids increased linearly with each level of Ca-salts supplementation from 3 to 9% of DM (Choi and Palmquist, 1996). Plasma triglycerides also were higher due to oil supplementation (3.5% of DM) compared with a basal diet (Loor et al., 1998). Higher arterial concentrations of triglycerides and free fatty acids in blood plasma could affect extraction (%) and uptake of fatty acids by the mammary gland. Using duodenal infusions of 16:0 and 18:0, to by-pass ruminal metabolism, it was shown that extraction and uptake of 16:0 and 18:0 from TGFA increased compared with control infusions of water (Enjalbert et al., 1998). Arterio-venous differences of TGFA were proportional to arterial concentration. Despite no changes in extraction, mammary uptake of *cis*9-18:1

was greater due to infusion of *cis*9-18:1. The extraction ratio for *trans*18:1 isomers averaged 53% and was not affected by treatments.

Effects on Milk Fat Yield and Fatty Acid Profiles

Few studies have combined estimates of plasma arterio-venous differences, fatty acid extraction, or fatty acid uptake by the mammary gland with the output of milk fat or fatty acid profiles. Annison et al. (1967) infused goats intravenously with ^{14}C-16:0, ^{14}C-*cis*9-18:1, or ^{14}C-18:0 and determined from arterio-venous differences that they were taken up by the mammary gland in amounts equivalent to 63-82% of the output of milk triglycerides. Work by West et al. (1972) confirmed that there was substantial transfer of radioactivity from radiolabeled (free acid or triglyceride) 16:0, *cis*9-18:1, or 18:0 into milk fat, after intravenous infusion of the acids. Based on the amount of radioactivity in milk fat after jugular infusion of radiolabeled *trans*9-18:1, it was also shown that *trans*18:1 isomers could be taken up by the mammary gland of goats fed supplemental soybean oil (Bickerstaffe et al., 1972). Extraction rate for *cis*-18:1 and *trans*-18:1 isomers from plasma triglycerides averaged 69%. *Trans*11-18:1 in arterial plasma triglycerides and free fatty acids averaged 58%, but was 86% of total *trans*18:1 isomers in milk fat. For most *trans*-18:1 isomers, however, their proportions in milk fat were a reflection of the proportions in blood plasma triglycerides and free fatty acids.

Feeding supplemental fat (2.5 or 4% of DM) to dairy cows increased uptake of triglycerides and non-esterified fatty acids by the mammary gland compared with controls (Wonsil, 1993; Cant et al., 1993). This increase was accompanied by greater concentrations and outputs of *cis*9-18:1 and 18:0, but lower concentrations and outputs of saturated 6:0 to 16:0 (Wonsil, 1993; Cant et al., 1993). Daily milk fat yield, however, was not affected by supplemental fat. The potential for greater milk fat output when mammary uptake of fatty acids is increased could be related to the fatty acid profile of the supplemental fat. Duodenal infusion of 16:0 resulted in greater extraction and uptake of 16:0, but also increased milk fatty acid output by 300 g/d compared with the control infusion (Enjalbert et al., 1998; Enjalbert et al., 2000). In contrast, infusion of *cis*9-18:1 increased its uptake by the mammary gland but did not improve milk fatty acid yield compared with the control. Yield of palmitic acid increased by an average of 270

g/d due to infusion of 16:0 compared with the control or *cis*9-18:1 infusions, and accounted for the overall increase in total fatty acid yields. Greater uptake of *cis*9-18:1 reduced 16:0 yield by 32 or 50% compared with control or 16:0 infusions. The substantial reduction in 16:0 due to greater uptake of *cis*9-18:1, suggests *cis*9-18:1 had a detrimental effect on *de novo* synthesis in the mammary gland.

Lipid Metabolism in the Mammary Gland

Overview

The extent to which dietary fat will alter milk fat and milk fat composition depends on the effects of the fat on ruminal biohydrogenation, microbial fatty acid synthesis, adipose tissue fatty acid release, and *de novo* synthesis of fatty acids in the mammary gland. In turn, the physical form, fatty acid composition, and amount of dietary fat could affect these. As shown with 16:0, saturated fats tend to increase milk fat concentration and yield (Sutton, 1989). It is generally believed that during biohydrogenation of 18:2n6 and 18:3n3, an intermediate(s) is produced that affects *de novo* synthesis in the mammary gland.

The first evidence that *trans*18:1 were involved in the low-fat milk syndrome was presented nearly 40 years ago (Moore and Williams, 1963). More than 50% of the total 18:1 in milk fat produced on a low-fiber diet supplemented with cottonseed oil were *trans*-18:1. Dietary *trans*-18:1 also can reduce milk fat concentration and yield (Wonsil et al., 1994). The reduction is caused by a decrease in the concentration of saturated fatty acids synthesized endogenously in the mammary gland. Evidence that other intermediates of biohydrogenation could potentially decrease milk fat synthesis was obtained when a conjugated linoleic acid mixture (CLA) reduced *de novo* synthesis of 16:0 and desaturation of 18:0 in mammary cell cultures (Dawson and Herbein, 1996).

Several studies have confirmed that specific isomers of *trans*-18:1 and CLA inhibit the amount and activity of lipogenic enzymes in the mammary gland of ruminants and rodents. Thus, metabolism of unsaturated fatty acids in the rumen is directly linked with lipogenesis in mammary tissue.

De Novo Fatty Acid Synthesis

Endogenous synthesis of saturated 6:0 to 16:0 in the mammary gland occurs in the cytosol, and involves two major steps. The initial step is the ATP- and bicarbonate-dependent carboxylation of acetyl-CoA to form malonyl-CoA, catalyzed by acetyl-CoA carboxylase (ACC). The second step is the conversion of acetyl-CoA and malonyl-CoA to palmitate, catlyzed by the fatty acid synthase (FAS) complex. Fatty acid synthase uses NADPH as the reducing equivalent to synthesize palmitate.

The reaction catalyzed by ACC takes place in two steps: (1) carboxylation of biotin and (2) transfer of the carboxyl to acetyl-CoA to form malonyl-CoA. ACC is a multienzyme protein, containing a variable number of identical subunits, each containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and transcarboxylase, as well as a regulatory allosteric site.

Mammalian FAS is a multi-enzyme complex that may not be subdivided without loss of activity, and the acyl carrier protein is part of this complex. The aggregation of all the enzymes of FAS into one multienzyme functional unit offers great efficiency and freedom from interference by competing reactions. Another advantage of a single multienzyme polypeptide is that synthesis of all enzymes in the complex is coordinated, since a single gene encodes it.

Palmitic acid is synthesized in seven sequential condensation reactions with intermediates attached to the acyl carrier protein. During each cycle, a new malonyl residue is incorporated until palmitate has been assembled. In the liver and adipose tissue, the final product of de novo synthesis is palmitate, liberated from the enzyme complex by thioesterase-1. The cytosol of mammary cells, however, contains a separate medium-chain acyl-thioester hydrolase, thioesterase-2. Thioesterase-2 terminates fatty acid synthesis after growth to 8 to 14 carbons, resulting in de novo synthesis of medium-chain fatty acids (Neville and Picciano, 1997). In the ruminant mammary gland, this enzyme is part of the FAS complex. Bovine milk fat contains short-chain fatty acids. Butyryl-CoA and hexanoyl-CoA are synthesized by mammary gland FAS via the malonyl-CoA pathway. Butyryl-CoA also originates from the conversion of β-hydroxybutyrate (produced during fermentation in the rumen) taken up by the lactating mammary gland.

Greater availability of long-chain fatty acids from the diet or adipose tissue causes a decrease in the percentage of saturated 6:0 to 16:0 in milk fat (Chilliard et al., 2000). A large portion of this reduction occurs as a result of greater uptake and secretion of long-chain fatty acids from the blood (Clapperton and Banks, 1985). Exogenous fatty acids compete for esterification with newly synthesized short-chain fatty acids in mammary cells, and the greater accumulation of short-chain fatty acids leads to feedback inhibition of lipogenic enzymes (Palmquist et al., 1993).

Trans-18:1 isomers, however, target ACC and FAS directly. Greater flow of total *trans*-18:1 isomers to the duodenum was negatively correlated with milk fat percentage and *de novo* fatty acid synthesis (Wonsil et al., 1994; Kalscheur et al., 1997b). However, an increase in the proportion of the *trans*10-18:1, not *trans*11-18:1, isomer in milk fat was correlated with the decreases observed (Griinari et al., 1998; Piperova et al., 2000). Lower activity and mRNA abundance for ACC and FAS were responsible for the reduction in lipid synthesis in the mammary gland due to greater concentrations of *trans*10-18:1 (Piperova et al., 2000).

Conjugated linoleic acid (CLA) isomers also appear to affect lipogenesis in the mammary gland. Abomasal infusion of a CLA mixture (*cis*9,*trans*11-18:2 plus *trans*10,*cis*12-18:2) reduced milk fat percentage and *de novo* fatty acids in milk fat (Loor and Herbein, 1998; Chouinard et al., 1999a). It was confirmed, however, that *trans*10,*cis*12-18:2 was the isomer responsible for the decrease (Baumgard et al., 2000b). This isomer is not a typical intermediate of 18:2n6 hydrogenation, but it might accumulate in the rumen under dietary conditions that reduce rumen pH and change bacterial populations (Piperova et al., 2000). Lactic acid (Kemp et al., 1975) and propionic acid (Verhulst et al., 1987) producing bacteria (Table 2) could produce *trans*10-18:1 and *trans*10,*cis*12-18:2 from hydrogenation of 18:2n6, and their numbers in the rumen increase due to low pH induced by feeding high-concentrate diets (Latham et al., 1972).

Desaturation of Long-Chain Fatty Acids

D^{9} *Desaturase*

Desaturation of fatty acids involves the enzymatic removal of hydrogen from a methylene group in an acyl chain, an energy-requiring step that requires an activated oxygen intermediate. Free fatty acids must be esterified to coenzyme A prior to desaturation via stearoyl-CoA desaturase.

Delta-9 desaturase is an iron-containing microsomal protein that catalyzes the critical committed step in the biosynthesis of monounsaturated fatty acids by introducing the first *cis* double bond in the Δ^9 position of the carbon chain. The desaturase system involves three enzyme components: cytochrome b_5, NADH-cytochrome b_5 reductase, and a desaturase. Only the terminal desaturase's activity is sensitive to changes in diet, hormonal balance, developmental processes, temperature changes, metals, alcohol, peroxisomal proliferators, and phenolic compounds (Tocher et al., 1998). The two electrons needed are transported through an electron-transport system that is composed of cytochrome b_5 and NADH-dependent cytocrhome b_5 reductase.

Delta-9 desaturase catalyzes the desaturation of a number of fatty acids. However, the preferred substrates (after binding to coenzyme A) are 16:0 and 18:0, which are converted to *cis*9-16:1 and *cis*9-18:1. Membrane fluidity is directly affected by the balance between 18:0 and *cis*9-18:1, and the changes in this ratio have been implicated in disease states including diabetes, obesity, hypertension, cancer, and neurological, vascular, and heart disease (Tocher et al., 1998). In bovine milk fat, a decrease in the ratios for 16:0 to *cis*9-16:1 and 18:0 to *cis*9-18:1 also have been used to interpret potential changes in desaturase activity due to dietary fatty acids (Griinari et al., 2000).

Some *trans*18:1 isomers can be substrates for Δ^9 desaturase. Desaturation of *trans*11-18:1 to *cis*9,*trans*11-18:2 was initially reported with liver homogenates from rats (Pollard et al., 1980). Infusion of *trans*11-18:1 into the abomasum of lactating cows also resulted in greater concentrations of *cis*9,*trans*11-18:2 in milk fat (Griinari et al., 2000). It was estimated than >50% of milk fat *cis*9,*trans*11-18:2 was desaturated endogenously from *trans*11-18:1 in the mammary gland. Because *trans*11-18:1 is the primary isomer arising during hydrogenation of 18:2n6 and 18:3n3, it has been postulated that its

desaturation in the mammary gland is the primary source of milk fat *cis*9,*trans*11-18:2 (Griinari et al., 2000). In growing mice, 11% of dietary *trans*11-18:1 was desaturated to *cis*9,*trans*11-18:2 (Santora et al., 2000). In lactating mice, dietary *trans*11-18:1 increased the *cis*9,*trans*11-18:2 content in blood plasma lipids, liver, carcass, mammary gland, and milk fat (Loor et al., 1999; Loor et al., 2000). The increase in *cis*9,*trans*11-18:2 in mammary gland and milk fat due to feeding *trans*11-18:1 to lactating mice, was associated with greater Δ^9 desaturase (Loor et al., 1999) and mRNA abundance (Lin et al., 2000).

Expression of the Δ^9 desaturase gene and activity of the enzyme are markedly reduced by *trans*10,*cis*12-18:2, not *cis*9,*trans*11-18:2, in rodent adipose, liver, or mammary gland tissue (Choi et al., 2000; Park et al., 2000; Lin et al., 2000). In contrast *trans*11-18:1 or *trans*10-18:1 did not affect Δ^9 desaturase activity in liver tissue (Park et al., 2000). Initial studies evaluating the involvement of CLA isomers in milk fat depression in dairy cows, indicated that 18:0 concentration in milk fat was substantially increased by abomasal infusion of CLA mixtures (Loor and Herbein, 1998; Chouinard et al., 1999a). Recent data indicated that, in addition to reducing milk fat percentage, *trans*10,*cis*12-18:2 also led to a substantial increase in 18:0 concentration (Baumgard et al., 2000b). The negative effect on desaturation in bovine mammary gland by *trans*10,*cis*12-18:2 might also be mediated by reductions in the transcription of the Δ^9 desaturase gene, which were proportional to concentration of the CLA *in vitro* (Lin, 2000).

D*6 and* D*5 Desaturase*

Activity of the Δ^6 desaturase has an important role in animals because it catalyzes the rate limiting step in the desaturation and elongation of the essential fatty acids 18:2n6 and 18:3n3 to 20:4n6 (arachidonic acid) and 22:6n3 (docosahexaenoic acid) (Tocher et al., 1998). Delta-5 desaturase activity is coupled with that of Δ^6 desaturase, because it catalyzes the desaturation of 20:4n3 and 20:3n6 to 20:5n3 (eicosapentaenoic acid) and 20:4n6.

Delta-6 desaturase is an iron-containing microsomal protein. The desaturase system involves three enzyme components: cytochrome b_5, NADH-cytochrome b_5

reductase, and a desaturase. The two electrons needed are transported through an electron-transport system that is composed of cytochrome b_5 and NADH-dependent cytochrome b_5 reductase. Activity of the desaturase is sensitive to changes in dietary protein and 18:2n6 availability. Despite similarities with Δ^9 desaturase, they are different enzyme proteins (Tocher et al., 1998).

Delta-5 desaturase also is an iron-containing protein. The desaturase system also involves three enzyme components: cytochrome b_5, NADH-cytochrome b_5 reductase, and a desaturase. Activity of the desaturase is sensitive to changes in dietary protein, 18:2n6, and 18:3n3 availability.

Data regarding the activity of delta-6 or delta-5 desaturase in the bovine mammary gland is lacking. However, using abomasal infusions to by-pass hydrogenation, it was shown that the concentrations of 20:4n6, 20:5n3, and 22:6n3 in milk fat increased in proportion to the amount of 18:2n6 or 18:3n3 available for uptake (Hagemeister et al., 1991; Hermansen, 1995; Loor and Herbein, 1998).

Conjugated linoleic acid (CLA) isomers have shown the potential for decreasing the activity of Δ^6 and(or) Δ^5 desaturase in the mammary gland of cows. It was demonstrated that 20:4n6 concentration in milk fat decreased substantially in proportion with CLA (*cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2) concentration in milk fat despite availability of exogenous 18:2n6 (Loor and Herbein, 1998). A similar response was observed in subcutaneous and omental adipose tissue of piglets reared by sows consuming a CLA mixture compared with 18:2n6 during lactation (Bee, 2000). At low concentrations (30 nmol), *cis*9,*trans*11-18:2 compared with *trans*10,*cis*12-18:2 reduced desaturation of 18:2n6 or 18:3n3 (60 nmol incubated) by decreasing the activity of Δ^6 desaturase in rat liver (Bretillion et al., 1999). It required four times the concentration of *cis*9,*trans*11-18:2 for *trans*10,*cis*12-18:2 to inhibit desaturation of 18:2n6.

Diet and Milk Fatty Acid Profiles

Pasture

The major fatty acids in pasture grasses and legumes are 18:2n6 and 18:3n3, which can account for up to 80% of total fatty acids (Hawke, 1973). Pasture fatty acid composition also varies during growth. Invariably, total fatty acid content of pasture

species was highest during the primary growth stage (spring), decreased steadily through the stemmy regrowth period, and increased sharply during the next leafy regrowth stage (fall) (Hawke, 1963; Hawke, 1973; Bauchart et al., 1984). At any stage of growth, however, grasses (~25 mg/g DM) contained more fatty acids than clovers (~19 mg/g DM) (Hawke, 1973). Concentrations of saturated fatty acids with 14 to 18-carbons increased, but 18:3n3 concentration decreased during growth. However, 18:2n6 concentration did not change appreciably.

The primary changes in the profiles of fatty acids due to grazing is the significant increase in concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat. Total *trans*-18:1 concentration in milk fat increased from 26.5 mg/g to 51 mg/g when cows fed a TMR were turned out to pasture (Precht and Molkentin, 1997). The concentration of *trans*11-18:1 increased from 9 to 29 mg/g when cows were fed the TMR or grazed mixed pastures. The concentrations of *trans* isomers with double bonds at positions 4 through 16 were not affected by pasture.

Concentrations of *cis*9,*trans*11-18:2 increased from 7 mg/g due to feeding a TMR to 14 mg/g when cows grazed (Precht and Molkentin, 2000). In addition, the concentration of *trans*11,*cis*15-18:2 was higher during grazing (5 mg/g) compared with feeding a TMR (2 mg/g). The distribution of grasses and legumes in pastures also affected the amounts of *cis*9,*trans*11-18:2 in milk fat. When cows grazed mixed pastures containing red clover, concentration of *cis*9,*trans*11-18:2 was 14 mg/g compared with 9 mg/g in milk fat from cows grazing pastures composed solely of grasses (Wu et al., 1998).

When compared with cows fed a TMR, grazing nearly doubled *cis*9,*trans*11-18:2 concentration in milk fat (Kelly et al., 1998b). In addition, 18:3n3 concentration also was 3-fold higher due to pasture compared with the TMR. The concentrations of saturated 6:0 to 16:0, however, decreased in response to grazing compared with feeding the TMR. The importance of pasture in enhancing the levels of *cis*9,*trans*11-18:2 was tested by offering grazing cows a grain supplement at two levels (Dhiman et al., 1999a). Cows consuming their entire daily intake from pasture had greater levels (22 mg/g) compared with cows consuming 12 (9 mg/g) or 6 (14 mg/g) kg/d of a grain

supplement. Concentrations of 18:3n3 also were higher for grazing cows compared with grain supplementation.

Oilseed supplementation to grazing cows also affected the profiles of fatty acids in milk fat. Grazing cows fed (3 kg/d) full-fat rapeseeds (61% of total fatty acids = *cis*9-18:1 and 19% = 18:2n6) had greater concentrations (22 mg/g total fatty acids) of *cis*9,*trans*11-18:2 compared with cows fed full-fat soybeans (17 mg/g) or controls (13 mg/g) (Lawless et al., 1998). The concentrations of saturated 6:0 to 16:0, however, were lower in milk fat from cows supplemented with both fat sources compared with controls.

Due to changes in pasture fatty acid profiles, seasonal variation in the concentrations of milk fatty acids also might be expected. These responses were evaluated from a large database (3,500 individual cows) in pastoral systems in New Zealand. Concentrations of *cis*9,*trans*11-18:2 averaged 12 mg/g during grazing in the spring, decreased to 9 mg/g in the summer, then increased to 13 mg/g during the autumn (Thomson and Van Der Poel, 2000). Oleic acid concentration followed a similar pattern and was 230 mg/g in the spring, decreased to 195 mg/g, then increased to 228 mg/g. In contrast, 18:0 concentration was 100 mg/g in the spring, increased to 130 mg/g during the summer, and decreased to 110 mg/g during the autumn. Concentrations of 4:0 to 16:0 did not change appreciably during the entire grazing season.

In milk fat from grazing cows consuming grass silage ad libitum, the concentration of *cis*9,*trans*11-18:2 averaged 15 mg/g during grazing of spring pastures (51% 18:3n3) compared with 8 mg/g when autumn pastures (41% 18:3n3) were grazed (Lawless et al., 1999). Concentrations of 18:3n3 also were higher during spring grazing compared with autumn. Results clearly indicate that the concentration of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat are substantially higher in response to pasture, and could vary due 18:3n3 content during the grazing season. Concentrations of *trans*11,*cis*15-18:2 also were proportional to 18:3n3 intake from pasture.

Supplemental Fat

The changes in milk fatty acid composition induced by feeding dietary fats have been typical. Specifically, dietary long-chain fatty acids (mainly unsaturated) have resulted in decreased proportions of short and medium chain fatty acids and increased proportions of unsaturated fatty acids. However, renewed interest in evaluating changes in *trans*-18:1 and CLA concentrations due to oil supplementation has resulted in a large body of literature within the past five years.

To evaluate changes in the profiles of *trans*-18:1, *cis*9,*trans*11-18:2, and fatty acids synthesized *de novo* in milk fat, oils containing different concentrations of *cis*9-18:1, 18:2n6, 18:3n3, 20:5n3, or 22:6n6 have been fed. A major effect of increasing unsaturated oil supplementation has been the marked decrease in the concentration of saturated fatty acids with 6 to 16 carbons.

As mentioned earlier, reduced concentrations of *de novo*-synthesized fatty acids occur partly because greater uptake of dietary fatty acids compete with endogenously-synthesized short- and medium- chain fatty acids for incorporation into milk fat triglycerides (Palmquist et al., 1993; Chilliard et al., 2000). This causes accumulation of short-chain fatty acids and feedback inhibition of lipogenic enzymes (Clapperton and Banks, 1985). Results from a recent study indicated that dietary *cis*9-18:1 was preferentially incorporated into the sn-2 position of the milk triglyceride at the expense of 16:0 (DePeters et al., 2001). The net result was lower concentrations of 16:0 but higher *cis*9-18:1 in milk fat.

High-oleic (75% *cis*9-18:1) sunflower oil supplementation to lactating cow diets increased *cis*9-18:1 in milk fat from 224 mg/g when the control diet was fed to 290 mg/g when the high-oleic oil was fed (Kalscheur et al., 1997a). In addition, the concentration of *trans*-18:1 was greater when the oil (118 mg/g) was fed compared with the basal diet (29 mg/g). Peanut oil (52% *cis*9-18:1) supplementation also increased (387 mg/g) *cis*9-18:1 in milk fat compared with feeding linseed oil (55% 18:3n3) (359 mg/g) (Kelly et al., 1998a). *Cis*9,*trans*11-18:2, however, was 13 mg/g in response to peanut oil compared with 17 mg/g when linseed oil was fed (Kelly et al., 1998a).

Stepwise supplementation (0.5 to 4% of DM) of soybean oil (50% 18:2n6) increased concentrations of 18:2n6 from 22 mg/g when the basal diet was fed to 35

mg/g at the highest level of oil addition (Dhiman et al., 2000). Concentrations of *cis*9,*trans*11-18:2 was 5 mg/g for the control diet compared with 20 mg/g for the highest level of oil supplementation. Feeding sunflower oil (65% 18:2n6) also increased 18:2n6 (45 mg/g) and *trans*-18:1 (110 mg/g) concentrations in milk fat compared with a basal diet (38 and 29 mg/g) (Kalscheur et al., 1997a).

Concentrations of *cis*9,*trans*11-18:2 when linseed oil (51% 18:3n3) was fed at 2 or 4% of DM were 20 mg/g compared with 5 mg/g for controls (Dhiman et al., 2000). When supplemented at 5.3% of DM concentration of *cis*9,*trans*11-18:2 only averaged 16 mg/g for linseed oil-supplemented diets compared with 24 mg/g due to feeding sunflower oil (Kelly et al., 1998a).

Marine oils contain higher amounts of 20:5n3 (11%) and 22:6n6 (11%), but lower amounts of *cis*9-18:1 (7%), 18:2n6 (1%), and 18:3n3 (3%) compared with vegetable oils (Donovan et al., 2000). Concentrations of *trans*18:1 and *cis*9,*trans*11-18:2, however, were increased by fish oil supplementation to dairy cows. *Trans*11-18:1 averaged 12 mg/g when the basal diet was fed, but it increased to 31, 41, 37, or 109 mg/g, respectively, when 1, 2, or 3% (DM basis) fish oil was fed. Similarly, *cis*9,*trans*11-18:2 concentration increased from 6 mg/g for controls to 16, 22, or 19 mg/g, respectively, when fish oil was fed at 1, 2, or 3%.

Results suggest that unsaturated fatty acids in vegetable or marine oils are hydrogenated in the rumen to a large extent. Oils with high 18:2n6 concentrations appear to increase *trans*-18:1 and *cis*9,*trans*11-18:2 to a greater extent compared with oils containing higher *cis*9-18:1, 18:3n3, or 20:5n3 and 22:6n6. Because fish oil contains only minor amounts of 18:2n6 or 18:3n3 and leads to significant increases in *trans*11-18:1 concentrations in milk fat, it seems that endogenous synthesis of *cis*9,*trans*11-18:2 from rumen-derived *trans*11-18:1 (Griinari et al., 2000) accounts for a large portion of the increase in CLA concentration.

Dietary Fatty Acids and Human Health

Detrimental Effects

Elevated consumption of saturated fatty acids (12:0, 14:0, and 16:0) has been implicated in a number of human diseases such as coronary heart disease (CHD), and

the main concern about excess saturated fatty acid intake is on their potential role for raising blood cholesterol (Kris-Etherton and Yu, 1997). Cholesterol is transported to tissues associated with plasma lipoproteins. Greater amounts of cholesterol deposited over time on the inner wall of arteries may result in atherosclerosis, which is regarded as a principal cause for coronary heart disease (Zyriax and Windler, 2000).

Blood cholesterol reflects the amount of three major classes of lipoproteins: very-low density lipoproteins (VLDL); low-density lipoproteins (LDL), which contains most of the cholesterol found in the blood; and high-density lipoproteins (HDL). Total serum cholesterol does not predict CHD well, since it has been shown that the HDL-cholesterol is the “good” cholesterol, being strongly and inversely related to CHD risk (Zyriax and Windler, 2000). In contrast, LDL-cholesterol seems to be the culprit and is associated with cholesterol deposits on artery walls. However, saturated fatty acids and dietary sources of dietary fatty acids vary in their effect on LDL-cholesterol levels. Butter and other dairy products (rich in 14:0) strongly increase LDL levels, beef fat (high in 16:0 and 18:0) increases LDL levels to a lesser degree, and cocoa butter (mostly 18:0) enhances LDL levels only slightly (Kris-Etherton and Yu, 1997; Zyriax and Windler, 2000). When 18:0 was provided at a level three times higher than in the basal diet, 18:0 reduced LDL-cholesterol but did not alter the ratio of LDL- to HDL-cholesterol (Kris-Etherton and Yu, 1997). It is apparent that all saturated fatty acids are not equal in their capacity to raise cholesterol, and it appears that 18:0 maybe neutral in its effect on serum LDL-cholesterol level.

Replacing monounsaturated for saturated fatty acids decreased plasma levels of total and LDL-cholesterol, while preserving HDL-cholesterol levels (Kris-Etherton and Yu, 1997). In addition, replacing saturated fatty acids (12:0 to 16:0) with *cis*-18:1 isomers and 18:2n6 was more effective in preventing the incidence of CHD compared with reducing the overall intake of fat (Kris-Etherton and Yu, 1997).

Trans fatty acids have drawn much attention due to their association with the risk of CHD. The vast majority of the *trans* fatty acids consumed by humans are produced during catalytic hydrogenation of vegetable oils, to form vegetable shortenings and margarine (Veldsink et al., 1997). They account for 3 to 6% of the daily fat intake in U.S. population (Hayakawa et al., 2000). *Trans*11-18:1 is the primary *trans*-18:1 isomer

produced during hydrogenation of dietary 18:2n6 and 18:3n3 in the rumen (Kepler and Tove, 1967). Its concentration also is significantly higher in milk or meat from ruminants. Of the *trans*18:1 isomers in partially-hydrogenated vegetable oils, *trans*9-, *trans*10-, and *trans*12-18:1 account for the majority of *trans* isomers.

Substantial data indicates that *trans*-18:1 from partially-hydrogenated vegetable oils negatively affect the risk of CHD. At isocaloric levels, a diet containing *trans*-18:1 increased LDL-cholesterol and reduced HDL-cholesterol compared with a diet containing *cis*9-18:1 (Hayakawa et al., 2000). The concentration of lipoprotein(a), which also is positively correlated with risk of CHD, also was increased by feeding the *trans* fatty acid diet.

Beneficial Effects

Evidence compiled during the last five years indicates that bovine milk fat contains a number of substances that are beneficial to human health (Parodi, 1999). Conjugated linoleic acid (CLA) has been shown to have many physiological effects. Most studies conducted to date with laboratory animals and humans have involved the use of CLA mixtures composed primarily of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 (Pariza et al., 1999). *Cis*9,*trans*11-18:2 is the primary intermediate during the hydrogenation of 18:2n6 in the rumen (Kepler and Tove, 1967). This isomer also can be synthesized endogenously by humans from *trans*11-18:1 (Adlof et al., 2000), the primary *trans*18:1 isomer produced during hydrogenation of 18:2n6 and 18:3n3 (Kepler and Tove, 1967). Dairy products contain significantly higher amounts of CLA and *trans*11-18:1, and their consumption increased concentrations of both fatty acids in blood plasma, adipose tissue, and human milk (Jiang et al., 1999; Park et al., 1999a).

Dietary CLA mixtures were shown to have anticarcinogenic effects in a large number of cancer models (Pariza et al., 1999). Feeding CLA also reduced the development of atherosclerosis in rabbits fed an atherogenic diet (Lee et al., 1994). Body fat content of mice was reduced by feeding CLA (Park et al., 1997). Prostaglandin (PGE_2) synthesis in mouse epidermis, which has been implicated in tumorigenesis, also was reduced by CLA (Kavanaugh et al., 1999). In obese-diabetic rats, feeding CLA normalized impaired glucose tolerance (Houseknecht et al.,1998).

When fed to overweight and obese subjects, feeding a CLA mixture at 3.4 or 6.8 g/d for up to 12 wk reduced body fat mass compared with placebo (Blankson et al., 2000). Using purified preparations of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2, it was shown that only the *trans*10,*cis*12-18:2 reduced carcass fat in mice (Park et al., 1999b). By inference, it could be assumed that this isomer is responsible for the observed reductions in lipogenesis when CLA mixtures are fed to humans.

In contrast, evidence indicates that *cis*9,*trans*11-18:2 is responsible for the protective effects against carcinogenesis found in early studies. Feeding butter fat containing mostly *cis*9,*trans*11-18:2 to rats (90% of total CLA) during the time of mammary gland development, reduced mammary epithelial mass by 22%, decreased the size and proliferation of terminal end buds by 30%, and inhibited mammary-induced tumor yield by 53% (Ip et al., 1999). Milk fat triglyceride-bound *cis*9,*trans*11-18:2 reduced growth and stimulated peroxidation of human mammary cancer cells more effectively compared with *trans*10,*cis*12-18:2 (O'Shea et al., 2000).

For lactating and non-lactating adults, beef and dairy product consumption are correlated with *cis*9,*trans*11-18:2 in blood plasma or milk (McGuire et al., 1999). Through the action of Δ^9 desaturase in tissues (Santora et al., 2000), *trans*11-18:1 in these foods could be an additional source of *cis*9,*trans*11-18:2 (Adlof et al., 2000). Results confirm the importance of intake in maintaining *cis*9,*trans*11-18:2 status in humans. In the U.S., intake of *cis*9,*trans*11-18:2 was estimated at only 50 mg/d. Because only minor increases in milk consumption (e. g. 2 servings/d or 55 mg/d additional *cis*9,*trans*11-18:2) were associated with a decrease in the risk of breast cancer, it may be important that humans increase their basal intakes of dairy products and beef (McGuire et al., 1999).

In summary, some of the saturated fatty acids found in milk fat are detrimental to human health, whereas unsaturated fatty acids may be beneficial. *Cis*9,*trans*11-18:2 has beneficial biological effects. Thus, it is important to produce dairy foods with less saturated fatty acids and more monounsaturated fatty acids and *cis*9,*trans*11-18:2. This goal can be achieved through dietary manipulation of the cow's diet.

CHAPTER 1

Biohydrogenation of Unsaturated Fatty Acids in Continuous Culture Fermenters During Digestion of Orchardgrass or Red Clover with Three Levels of Ground Corn Supplementation.

ABSTRACT

Digestibility of diet components and outputs of intermediates in pathways for biohydrogenation of unsaturated fatty acids were determined in a continuous culture of mixed rumen microorganisms using two forages with three levels of ground corn as substrates. Orchardgrass or red clover harvested and frozen during spring or fall served as the primary substrate for fermentation. During 10-d incubations, fermenters were fed either thawed forage (50 g DM/d), forage (42 g/d) plus 8 g/d corn, or forage (34 g/d) plus 16 g/d corn. The effluents from the last 3-d of incubation were composited for analyses. Inputs of oleic (18:1), linoleic (18:2), and α-linolenic (18:3) acid from orchardgrass averaged 14, 142, and 439 mg/d, respectively, or 38, 117, and 235 mg/d from clover when forage was the only input. Replacing some of the forage with corn resulted in lower 18:3 input, but greater 18:1 and 18:2 inputs. As corn input increased from 0 to 16 g/d, starch input increased from 5 to 27% of total DM. Corn input resulted in lower pH, greater microbial DM yield, and improved digestibility of DM, NDF, CP, and NSC. Overall, the apparent hydrogenation (%) of 18:1, 18:2, and 18:3 was greater for orchardgrass compared with clover. Hydrogenation of 18:1 and 18:2 increased linearly due to corn input regardless of forage. Apparent hydrogenation of 18:3, however, decreased as corn input increased. As a result, output of the *trans*11,*cis*15-18:2 intermediate in 18:3 hydrogenation also decreased. Average output of the *cis*9,*trans*11-18:2 intermediate in 18:2 hydrogenation was greater for clover (1.3 mg/d) compared with orchardgrass (0.6 mg/d), but corn input with either forage increased *cis*9,*trans*11-18:2 output by approximately 205%. Output of *trans*11-18:1, an intermediate in 18:2 and 18:3 hydrogenation, was greater from orchardgrass compared with clover (174 vs 90 mg/d), but corn increased *trans*11-18:1 output only from clover fermentations. Output of *trans*10-18:1 was greater in response to orchardgrass compared with clover

(10 vs 4 mg/d), but corn addition doubled the output regardless of forage type. Output of *trans*10,*cis*12-18:2, which did not differ due to forage type, increased 2-fold in response to corn. Results indicated rates of ruminal production of hydrogenation intermediates vary with type and unsaturated fatty acid content of forage input. When inputs of 18:2 plus 18:3 were less than 0.9% of total DM (clover), apparent hydrogenation was low (87%). When 18:2 plus 18:3 inputs were from 1.2 to 1.5% of total DM (orchardgrass) apparent hydrogenation averaged 96%. Despite greater hydrogenation, however, incremental additions of 18:1 and 18:2 from corn increased outputs of *trans*10-18:1, *trans*11-18:1, *trans*10,*cis*12-18:2, and *cis*9,*trans*11-18:2 in effluents.

INTRODUCTION

Linoleic (18:2n6) acid and α-linolenic (18:3n3) acid account for up to 80% of total fatty acids in mixed pastures (Hawke, 1973). Incomplete biohydrogenation of 18:2n6 and 18:3n3 in the rumen results in flow of *trans*11-18:1 and *cis*9,*trans*11-18:2 (CLA) to the intestine for absorption into blood. The CLA in bovine milk fat is derived from rumen fermentation and *trans*11-18:1 desaturation to CLA in mammary tissue (Kelly et al., 1998a; Griinari et al., 2000). Concentrations of *trans*11-18:1 and CLA are greater in milk fat from grazing cows compared with those fed a typical TMR (Kelly et al., 1998b; Dhiman et al., 1999a). Total fatty acid, 18:2n6, and 18:3n3 of grasses and legumes, however, are different (Hawke, 1973). Thus, there is a possibility that distribution of grasses and legumes in pastures may affect amounts of biohydrogenation intermediates available for absorption. For example, concentration of CLA in milk fat was 50% higher when cows grazed pastures containing a mixture of red clover and grasses compared with an all grass pasture (Wu et al., 1998).

Orchardgrass and red clover are forage species commonly grazed by dairy cattle in the United States. However, the extent of ruminal degradation of CP and NDF from legumes versus grasses varies and can be affected by stage of maturity (Hoffman et al., 1993). These differences could potentially affect bacterial growth, digestibility of dietary inputs, and modify the extent of biohydrogenation.

By replacing a fraction of herbage with supplemental grain, the availability of substrates for energy production by microbes is improved (Bach et al., 1999). Grains also contain significant amounts of unsaturated fatty acids, which along with starch could affect the extent of lipolysis and the production of biohydrogenation intermediates in the rumen (Gerson et al., 1985). The higher rate of rumen turnover in grazing cows (Berzaghi et al., 1996), compared with those fed typical diets, and the relative high content of polyunsaturated fatty acids in mixed pastures and grains provide ideal conditions for biohydrogenation intermediates to accumulate and flow to the small intestine for absorption.

In a classical study, Polan et al. (1964) showed that, among twenty pure cultures of rumen bacteria, only certain strains of *Butyrivibrio fibrisolvens* could hydrogenate

18:2n6 to an 18:1 isomer (probably *trans*11-18:1), but not to 18:0. Presumably, other bacterial species in the rumen were responsible for completing the hydrogenation by converting 18:1 isomers to 18:0. Under "normal" conditions, *B. fibrisolvens* accounts for 30% of total rumen bacterial species (Latham et al., 1972). In the first step of the predominant pathway for biohydrogenation of 18:2n6, *cis*9,*cis*12-18:2 is isomerized to *cis*9,*trans*11-18:2 (Kepler and Tove, 1967). Sequential reductions of the double bonds at carbons 9 and 11 yield *trans*11-18:1 then 18:0, respectively, as major products. The major pathway for 18:3n3 biohydrogenation *in vitro* involves an initial isomerization to a conjugated triene (*cis*9,*trans*11,*cis*15-18:3), followed by reductions of the double bonds at carbons 9, 15, and 11 to yield *trans*11,*cis*15-18:2, *trans*11-18:1, then 18:0, respectively (Wilde and Dawson, 1966; Kepler and Tove, 1967).

Bacterial species other than *B. fibrisolvens* also were shown to isomerize and hydrogenate unsaturated fatty acids to various end products. A *Selenomonas ruminantium* strain hydrogenated 18:2n6 primarily to *trans*11-18:1 and *trans*9-18:1 (Fujimoto et al., 1993). In contrast, a *Ruminococcus* strain hydrogenated 18:2n6 and 18:3n3 to *trans*10-18:1 (48% of total fatty acids) and *trans*11-18:1 (49%) (Kemp et al., 1975). The bacteria also produced *cis*-18:1 isomers, but they accounted for only 3% of total fatty acid end products. The *cis*10-, *cis*9-, and *cis*11- isomers of 18:1 accounted for, 45, 35, and 20% of total *cis*-18:1. *Eubacterium* strain W461 hydrogenated 18:2n6 to *trans*11- (33% of total fatty acids), *trans*9- (1%), *trans*10- (9%), and *trans*12- (6%); whereas, *cis*11-18:1 accounted for 43% of total fatty acids (Kemp et al., 1975). Incubating 18:3n3 with this bacterium resulted in the formation of *trans*11- (33% of total fatty acids), *trans*9- (1%), *trans*10- (10%), and *trans*12-18:1 (8%), but *cis*11-18:1 accounted for 48% of total fatty acids (Kemp et al., 1975). *Eubacterium* strain F2/2 hydrogenated 18:3n3 to *trans*11,*cis*15-18:2 (95%) exclusively, but a *Fusobacterium* strain hydrogenated 18:3n3 to *cis*15- (85% of total fatty acids), *trans*13- (1%), *trans*14- (4%), and *trans*15-18:1 (10%). Oleic acid (*cis*9-18:1) was hydrogenated to 18:0, isomerized to *trans*10-18:1, or not hydrogenated at all (Kemp et al., 1975; Mortimer and Niehaus, 1972). Interestingly, lactic acid producing bacteria favored the accumulation of *trans*-18:1 isomers (95% of total fatty acids), with *trans*10 and *trans*11 in various proportions accounting for all *trans*-18:1 isomers produced (Kemp et al., 1975).

Conjugated 18:2 isomers (*cis*9,*trans*11-18:2 primarily) in these studies were always found in low concentrations.

As indicated by the profiles of *trans*-18:1 and conjugated 18:2 isomers found in rumen fluid and milk fat, rumen microorganisms may possess several specific *cis*,*trans* isomerases (Griinari and Bauman, 1999). The diet of the cow directly affects the composition of the rumen microflora and may lead to preferential accumulation of certain 18:1 and 18:2 isomers. Feeding a high-concentrate/low-forage diet (80:20) severely reduced (from 29 to 2% of total isolates) the proportion of *B. fibrisolvens* while increasing the proportions of *Selenomonas* (6.9 to 19%), lactogenic (6 to 20%), and propionogenic (6 to 14%) bacteria in rumen fluid (Latham et al., 1972). A *Propionibacterium* strain isomerized 18:2n6 to *trans*10,*cis*12-18:2 and hydrogenated a portion of it to *trans*10-18:1 (Verhulst et al., 1987). The *trans*10,*cis*12-18:2 accounted for >50%, and *trans*10-18:1 accounted for 10%, of total fatty acid end products.

When the concentration of *trans*10-18:1 or *trans*10,*cis*12-18:2 increased in milk fat, due to feeding a low-fiber diet (Griinari et al., 1998) or infusing the CLA into the abomasum (Baumgard et al., 2000b), milk fat percentage and medium chain fatty acid concentrations were depressed. Lower milk fat percentages also were observed when grazing cows were supplemented with increasing levels of ground corn (Berzaghi et al., 1996; Reis and Combs, 2000), raising the possibility that the profiles of hydrogenation intermediates might have changed due to higher grain input.

Milk and dairy products contain many isomeric forms of 18:1 and 18:2 derived from microbial pathways for hydrogenation of unsaturated fatty acids in the rumen. *Trans* isomers of 18:1, *cis*9,*trans*11-18:2, and *trans*10,*cis*12-18:2 in dairy products are examples of hydrogenation intermediates that may have implications in human health (Parodi, 1999). Thus, a better understanding of microbial hydrogenation of unsaturated fatty acids in the rumen may benefit our knowledge of human nutrition and health as well as that of the cow. Continuous culture fermenters provide a means for quantifying the production of isomers of 18:1 and 18:2 during hydrogenation of dietary unsaturated fatty acids. The objective of this study was to evaluate the production of biohydrogenation intermediates when red clover or orchardgrass alone or with three levels of supplemental corn grain were incubated in continuous culture fermenters.

MATERIALS AND METHODS

Continuous Culture System

The dual-flow continuous culture system was described by Stern and Hoover (1990). Ruminal contents were obtained weekly for 7-wk from two dry, grazing Holstein cows via a rumen cannula prior to incubations. Cows also received grass hay and a concentrate mixture containing (DM basis) dry shelled corn (75%), soybean meal (24%; 48% CP), and a vitamin/mineral mix (1%). Rumen contents were collected 3 h after feeding and strained through 1 layer of cheesecloth. One liter of rumen fluid and 200 mL of warm buffer (2.2 g/L Na_2HPO_4, 5 g/L $NaHCO_3$, 0.6 g/L KCl, 1.6 g/L $KHCO_3$, and 0.2 g/L urea; Weller and Pilgrim, 1974) were added to each of eight fermenters and were maintained at 39 °C during incubations. The pH for all fermenters was not controlled during fermentations. The liquid turnover rate was maintained at 0.18/h using the buffer solution. The solids turnover rate was 0.07/h, which provided a mean solids retention time of 14.3 h. The liquid turnover rate and solids turnover rate were chosen to resemble the fractional passage rate of digesta from the rumen of cows grazing pasture with or without corn grain supplementation (Berzaghi et al., 1996).

Diets and Feeding

Red clover and orchardgrass were harvested in October (Fall), 1996, and May (Spring), 1997, from the same field at the Virginia Tech Dairy Center. After harvesting, forages were placed in plastic bags and immediately frozen (-40 °C). Subsequently, forages were mixed with dry ice, ground through a 5 mm screen in a Wiley Mill (Thomas-Wiley Laboratory Mill), and remained frozen for transport to West Virginia University. Upon arrival, a composite sample of orchardgrass or clover was made from the bags (6 bags per forage) used for shipping. Each composited sample was analyzed for chemical composition in duplicate. The remaining forage was stored frozen. Corn grain was ground through a 4 mm screen in a Wiley Mill and analyzed in duplicate. At the beginning of each of the 10-d fermentation periods, forages were analyzed for DM content and enough forage for each day of feeding was kept at 4 °C until feeding time. Fermenters were manually fed 25 g of DM at 0800 and 1800 h. Forages were fed

in the following combinations with ground corn: (g DM/day) 50 g forage, 42 g forage plus 8 g corn, or 34 g forage plus 16 g corn. Quadruplicate fermentations of each diet and combination were conducted during a 10-d period, with the first 7 d serving as an equilibration period and the last 3 d for sample collection into containers held at 4 °C.

Sampling, Measurements, and Analyses

On d 7, Chemtrix pH meters (type 45 AR) (Chemtrix, Hillsboro, OR) were used to monitor pH every 2 h from 0 to 10 h after the 0800 h feeding. Three 24-h collections during d 8 through 10 of each period provided 3 L of effluent, which was mixed to obtain a 1 L sample for analyses. On d 10, the composited effluents were allowed to settle and two 250 mL samples of the fluid layer were collected for microbial harvesting. Fluid from each fermenter was initially centrifuged at 200 $\times$ *g* for 20 min to remove protozoa and feed particles. The remaining supernatant fluid was again centrifuged at 30,000 $\times$ *g* for 15 min. After centrifugation, the liquid portion was discarded and the bacterial pellet resuspended and recentrifuged twice at 30,000 $\times$ *g* for 15 min, first using saline then using 50% methanol. The final pellet was dispersed in water, lyophilized, and stored for analyses.

Effluent DM was determined by centrifuging a 40 g sample of effluent at 30,000 $\times$ *g* for 45 min and drying the precipitated residue at 102 °C for 24 h. Forage and ground corn DM also was determined by drying at 102 °C for 24 h. The ADF and NDF concentrations in forages, ground corn, and effluents were determined as described by Van Soest et al. (1991). Nonstructural carbohydrate (NSC) content of the diet and effluents was estimated with the enzymatic procedure of Smith (1969) with modification for use of ferricyanide as a colorimetric indicator. Total N in forages, ground corn, effluents, and bacterial samples were determined according to AOAC (1975). Digestibility of dietary DM, NDF, ADF, CP, and NSC was calculated using the equation: digestibility (%) = [(input [g] - output [g])/input [g])] $\times$ 100.

Lipids were extracted from freeze-dried effluents (500 mg), ground corn, or forages with chloroform/methanol (2:1, vol/vol). Fatty acids in forages, corn, and effluents were directly methylated using 0.5 N NaOH in methanol (Park and Goins, 1994). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard.

Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters of fatty acids (0.5 μL methyl esters in hexane injected at a 70:1 split ratio) were separated on a 100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88,Chrompack, Middelburg, The Netherlands).

The injector temperature was maintained at 250 °C and the detector temperature maintained at 255 °C. The initial oven temperature was 70 °C (held for 1 min) and was programmed to increase 5 °C/min to 100 °C (held for 2 min), 10 °C/min to 175 °C (held for 40 min), and 5 °C/min to a final temperature of 225 °C (held for 15 min). Hydrogen was the carrier gas.

Fatty acid output (mg/d) was calculated by multiplying fatty acid concentration (μg/g DM) in effluents by total DM output, which was estimated from DM digestibility. Total fatty acid recovery ([output ÷ input] × 100) for all diet combinations averaged 101%.

Statistical Analysis

Data for digestibility, microbial yield, apparent biohydrogenation, and fatty acid output are reported as Least squares means ± SEM. Four replicate fermentations of each treatment were analyzed as a completely randomized design using the MIXED procedure of SAS (2000). Main effects in the model included season, forage type, corn supplementation level, and their interactions. Overall differences due to season, forage type, corn level, linear or quadratic effects due to corn supplementation, or two-way interactions between forage type and corn supplementation were considered to be significant when $P \leq 0.05$. However, all *P* values are presented in tables.

RESULTS AND DISCUSSION

The chemical composition of forages varied due to type and season (Table 1.1). Spring forages had numerically lower concentrations of ADF and NDF compared with fall forages. Total fatty acid concentration in orchardgrass was approximately 50% higher than that of red clover regardless of season. Oleic and linoleic acid accounted

for 4 and 18% of total fatty acids in forages compared with 24 and 63% in corn grain. Linolenic acid, however, accounted for 45% of total fatty acids in forages compared with 1% in corn grain. Among forages, concentrations of *cis*9-18:1 and 18:2n6 in red clover were higher compared with orchardgrass, regardless of season. Linolenic acid concentration, however, was higher in orchardgrass compared with red clover regardless of season. Overall, fatty acid profiles of forages were consistent with previous reports. Total fatty acid content of pasture species were highest during the primary growth stage (spring), decreased steadily through the stemmy regrowth period, and increased sharply during the next leafy regrowth stage (fall) (Hawke, 1973; Bauchart et al., 1984; Dewhurst et al., 2001). Linolenic acid concentration followed the same trend during growth, whereas linoleic acid concentration did not change appreciably. Grasses, however, contain more fatty acids than legumes at any stage of growth (Hawke, 1973).

Due to the replacement (0 to 16 g/d) of forage DM with corn grain (Table 1.2), input of CP into fermenters decreased and input of energy in the form of starch increased (Table 1.3). Linolenic acid input decreased at the expense of greater inputs of oleic and linoleic acid as corn replaced portions of forage (Table 1.3). These conditions closely reflect a typical grazing situation in which cows reduce their intake of pasture to accommodate a fixed amount of supplemental grain. Furthermore, it allowed for the examination of patterns for biohydrogenation of unsaturated fatty acids during digestion, when variable amounts of substrates were available.

Digestibility of Chemical Components, Microbial DM yield, and pH

Digestion (percentage) of NDF, ADF, and CP was higher when orchardgrass was the forage source (65, 61, and 73%, respectively) compared with red clover (52, 56, and 57%) (Table 1.4). No differences in DM or NSC digestion due to forage type, however, were apparent. Across seasons, DM, NDF, and ADF in spring forages were digested to a greater extent than those in fall forages. Greater digestion of chemical components from orchardgrass compared with red clover could be partly explained by higher microbial DM yield, which resulted from a greater efficiency of microbial N production. Differences in the rate of CP degradation between forages could have affected bacterial

growth. At similar maturity, the soluble CP fraction from orchardgrass compared with red clover is more degradable (45 vs 35%) (Hoffman et al., 1993). Lignin content of orchardgrass also is lower than red clover, and it was shown to be a factor which accounted for the higher ruminal degradability of fiber from orchardgrass (Hoffman et al., 1993; Varga and Hoover, 1983). As forages mature, lignin content also increases and could lead to lower digestibility of DM and fiber (Hoffman et al., 1993). Such an effect could explain the lower digestibility of DM and NDF when fall forages were the input.

Higher input of starch, as corn input increased, enhanced microbial DM production when fed with either forage, and led to higher digestibility of DM, NDF, CP, and NSC despite a lower effluent pH. Digestion of NDF is sensitive to reductions in rumen pH (Bach et al., 1999), but the observed decrease in pH was relatively small. Overall, changes in digestion of chemical components were similar to previous observations obtained in continuous culture fermentations of pasture alone or with corn grain supplementation and in grazing cows supplemented daily with 0 to 9.1 kg corn grain (Bach et al., 1999; Reis and Combs, 2000).

Apparent Biohydrogenation of Dietary cis9-18:1, 18:2n6, and 18:3n3

Lipolysis of dietary unsaturated fatty acids is a prerequisite for efficient hydrogenation, because incubation of triglyceride-bound substrate compared with the free fatty acid form increased concentrations of 18:0 and lowered concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 to a greater extent (Noble et al., 1974). Apparent biohydrogenation (%) of unsaturated fatty acids assumes that changes in the proportions of 18:0, 18:1, 18:2, or 18:3 flowing to the small intestine are not due to degradation, but a function of microbial hydrogenation (Wu and Palmquist, 1991).

Monogalactolipids are the most abundant complex lipids in pastures and seem to be readily hydrolyzed and hydrogenated in the rumen. Lipolysis and biohydrogenation of purified ryegrass ^{14}C-monogalactolipids (12% 18:2n6 and 53% 18:3n3) in rumen fluid from grazing cows was almost complete after only 1 h of incubation (Singh and Hawke, 1979). This led to a rapid increase in the concentration of ^{14}C-fatty acids in the free fatty acid fraction (70%) compared with the monogalactolipid fraction (22%). The distribution

of radioactivity in the free fatty acids produced during incubation, was primarily found in 18:0 and *trans*-18:1 isomers. The degree of maturity of fresh forage also could affect lipolysis and hydrogenation. Lypolysis and hydrogenation were greater in rumen fluid from sheep fed immature forage compared with mature forage (Gerson et al., 1986). Immature forage had a higher ratio of leaf tissue to stem tissue and greater concentrations of CP, total fatty acids, and 18:3n3. Thus, the extent of lipolysis and hydrogenation of fresh forage must be related to conditions which enhance microbial yield and(or) metabolism, as well as the availability of unsaturated fatty acids.

In the present study, overall apparent hydrogenation of total dietary unsaturated fatty acids in forages (with no corn input) was higher for orchardgrass (95%) compared with red clover (84%) (Table 1.5). Across seasons, however, only the input of spring red clover resulted in higher hydrogenation of total unsaturated fatty acids compared with fall red clover. Apparent hydrogenation was proportional to the input of unsaturated fatty acids which was higher for orchardgrass compared with red clover (Table 1.3). Greater microbial DM yield (Table 1.4) due to input of orchardgrass compared with red clover also could have enhanced the number of bacterial species capable of hydrogenation. The greater extent of hydrogenation when spring red clover was the input, compared with fall red clover, also was a function of unsaturated fatty acid input. However, the lack of a similar response with orchardgrass suggests that apparent hydrogenation was near its maximum when unsaturated fatty acid input accounted for as little as 1.1% of total DM input. Our results might explain the higher rates of hydrogenation observed in rumen fluid collected during grazing in summer compared with winter (Polan et al., 1964).

There were marked differences in the hydrogenation of *cis*9-18:1, 18:2n6, and 18:3n3 due to forage, season, and corn supplementation. Compared with the hydrogenation of 18:2n6 and 18:3n3, hydrogenation of *cis*9-18:1 was always lower regardless of forage, season, and corn input. The greater input of oleic acid from clover compared with orchardgrass could account for the higher hydrogenation of *cis*9-18:1. Despite minor differences in *cis*9-18:1 input across seasons, input of spring forages also increased *cis*9-18:1 hydrogenation. Hydrogenation of *cis*9-18:1 to 18:0 in other studies ranged from 40 to 90%, but some organisms could not hydrogenate it to any extent

(Polan et al., 1964; Kemp et al., 1975; Kemp et al., 1984a). Overall, apparent hydrogenation of 18:2n6 and 18:3n3 was higher for orchardgrass compared with red clover. Similar to hydrogenation of *cis*9-18:1, fermentation of spring red clover, compared with fall red clover, resulted in greater hydrogenation of 18:3n3. The 18:3n3 (Table 1.3) content of spring red clover was greater than fall red clover, and could have contributed to its higher degree of hydrogenation. Linoleic and linolenic acid hydrogenation to 18:0 in rumen fluid proceeds very rapidly, such that during the first 5 h of incubation 75% of 18:2n6 and 27% of 18:3n3 were converted to 18:0 (Wilde and Dawson, 1966; Noble et al., 1974; Singh and Hawke, 1979).

Incremental (0 to 16 g/d) input of corn grain with forages, substantially increased the input of *cis*9-18:1 and 18:2n6 while it decreased the input of 18:3n3 into fermenters. Consequently, apparent hydrogenation of *cis*9-18:1 and 18:2n6 increased substantially, but hydrogenation of 18:3n3 (primarily in red clover) decreased. The extent of the increase in hydrogenation of *cis*9-18:1 and 18:2n6 was more pronounced for red clover compared with orchardgrass, providing additional evidence that the hydrogenation efficiency of microbes at the higher input of unsaturated fatty acids from orchardgrass was almost maximal. Thus, as shown previously, at higher inputs of unsaturated fatty acids there could be greater accumulation of *trans*-18:1 and conjugated 18:2 intermediates (Polan et al., 1964; Noble et al., 1974). Because overall biohydrogenation increased as input of CP decreased (as corn replaced portions of forage) our results indicated apparent hydrogenation of individual unsaturated fatty acids was primarily a function of the amount of each unsaturated fatty acid entering the fermenter on a daily basis.

Our estimates for biohydrogenation of *cis*9-18:1, 18:2n6, and 18:3n3 were similar to those previously reported for ruminants fed a basal diet or the basal diet supplemented with unsaturated oils. Increases in the hydrogenation of *cis*9-18:1 (38 to 73%), 18:2n6 (70 to 95%), and 18:3n3 (89 to 98%) were observed when the oil content of the diet increased (Murphy et al., 1987; Doreau and Chilliard, 1997; Wachira et al., 2000). Compared with pasture-fed ruminants, our estimates of 18:2n6 and 18:3n3 hydrogenation were slightly higher than previously reported. In sheep fed fresh lucerne (18% 18:2n6 and 40% 18:3n3; pre-bloom), apparent hydrogenation of 18:2n6 and

18:3n3 averaged 61 and 91% (Doreau and Poncet, 2000). When the same forage was fed as hay, effectively reducing total fatty acid and 18:2n6 and 18:3n3 intake, the degree of hydrogenation of 18:3n3, but not 18:2n6, decreased.

Along with estimates of dietary unsaturated fatty acid hydrogenation, the outputs of intermediates arising during this process can be used to establish precursor-product relationships in response to changes in the ratios of *cis*9-18:1, 18:2n6, and 18:3n3 in the diet. In addition, the level of starch fed also could affect lipolysis and hydrogenation. Results from *in vitro* studies with rumen fluid from sheep fed increasing levels of wheat starch (12 to 31% of DM) indicated that lipolysis and hydrogenation of 18:2n6 decreased linearly (Gerson et al., 1985). The overall result was a reduction in the concentration of unesterified 18:0, but the accumulation of 18:1 isomers. Our results indicate that there was accumulation of specific 18:1 and 18:2 isomers, as well as 18:0, as the level of corn grain input increased. These relationships will be discussed in the following sections.

Outputs of Medium-Chain Fatty Acids and Biohydrogenation Intermediates

Overall, total fatty acid output in effluents from fermenters given orchardgrass was 52% higher compared with those from red clover (Table 1.6), which was consistent with the higher total fatty acid content of orchardgrass (Table 1.3). Recoveries (output ÷ input × 100) of total 18-carbon fatty acids in effluent DM averaged 101% when forages were the inputs, regardless of season, and 95% when the daily DM contained 8 or 6 g of corn DM. Duodenal fatty acid flow in ruminants fed fresh pasture ranged between -33 to 120% of input (Bauchart et al., 1984; Doreau and Ferlay, 1994; Doreau and Poncet, 2000). Negative flows were associated with higher intake of forage fatty acids from immature pasture during the spring, but positive flows were found when mature pasture contained 67% less fatty acids (Bauchart et al., 1984).

Rumen microbes can synthesize fatty acids containing 10 to 14 carbons from acetate or glucose, and 15:0 and 17:0 from propionate or valerate (Jenkins, 1994). In addition, 14:1 and 16:1 isomers can be synthesized via an anaerobic pathway. Overall, outputs of 10:0, 12:0, and 14:0 were greater for spring versus fall forages, due primarily to high outputs from spring red clover (Table 1.6). Forages were the primary source of

14:0 in the total DM input during fermentation (Tables 1.2 and 1.3). Thus, type of forage and season affected 14:0 output. Compared with 14:0 input, however, overall output of 14:0 was 23% higher. All of the 15:0 in the effluent apparently was synthesized during fermentation. Output of 15:0 varied due to season and type of forage, but numerical differences were small.

Our results suggested a portion of 16:0 in the DM input was lost due to desaturation and(or) degradation (Mackie et al., 1991). Disappearance of dietary 16:0 from orchardgrass during fermentation *in vitro* was previously observed (Wu and Palmquist, 1991). End products of 16:0 oxidation by rumen microbes could include isomers of 15-, 13-, or 11-carbon fatty acids in addition to short-chain volatile fatty acids (Emmanuel, 1978; Jenkins, 1994). Although outputs of *cis*9-14:1 or *cis*9-16:1 were not affected by forage, output of *trans*9-18:1 was greater for orchardgrass compared with red clover, primarily due to input of fall orchardgrass. Anaerobic bacteria can synthesize 14:1 or 16:1 isomers by dehydration of *de novo* synthesized 10:0, via β,γ-desaturase, to 10:1 followed by elongation to 14:1 or 16:1 isomers (Mackie et al., 1991).

Overall, the profiles of 14:0, 15:0, 16:0, and 17:0 in effluents regardless of forage or season were similar to those found previously in rumen fluid from cows fed fresh grass/clover pasture (Hawke and Robertson, 1964). Addition of corn to the DM input led to greater outputs of 14:0, 15:0, 16:0, *trans*9-16:1, and 17:0, suggesting *de novo* microbial fatty acid synthesis was not affected by the higher input of dietary fatty acids.

Across forage and season, *cis*9-18:1 plus 18:2n6 plus 18:3n3 accounted for 66% and 18:0 only 3% of total fatty acid input (Table 1.3). Due to biohydrogenation, *cis*9-18:1, 18:2n6, and 18:3n3 were converted primarily to 18:0 and *trans*-18:1 isomers (Table 1.6). Outputs of total *cis*-18:1 isomers accounted for approximately 4% (orchardgrass) or 8% (red clover) of total fatty acid output. Similarly, total isolated 18:2 isomers accounted for 4% (orchardgrass or 12% (red clover) of total fatty acid output. Conjugated 18:2 isomers, however, accounted for only 1% of total fatty acid output.

Outputs of *trans*-18:1 and 18:0 in effluents from fermenters fed orchardgrass as the input, were 80 and 134% greater compared with those fed red clover. In contrast, outputs of *cis*-18:1, isolated 18:2, and 18:3n3 were 12, 158, and 83% greater when red clover was fed. Input of spring forages, but not fall forages, resulted in higher outputs of

trans-18:1, but input of spring red clover also resulted in greater output of isolated 18:2 isomers (higher *trans*11,*cis*15-18:2 output). These data confirm that the efficiency of apparent hydrogenation was greater when orchardgrass, due to higher 18:2n6 and 18:3n3 input, was the source of DM input. Stearic acid and *trans*-18:1 isomers were the major end products of hydrogenation of 18:2n6 and 18:3n3 in ryegrass. However, there was small accumulation of *cis*-18:1, total 18:2, and 18:3n3 respectively (Singh and Hawke, 1979).

Replacing portions of either forage with corn grain, regardless of season, caused an average increase of 64% in the output of 18:0 (Table 1.6). The extent of the increase, however, was more pronounced when corn grain was fed with red clover. Because 18:3n3 input decreased, as corn replaced portions of forage (Table 1.3), the increase in 18:0 output most likely resulted from complete hydrogenation of *cis*9-18:1 and 18:2n6 in the corn and forage. The lack of change in output of isolated 18:2 isomers, despite increasing 18:2n6 output as corn replaced portions of forage, suggested most of the additional 18:2n6 input was partially or completely hydrogenated. In contrast, total output of *cis*-18:1 isomers increased as the amount of corn grain (the primary source of *cis*9-18:1) in the daily input of DM increased.

Despite the greater input of 18:2n6 from corn, output of total *trans*-18:1 isomers and conjugated 18:2 isomers only increased by 9 and 34% with orchardgrass compared with increases of 65 and 117% when corn was added with red clover. This response was more pronounced when spring forages plus corn were fed. The higher efficiency of hydrogenation of 18:2n6 from orchardgrass plus corn (96%) compared with red clover plus corn (85%), resulted in greater 18:0 output but only moderate production of *trans*-18:1 and conjugated 18:2 isomers.

To further evaluate production of intermediates in biohydrogenation pathways, isolated 18:2 isomers, conjugated 18:2 isomers, *cis*-18:1 isomers, and *trans*-18:1 isomers are listed individually in tables 7 and 8. In addition, Figure 1.1 depicts the primary pathways for hydrogenation of dietary *cis*9-18:1, 18:2n6, and 18:3n3 during fermentation. The pathways were developed based on the relative outputs of hydrogenation intermediates in response to input of unsaturated fatty acids from forage and corn.

Outputs of Isolated and Conjugated Isomers of 18:2

Although 18:2n6 was the only isolated 18:2 isomer provided as input, it (*cis*9,*cis*12-18:2) accounted for only 25% of total isolated 18:2 isomers in the effluent when orchardgrass or red clover was fed (Table 1.7). This was equivalent to 3% of total fatty acids in effluents. Overall, output of *cis*9,*cis*12-18:2 was greater (22 vs 10 mg/d) from red clover compared with orchardgrass. Effluents also contained *cis*9,*trans*12-18:2 (0.5 to 0.6 mg/d), *trans*9,*cis*12-18:2 (0.9 to 2 mg/d), and *trans*9,*trans*12-18:2 (0.5 to 0.6 mg/d), but they accounted for only 2 to 9% of total isolated 18:2 isomers. These *cis*/*trans*, *trans*/*cis*, and *trans*/*cis* isomers were most likely produced as a result of the isomerization of dietary *cis*9,*cis*12-18:2 during fermentation (Kemp et al., 1984b).

Output of *trans*11,*cis*15-18:2 did not result from *cis*9,*cis*12-18:2 isomerization, because it is an intermediate in the isomerization and hydrogenation of dietary 18:3n3 (Wilde and Dawson, 1966). The percentage of total radioactivity found in *trans*11,*cis*15-18:2 when ^{14}C-α-18:3n3 was incubated with a wide range of rumen bacteria, ranged from 25 to 100% (Hazlewood et al., 1976). Also, incubating increasing amounts of 18:3n3 with rumen fluid more than doubled *trans*11,*cis*15-18:2 concentration (Body, 1976). In our study, output represented 65 to 73% of total isolated 18:2 isomers or 3 to 12% of total fatty acid output (Figure 1.1). To our knowledge, this is the first study reporting outputs of *trans*11,*cis*15-18:2 during long term digestion of fresh forage. Compared with orchardgrass, clover yielded greater output of *trans*11,*cis*15-18:2 (68 vs 24 mg/d).

Input of increasing levels of corn grain with either forage increased the output of *cis*9,*cis*12-18:2 linearly but decreased the output of *trans*11,*cis*15-18:2 (Figure 1.1). At the highest level of corn input with orchardgrass or red clover, *cis*9,*cis*12-18:2 output had increased by 6 or 29 mg/d. In contrast, output of *trans*11,*cis*15-18:2 decreased by 9 to 29 mg/d when orchardgrass or red clover were fed with corn. Similar to our results, a greater proportion of the 18:2n6 input was recovered during *in vitro* incubations of rumen contents with increasing levels of 18:2n6 (Polan et al., 1964; Noble et al., 1974). Overall, our results indicated that *trans*11,*cis*15-18:2 was the major 18:2 isomer produced during hydrogenation of 18:3n3 in forages. Dietary 18:2n6, whether derived from forage or ground corn, was isomerized and hydrogenated significantly. As

discussed below, however, 18:2n6 hydrogenation also led to substantial accumulation of conjugated 18:2 isomers.

Formation of a *cis*9,*trans*11 conjugated 18:2 intermediate is a prerequisite for the biohydrogenation of *cis*9,*cis*12-18:2 to 18:0 (Kepler and Tove, 1967), but also could arise during hydrogenation of *cis*9,*cis*12,*cis*15-18:3 to 18:0 (Wilde and Dawson, 1966). Linoleate-*cis*12,*trans*11-isomerase (EC 5.2.1.5) is responsible for this isomerization step, and has a specific requirement for the *cis*9,*cis*12 diene configuration (Kepler et al., 1971). Conjugated 18:2 isomers, however, appear to be transient intermediates during hydrogenation because their concentrations are very low (0.3 to 1.3%) compared with *trans*-18:1 or 18:0 (Fellner et al., 1997). During incubations with pure cultures of rumen bacteria or rumen fluid, hydrogenation of *cis*9,*cis*12-18:2 yielded 18:0 (40 to 70%) and *trans*11-18:1 (14 to 100%) as major end products (Kemp et al., 1984b; Fellner et al., 1997). In contrast, hydrogenation of *cis*9,*cis*12,*cis*15-18:3 in mixed rumen fluid resulted in equal proportions of *trans*-18:1 (40%) and 18:0 (38%) as major products (Wilde and Dawson, 1966; Singh and Hawke, 1979).

Outputs of conjugated 18:2 isomers in the present study (Table 1.7) accounted for 0.7 to 1.0% of total fatty acid output, and were doubled than previously reported in effluents from fermenters fed a mixture of hay and concentrate (Fellner et al., 1997). Among the conjugated 18:2 isomers, *cis*9,*cis*11-18:2 and *trans*11,*trans*13-18:2 were the predominant isomers. Overall, their outputs accounted for 28 to 39% of total conjugated 18:2 isomers when forage was the only DM input. The output of a mixture of *trans*,*trans*-18:2 isomers (*trans*8,*trans*10, *trans*9,*trans*11, and *trans*10,*trans*12-18:2) represented 9 to 15% of total conjugated 18:2 isomer output. In an earlier study, it was reported that approximately 41% of total conjugated isomers consisted of a mixture of *trans*,*trans*-18:2 isomers when fermenters were fed a mixed diet (Fellner et al., 1997). It could be possible that the *trans*,*trans*-18:2 isomer reported by Fellner et al. (1997) also contained the *trans*11,*trans*13-18:2, as well as other isomers with a conjugated *trans*,*trans* configuration. *Cis*9,*trans*11-18:2 output accounted for 9 or 23% of total conjugated 18:2 isomers derived from orchardgrass or red clover (Figure 1.1). Red clover fermentations resulted in greater output of *cis*9,*trans*11-18:2 (1.3 mg/d compared

with ochardgrass 0.62 mg/d), and these values were similar to those in effluents from fermenters fed a mixed diet (Qiu et al., 2000).

*Trans*10,*cis*12-18:2 was previously reported to account for 17% of total conjugated 18:2 isomers in fermenter effluents fed a mixed diet (Fellner et al., 1997). However, in our study it only accounted for 7% of total conjugated 18:2 isomers when either forage was fed. Production of conjugated isomers other than *cis*9,*trans*11-18:2 suggests that the rumen ecosystem contains *cis*,*trans* isomerases in addition to the *cis*12,*trans*11-isomerase (EC 5.2.1.5). The *trans*10,*cis*12-18:2 could arise from isomerization of 18:2n6 or 18:3n3 via a *cis*9,*trans*10-isomerase (Griinari and Bauman, 1999). Low rumen pH and availability of unsaturated fatty acids appear to be the major factors responsible for the synthesis of isomers with a *trans*10 double bond (Piperova et al., 2000).

Corn input with either forage increased the overall outputs (Figure 1.1) of *cis*9,*trans*11-18:2, *trans*10,*cis*12-18:2, and *trans*,*trans*-18:2 by 201, 187, and 171%, respectively. In contrast, the overall output of *cis*9,*cis*11-18:2 decreased 33% when corn was added to orchardgrass, but not red clover. Concentrations of *cis*9,*trans*11-18:2 in effluents from forage plus corn were similar to those found in omasal contents of grazing cows deriving 40% of daily DMI from supplemental grain (Wu et al., 1998). Corn provided additional 18:2n6 and decreased effluent pH, conditions which may have stimulated production of *trans*10,*cis*12-18:2 in the present study because supplementing 18:2n6 to a mixed diet during fermentation decreased the concentration of *trans*10,*cis*12-18:2 but increased *cis*9,*trans*11-18:2 (Fellner et al., 1997). When high-concentrate/low forage (80:20) diets were fed, the ratio of cellulolytic (primarily *B. fibrisolvens*) to propionogenic, lactogenic, and amylolitic bacteria in the rumen was severely reduced (Latham et al., 1972). *In vitro*, some strains of *Propionibacterium* isomerized and hydrogenated 18:2n6 to *trans*10,*cis*12-18:2 (>50% of total fatty acids) (Verhulst et al., 1987). The extent to which pH was decreased in our study (-0.11 pH units) was minimal, but may have increased the growth of starch fermenting bacteria as noted previously in grazing cows supplemented with increasing amounts of grain (Elias et al., 1996). Corn addition to either forage increased bacterial DM yield by an average

of 53% in the present study. Amylolitic and(or) propionogenic bacteria may have accounted for a proportion of this increase.

Outputs of cis and trans Isomers of 18:1

Oleic acid was the only 18:1 isomer detected in forages and corn grain (Table 1.1), and amount of *cis*9-18:1 entering the fermenters (Table 1.3) varied with the ratio of forage to corn grain (Figure 1.1). Amount of *cis*9-18:1 in effluents was greater during red clover fermentation, especially fall red clover, compared with orchardgrass (Table 8). The overall output of *cis*9-18:1, however, accounted for only 29 to 45% of total *cis*-18:1 isomers in effluents from orchardgrass or red clover. Although not affected by forage type or corn, overall outputs of *cis*11-, *cis*13-, and *cis*15-18:1 accounted for up to 53% of total *cis*-18:1 isomers. Output of *cis*13-18:1 and *cis*15-18:1, however, were greater when spring forages were fermented. *In vitro* studies with pure cultures indicated only a small fraction of *cis*9-18:1 was isomerized to other *cis*-18:1 isomers (Kemp et al., 1984a). In contrast, isomerization and hydrogenation of *cis*9,*cis*12-18:2 or *cis*9,*cis*12,*cis*15-18:3 can potentially result in the formation of several *cis*-18:1 isomers. With 18:2n6 as the substrate, *cis*11-18:1 and *cis*12-18:1 accounted for 44 and 5% of total fatty acids recovered (Hazlewood et al., 1976). Although, *cis*-18:1 isomers were less than 3% of total fatty acids after incubation of 18:2n6 or 18:3n3 with pure cultures of rumen bacteria, *cis*10-, *cis*9-, and *cis*11-18:1 accounted for 45, 35, and 20% of total *cis*-18:1 isomers (Kemp et al., 1975). A major isomer produced during hydrogenation of 18:3n3 in rumen fluid was *cis*15-18:1, which increased from 0 to 32% of total fatty acid end products and was proportional to the amount of 18:3n3 provided as substrate for incubations (Body, 1976).

Eight *trans*-18:1 isomers were identified in effluents, and they accounted for 25 to 30% of total fatty acid output from red clover or orchardgrass fermentations. *Trans*11-18:1, however, represented 61 to 66% of total *trans*-18:1 isomers (Figure 1.1). Each of the remaining isomers accounted for 2 to 12% of total *trans*-18:1 isomers. Consistent with the greater apparent hydrogenation of 18:2n6 or 18:3n3 (Table 1.5), outputs of all *trans*-18:1 isomers, except *trans*15-18:1, were greater from orchardgrass than from red clover fermentations. In addition, spring forages resulted in greater output of all *trans*-

18:1 isomers, except *trans*16-18:1, compared with fall forages. The difference due to season, however, was more evident when red clover was fermented.

Corn grain addition to red clover, regardless of season, resulted in greater outputs of all *trans*-18:1 isomers. Increases in outputs of *trans*9-, *trans*10-, and *trans*12-18:1, however, also were evident when corn was added to orchardgrass. The greater outputs of all *trans*-18:1 isomers from red clover, were a function of higher input and apparent hydrogenation of *cis*9-18:1 and 18:2n6. It appears, however, that *trans*9-, *trans*10-, and *trans*12-18:1 are major *trans* isomers resulting from isomerization of *cis*9-18:1 in the rumen. *Trans*10-18:1 represented 25% of total fatty acids during incubation of *cis*9-18:1 *in vitro* with a *pseudomonad* strain, but its concentration increased more than 2.5-fold when the pH decreased gradually from 7 to 5 (Mortimer and Niehaus, 1972). Using ^{13}C-*cis*9-18:1, it was conclusively shown that *cis*9-18:1 in the rumen could be isomerized to *trans*11-, *trans*10-, or *trans*12-18:1, with the latter two being the predominant isomers (Jenkins, personal communication). Our data demonstrated that output of *trans*10-18:1 was linearly related to *cis*9-18:1 and *cis*9,*cis*12-18:2 input (Figure 1.2) from corn grain. Taking into account that production of *trans*10,*cis*12-18:2 also increased in response to 18:2n6 input (Table 1.7), it could be possible that incomplete hydrogenation of *trans*10,*cis*12-18:2 contributed to the output of *trans*10-18:1 resulting from isomerization of *cis*9-18:1 to *trans*10-18:1 (Figure 1.1).

The greater input of 18:2n6 with corn grain, apparently was a driving force leading to greater outputs of *trans*-18:1 isomers, particularly *trans*11-18:1 (Figure 1.1). Upon hydrogenation of 18:2n6 and 18:3n3 in pure cultures of rumen bacteria, concentrations of *trans*9-18:1 through *trans*15-18:1 were significantly increased (Kemp et al., 1975; Hazlewood et al., 1976). However, *trans*11-18:1 was by far (20 to 100% of total fatty acids) the predominant fatty acid produced during hydrogenation. In our study, outputs of *trans*-18:1 isomers were probably enhanced by the rapid rate of liquid and solid outflow from fermenters meant to resemble rumen kinetics during grazing. Duodenal flows of up to 10 isomers of *trans*-18:1 also were detected when lactating goats were fed supplemental soybean oil (Bickerstaffe et al., 1972). *Trans*6/7/8, *trans*9, *trans*10, *trans*11, *trans*12, *trans*13/14, *trans*15, and *trans*16-18:1 accounted for 2, 2, 4, 57, 6, 14, 7, and 9%, respectively, of total *trans*-18:1 flow (8.3 g/d) in their study. In

terms of proportions, outputs of individual *trans*-18:1 isomers in our study were consistent with these values.

CONCLUSIONS

Greater microbial DM yields were associated with higher digestibility of DM, NDF, CP, and NSC. The efficiency of apparent hydrogenation of *cis*9-18:1, 18:2n6, or 18:3n3 during fermentation varies with type of forage and season, due to differences in types and amounts of unsaturated fatty acids. Linolenic acid is the primary substrate for hydrogenation when forages are fed, and results in greater production of *trans*11,*cis*15-18:2 and *trans*11-18:1 during fermentation (Figure 1.1). Replacing corn (starch) for portions of forage, provides more *cis*9-18:1 plus 18:2n6 during fermentation and these become the primary substrates for hydrogenation. As a result, the outflow of *trans*11,*cis*15-18:2 from the rumen decreases, but outflows of *trans*10-18:1, *trans*11-18:1, *cis*9,*trans*11-18:2, or *trans*10,*cis*12-18:2 increase (Figure 1.1). Depending on the amount of supplemental grain provided, however, concentrations of *trans*10-18:1 or *trans*10,*cis*12-18:2 in milk fat could increase and might cause a reduction in milk fat percentage.

Table 1.1. Composition of forages and ground corn [1].

	Orchardgrass		Red clover		
	Fall	Spring	Fall	Spring	Corn grain
	% of DM				
ADF	24.6	20.8	23.9	20.2	3.3
NDF	46.4	41.6	31.0	27.4	13.4
CP	23.5	22.6	20.4	22.4	9.9
Sugars [2]	12.6	20.1	13.1	11.6	1.1
Starch	4.7	7.6	4.5	4.5	72.2
Ash	9.8	8.5	9.9	8.9	1.5
EE	4.1	4.1	2.3	2.7	3.8
Total fatty acids	1.7	1.9	1.0	1.4	3.3
	mg/g total fatty acids				
14:0	12.3	12.8	11.0	13.6	0.5
16:0	278.5	294.4	310.5	241.6	102.1
18:0	26.2	27.9	48.1	43.5	18.5
*cis*9-18:1	14.2	14.8	80.0	52.1	238.9
18:2n6	156.0	164.8	214.0	190.6	626.6
18:3n3	512.9	485.3	336.3	458.7	13.4

[1] Six samples of each forage were composited and analyzed in duplicate. However, a single sample of corn grain was analyzed in duplicate.
[2] Sugars = soluble sugars extracted from feeds by stirring in water at 39 °C for 1 h.

Table 1.2. Composition of diets [1].

	Orchardgrass						Red clover					
	Fall			Spring			Fall			Spring		
	Corn (g)						Corn (g)					
	0	8	16	0	8	16	0	8	16	0	8	16
	% of DM											
NDF	46.4	41.1	35.8	41.6	37.1	32.6	31.0	28.2	25.4	27.4	25.2	22.9
CP	23.5	21.3	19.2	22.6	20.6	18.5	20.4	18.7	17.0	22.4	20.4	18.4
Sugars [2]	12.6	10.8	8.9	20.1	17.1	14.0	13.1	11.2	9.3	11.6	9.9	8.2
Starch	4.7	15.5	26.3	7.6	17.9	28.3	4.5	15.3	26.2	4.5	15.3	26.2
Total fatty acids	1.7	1.9	2.2	1.9	2.1	2.3	1.0	1.3	1.7	1.4	1.7	2.0
	mg/g total fatty acids											
14:0	12.3	9.0	6.5	12.8	9.7	7.1	11.0	6.6	4.3	13.6	9.4	6.5
16:0	290.7	238.5	199.1	307.2	254.9	213.3	321.6	230.7	182.2	255.2	206.1	172.9
18:0	26.2	24.0	22.4	27.9	25.5	23.6	48.1	35.9	29.3	43.5	35.4	30.0
*cis*9-18:1	14.2	76.6	123.6	14.8	72.1	117.6	80.0	145.9	181.2	52.1	112.1	152.8
18:2n6	156.0	286.6	385.2	164.8	282.7	376.6	214.0	385.1	476.6	190.6	330.7	425.6
18:3n3	512.9	374.3	269.7	485.3	364.8	268.8	336.3	202.4	130.8	458.7	315.6	425.6

[1] Forages were fed in the following combinations with ground corn (g DM/day): 50 g forage, 42 g forage plus 8 g corn, or 34 g forage plus 16 g corn.
[2] Sugars = soluble sugars extracted from feeds by stirring in water at 39 °C for 1 h.

Table 1.3. Daily input of fiber, crude protein, carbohydrates, and fatty acids [1].

	Orchardgrass						Red clover					
	Fall			Spring			Fall			Spring		
	Corn (g)						Corn (g)					
	0	8	16	0	8	16	0	8	16	0	8	16
	g/d											
NDF	23.2	20.8	18.4	20.8	18.8	16.8	15.5	14.4	13.2	13.7	12.8	12.0
CP [2]	11.8	10.5	9.2	11.3	10.1	8.9	10.2	9.2	8.2	11.2	10.0	8.9
Sugars [3]	6.3	5.4	4.5	10.1	8.5	7.0	6.6	5.6	4.6	5.8	5.0	4.1
Starch	2.4	7.8	13.2	3.8	9.0	14.1	2.3	7.7	13.1	2.3	7.7	13.1
Total fatty acids	0.83	0.97	1.10	0.93	1.05	1.17	0.48	0.65	0.84	0.67	0.83	0.99
	mg/d											
14:0	10.2	8.7	7.2	11.9	10.1	8.3	5.0	4.3	3.6	9.2	7.8	6.5
16:0	231.1	221.5	211.9	273.8	257.3	240.9	139.7	144.7	149.7	162.7	164.0	165.3
18:0	21.7	23.2	24.7	26.0	26.8	29.8	21.7	23.2	24.7	29.3	29.6	29.8
*cis*9-18:1	11.8	73.9	136.0	13.8	75.6	137.4	41.0	94.3	152.5	35.1	93.5	151.9
18:2n6	129.5	276.6	423.7	153.3	296.6	439.9	106.3	248.7	401.2	128.4	275.7	423.0
18:3n3	425.7	361.2	296.7	451.3	382.7	314.0	161.3	130.7	110.1	308.9	263.1	217.2

[1] Forages were fed in the following combinations with ground corn (g DM/day): 50 g forage, 42 g forage plus 8 g corn, or 34 g forage plus 16 g corn.
[2] Sugars = sucrose, fructans, fructose, glucose, and lactose.
[3] Addition of buffer into fermenters provided 0.47 g urea-N/d.

Table 1.4. Digestibility of chemical components, yield of microbial DM and N, and pH in rumen fermenters fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g corn grain replacing equal portions of forage DM.

	Orchardgrass						Red clover									
	Fall			Spring			Fall			Spring						
	Corn (g)						Corn (g)							Overall P [1]		
	0	8	16	0	8	16	0	8	16	0	8	16	SE	S	F	C
Digestibility	%															
DM [2, 3]	52	57	70	68	78	81	57	66	75	66	75	80	4	0.01	0.33	0.01
NDF [2, 3]	59	63	63	71	73	74	48	44	55	56	57	61	2	0.01	0.01	0.01
ADF [3]	58	59	59	64	69	66	50	52	55	61	61	64	2	0.01	0.01	0.07
CP [2, 3]	75	80	93	71	74	98	54	58	70	60	65	68	4	0.66	0.01	0.01
NSC [2, 3]	51	64	67	68	69	69	62	61	76	50	58	66	3	0.97	0.44	0.01
Microbial																
DM, g/d [2, 3]	10	12	16	10	12	15	7	8	10	8	10	11	0.3	0.57	0.01	0.01
Efficiency [4]	41	44	39	32	29	37	27	26	29	24	25	24	1.4	0.01	0.01	0.39
pH [2, 3]	7.06	7.00	6.89	6.88	6.94	6.89	6.88	6.87	6.81	6.98	6.85	6.76	0.03	0.01	0.01	0.01

[1] Overall effect due to season (S), forage type (F), or corn supplementation (C).
[2] Linear effect of orchardgrass by corn interaction was significant ($P < 0.05$).
[3] Linear effect of clover by corn interaction was significant ($P < 0.05$).
[4] Efficiency of microbial N production = g microbial N/kg DM digested.

Table 1.5. Apparent biohydrogenation[1] of dietary oleic, linoleic, and linolenic acid in rumen fermenters fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g corn grain replacing equal portions of forage DM.

	Orchardgrass						Red clover									
	Fall			Spring			Fall			Spring						
	Corn (g)						Corn (g)							Overall P [2]		
	0	8	16	0	8	16	0	8	16	0	8	16	SE	S	F	C
	%															
*cis*9-18:1 [3, 4]	21.2	76.0	80.3	31.2	77.7	85.6	47.6	62.7	73.4	62.7	70.6	72.1	2.1	0.01	0.04	0.01
18:2n6 [3, 4]	93.5	95.1	95.5	94.4	95.5	96.8	83.3	84.0	86.9	80.7	83.3	85.5	0.9	0.50	0.01	0.01
18:3n3 [4]	97.6	97.2	96.7	97.4	97.5	97.7	87.8	84.9	83.0	93.0	90.8	91.2	0.5	0.01	0.01	0.01
Total	95.1	94.2	93.5	95.2	94.8	95.4	81.2	80.1	83.1	87.4	84.6	84.5	0.7	0.01	0.01	0.04

[1] Biohydrogenation (%) = 100 - [(18:1, 18:2, or 18:3n3 output [mg/d]/total 18-carbon output [mg/d])/(*cis*9-18:1, 18:2n6, or 18:3n3 input [mg/d]/total 18-carbon input [mg/d]) × 100].
[2] Overall effect due to season (S), forage type (F), or corn supplementation (C).
[3] Linear effect of orchardgrass by corn interaction was significant ($P < 0.05$).
[4] Linear effect of clover by corn interaction was significant ($P < 0.05$).

Table 1.6. Outputs of fatty acids in effluents from rumen fermenters fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g corn grain replacing equal portions of forage DM.

	Orchardgrass						Red clover									
	Fall			Spring			Fall			Spring						
	Corn (g)						Corn (g)							Overall P [1]		
	0	8	16	0	8	16	0	8	16	0	8	16	SE	S	F	C
	mg/d															
10:0	0.20	0.23	0.36	0.27	0.05	0.16	0.09	0.17	0.17	1.89	1.53	0.79	0.33	0.01	0.01	0.58
12:0 [2]	6.4	3.3	4.3	5.9	3.3	3.4	2.3	2.6	2.8	5.5	5.2	5.1	0.8	0.01	0.24	0.03
14:0 [2, 3]	6.8	10.7	12.9	12.8	11.0	13.2	5.1	6.2	9.3	19.8	20.7	22.5	1.3	0.01	0.01	0.01
*cis*9-14:1	3.3	0.19	0.10	0.24	0.29	0.24	0.14	0.10	0.23	0.28	0.20	0.15	0.93	0.39	0.31	0.35
15:0 [2, 3]	7.4	11.3	12.7	12.1	12.9	13.5	7.8	9.6	12.4	7.5	10.2	12.5	1.0	0.05	0.01	0.01
16:0 [2, 3]	136.8	144.4	163.1	167.5	159.0	177.3	82.5	100.6	125.0	115.8	131.0	158.3	8.2	0.01	0.01	0.01
*cis*9-16:1	8.2	7.2	14.9	8.7	3.7	5.4	5.9	3.8	5.3	5.4	5.1	5.3	3.6	0.36	0.18	0.53
*trans*9-16:1	8.3	7.9	8.7	5.5	4.2	4.5	4.2	2.3	2.6	3.7	3.6	3.1	0.7	0.01	0.01	0.19
17:0	4.3	4.8	5.5	5.4	4.8	5.1	5.3	6.0	6.4	5.1	5.6	4.1	0.5	0.19	0.14	0.74
18:0 [2, 3]	351.8	396.6	458.1	307.6	406.0	486.5	128.3	183.2	256.0	153.8	194.7	261.0	16.3	0.54	0.01	0.01
18:1 total																
cis [2, 3]	34.1	41.3	53.0	35.2	42.1	43.1	42.3	57.8	66.6	37.5	61.8	71.8	3.8	0.78	0.01	0.01
trans [2, 3]	235.8	233.8	271.8	290.0	312.7	301.1	118.0	139.4	208.1	175.2	206.1	277.3	11.2	0.01	0.01	0.01
18:2 total																
isolated	33.7	36.5	35.4	38.3	38.8	29.4	60.9	71.3	76.2	124.5	104.5	110.3	7.8	0.01	0.01	0.95
conjugated [3]	6.0	6.5	9.7	7.3	8.6	8.2	4.3	7.1	11.5	6.8	8.9	14.3	1.4	0.08	0.19	0.01
18:3n3 [2, 3]	11.5	10.2	9.4	12.6	10.1	6.9	22.4	19.3	17.1	22.0	22.2	17.2	1.5	0.82	0.01	0.01
Total [2, 3, 4]	855.3	918.5	1059.1	917.5	1019.2	1099.7	485.6	615.7	796.7	683.3	787.2	959.0	40.6	0.01	0.01	0.01

[1] Overall effect due to season (S), forage type (F), or corn supplementation (C).
[2] Linear effect of orchardgrass by corn interaction was significant ($P < 0.05$).
[3] Linear effect of clover by corn interaction was significant ($P < 0.05$).
[4] Total = 10:0 to 20:5.

Table 1.7. Outputs of isolated and conjugated 18:2 isomers in effluents from rumen fermenters fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g corn grain replacing equal portions of forage DM.

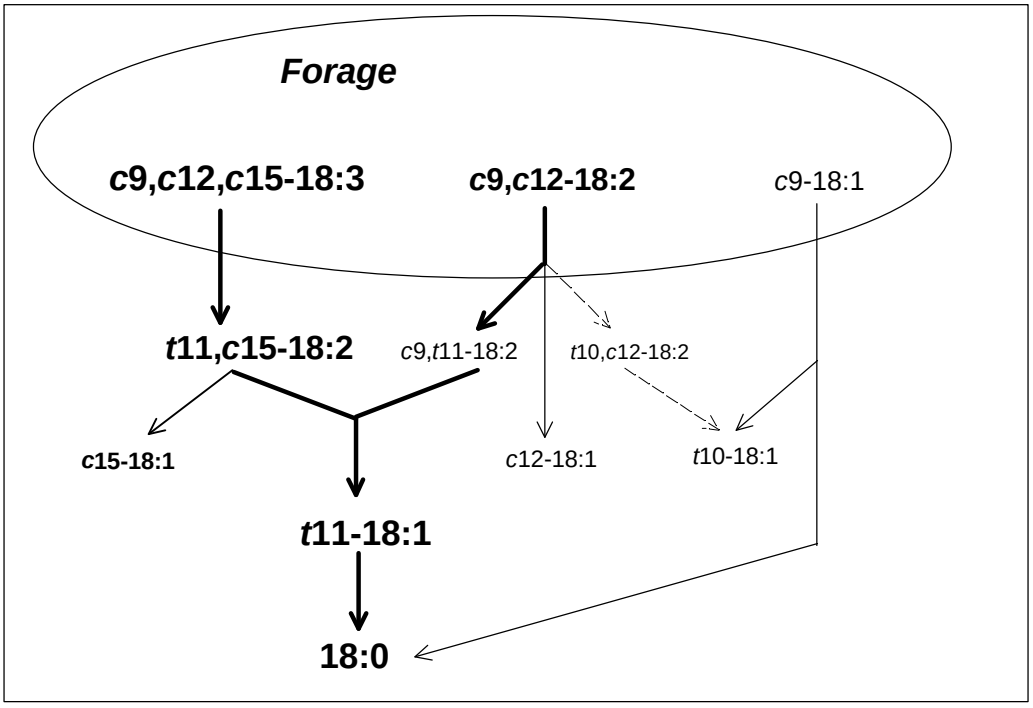
	Orchardgrass						Red clover									
	Fall			Spring			Fall			Spring						
	Corn (g)						Corn (g)							Overall P [1]		
	0	8	16	0	8	16	0	8	16	0	8	16	SE	S	F	C
	mg/d															
Isolated 18:2																
*c*9,*c*12 [2, 3]	9.7	13.5	18.1	9.3	14.2	13.4	19.3	39.1	48.0	25.3	42.4	55.2	2.4	0.32	0.01	0.01
*c*9,*t*12	0.52	0.37	0.36	0.47	0.51	0.36	0.42	0.39	0.42	0.72	0.46	0.92	0.1	0.01	0.04	0.35
*t*9,*c*12 [2]	1.8	1.3	1.2	2.2	1.8	1.4	0.42	0.45	0.58	1.4	1.2	0.96	0.2	0.01	0.01	0.02
*t*9,*t*12	0.48	0.47	0.61	0.76	0.58	0.58	0.39	0.38	0.52	0.73	0.59	0.69	0.1	0.01	0.68	0.37
*t*11,*c*15 [2, 3]	21.3	21.4	14.7	25.7	22.0	13.4	40.2	31.1	26.4	95.9	60.5	52.4	4.4	0.01	0.01	0.01
Conjugated 18:2																
*c*9,*t*11 [2, 3]	0.51	0.89	2.5	0.73	1.0	1.4	0.97	1.8	2.7	1.6	2.3	4.4	0.3	0.10	0.01	0.01
*c*9,*c*11 [2]	1.8	1.2	1.0	1.8	1.6	1.3	1.1	1.1	1.2	1.4	1.3	1.6	0.1	0.01	0.08	0.03
*t*10,*c*12 [2, 3]	0.28	0.87	1.3	0.57	0.64	0.89	0.32	0.49	1.0	0.49	0.52	1.5	0.2	0.70	0.71	0.01
*t*11,*t*13 [3]	2.7	2.7	3.4	3.5	4.5	3.5	1.3	2.3	4.3	2.5	3.2	3.9	0.9	0.21	0.43	0.19
t,*t* [2, 3, 4]	0.51	0.67	1.4	0.65	0.69	1.1	0.76	1.3	2.5	0.93	1.4	2.9	0.3	0.71	0.01	0.01

[1] Overall effect due to season (S), forage type (F), or corn supplementation (C).
[2] Linear effect of orchardgrass by corn interaction was significant ($P < 0.05$).
[3] Linear effect of clover by corn interaction was significant ($P < 0.05$).
[4] Coelution of t8,t10-18:2, t9,t11-18:2, and t10t12-18:2.

Table 1.8. Outputs of *cis* and *trans* isomers of 18:1 in effluents from rumen fermenters fed (50 g DM/d) orchardgrass or red clover with 0, 8, or 16 g corn grain replacing equal portions of forage DM.

	Orchardgrass						Red clover									
	Fall			Spring			Fall			Spring						
	Corn (g)						Corn (g)							Overall P [1]		
	0	8	16	0	8	16	0	8	16	0	8	16	SE	S	F	C
	mg/d															
Cis																
9 [2, 3]	10.7	17.6	25.4	10.3	17.8	18.8	22.8	34.5	37.0	13.4	25.2	37.7	2.0	0.01	0.01	0.01
11	6.8	6.4	10.0	7.9	7.9	8.1	7.8	7.4	8.6	8.0	10.6	11.2	1.2	0.14	0.15	0.12
12 [2, 3]	2.7	4.4	6.5	2.5	4.8	5.7	3.3	4.9	10.6	3.3	6.2	8.6	0.8	0.60	0.01	0.01
13 [2]	1.6	1.7	2.4	1.2	1.7	1.9	1.3	1.1	1.3	2.2	2.3	2.2	0.3	0.04	0.86	0.19
15 [2]	12.0	11.5	8.4	12.9	9.9	8.4	7.4	9.5	9.2	10.9	17.7	12.3	1.6	0.02	0.50	0.11
Trans																
6,7,8 [3]	9.0	9.1	11.5	11.6	12.8	11.7	2.9	3.8	5.9	4.9	5.5	7.9	1.0	0.01	0.01	0.02
9 [2, 3]	5.9	5.9	8.0	7.3	8.1	8.1	2.1	2.7	3.9	3.5	3.7	4.8	0.4	0.01	0.01	0.01
10 [2, 3]	8.5	11.7	18.2	10.8	13.5	19.0	3.0	4.7	9.4	5.0	6.2	10.4	1.1	0.02	0.01	0.01
11 [3]	149.1	143.0	167.4	198.9	210.1	197.1	70.9	89.6	127.6	109.2	131.5	182.0	10.0	0.01	0.01	0.01
12 [2, 3]	10.4	11.6	13.7	11.4	13.3	14.0	6.3	7.3	12.2	9.1	10.5	14.4	0.8	0.01	0.01	0.01
13,14 [3]	28.8	29.5	29.5	29.8	32.5	29.0	15.9	17.5	26.9	23.4	27.4	31.4	2.4	0.01	0.01	0.02
15 [3]	13.7	13.8	14.1	13.2	13.7	13.4	9.4	10.9	13.6	12.0	15.2	16.4	1.0	0.01	0.29	0.01
16 [3]	10.7	10.2	9.4	9.1	9.5	9.2	5.3	5.3	7.0	6.5	7.4	7.4	0.6	0.54	0.01	0.71

[1] Overall effect due to season (S), forage type (F), or corn supplementation (C).
[2] Linear effect of orchardgrass by corn interaction was significant ($P < 0.05$).
[3] Linear effect of clover by corn interaction was significant ($P < 0.05$).
[4] Quadratic effect of clover by corn interaction was significant ($P < 0.05$).



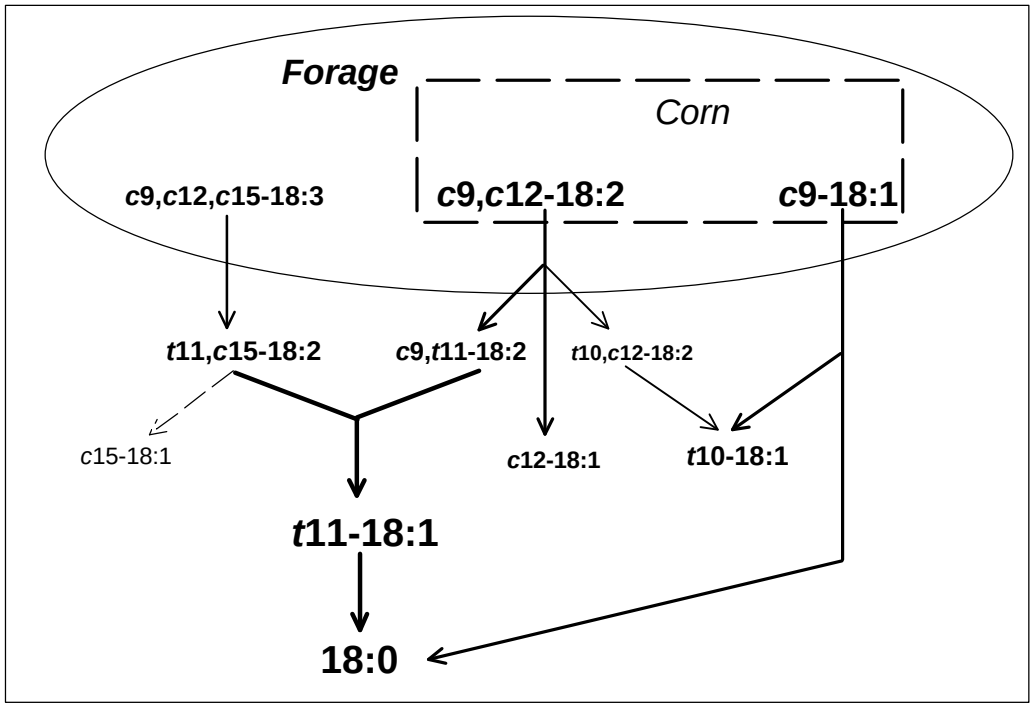


Figure 1.1. Major pathways for hydrogenation of dietary *c*9-18:1, *c*9,*c*12-18:2, and *c*9,*c*12,*c*15-18:3. Forages were fed (50 g DM/d) alone, or in combination with 0, 8, or 16 g DM from corn grain replacing equal portions of forage. Bold typeface and large font were used to denote primary substrates and products during fermentation. Dashed or thin lines and small font indicate secondary pathways.

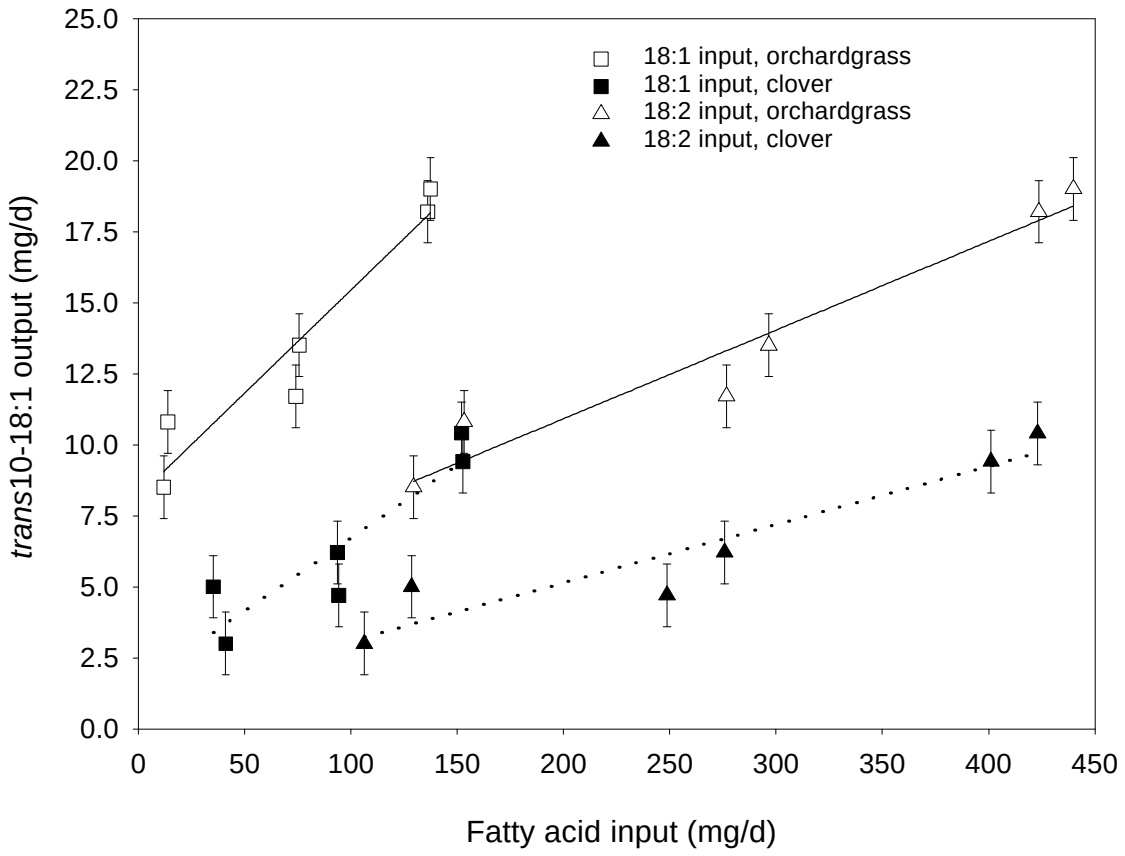


Figure 1.2. Output of *trans*10-18:1 in response to input of *cis*9-18:1 and 18:2n6. Regressions for *cis*9-18:1 input versus *trans*10-18:1 output were: 8.2 + 0.007 (*cis*9-18:1 input) for orchardgrass and 1.6 + 0.05 (*cis*9-18:1 input) for red clover. Regressions for 18:2n6 input versus *trans*10-18:1 output were: 4.7 + 0.03 (18:2n6 input) for orchardgrass and 1.04 + 0.02 (18:2n6 input) for red clover. All regressions were significant ($P < 0.05$).

CHAPTER 2

*Trans*18:1 and 18:2 Isomers in Blood Plasma and Milk Fat of Grazing Cows Fed a Grain Supplement Containing Solvent-Extracted or Mechanically-Extracted Soybean Meal.

ABSTRACT

Thirty Holstein cows grazing mixed clover-grass pastures were fed a grain supplement containing solvent-extracted soybean meal (SES, 15 mg 18:2n6/g of DM), or mechanically-extracted soybean meal (MES, 24 mg 18:2n6/g of DM, SoyPlus®) to determine if dietary 18:2n6 intake influenced ruminal hydrogenation of unsaturated fatty acids. Cows were managed as one group in an intensive rotational grazing system from May through July, but groups of 10 cows each were group-fed their assigned grain supplement after each milking. The daily allotment of supplement (6.7 kg) contained ground corn plus 1.7 kg SES, 1.9 kg MES, or 1.9 kg MES plus 30 g liquid methionine hydroxy analog (Alimet ®) (MESM). Total fatty acid content (% of DM in grass and clover) of pasture was greater in May (2.2 and 1.6%), but decreased through June (1.9 and 1.5%) and July (1.7 and 1.5%). Concentration of 18:3n3 was higher in grass compared with clover (532 vs. 454 mg/g total fatty acids). Estimated intake of 18:2n6 from pasture plus grain supplements was higher when MES or MESM (205 g/d) was fed compared with SES (144 g/d). Milk yield was not affected by treatment and averaged 32 kg/d. Concentrations and yields of milk components also were not affected by supplements. Total blood plasma fatty acids on wk 4 were higher due to MESM (2.0 mg/mL) compared with MES (1.1 mg/mL), or SES (1.1 mg/mL), but cows fed MESM or MES had greater concentrations of 18:2n6, *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 compared with cows fed SES. The additional amount of *trans*11-18:1 was found exclusively in plasma triglycerides. In contrast, the additional amount of *cis*9,*trans*11-18:2 was found in phospholipids and free fatty acids. Daily yields of 18:0, *cis*9-18:1, 18:2n6, and 18:3n3 in milk fat were 16, 32, 6, and 1 g/d, respectively, greater for cows fed MES or MESM compared with SES. Similarly, MES or MESM increased daily yields of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat by 8 and 4 g/d compared with SES. Yields of *trans*10,*cis*12-18:2 and *trans*11,*cis*15-18:2, however, were not

affected by treatment. Cows fed grain supplement plus methionine (MESM) had elevated concentrations of most fatty acids in blood plasma. Yields of individual fatty acids in milk fat, however, were not affected by supplemental methionine. Results indicated the yield of *trans*11-18:1, *cis*9,*trans*11-18:2, 18:2n6, and 18:3n3 in milk fat of pasture-fed cows can be enhanced by feeding mechanically-extracted, rather than solvent-extracted soybean meal.

INTRODUCTION

Milk fat is the richest natural dietary source of the *cis*9,*trans*11 isomer of conjugated linoleic acid (CLA), which is considered to be a potent anticarcinogen (Ip et al., 1999). In the rumen, *cis*9,*trans*11-18:2 results primarily from isomerization of dietary 18:2n6 during the first step of the biohydrogenation process (Kepler and Tove, 1967). Subsequent reductions of the double bonds at carbons 9 and 11 yield *trans*11-18:1 and 18:0, respectively, as major products (Polan et al., 1964). Dietary 18:3n3 also undergoes biohydrogenation by being first isomerized to a conjugated triene (*cis*9,*trans*11,*cis*15-18:3), followed by reductions of the double bonds at carbons 9, 15, and 11 to yield *trans*11,*cis*15-18:2, *trans*11-18:1, and 18:0, respectively (Wilde and Dawson, 1966). Biohydrogenation of 18:2n6 or 18:3n3 also can result in variable production of *trans*18:1 isomers (with double bonds at positions 9, 10, and 12) and *cis*18:1 isomers (with double bonds at positions 9, 12, and 15) (Kemp et al., 1975).

In pasture grasses and legumes, 18:2n6 or 18:3n3 account for 17 and 60% of total fatty acids (Hawke, 1973). Milk from grazing cows contains more *trans*-fatty acids than milk from cows fed a TMR (Jahreis et al., 1997). *Trans*11-18:1 accounted for 55% of total *trans*-18:1 isomers in milk fat from grazing cows compared with 33% in milk from cows fed a TMR (Precht and Molkentin, 1997). Concentrations of *trans*18:1 isomers with double bonds at other positions (4 through 10 and 12 through 16), however, did not differ due to diet. Concentration of *cis*9,*trans*11-18:2 in milk from grazing cows also was greater compared with milk from cows fed a TMR (Kelly et al., 1998b). However, supplementing a TMR with unsaturated oils containing primarily 18:2n6, rather than 18:3n3 or *cis*9-18:1 also elevated concentration of *cis*9,*trans*11-18:2 in milk (Kelly et al., 1998a).

Supplementing daily pasture intake with full-fat soybeans or rapeseed increased *cis*9,*trans*11-18:2 in milk fat (Lawless et al., 1998), indicating addition of 18:2n6 in the diet will yield more *cis*9,*trans*11-18:2 in milk fat. Oil seeds are typically processed to remove the oil, but the method used to process oilseeds for lactating cows may influence *cis*9,*trans*11-18:2 content in milk fat. For example, feeding extruded cottonseed or soybeans increased *cis*9,*trans*11-18:2 concentration in milk fat 100%

compared with controls (Dhiman et al., 1999a). Lipid content of mechanically-extracted soybean meal (SoyPlus®) is nearly 3-fold greater than that of solvent-extracted soybean meal, thus it can provide more 18:2n6 for biohydrogenation in the rumen and may alter fatty acid composition of milk fat.

Methionine and lysine were considered the most limiting amino acids for milk production in dairy cows grazing high-quality pastures, because extensive degradation of ingested pasture protein may limit the supply of protein and amino acids to the small intestine (Beever and Siddons, 1986). When cows were fed equal amounts of ryegrass and white clover pasture, amino acid-N flow to the duodenum was higher compared with feeding fresh ryegrass alone (Kolver et al., 1999). However, methionine flow was only 71% of its absorbable requirement and may have limited milk production. Methionine is essential for synthesis and secretion of milk proteins and deficiency reduced β-casein mRNA (Wu et al., 1999). In addition to serving as a methyl donor for methylation and synthesis of DNA, methionine is required for synthesis of membrane phospholipids and plasma lipoproteins (Cantoni, 1975). Methionine also influences lipid metabolism in the mammary gland, as indicated by higher milk fat percentage (Polan et al., 1970; Huber et al., 1984) and concentrations of *cis*9-16:1, 18:0, or *cis*9-18:1 in milk fat (Canale et al., 1990; Sevi et al., 1998) when lactating cows were fed rumen-protected methionine.

A supplemental source of dietary energy and RUP may be necessary to meet metabolizable energy and protein requirements of high-producing grazing cows. Hypothetically, a supplement containing corn grain combined with mechanically-extracted soybean meal (RUP = 60% of CP) should meet the dietary requirements of a grazing cow more closely than a supplement containing corn grain plus solvent-extracted soybean meal (RUP = 43% of CP). The methionine content of soybean meal, however, may be limiting (Casper et al., 1987). Thus, addition of rumen-protected methionine to soybean meal may benefit the high producing grazing cow. The objectives of this study were to evaluate the above hypothesis with respect to 1) milk production and component yields, 2) changes in concentrations of *cis* and *trans* isomers of 18:1 and *cis*,*trans* isomers of 18:2 in blood plasma and their yields in milk fat.

MATERIALS AND METHODS

Grazing Management and Experimental Design

An 11 ha mixed-pasture field at the Virginia Tech Dairy Center was subdivided into four paddocks of approximately 2.7 ha each, and used in an intensive rotation system. The predominant species in swards were orchardgrass (*Dactylis glomerata*), white clover (*Trifolium repens*), and red clover (*Trifolium pratense*). The proportions of Kentucky bluegrass (*Poa pratensis*) were small compared with the above species. The relative proportions of major species varied across paddocks and throughout the grazing season. During grazing, an electrified nylon string separated grazing areas within a paddock. Water was available at all times in pasture fields. Cows grazed between 0400 and 1000 h and 1500 and 2200 h. At other times, cows were kept in a dirt lot with access to orchardgrass hay and water for ad-libitum consumption. Stubble remaining in pasture fields after a grazing session was either clipped or grazed by dry cows.

Thirty lactating Holstein cows between 17 and 119 DIM were used for a 12- wk study from May through July 1998. Cows were initially grouped and fed a TMR for 2 wk. To obtain an estimate of group-fed DMI prior to grazing, the amounts of feed offered and refused during the last 4 d were recorded. Cows then were adapted to grazing for 7 d (wk 0) by allowing them access to pasture fields and the TMR while in confinement for equal periods of time each day. At the end of the adjustment period, ten cows were randomly assigned to one of three groups on the basis of milk production. Each group was offered 6.7 kg/d (DM basis) of a supplement (Table 2.1) containing corn grain combined with solvent-extracted soybean meal (SES), mechanically-extracted soybean meal (SoyPlus®, West Central Cooperative, Ralston, IA) (MES), or mechanically-extracted soybean meal plus 30 g liquid methionine hydroxy analog (Alimet ®, Novus® Intl., Inc., St. Louis, MO) (MESM). Cows were group-fed their assigned supplement in equal proportions after milking at 0100 and 1300 h. Supplement refusals were negligible.

Sample Collection and Analyses

Milk production was recorded electronically at each milking throughout the study. At 2-wk intervals from 0 through 12 wk, a 30 mL aliquot of milk was collected in a vial containing Bromopol (D & F Control Systems, San Ramon, CA) at 1300 h. Milk was analyzed for fat, protein, lactose, and SNF content by infrared analysis with a 4-channel spectrophotometer (Virginia Dairy Herd Improvement Association). At 4-wk intervals (wk 0, 4, 8, and 12), an additional aliquot of milk was collected without Bromopol, then stored at -20 °C. Subsequently, samples were thawed at room temperature and centrifuged at 10,000 × *g* for 1 h to isolate milk fat for fatty acid analysis.

Throughout the study, a minimum of six samples of grass and clover from a paddock were collected by tracing a diagonal transect across the area available prior to grazing. Quadrats (0.25 m^2 each) were used to obtain the samples, leaving a stubble of ~5 cm. Grass and clover were separated, weighed, and dried in a forced-air oven at 55 °C until constant weight. A sample of each supplement mixture was collected after mixing each batch (weekly basis), then oven-dried at 55 °C. Dried samples were stored in sealed plastic containers. Equal amounts of dried grass or clover were combined according to paddock to determine chemical composition and fatty acid profiles. After compositing by paddock, there were five samples of each forage from May, June, and July for analyses (Table 2.2). Dried forages and supplements were ground through a 2 mm screen (Thomas-Wiley Laboratory Mill), then through a 1 mm screen (Cyclotec mill, Tecator 1093, Hoganas, Sweden) prior to analyses for ADF and NDF (Van Soest et al., 1991), total N (AOAC, 1990), and fatty acids. Net energy (NE_L) content of each supplement was estimated with individual values for ground corn and solvent-extracted soybean meal (NRC, 1989). For mechanically-extracted soybean meal, NE_L values used were those specified by the manufacturer. The RUP content of each supplement also was estimated as outlined for NE_L.

Pasture intake (Table 2.3) was estimated (Dhiman et al., 1999a) using the equation: kg DM/d = [(Mcal milk energy output/d + Mcal energy spent for maintenance and production/d [NRC, 1989]) - Mcal NE_L intake from supplement/d]/Mcal NE_L in mixed pastures/kg DM. The energy requirements for maintenance and production were corrected for the loss (wk 0 through 4) or gain (wk 4 through 12) of body weight. Milk

energy output was estimated using the equation of Tyrrell and Reid (1965). Body weights used to determine energy requirements for maintenance and production (NRC, 1989) were determined at 0, 4, 8, and 12 wk. The NE_L content available in mixed pastures was estimated as described by Undersander et al. (1993): NE_L (Mcal/kg DM) = 1.088 - 0.013 $\times$ ADF, where ADF = concentration of ADF in mixed pastures.

Intakes of chemical components from pastures were estimated using the concentrations of NDF, ADF, CP, and fatty acids from grass and clover samples collected during 2 d prior to milk sample collection on wk 4, 8, and 12. The relative proportions of grass and clover in pastures were determined based on dry weights of each sample in the pasture and used to calculate the overall chemical composition in mixed pastures. It was assumed that each cow consumed 6.7 kg supplement daily. The concentrations of NDF, ADF, CP, and fatty acids in each supplement were used to estimate their intake. Total intake of chemical components represented the sum of their intakes from pasture plus the supplement.

Blood samples (10 mL) were obtained during wk 0 and 4 from the jugular vein. After collection, blood was transferred to tubes containing 286 IU heparin in 100 µL of sterile saline and centrifuged at 3,000 $\times$ *g* for 15 min for harvesting plasma. Lipids were extracted from plasma (2 mL), herbage, and supplements with chloroform/methanol (2:1, vol/vol). Blood plasma lipid fractions (cholesterol esters, phospholipids, triglycerides, and free fatty acids) were isolated (Kaluzny et al., 1985) from lipid extracts using Bond Elut® aminopropyl disposable columns (Analytichem International, Harbor City, CA).

Fatty acids in milk fat and lipid extracts from blood plasma, plasma lipid fractions, herbage, and supplements were directly methylated using 0.5 N NaOH in methanol (Park and Goins, 1994). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters of fatty acids in blood plasma lipid fractions were separated using a 30 m × 0.25 mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellefonte, PA). Methyl esters in the remaining samples were separated using a

100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88,Chrompack, Middelburg, The Netherlands).

For milk, herbage, and supplement fatty acid analysis (1μL methyl esters in hexane injected at a 100:1 split ratio), the injector and detector temperatures were maintained at 255 °C. The initial column temperature was held at 175 °C for 42 min, increased 20 °C/min to 215 °C (held for 10 min), then increased at 10 °C/min to a final temperature of 225 °C (held for 11 min). Hydrogen was the carrier gas.

Analysis of total fatty acids in blood plasma required injection of 2 μL methyl esters (splitless). The injector temperature was maintained at 150 °C and the detector temperature at 255 °C. The purge valve on the GC was closed for 1.5 min after sample injection. The initial column temperature was 40 °C (held for 1.5 min), then increased at 40 °C/min to 100 °C (held for 10 min), 25 °C/min to 175 °C (held for 70 min), and 10 °C/min to a final temperature of 220 °C (held for 20 min). Ultra pure helium was the carrier gas.

Analysis of fatty acids in plasma lipid fractions required injection of 2 μL methyl esters in hexane (30:1 split ratio). The injector temperature was maintained at 225 °C and the detector temperature at 275 °C. The initial column temperature was 205 °C (held for 12 min) and was programmed to increase 2 °C/ min to a final temperature of 220 °C (held for 2 min).

Statistical Analysis

Data are reported as Least squares means ± SEM. Forage chemical composition, milk production and composition, intake, and milk fatty acid data were analyzed using the MIXED procedure of SAS with repeated measures (2000). Plasma fatty acid data were analyzed using the MIXED procedure of SAS without repeated measures. Observations at wk 0 served as covariate for observations at wk 2, 4, 6, 8, and 12. The model for statistical analysis of body weight, DMI, milk yield, milk component percentages and yields, and milk fatty acid yields included: covariate adjustment, supplement (SES, MES, or MESM), week, supplement by week interaction, cow within supplement, and residual error. The model for statistical analysis of forage

chemical composition included: forage species, date of collection in each month, species by date interaction, paddock, and residual error. The model for statistical analysis of blood plasma fatty acids included: covariate adjustment, supplement (SES, MES, or MESM), cow within supplement, and residual error. For all data, except forage chemical composition, orthogonal contrasts were used to determine differences due to supplements. Differences were designated as significant at $P \leq 0.05$. However, all P values are presented in tables. The orthogonal contrast presented in tables and figures are MES + MESM versus SES, and MES versus MESM.

RESULTS AND DISCUSSION

Chemical Composition of Pastures and Supplements

Supplements containing mechanically-extracted soybean meal (MES or MESM), compared with solvent-extracted soybean meal (SES), had higher RUP concentration, total fatty acid content, and provided more 18:2n6 and 18:3n3 (Table 2.1). Mechanical extraction (expeller extraction) of soybeans (SoyPlus®) involves heating of cracked dried beans and extraction of oil inside expeller presses due to pressure created by a central revolving mechanism (Shaver, 1999). Solvent extraction also involves heating of soybeans but at lower temperatures for smaller periods of time, and the oil is removed by extraction with hexane. Thus, the resulting meal from the expeller process has a higher RUP and oil content compared with solvent-extracted meals.

Chemical composition of grass and clover in mixed pastures differed (Table 2.2). Grass had greater concentrations of NDF, ADF, and total fatty acids compared with clover, but CP concentration was higher in clover. Values for NDF, ADF, and CP, were in agreement with results obtained during previous grazing studies conducted by our laboratory (Polan, 1997). Although concentrations of NDF, ADF, and CP did not change between May and July, total fatty acid content decreased from May through July regardless of species. Despite this gradual decrease, total fatty acid, 14:0, and 18:3n3 content of grasses was always higher compared with clover. Clover, however, contained more 16:0, 18:0, *cis*9-18:1, and 18:2n6 compared with grass. As grazing progressed from May through July, the concentrations of saturated fatty acids and

18:2n6 increased, but 18:3n3 concentration decreased regardless of species. In previous studies, total concentration and profiles of fatty acids in grasses and clover during grazing were shown to change from spring through fall. Invariably, total fatty acid content of pasture species was highest during the primary growth stage (spring), decreased steadily through the stemmy regrowth period, and increased sharply during the next leafy regrowth stage (fall) (Hawke, 1973; Bauchart et al., 1984; Dewhurst et al., 2001). At any stage of growth, however, grasses contained more fatty acids than clovers (Hawke, 1973). Concentrations of saturated fatty acids with 14 to 18-carbons increased, but 18:3n3 concentration decreased during growth. However, 18:2n6 concentration did not change appreciably.

Monogalactolipids are the major lipids in forages, and are primarily found in chloroplasts of leaf tissue (Hawke, 1973). Due to greater chloroplast concentration, green leaves from immature growing forage contains a higher lipid concentration compared with green leaves from mature forage (Hawke, 1973). The apparent decrease in lipid content is due to an increase in fiber content in leaves from mature plants. Environmental temperature also affects forage lipid content and 18:3n3 concentration, both being higher at 15 to 20 °C compared with 30 °C (Hawke, 1973). It has been suggested that environmental light intensity is an important determinant of unsaturated fatty acid synthesis in forages. However, it is difficult to differentiate between changes due to maturity versus light intensity as plants age (Hawke, 1973).

Intake of Chemical Components

Although average body weights appeared similar during the preliminary period (TMR) and the 12-wk grazing period (Table 2.3), cows lost an average of 15 kg between wk 0 and wk 4, then gained an average of 8 kg from wk 4 through 12 (data not shown). Despite the similar NE_L content of good quality pastures and typical TMR, grazing cows mobilize more adipose tissue to meet their energy requirements, because they can not consume enough forage DM (Polan, 1997; Holden et al., 1994). However, after a period of adaptation to grazing and supplementation with grain or preserved forages, cows gradually gain weight and improve body condition score (Polan, 1997; Holden et al., 1994).

Overall, calculated pasture intake (Table 2.3) during the study was not affected by supplements and averaged 10 kg/d. However, calculated pasture intake was highest at wk 4 and declined by wk 8 (Figure 2.1). Rainfall during May sustained high rates of pasture growth, but as the study progressed limited rainfall eventually compromised normal growth of the pastures. Thus, higher intake of pasture at wk 4 compared with wk 8 or 12 appeared to be consistent with availability of forage DM in our paddocks. Due primarily to a fixed intake of supplement (6.7 kg/d), overall averages for total DMI and intakes of NDF, ADF, or CP did not differ during grazing periods (Table 2.3). Compared with the preliminary period, however, estimated DMI during grazing averaged 8 kg/d lower. Due to the greater total fatty acid content of mechanically-extracted soybean meal, MES or MESM increased total fatty acid intake by 26% compared with feeding SES. In addition, intakes of 16:0, *cis*9-18:1, 18:2n6, and 18:3n3 were 12, 3, 61, and 15 g/d, respectively, greater when MES or MESM were fed compared with SES.

Grain supplements were the primary source of *cis*9-18:1 and 18:2n6 during grazing. Thus, intake of *cis*9-18:1 and 18:2n6 was relatively constant during the grazing period. In contrast, pasture was the primary source of 18:3n3. Calculated intake of 18:3n3 declined from 135 g/d at wk 4 to 70 g/d at wk 12 due to declining pasture intake.

Milk Production, Composition, and Component Yields

Cows averaged 47 kg milk/d during the preliminary period, but during grazing milk production declined steadily through wk 8, then appeared to level off until wk 12 (Figure 2.1). Overall, there was a tendency ($P = 0.08$) for cows fed MES or MESM to produce approximately 2 kg more milk per day than cows fed SES (Table 2.4). Percentages and yields of milk components, except SNF, did not differ due to treatments. The greater yield of SNF by cows fed MES or MESM was due to non-significant ($P = 0.07$) increases in protein and lactose yield.

Relative to published amino acid requirements, methionine and lysine (in this order) were identified as the most limiting amino acids for milk production by cows grazing mixed pastures (Kolver et al., 1998; Pacheco-Rios et al., 1998; Wu et al., 1997). Leucine, arginine, and histidine also were found to be co-limiting. Methionine deficiency

is not surprising, because its concentration in rumen microbial protein and in most feed proteins is low. According to the Cornell-Penn-Miner model (data not shown), addition of methionine hydroxy analog to MES in this study should have alleviated the methionine deficiency of the diet, but lysine then became the most limiting amino acid. Estimated N intake (CP intake $\times$ 0.16) averaged 616 g/d for MES or MESM and 560 g/d for SES-supplemented cows. However, the N contribution from RUP, including methionine hydroxy analog in MESM, was 50 g/d higher for cows fed MES or MESM compared with SES (138 vs 89 g/d). The lack of response to supplemental methionine (MESM) suggests that lysine or other amino acids were limiting or affecting the physiological utilization of methionine for milk production. Also energy intake was shown to be the limiting factor preventing greater milk production in high-yielding grazing cows fed high versus low supplemental RUP (Hongerholt and Muller, 1998).

Plasma Fatty Acid Profiles

Fatty acid concentrations in isolated blood plasma phospholipids on wk 4 of the study were greater for cows fed MES or MESM compared with SES (Table 2.5). Concentrations of fatty acids in other plasma lipid fractions and the sum of all fatty acids recovered in lipid fractions, however, did not differ due to treatment. When fatty acids in blood plasma were analyzed without isolating lipid fractions (as noted above), the sum of all fatty acids (1,013 μg/L in Table 2.6) was numerically greater than the sum of all fatty acids in the lipid fractions (647 μg/L in Table 2.5). A common problem with use of solid-phase extraction columns for separation of lipid fractions has been overloading of column with lipid (Kaluzny et al., 1985). If the amount of total blood lipid applied to the solid-phase columns were too high, recovery of phospholipids and cholesterol esters most likely would have been incomplete and underestimated in Table 2.5. In the event that recovery of the total amount of a fraction was low, the distribution of fatty acids in that fraction should still be valid (Agren et al., 1992) (Figure 2.2).

Direct analysis of fatty acids in plasma (without isolating lipid fractions) provides a valid quantification of individual fatty acids and the sum of all fatty acids in blood (Table 2.6). Supplemental methionine hydroxy analog apparently increased the amount of nearly all fatty acids in plasma. Methionine hydroxy analog (0.2% of concentrate DM)

increased plasma triglyceride concentrations in lactating cows (Huber et al., 1984). When rats were fed a soybean protein-based diet supplemented with methionine (0 to 8% of diet DM), plasma VLDL, cholesterol ester, and phospholipid concentrations increased linearly (Sugiyama et al., 1998). Because methionine promotes the synthesis of phosphatidyl choline, which is essential for hepatic VLDL synthesis and secretion (Gruffat et al., 1996), it could be possible that such an effect led to higher total circulating plasma fatty acids in cows fed MESM. Despite the slow rate of VLDL export from the lactating bovine liver, intravenous infusion of methionine plus lysine increased net hepatic VLDL output (Durand et al., 1992).

Despite the greater amounts of fatty acids in plasma of cows fed MESM, comparison of responses to solvent-extracted versus mechanically-extracted soybean meal can be made on the basis of fatty acid proportions in blood plasma. The following discussion focuses on plasma fatty acid profiles in terms of proportions. Fatty acid proportions can be calculated from data in Table 2.6.

The profiles of biohydrogenation intermediates in blood plasma were affected by differences in the 18:2n6 or 18:3n3 content of the diets. Compared with SES, feeding MES or MESM increased the proportions of *trans*11-18:1 and *cis*9,*trans*11-18:2 in blood plasma by 63 and 69%, respectively. Changes in the concentrations of these hydrogenation intermediates in plasma fatty acids due to supplemental 18:2n6 (MES or MESM), corresponded with alterations in ruminal biohydrogenation when 18:2n6 availability increases (Loor et al., unpublished). Previous (Chapter 1) results also indicated that the efficiency of apparent hydrogenation of 18:2n6 decreased as the level of 18:2n6 supplementation to pasture diets increased. As a result, production of *trans*11-18:1, *cis*9,*trans*11-18:2, and 18:2n6 in rumen fermenters was enhanced.

The higher proportions of *trans*11-18:1, *cis*9,*trans*11-18:1, and 18:2n6 in plasma due to feeding MES or MESM, were also apparent in blood plasma lipid fractions (Figure 2.2). Concentration of *trans*11-18:1 was elevated in triglycerides, whereas *cis*9,*trans*11-18:1 increased in phospholipids and free fatty acids. Concentrations of 18:2n6 in phospholipids, triglycerides, and cholesterol esters were elevated in response to MES or MESM. Under basal conditions, 18:2n6 and 18:3n3 in blood plasma of ruminants are selectively incorporated into plasma cholesterol esters and phospholipids

because they are the major substrates for lecithin:cholesterol acyl transferase (Noble et al., 1969; Noble et al., 1972). Concentrations of dietary or ruminally-derived fatty acids in the major plasma lipid fractions are directly proportional to the amounts of fatty acids absorbed from the small intestine (Christie, 1981). In this study, concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 in lipid fractions after 4 wk of grazing were substantially increased compared with concentrations during the preliminary period (TMR), despite lower total fatty acid intake (Table 2.3) during grazing. Thus, plasma fatty acid profiles provided evidence of incomplete hydrogenation of unsaturated fatty acids in pasture.

The 2.5-fold greater concentration (Table 2.6) and proportion of *trans*11,*cis*15-18:2 in plasma during grazing, compared with the preliminary TMR, indicated hydrogenation of 18:3n3 in pasture was not complete. As shown previously (Chapter 1), lower apparent hydrogenation of 18:3n3 was favorable for the production of this isomer. As indicated below, plasma profiles of hydrogenation intermediates may be a good indicator of their yields in milk fat.

Milk Fatty Acid Yields

Feeding MES or MESM compared with SES did not affect the yield of total milk fatty acids, yields of individual saturated 6:0 to 16:0, or the sum of 6:0-16:0 (Table 2.7). Saturated fatty acids with 6 to 16 carbons can be synthesized *de novo* in the mammary gland, and accounted for approximately half of the total fatty acid yield. Fatty acids with 6 to 16 carbons also accounted for approximately 50% of total fatty acid yield in milk during the preliminary period (TMR). It has been suggested that during the transition from a TMR to grazing, release of fatty acids (endogenous) from adipose tissue due to lower dietary energy intake, results in a lower rate of *de novo* fatty acid synthesis in mammary tissue (Palmquist et al., 1993). After the transition to pasture, the concentration or yield of 6- to 16-carbon fatty acids in milk fat did not differ for cows consuming pasture alone or pasture plus various supplement mixtures (Mackle et al., 1997; Dhiman et al., 1999a). A portion of the 16:0 in milk fat is derived from the diet. Despite greater intake of 16:0 by cows fed MES or MESM compared with SES (Table 2.3), 16:0 yield in milk fat did not differ. The remainder of the 16:0 in milk fat is from *de*

novo synthesis, thus 16:0 yield in milk fat should not be expected to be a reflection of 16:0 intake. For example, grazing cows fed a supplement containing rapeseed (61% *cis*9-18:1) had a lower yield of 16:0 in milk fat (Lawless et al., 1998). The lower yield was associated with increased amounts of *cis*9,*trans*11-18:2 and *trans*11-18:1 in milk fat.

Overall yields of 18:0, *cis*9-18:1, 18:2n6, and 18:3n3 during the 12-wk study were 16, 18, 25, and 20%, respectively, greater in response to MES or MESM compared with SES. Despite extensive desaturation of 18:0 to *cis*9-18:1 in the mammary gland (Enjalbert et al., 1998), higher yields of 18:0 in milk fat during grazing can be expected because it is a major end product of hydrogenation of 18:2n6 or 18:3n3 (Chapter 1). Hydrogenation of 18:2n6 and 18:3n3 in fresh forage increased the output of 18:0, making it the primary fatty acid produced during ruminal fermentation (Chapter 1). Supplementing a pasture diet with corn grain provides additional *cis*9-18:1 and 18:2n6, which might further increase the outflow of 18:0, *cis*9-18:1, and 18:2n6 from the rumen (Chapter 1). The amount of *cis*9-18:1, 18:2n6, and 18:3n3 escaping hydrogenation in the rumen increased as the efficiency of hydrogenation decreased (Chapter 1). During the 12-wk of grazing in this study, intake of 18:2n6 and 18:3n3 was greater for cows fed MES or MESM compared with SES (Table 2.3).

Yields of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in milk fat gradually increased from wk 0 through 8 (Figure 2.3). Their elevated concentrations in blood plasma (Table 2.6) and milk fat (data not shown), confirmed that pasture intake was a major factor driving this increase. These fatty acids are the major intermediates in 18:2n6 and 18:3n3 hydrogenation in the rumen (Wilde and Dawson, 1966; Kepler and Tove, 1967), especially when fresh forage is fed. Outputs of *trans*11-18:1 and *trans*11,*cis*15-18:2 accounted for 64 and 71% of total *trans*-18:1 and isolated 18:2 isomers during digestion of 18:2n6 and 18:3n3 from fresh forage in continuous culture fermenters (Chapter 1).

Greater intake of 18:2n6 by cows fed MES or MESM increased the yield of *trans*11-18:1 by 23% compared with feeding SES (Table 2.7). Feeding MES or MESM also increased *trans*6/7/8-18:1 yield by 17% compared with feeding SES, but decreased *trans*13/14-18:1 yield by 13%. Accumulation of *trans*-18:1 isomers in the rumen during

hydrogenation of pasture lipids is a typical response (Singh and Hawke, 1979). Due to the inability of the capillary column to separate the *trans*-6, -7, -8, -13, and -14 isomers, however, changes in the yields of the individual isomers could not be determined. Overall, the proportions of individual *trans*-18:1 isomers in milk fat were similar to previous reports with grazing cows supplemented with grain mixtures or oilseeds (Precht and Molkentin, 1997; Lawless et al., 1998; Jahreis et al., 1997), and confirmed that *trans*11-18:1 is the major isomer resulting from hydrogenation of 18:2n6 or 18:3n3 in pastures or grain supplements (Chapter 1).

The greater 18:2n6 intake from MES or MESM also increased yields of *cis*9,*trans*11-18:2 and *cis*9,*trans*12-18:2 in milk fat (Table 2.7). A portion of the additional *cis*9,*trans*12-18:2 in milk fat was likely a result of isomerization of 18:2n6 during fermentation (Kemp et al., 1984b). Higher yields of *cis*9,*trans*11-18:2 also were found when grazing cows were supplemented with various levels of grain or oilseed supplements (Kelly et al., 1998b; Dhiman et al., 1999a; Lawless et al., 1998). Furthermore, *cis*9,*trans*11-18:2 concentration and yield in milk fat was up to 1.5-fold greater as cows derived increasing portions of their daily DMI from pasture (Dhiman et al., 1999a).

The major conjugated intermediate arising from isomerization of dietary 18:2n6 during the first step of the biohydrogenation process is *cis*9,*trans*11-18:2 (Kepler and Tove, 1967). During digestion of fresh forage in continuous cultures, however, it accounted for only 9 to 22% of conjugated 18:2 isomer output or 0.07 to 0.21% of total fatty acid output (Chapter 1).

Rumen-derived *cis*9,*trans*11-18:2 undoubtedly contributes to the pool of *cis*9,*trans*11-18:2 secreted in milk fat. In a recent study, however, it was estimated that 64% of the *cis*9,*trans*11-18:2 in milk fat is synthesized endogenously from *trans*11-18:1 via Δ^9 desaturase (Griinari et al., 2000). Based on our previous findings, flow of *trans*11-18:1 to the duodenum of pasture-fed cows could account for 15 to 22% of total fatty acid flow (Chapter 1). Given the lower ratios of *cis*9,*trans*11-18:2 to *trans*11-18:1 in rumen fermenter effluent (1:300 to 1:80) compared with plasma (1:5) or milk fat (1:4 to 1:3) in the present study, it would appear that a significant portion of rumen-derived *trans*11-18:1 in grazing cows is desaturated to *cis*9,*trans*11-18:2. A portion of the

*trans*11-18:1 may be desaturated in the enterocyte during absorption (Bickerstaffe and Annison, 1969), but the remainder may be desaturated in adipose tissue or the mammary gland. Activity of Δ^9 desaturase in adipose tissue of grazing beef cattle was 73% higher compared with feedlot cattle (Yang et al., 1999). If mammary Δ^9 desaturase activity resembles that of adipose tissue, *trans*11-18:1 may indeed be the major source of *cis*9,*trans*11-18:2 in milk fat of grazing cows.

On the basis of their profiles in rumen fluid and milk fat, it is thought that conjugated 18:2 isomers other than *cis*9,*trans*11-18:2 arise via several microbial *cis*,*trans* isomerases (Griinari and Bauman, 1999). It follows then, that a *cis*9,*trans*10 isomerase can isomerize 18:2n6 to *trans*10,*cis*12-18:2 (Griinari and Bauman, 1999). Low rumen pH and availability of 18:2n6 appear to be the major factors responsible for the synthesis of this isomer (Piperova et al., 2000; Chapter 1). Although not affected by the grain supplements fed in this study, the yield of *trans*10,*cis*12-18:2 in milk fat increased gradually from wk 0 through wk 12, when it was 5-fold greater (data not shown). Thus, it appears that rumen conditions for the production of this isomer became more favorable as the study progressed. The major factors contributing to the greater yield of *trans*10,*cis*12-18:2 could have been the constant supply of 18:2n6 and starch from supplements, because they represented a greater portion of total DMI over time due to the gradual decrease in pasture intake (Figure 2.1). Corn input (0 to 32% of DM) with orchardgrass or red clover increased the output of *trans*10,*cis*12-18:2 in fermenter effluents by 187% compared with inputs of forages alone (Chapter 1). Certain propionogenic bacteria can isomerize 18:2n6 to *trans*10,*cis*12-18:2 (Verhulst et al., 1987), and their proportions in the rumen of grazing cows supplemented with grain mixtures increased in proportion with the level of supplementation (Elias et al., 1996).

Compared with feeding a TMR during the preliminary period, grazing caused a 2.5-fold increase in *trans*11,*cis*15-18:2 yield regardless of supplement. A similar increase in concentration was observed in milk fat from grazing cows compared with cows fed preserved forages (Precht and Molkentin, 2000). We previously confirmed that incomplete hydrogenation of 18:3n3 from orchardgrass or red clover in rumen fermenters resulted in production of *trans*11,*cis*15-18:2, which could account for up to 15% of total fatty acid ouflow from the rumen (Chapter 1).

CONCLUSIONS

Cows grazing mixed pastures, especially in the spring, have a greater intake of 18:3n3 compared with cows fed a TMR. Incomplete ruminal hydrogenation of 18:2n6 and 18:3n3 during grazing, however, leads to greater concentrations of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in blood plasma and greater yields in milk fat. Yields of *cis*9-18:1, *trans*11-18:1, 18:2n6, *cis*9,*trans*11-18:2, *trans*11,*cis*15-18:2, and 18:3n3 in milk fat of grazing cows can be increased by feeding mechanically-extracted, rather than solvent-extracted, soybean meal as part of a grain supplement.

Table 2.1. Composition of supplements [1].

	SES	MES	MESM
Ingredients		% of DM	
Ground corn [2]	66.9	60.8	60.5
Soybean meal, 48%CP [3]	25.0	0.0	0.0
SoyPlus [4]	0.0	31.1	31.1
Mineral/vitamin mix [5]	6.6	6.7	6.7
$NaHCO_3$	1.5	1.4	1.2
Alimet [6]	0.0	0.0	0.5
Chemical composition			
CP, % of DM	19.2	21.1	21.4
RUP, % of CP	43.1	60.2	60.8
NE_L, Mcal/kg DM	1.95	2.01	2.01
Total fatty acids, % of DM	2.5	3.6	3.6
		mg/g of total fatty acids	
16:0	105	109	109
18:0	47	28	28
*cis*9-18:1	214	159	159
18:2n6	612	660	660
18:3n3	22	43	43

[1] Twelve samples (collected weekly) of each supplement were composited and analyzed in duplicate.
[2] Ground corn: CP = 100 g/kg, RUP = 600 g/kg CP, fatty acids = 34 g/kg, and NE_L = 1.96 Mcal/kg.
[3] Solvent extracted soybean meal: CP = 500, RUP = 340 g/kg CP, fatty acids = 13 g/kg, and NE_L = 1.93 Mcal/kg.
[4] SoyPlus® (West Central Cooperative, Ralston, IA): CP = 483 g/kg, RUP = 600 g/kg CP, fatty acids = 49 g/kg, and NE_L = 2.10 Mcal/kg.
[5] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA): salt (38-48 g/kg), $NaHCO_3$ (180 g/kg), Ca (145-174 g/kg), P (65 g/kg), Cl (58 g/kg), S (32 g/kg), Mg (22 g/kg), K (35 g/kg), Mn (1 g/kg), Zn (1 g/kg), Fe (0.3 g/kg), Cu (0.1 g/kg), I (0.02 g/kg), Co (0.003 g/kg), Se (0.005 g/kg), F (0.65 g/kg), retinyl acetate (0.36 g/kg), cholecalciferol (0.01 g/kg), dl-α-tocopherol acetate (0.59 g/kg).
[6] Alimet®(Novus® Intl., Inc., St. Louis, MO): 890 g DL-Methionine hydroxy analog/kg DM.

Table 2.2. Composition of clover and grass in mixed pastures [1].

	Grass			Clover				Effects		
	May	June	July	May	June	July	SE	Species	Month	Species by month
	% of DM									
NDF	65.5	64.9	63.8	25.5	27.5	28.4	1.1	0.01	0.78	0.15
ADF	29.9	29.6	29.0	19.7	22.4	22.9	0.8	0.01	0.24	0.04
CP	21.1	21.9	21.6	25.5	25.2	24.5	0.7	0.01	0.80	0.58
Total fatty acids	2.2	1.9	1.7	1.6	1.5	1.5	0.1	0.01	0.01	0.01
	mg/g of total fatty acids									
14:0	5.3	7.1	6.5	5.1	5.5	5.7	0.3	0.01	0.01	0.01
16:0	192.1	211.3	224.4	229.5	244.1	252.3	13.1	0.01	0.01	0.23
*cis*9-16:1	1.9	3.8	4.3	3.3	3.6	3.5	0.7	0.93	0.04	0.17
18:0	16.3	17.7	19.8	33.8	35.5	35.7	1.7	0.01	0.03	0.42
*cis*9-18:1	22.1	17.6	22.1	35.8	37.7	38.6	3.7	0.01	0.09	0.23
18:2n6	203.9	205.5	223.6	211.1	226.1	233.6	7.3	0.01	0.01	0.26
18:3n3	558.5	536.9	501.1	481.6	448.2	431.9	16.1	0.01	0.01	0.38

[1] Samples were collected throughout May, June, and July from each of four fields prior to being grazed. After compositing by field, 15 samples of clover and grass were used for chemical analyses. Values shown in the table are the average of five observations for May, June, or July.

Table 2.3. Average body weight and estimated intake of DM, chemical components, and fatty acids by grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM) [1].

		Supplements				Contrasts	
	TMR [2]	SES	MES	MESM	SE	MES+MES vs SES	MESM vs MES
Body weight, kg	550	542	545	548	9.7	0.95	0.62
Intake	———	kg/d	———				
Pasture [3]	. . .	9.8	10.5	10.7	0.6	0.27	0.82
Supplement	. . .	6.7	6.7	6.7	. . .	. . .	. . .
Total DMI	24.6	16.5	17.2	17.4	. . .	. . .	. . .
NDF	7.7	5.1	5.5	5.6	0.3	0.28	0.84
ADF	5.3	2.5	2.7	2.8	0.2	0.27	0.83
CP	4.4	3.5	3.8	3.9	0.1	0.07	0.75
Total fatty acids	1.1	0.35	0.44	0.44	0.01	0.01	0.80
	———	g/d	———				
16:0	126.4	58.8	70.8	71.7	2.6	0.01	0.81
18:0	16.8	12.1	11.3	11.4	0.3	0.07	0.81
*cis*9-18:1	183.1	40.1	43.0	43.1	0.3	0.01	0.79
18:2n6	611.6	144.1	204.1	205.2	2.6	0.01	0.77
18:3n3	67.3	91.1	104.7	106.6	5.5	0.05	0.82

[1] Values are the average of means obtained at wk 4, 8, and 12 during grazing.
[2] TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.
[3] Pasture intake: kg DM/d = [(Mcal milk energy output/d + Mcal energy spent for maintenance and production/d [NRC, 1989]) - Mcal NE_L intake from supplement/d]/Mcal NE_L in mixed pastures/kg DM.

Table 2.4. Milk production, composition, and component yields by grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM) [1].

		Supplements				Contrasts	
	TMR [2]	SES	MES	MESM	SE	MES+MESM vs SES	MESM vs MES
Milk, kg/d	46.8	30.5	32.5	32.6	1.3	0.08	0.95
Composition			%				
Fat	3.66	3.29	3.28	3.30	0.11	0.96	0.86
Protein	2.86	2.74	2.73	2.72	0.05	0.79	0.91
Lactose	4.91	4.72	4.72	4.71	0.05	0.90	0.88
SNF	8.52	8.17	8.20	8.17	0.11	0.75	0.53
Yield			kg/d				
Fat	1.72	1.01	1.06	1.08	0.06	0.23	0.74
Protein	1.34	0.83	0.88	0.89	0.04	0.07	0.84
Lactose	2.30	1.43	1.54	1.54	0.07	0.07	0.97
SNF	3.98	2.48	2.50	2.51	0.11	0.05	0.94

[1] Values are the average of means obtained at wk 2, 4, 6, 8, 10, and 12 during grazing.
[2] TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

Table 2.5. Concentrations of fatty acids in lipid fractions of plasma from grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM) [1].

		Supplements				Contrasts	
	TMR [2]	SES	MES	MESM	SE	MES+MES vs SES	MESM vs MES
	——————— μg/mL ———————						
Lipid fractions							
Phospholipid	303	268	283	306	13	0.05	0.26
Cholesterol ester	268	248	241	274	10	0.11	0.19
Free fatty acid	39	94	47	51	2	0.29	0.26
Triglyceride	34	32	17	19	1	0.15	0.24
Total	634	576	608	647	25	0.22	0.32

[1] Values are the means obtained at wk 4 during grazing.
[2] TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

Table 2.6. Fatty acid concentrations in blood plasma of grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM) [1].

		Supplements				Contrasts	
	TMR [2]	SES	MES	MESM	SE	MES+MES vs SES	MESM vs MES
Fatty acid	———	———	μg/mL	———	———		
14:0	2.6	0.6	0.7	1.1	0.1	0.04	0.03
*cis*9-14:1	1.1	0.3	0.4	0.7	0.1	0.04	0.02
16:0	105	63	63	116	11.7	0.05	0.01
*cis*9-16:1	24	8.6	8.2	16	1.1	0.04	0.01
18:0	125	82	78	146	10.6	0.04	0.01
Cis 18:1							
9	64	47	43	75.3	7.8	0.20	0.01
11	4.7	2.7	2.7	4.8	0.5	0.13	0.01
12	11	2.3	2.2	3.8	0.3	0.12	0.01
13	0.8	0.6	0.4	0.6	0.2	0.65	0.44
15	1.3	0.8	1.3	1.3	0.1	0.12	0.01
Trans 18:1							
6,7,8	0.8	0.04	0.2	0.5	0.1	0.07	0.09
9	0.9	0.6	0.4	0.4	0.2	0.21	0.63
10	1.2	0.0	0.0	0.1	0.1	0.60	0.36
11	4.4	5.0	5.9	10	1.1	0.02	0.01
12	1.2	1.0	0.4	0.7	0.2	0.11	0.37
13,14	7.0	2.0	1.9	3.2	0.4	0.33	0.02
16	1.6	0.6	0.6	1.2	0.1	0.01	0.01
Isolated 18:2							
*c*9,*c*12	470	237	261	484	10	0.03	0.01
*t*9,*t*12	0.5	0.3	0.3	0.3	0.02	0.42	0.06
*c*9,*t*12	0.8	0.0	0.0	0.2	0.1	0.48	0.44
*t*9,*c*12	1.1	0.0	0.03	0.1	0.04	0.31	0.70
*t*11,*c*15	0.4	1.9	1.7	3.1	0.1	0.01	0.01
Conjugated 18:2							
*c*9,*t*11	0.9	1.1	1.2	1.9	0.2	0.01	0.01
*t*10,*c*12	0.01	0.0	0.0	0.0	0.0	. . .	. . .
18:3n3	53	54	46	80	8.1	0.36	0.01
20:3n3	20	9.5	9	17	2.1	0.08	0.01
20:4n6	22	14	13	25	2.0	0.04	0.01
20:5n3	24	18	16	27	1.7	0.05	0.01
Total	943	551	554	1013	98	0.05	0.01

[1] Values are the average of means obtained at wk 4 during grazing.

[2] TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

Table 2.7. Milk fatty acid yields by grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM) [1].

		Supplements				Contrasts	
	TMR [2]	SES	MES	MESM	SE	MES+MES vs SES	MESM vs MES
	g/d						
4:0	89.0	55.7	65.5	58.6	3.2	0.53	0.14
6:0	44.9	26.9	28.7	27.4	1.2	0.73	0.44
8:0	23.5	17.1	15.0	14.5	1.9	0.33	0.86
10:0	49.6	28.8	30.6	29.0	1.6	0.95	0.46
12:0	48.1	28.4	29.4	27.9	1.5	0.82	0.50
14:0	151.3	91.6	92.7	91.5	3.8	0.98	0.83
*cis*9-14:1	9.1	5.8	6.4	6.6	0.5	0.66	0.89
16:0	402.2	222.2	223.3	219.2	9.2	0.88	0.76
6:0-16:0	719.6	414.8	418.0	410.7	16.8	0.75	0.55
*cis*9-16:1	18.1	10.7	11.3	11.5	0.7	0.70	0.84
*trans*9-16:1	3.8	3.6	3.9	3.9	0.2	0.33	0.84
18:0	166.9	97.0	113.8	111.1	4.8	0.05	0.75
Cis 18:1							
9	275.9	170.5	199.6	204.3	10.4	0.04	0.75
11	8.4	4.2	4.9	4.5	0.3	0.14	0.30
12	11.1	2.5	2.2	2.1	0.2	0.25	0.88
13	2.3	0.9	1.1	1.2	0.1	0.15	0.41
15	3.2	1.8	1.9	2.2	0.1	0.09	0.13
Trans 18:1							
6,7,8	4.5	2.3	2.7	2.7	0.01	0.01	0.95
9	4.2	2.4	2.5	2.6	0.01	0.14	0.41
10	7.7	2.8	2.5	2.8	0.3	0.78	0.61
11	17.8	35.1	42.6	43.9	1.7	0.01	0.59
12	9.4	3.9	3.7	3.7	0.2	0.40	0.84
13,14	18.3	7.5	6.5	6.4	0.3	0.03	0.76
16	7.3	3.2	3.0	3.0	0.2	0.45	0.92
Isolated 18:2							
*c*9,*c*12	38.8	22.5	28.2	28.2	0.7	0.01	0.97
*c*9,*t*12	0.2	0.7	1.2	1.3	0.01	0.01	0.12
*t*9,*c*12	0.9	1.0	1.3	0.4	0.2	0.47	0.24
*t*9,*t*12	4.9	2.9	2.6	3.0	0.1	0.15	0.06
*t*11,*c*15	0.7	4.1	3.6	4.1	0.3	0.35	0.22
Conjugated 18:2							
*c*9,*t*11	5.1	8.9	12.1	13.6	0.6	0.01	0.13
*t*10,*c*12	0.4	0.7	0.7	0.9	0.1	0.55	0.28
18:3n3	5.7	4.4	5.5	5.0	0.2	0.05	0.17
20:3n3	1.5	0.8	0.9	0.9	0.05	0.13	0.54
20:4n6	2.0	1.1	1.2	1.2	0.06	0.21	0.94
20:5n3	1.2	0.7	0.6	0.7	0.04	0.73	0.49
Total	1429.8	812.5	887.2	881.4	34.1	0.09	0.91

[1] Values are the average of means obtained at wk 4, 8, and 12 during grazing.
[2] TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

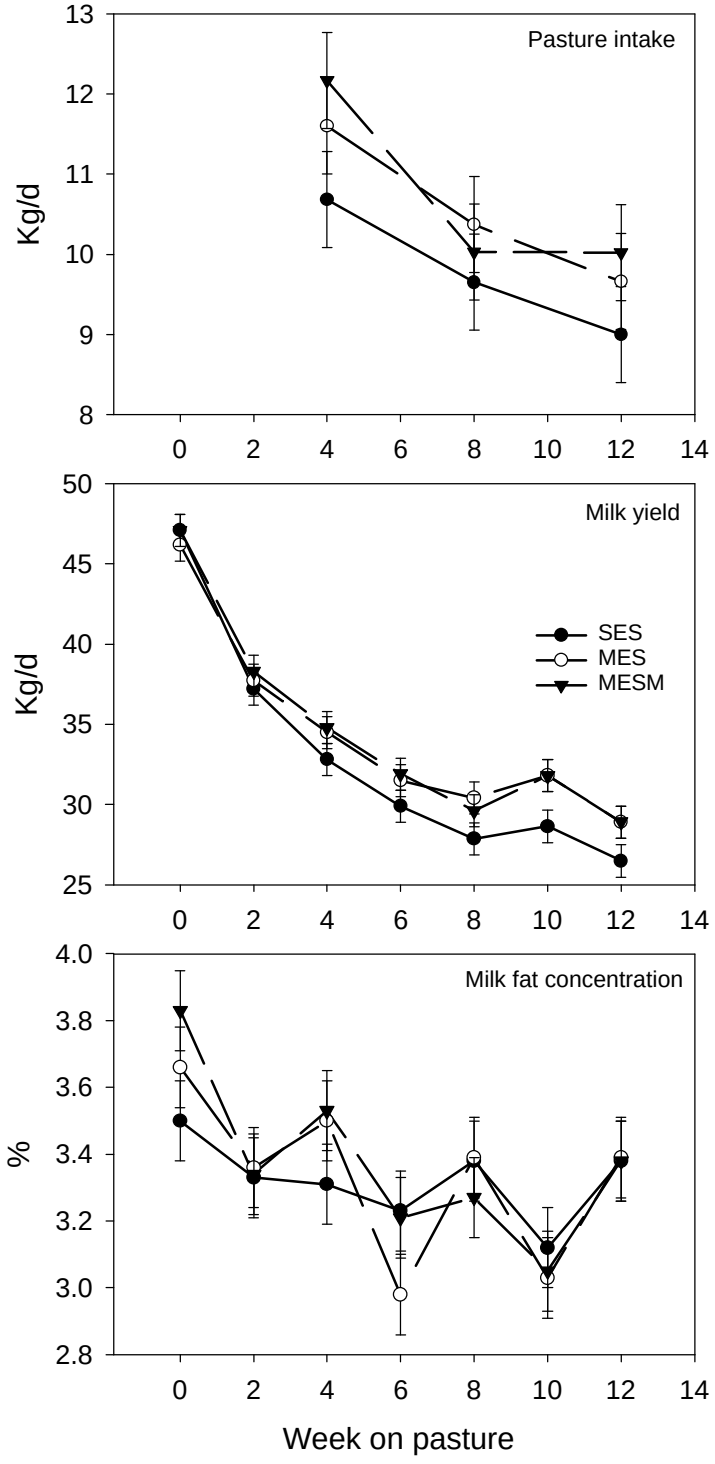


Figure 2.1. Intake of pasture, milk yield, and milk fat concentration in grazing cows supplemented with solvent-extracted soybean meal (SES), mechanically-extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM).

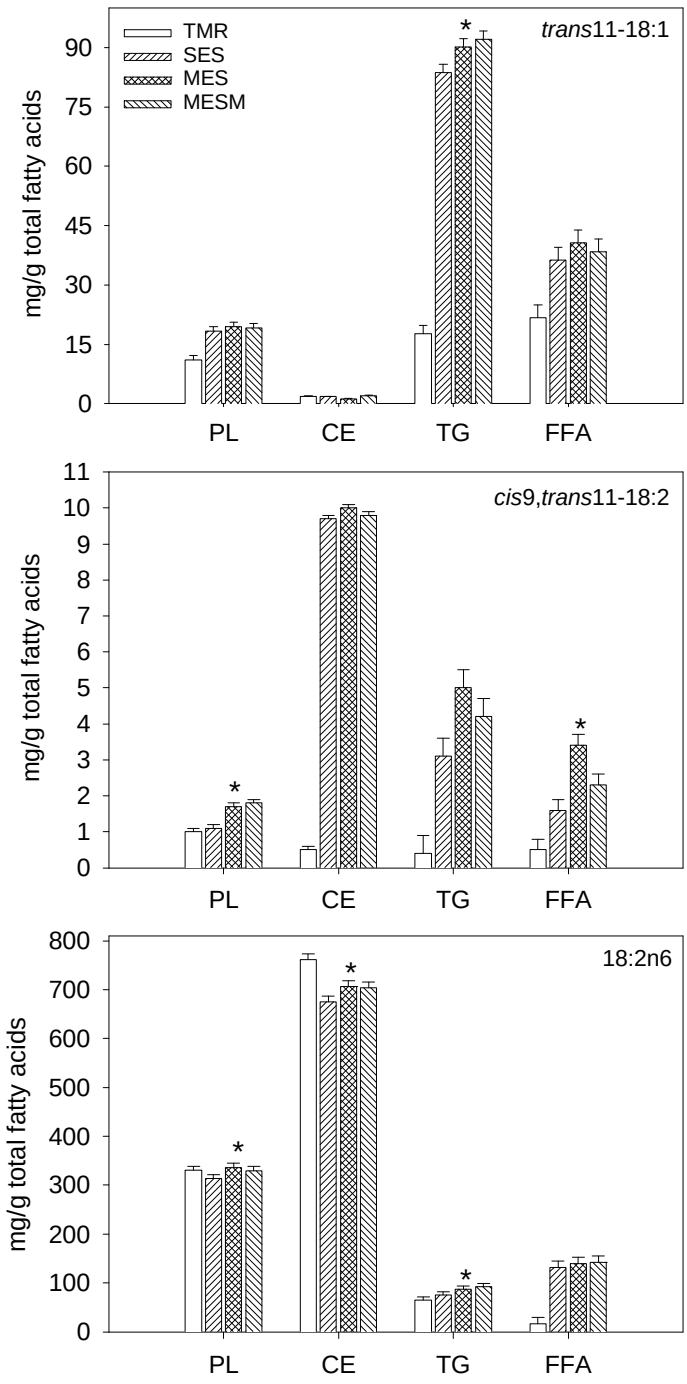


Figure 2.2. Distribution of *trans*11-18:1, *cis*9,*trans*11-18:2, and 18:2n6 in blood plasma phospholipids (PL), cholesterol esters (CE), triglycerides (TG), or free fatty acids (FFA) from grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM). Values for TMR are shown for comparison only. Asterisks denote differences ($P < 0.05$) due to feeding MES or MESM versus SES.

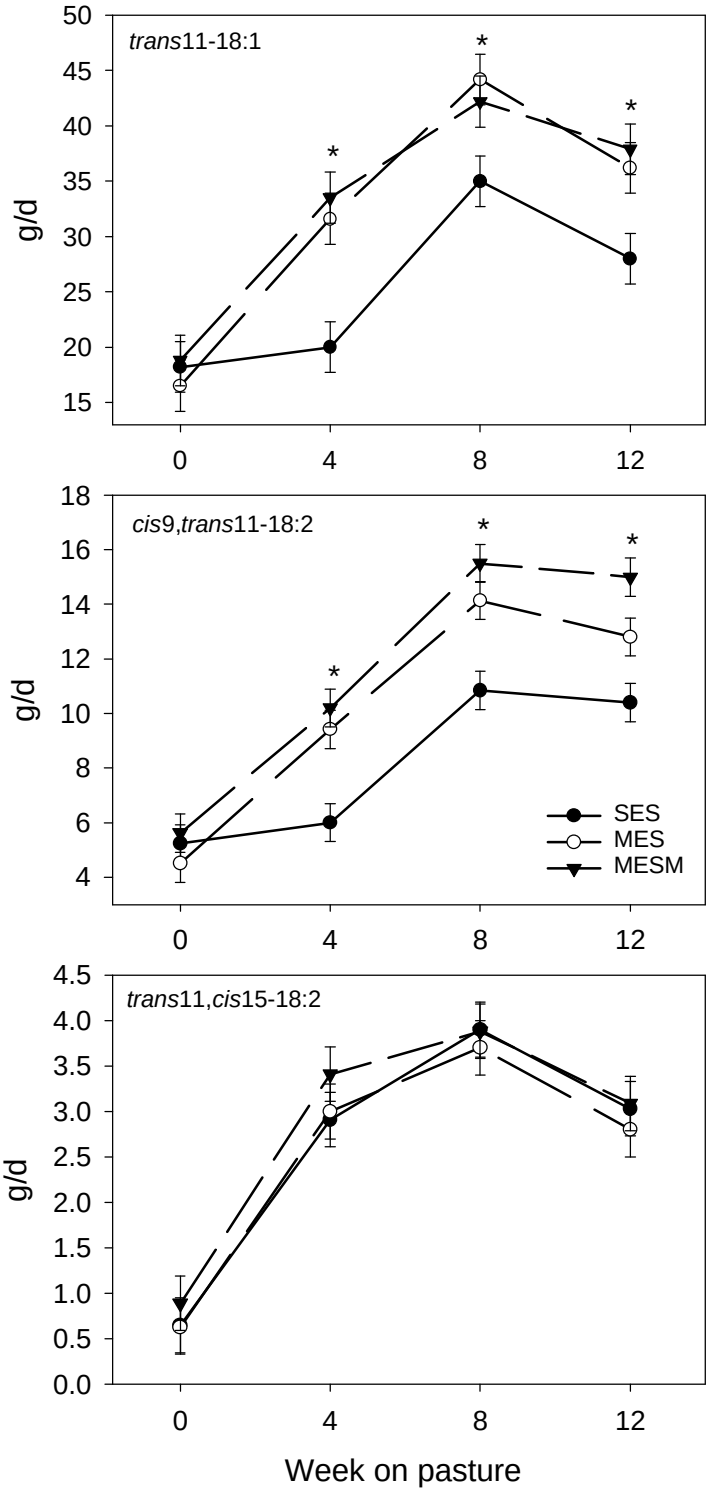


Figure 2.3. Yields of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in milk fat from grazing cows supplemented with solvent-extracted soybean meal (SES), mechanically-extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM). Asterisks denote differences ($P < 0.05$) due to feeding MES or MESM versus SES.

CHAPTER 3

Alterations in Plasma and Milk Fatty Acid Profiles of Lactating Cows in Response to Ruminal Infusion of a Conjugated Linoleic Acid Mixture

ABSTRACT

*Cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 are produced in the rumen during hydrogenation of dietary polyunsaturated fatty acids, and could affect lipid metabolism in the mammary gland. To determine blood plasma profiles and yields of milk fatty acids in response to a conjugated linoleic acid mixture (CLA; 41% *cis*9,*trans*11-18:2 and 44% *trans*10,*cis*12-18:2), CLA was infused into the rumen of lactating cows. Four Holstein cows fed a basal diet were infused for 48 h with doses of 0, 45, 90, or 180 g CLA/d into the rumen. Treatments were randomly assigned in a 4×4 Latin square with 4-d experimental periods, and a 7-d transition between periods. Milk samples were obtained at -12 and 0 h before infusion, at 12, 24, 36, and 48 h during infusion and at 60, 72, 84, and 96 h after infusion. Milk yield and DMI were not affected by treatment. Milk fat concentration was 12% lower, causing an 18% decrease in fat yield, when 180 g CLA/d was infused. Concentration of *trans*11-18:1 in blood plasma increased in proportion to CLA dose. *Trans*10-18:1 concentration in blood plasma also increased, and was 240% greater when CLA was infused at 180 g/d. *Trans*10,*cis*12-18:2 was strictly a function of exogenous CLA input into the rumen, and ranged from 0.2 to 0.7 mg/g of total plasma fatty acids. Yields of saturated 6:0 to 16:0 in milk fat decreased by 87 g/d when 180 g CLA/d was infused. Stearic acid concentration and yield increased by 25 and 6%, but *cis*9-18:1 yield decreased, in response to increasing dose of CLA. Yields of *trans*11-18:1 and *cis*9,*trans*11-18:2 increased in proportion to CLA dose infused. Milk fat yields of *trans*10-18:1 and *trans*10,*cis*12-18:2 also increased in proportion to CLA input. Lower normalized ratios for 18:0 to *cis*9-18:1 and *trans*11-18:1 to *cis*9,*trans*11-18:2 in milk fat when CLA was infused indicated CLA reduced desaturase activity in the mammary gland. Results indicated enhanced flow

of *trans*10-18:1 or *trans*10,*cis*12-18:2 from the rumen can decrease milk fat yield by reducing *de novo* synthesis and desaturation.

INTRODUCTION

The *cis*9,*trans*11 isomer of conjugated linoleic acid (CLA), accounts for nearly 95% of total CLA found in milk fat (Parodi, 1999). *Cis*9,*trans*11-18:2 results from isomerization, via *cis*12,*trans*11-isomerase (Kepler et al., 1970), of dietary 18:2n6 by rumen microorganisms during the first step of the biohydrogenation process (Kepler and Tove, 1967). Accumulation of *trans*11-18:1 and *cis*9,*trans*11-18:2 *in vitro*, however, was lower when triglyceride-bound 18:2n6 was the substrate compared with the free fatty acid (Noble et al., 1974). *Trans*11-18:1 and *cis*9-18:1 were found to be competitive inhibitors of linoleate isomerase activity (Kepler et al., 1970). If present in large amounts, *trans*11-18:1 or *cis*9-18:1 could alter the profiles of intermediates produced in the rumen, thereby altering the amounts available for absorption in the small intestine.

Diet affects the individual profiles of *trans*-18:1 or conjugated 18:2 isomers produced during fermentation. Feeding supplemental soybean oil resulted in greater duodenal flows of *trans*-18:1 with double bonds at positions 6 through 16 (Bickerstaffe et al., 1972). The output of *cis*9,*trans*11-18:2 in effluents from rumen fermenters fed fresh forage ranged from 9 to 23% of total conjugated-18:2 isomer output, and averaged 17% during digestion of a mixed diet plus supplemental 18:2n6 (Fellner et al., 1997; Chapter 1). *Trans*10,*cis*12-18:2 accounted for 7 or 16% of total conjugated isomer output when fresh forage or the mixed diet were the DM input (Fellner et al., 1997; Chapter 1). Outputs of *cis*9,*cis*11-18:2, *trans*11,*trans*13-18:2, and a mixture of *trans*,*trans*-18:2 isomers were predominant regardless of diet fed (Fellner et al., 1997; Chapter 1).

In terms of mammary lipid metabolism, identification of the precursors which give rise to the production of 18:1 and 18:2 isomers with a *trans*10 double bond in the rumen is of interest because these isomers may depress milk fat synthesis (Griinari et al., 1998; Piperova et al., 2000). It is well established that production of *trans*10-18:1 in the rumen is enhanced when high-grain diets containing supplemental oil are fed to dairy cows (Griinari et al., 1998; Piperova et al., 2000). However, it is not clear if *trans*10,*cis*12-18:2 is a required precursor for the formation of *trans*10-18:1 (Griinari

and Bauman, 1999). We have shown (Chapter 1) that production of *trans*10-18:1 in rumen fermenters is directly proportional to *cis*9-18:1 input from corn grain, but corn grain also contains substantial amounts of 18:2n6. One way to verify if *trans*10-18:1 can be formed from hydrogenation of *trans*10,*cis*12-18:2 *in vivo* is to enhance the availability of the CLA in the rumen by infusing a mixture of CLA, which contains substantial amounts of *trans*10,*cis*12-18:2. Our objective was to evaluate the extent of hydrogenation of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 in response to doses of a CLA mixture infused into the rumen.

MATERIALS AND METHODS

Animals and Diets

Four early-lactation primiparous Holstein cows (between 48 and 60 DIM) were utilized in a 4 × 4 Latin square design with four 4-d periods to evaluate responses to 0, 45, 90, or 180 g CLA infused continuously into the rumen for 2-d. During infusion, cows were housed in a tie-stall barn and their basal diet was prepared and offered in equal amounts at 1400 and 0200 h daily. Feed refusals were removed daily at 1200 h and 0100 h and weighed. Daily feed allotment was calculated to allow 5 to 10% feed refusals. Cows were milked each day at 1300 and 0100 h.

This basal diet was formulated using Dair4 (Stallings et al., 1985) to meet or exceed nutrient requirements of cows producing 34 kg milk and consuming 19 kg of DM daily. The concentrate portion of the diet was mixed in 500 kg batches, stored in sealed plastic containers, and removed as needed to mix with the forage on a daily basis (Table 1). The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

CLA infusion

Conjugated linoleic acid (CLA-90, Natural Lipids, Norway) contained 90% CLA, with *cis*9,*trans*11-18:2 (410 mg/g) and *trans*10,*cis*12-18:2 (440 mg/g) being the primary isomers.

The CLA mixture (0, 45, 90, or 180 g CLA/d) was emulsified in skim milk to ensure a uniform supply of CLA during the 48-h infusion. Emulsions were prepared the day prior to infusion by combining the desired amount of CLA with 0.23 g glycerol (Eastman Kodak Co., Rochester, NY)/g CLA and 0.12 g soy lecithin powder (Refined, Alfa®, Ward Hill, MA)/g CLA in 972.5 mL skim milk at room temperature. The mixture was homogenized at 12,000 rpm for 2 min with a Polytron® PT 10/35 homogenizer (Brinkmann Instruments, Westbury, NY), and checked for the presence of clumps before stirring at medium-to-high speed for 30 min at room temperature. Emulsions were dispensed into 1 L Viaflex® plastic bags (Baxter Corporation, Deerfield, IL) and stored at 4 °C until infusion. Abomasal infusion of CLA began at 1400 h in each period.

During infusion, bags containing CLA emulsions were attached to a flat platform on a wrist-action shaker (Burrell Corporation, Pittsburgh, PA) set at low speed. Emulsions were infused via Tygon® tubing (1.6 mm i.d., 0.8 mm wall; Fisher Scientific Co., Pittsburgh, PA) that passed through a Harvard Peristaltic pump (55-1762; Harvard Apparatus, South Natick, MA). Flow from the pump was via Tygon® tubing (3.2 mm i.d., 1.6 mm wall) that passed through the rumen cannula and into the rumen. A perforated Nalgene® plastic bottle (60 mL) was attached to the end of the tubing. The tubing was primed with 15 mL infusate at the start of infusion, and flow rate was set at 41.7 mL/h.

Sampling, Measurements, and Analysis

Forages and concentrate were sampled during the last day of each experimental period. Samples were dried in a forced-air oven at 60 °C, then stored in sealed plastic containers. Equal amounts of samples from each period were combined to determine chemical composition. In preparation for analyses, dried forages and concentrate were ground first through a 2 mm screen (Thomas-Wiley Laboratory Mill), then through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Forages and concentrates were analyzed for ADF and NDF (Van Soest et al., 1991) and total N (AOAC, 1975).

Milk was collected in a stainless steel bucket, weighed, and thoroughly mixed prior to obtaining samples at each milking from -12 h to 96 h relative to the start of infusion. A 30 mL aliquot was collected in a 50 mL vial containing Bronopol (milk preservative; D & F Control Systems, San Ramon, CA) immediately after milking. Milk was analyzed for milk fat, protein, and SNF by infrared analysis with a 4-channel spectrophotometer (Virginia Dairy Herd Improvement Association). An additional aliquot of milk without Bronopol also was collected, then frozen at -20 °C. Subsequently, samples were thawed at room temperature and centrifuged at 10,000 × g for 1 h to isolate milk fat.

Blood samples (10 mL) were obtained from the coccygeal artery immediately after the collection of milk samples. After collection, blood was transferred to tubes containing 286 IU heparin in 100 µL of sterile saline and centrifuged at 3,000 × *g* for 15 min for harvesting plasma.

Plasma lipids were extracted with chloroform/methanol (2:1, vol/vol). Fatty acids in forages, concentrates, milk fat, and blood plasma lipids were methylated by *in situ* transesterification with 0.5N methanolic NaOH as described by Park and Goins (1994). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters of fatty acids were separated on a 100 m × 0.25 mm i.d. fused silica capillary column (CP-Sil 88,Chrompack, Middelburg, The Netherlands).

For milk, forage, and concentrate fatty acid analysis (0.5 µL methyl esters in hexane injected at a 35:1 split ratio) the injector temperature was maintained at 250 °C and the detector temperature was maintained at 255 °C. The initial oven temperature was 70 °C (held for 1 min) and increased 5 °C/min to 100 °C (held for 2 min), 10 °C/min to 175 °C (held for 40 min), and then increased 5 °C/min to a final temperature of 225 °C (held for 15 min). Hydrogen was the carrier gas.

Analysis of blood plasma fatty acids required injection of 2 µL methyl esters (splitless). The injector temperature was maintained at 150 °C and the detector temperature at 255 °C. The purge valve on the GC was closed for 1.5 min after

sample injection. The initial column temperature was 40 °C (held for 1.5 min) and increased 40 °C/min to 100 °C (held for 10 min), 25 °C/min to 175 °C (held for 70 min), and then increased 10 °C/min to a final temperature of 220 °C (held for 20 min). Ultra pure helium was the carrier gas.

Statistical Analysis

Data for DM and fatty acid intake, milk production and composition, plasma fatty acid profiles, milk fatty acid yields, and normalized ratios of milk fatty acids are reported as Least squares means ± SEM. All data, except plasma fatty acid profiles, were analyzed as a 4 × 4 Latin square with repeated measures using the MIXED procedure of SAS (2000). Observations obtained at -12 and 0 h were averaged and served as a covariate for observations at 12, 24, 36, 48, 60, 72, 84, or 96 h. Main effects in the model included covariate adjustment, cow, period, CLA dose, time, time by CLA dose interaction, and residual error. For plasma fatty acid profiles, main effects in the model included cow, period, CLA dose, and residual error. Linear and quadratic contrasts were used to determine differences due to CLA infusion. Overall differences between treatment means were considered to be significant when $P \leq 0.05$. However, all *P* values are presented in tables.

RESULTS

Diet Composition

Total fatty acid content of the basal diet was 3% (Table 3.1). Linoleic acid accounted for 50% of total fatty acids, and *cis*9-18:1 and 18:3n3 for 26 or 7% of total fatty acids. The primary sources of fatty acids were SoyPlus® and ground corn, which were the primary sources of supplemental 18:2n6. Forages contributed primarily 18:3n3.

Fatty Acid Intake, DMI and Milk Production

Estimated intake of total fatty acids increased in proportion with CLA dose due to the combination of the amounts of CLA infused (Table 3.2) and variations in DMI

(Table 3.3). Daily DMI and milk yields were not affected by dose of CLA, and averaged 19 or 31 kg/d (Table 3.3). Because of numerically lower milk yield as the dose of CLA infusion increased, yields of protein, lactose, and SNF in milk also decreased. Milk fat percentage and yield decreased by 13 and 16% due primarily to CLA infusion at 180 g/d.

Blood Plasma Fatty Acid Profiles

Overall, total plasma fatty acid concentrations did not differ due to CLA dose and averaged 1,132 μg/mL at the end of the 48-h ruminal infusion (Table 3.4). Among 18:1 isomers derived from hydrogenation of exogenous *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2, concentrations of *trans*10-18:1 and *trans*11-18:1 increased in proportion to dose of CLA infused. Concentrations of *trans*10,*cis*12-18:2 also were proportional to CLA dose, and averaged 0, 0.2, 0.5, or 0.7 μg/mL when 0, 22, 44, or 88 g *trans*10,*cis*12-CLA/d were infused.

Milk Fatty Acid Yields

Total milk fatty acid yield relative to basal levels decreased 13% when the 180 g CLA/d dose was infused for 48 h (Table 3.5). The decrease was primarily due to a 22% reduction in yields of saturated fatty acids with 6 to 16 carbons. The yield of 18:0, however, increased due to infusion of CLA. In contrast, yield of *cis*9-18:1 decreased when the dose of CLA was 180 g. All doses of CLA increased the yields of most *trans*-18:1 isomers compared with the control infusion without CLA. Compared with the control infusion, yields of *cis*9,*trans*11-18:2 were only 6% greater when 90 or 180 g CLA were infused. Supplemental CLA was the primary source of *trans*10,*cis*12-18:2 in the rumen, and resulted in yields of 0.2, 0.6, and 1 g/d when 22, 44, or 88 g *trans*10,*cis*12-CLA/d, respectively, were infused.

Normalized Ratios of Milk Fatty Acids

Normalized ratios (mg/g product/[mg/g substrate + mg/g product]) were estimated to assess the extent of desaturation of specific fatty acids during milk fat synthesis (Palmquist and Santora, 1999). The ratios of 14:0 to *cis*9-14:1, 18:0 to *cis*9-

18:1, and *trans*11-18:1 to *cis*9,*trans*11-18:2 decreased due to CLA infusion, primarily at the 180 g dose (Table 3.6). The lower ratios suggested exogenous *trans*10,*cis*12-18:2 reduced the amount and(or) activity of Δ^9 desaturase in the mammary gland (Chapter 4).

DISCUSSION

Our experiment evaluated the quantitative significance of ruminal availability of *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 on their secretion in milk fat. Plasma fatty acid profiles and milk fatty acid yields were used to assess changes in the production of hydrogenation intermediates. Milk fatty acid data also provided the means to evaluate apparent changes in lipogenesis and desaturation in the mammary gland due to exogenous CLA. Daily DMI or milk yields were not affected by CLA dose.

Despite CLA infusion, the concentration of *cis*9,*trans*11-18:2 in blood plasma did not increase. Concentration of *trans*11-18:1 in plasma, however, increased with each increment of CLA infused (Table 3.4). Yields of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat increased in proportion to CLA dose (Table 3.5). The greater availability of exogenous *cis*9,*trans*11-18:2 may have overcome the capacity for microbes to hydrogenate it completely. Polan et al., (1964) first noted that hydrogenation of 18:2n6 to 18:0 in rumen fluid decreased linearly as the concentration of 18:2n6 in the incubation increased. Isomers of 18:1, however, accumulated up to the point where concentration of 18:2n6 was 3-fold greater. When 18:2n6 concentration was 8-fold greater, hydrogenation was only 12% (Polan et al., 1964). A recent study confirmed that *Butirivibrio fibrisolvens* A38 produced significant amounts of *cis*9,*trans*11-18:2 when the concentration of 18:2n6 was high enough to inhibit hydrogenation of 18:1 isomers to 18:0 (Kim et al., 2000). Because *trans*11-18:1 could be desaturated to *cis*9,*trans*11-18:2 in the mammary gland (Griinari et al., 2000), it could also serve as an alternate source for endogenous synthesis of *cis*9,*trans*11-18:2. However, the lower ratio of *trans*11-18:1 to *cis*9,*trans*11-18:2 (Table 3.6) in response to 180 g CLA suggests that desaturation of rumen-derived *trans*11-18:1 $\Rightarrow$ *cis*9,*trans*11-18:2 was inhibited, possibly by greater uptake of *trans*10,*cis*12-18:2 (Chapter 4).

*Trans*12-18:1 and *trans*13/14-18:1 yields in milk fat increased in proportion to CLA dose. Exogenous *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 accounted for 85% of total CLA isomers infused, and it could be possible that *trans*12-18:1 and *trans*13/14-18:1 were derived from the isomerization of end products which accumulated during hydrogenation. *Trans*-18:1 isomers produced during hydrogenation studies with *B. fibrisolvens* reflected the double bond positions of the substrates. Thus, hydrogenation of 18:2n6 led to production of *trans*11-18:1, primarily, but *trans*9-18:1 also accumulated (Kepler et al., 1966). Incubating a mixture of *cis*9,*trans*11-18:2 (39% of total fatty acids), *trans*10,*cis*12-18:2 (3%), and *cis*8,*trans*10-18:2 (54%) resulted in accumulation of *trans*8-18:1 (27%), *trans*9-18:1 (7%), *trans*10-18:1 (10%), *trans*11-18:1 (46%), and *trans*12-18:1 (9%) (Kepler et al., 1966). Although *B. fibrisolvens* accounts for a large number of total rumen bacteria, numerous isomers are produced during hydrogenation of unsaturated fatty acids by the mixed rumen microflora (Kemp et al., 1975).

Infused CLA was the only source of *trans*10,*cis*12-18:2 in blood plasma or milk fat. However, concentrations of *trans*10-18:1 and *trans*10,*cis*12-18:2 in plasma and yields in milk fat increased in proportion to CLA infused. As indicated by Kepler et al. (1966) hydrogenation of *trans*10,*cis*12-18:2 could lead to the formation of *trans*10-18:1. Similar to *cis*9,*trans*11-18:2, availability of *trans*10,*cis*12-18:2 was large enough to prevent complete hydrogenation.

Milk fat percentage and yield decreased significantly when 180 g CLA were infused relative to basal levels. Yields of saturated 6:0 to 16:0 in milk fat also decreased. This response led to a reduction in total fatty acid yields (Table 3.5). Lower fat concentration and yields of medium-chain fatty acids were previously observed when the concentrations of *trans*10-18:1 (Piperova et al., 2000) or *trans*10,*cis*12-18:2 (Baumgard et al., 2000b) in milk fat increased. The reduction in milk fat percentage due to *trans*10-18:1 was directly proportional to lower fatty acid synthase and acetyl-CoA carboxylase activities in mammary tissue (Piperova et al., 2000). Based on the level reported to decrease milk fat synthesis, however, *trans*10,*cis*12-18:2 appears to be a more potent inhibitor than *trans*10-18:1. The greater yields of *trans*10-18:1 and *trans*10,*cis*12-18:2 observed at the 180 g CLA dose,

were proportional to lower milk fat percentage, lower milk fat yield, and reduced yields of medium-chain fatty acids.

Opposite to the response for medium-chain fatty acids, yield of 18:0 in milk fat increased with each dose of CLA infused. Despite this increase, the yield of *cis*9-18:1 (a product of 18:0 desaturation) was markedly lower when 180 g CLA was infused (Table 3.5). The lower ratio of 18:0 to *cis*9-18:1 suggested that desaturation of 18:0 ⇒ *cis*9-18:1 in response to infusion with 180 g CLA, was impaired. Ratios of fatty acid pairs affected by Δ^9 desaturase activity have been previously used to estimate the potential effect of exogenous fatty acids on desaturation. Inhibiting the activity of Δ^9 desaturase, by infusing sterculic acid into the abomasum, decreased the ratios of 14:0 ⇒ *cis*9-14:1, 18:0 ⇒ *cis*9-18:1, or *trans*11-18:1 ⇒ *cis*9,*trans*11-18:2 in milk fat (Griinari et al., 2000). An increase in *trans*10,*cis*12-18:2 concentration in milk fat (by infusing the isomer into the abomasum) also decreased the above ratios (Baumgard et al., 2000b). In the presnent study, ratios also were lower when CLA was infused at the rate of 180 g/d (Table 3.6). Overall, results confirmed that greater availability of *trans*10,*cis*12-18:2 could decrease lipogenesis and desaturation of long-chain fatty acids in the mammary gland.

CONCLUSIONS

*Trans*10,*cis*12-18:2 was not detected in blood plasma or milk fat unless the CLA mixture was infused, suggesting it is not a typical intermediate of 18:2n6 hydrogenation. *Trans*10-18:1, however, was detected and yield of *trans*10-18:1 was proportional to the amount of CLA mixture infused. Thus, under basal conditions in the rumen, *trans*10-18:1 could arise primarily from isomerization of *cis*9-18:1 ⇒ *trans*10-18:1 (Mortimer and Niehaus, 1972) rather than isomerization/hydrogenation of 18:2n6. Due to high susceptibility for hydrogenation, the production of *trans*10,*cis*12-18:2 in the rumen must be at least 22 g/d before it is detectable in blood plasma and milk fat. Furthermore, production must range between 45 and 89 g/d to potentially cause lower milk fat percentage and desaturation in the mammary gland.

Table 3.1. Composition of basal diet [1].

Ingredient	% of DM
Alfalfa silage	31.5
Corn silage	13.6
Orchardgrass hay	7.0
Ground corn	35.1
Soybean meal, 48%CP	8.0
SoyPlus [2]	3.4
Mineral/vitamin mix [3]	0.7
Limestone	0.5
Dicalcium phosphate	0.2
Chemical composition	
NDF	30.7
ADF	19.5
CP	17.1
Total fatty acids	3.0
	mg/g of total fatty acids
12:0	2
14:0	1
16:0	134
*cis*9-16:1	2
18:0	34
*cis*9-18:1	262
18:2n6	499
18:3n-3	66

[1] Four samples (collected in each period) of forages and supplements were composited and analyzed in duplicate.

[2] SoyPlus® (West Central Cooperative, Ralston, IA): CP = 483 g/kg DM, fatty acids = 48 g/kg DM.

[3] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA): salt (38-48 g/kg), $NaHCO_3$ (180 g/kg), Ca (145-174 g/kg), P (65 g/kg), Cl (58 g/kg), S (32 g/kg), Mg (22 g/kg), K (35 g/kg), Mn (1 g/kg), Zn (1 g/kg), Fe (0.3 g/kg), Cu (0.1 g/kg), I (0.02 g/kg), Co (0.003 g/kg), Se (0.005 g/kg), F (0.65 g/kg), retinyl acetate (0.36 g/kg), cholecalciferol (0.01 g/kg), dl-α-tocopherol acetate (0.59 g/kg).

Table 3.2. Estimated daily fatty acid intake by cows infused continuously into the rumen for 2 d with 0, 45, 90, or 180 g/d of a conjugated linoleic acid (CLA) mixture [1].

	CLA (g/d)					Effect [2]		
	0	45	90	180	SE	CLA	L	Q
	g/d							
14:0	0.8	0.8	0.8	0.8	0.04	0.33	0.67	0.54
16:0	73.6	78.7	74.4	73.6	3.4	0.33	0.67	0.55
*cis*9-16:1	1.1	1.2	1.1	1.1	0.03	0.33	0.67	0.56
18:0	18.0	23.1	26.2	23.8	0.8	0.01	0.01	0.29
*cis*9-18:1	137.3	146.2	138.6	137.3	7.0	0.39	0.89	0.48
18:2 isomers								
*c*9,*c*12	264.4	282.8	267.1	264.6	13.8	0.32	0.66	0.44
*c*9,*t*11	0.0	20.4	40.8	81.5	. . .	. . .	. . .	. . .
*t*10,*c*12	0.0	21.8	43.7	87.5	. . .	. . .	. . .	. . .
*c*9,*c*11	0.0	0.8	1.5	3.0	. . .	. . .	. . .	. . .
*c*10,*c*12	0.0	0.5	1.0	2.0	. . .	. . .	. . .	. . .
t,*t* [4]	0.0	0.9	1.9	3.8	. . .	. . .	. . .	. . .
other	0.0	0.6	1.1	2.2	. . .	. . .	. . .	. . .
18:3n3	19.8	20.5	19.4	19.5	1.0	0.33	0.67	0.45
Total	558.8	605.1	599.2	651.2	19.1	0.01	0.01	0.82

[1] Values are the average of means obtained during CLA infusion, and include the daily amount of CLA isomers infused.

[2] Effects due to CLA and linear (L) or quadratic (Q) effects of CLA dose were calculated on means obtained from 12 through 96 h after infusion.

[3] *trans*9,*trans*11 + *trans*10,*trans*12.

Table 3.3. DMI and milk production, composition, and component yields by cows infused continuously into the rumen for 2 d with 0, 45, 90, or 180 g/d of a conjugated linoleic acid (CLA) mixture [1].

	CLA (g/d)					Effect [2]		
	0	45	90	180	SE	CLA	L	Q
	kg/d							
DMI	18.6	19.4	18.4	18.4	0.8	0.33	0.67	0.45
Milk yield	32.0	31.4	31.6	30.7	0.9	0.08	0.06	0.54
Composition	%							
Fat	3.53	3.61	3.57	3.11	0.10	0.01	0.02	0.01
Protein	2.82	2.80	2.83	2.80	0.05	0.44	0.50	0.56
Lactose	4.68	4.65	4.66	4.64	0.04	0.03	0.01	0.85
SNF	8.25	8.19	8.24	8.19	0.07	0.07	0.03	0.90
Yield	kg/d							
Fat	1.14	1.12	1.14	0.95	0.04	0.01	0.01	0.01
Protein	0.90	0.88	0.90	0.84	0.02	0.01	0.01	0.13
Lactose	1.50	1.46	1.48	1.41	0.04	0.02	0.01	0.31
SNF	2.64	2.58	2.62	2.46	0.05	0.02	0.01	0.24

[1] Values are the average of means obtained from 12 through 96 h after the start of the infusion.
[2] Effects due to CLA and linear (L) or quadratic (Q) effects of CLA dose were calculated on means obtained from 12 through 96 h after infusion.

Table 3.4. Fatty acid concentrations in plasma from cows infused continuously into the rumen for 2 d with 0, 45, 90, or 180 g/d of a conjugated linoleic acid (CLA) mixture [1].

	CLA (g/d)					Effect [2]		
	0	45	90	180	SE	CLA	L	Q
	μg/mL							
14:0	4.6	4.1	5.3	4.1	0.4	0.26	0.25	0.57
*cis*9-14:1	2.1	1.1	2.6	1.6	1.3	0.66	0.29	0.86
16:0	115	111	134	100	17	0.40	0.25	0.74
*cis*9-16:1	16	12	17	12	2	0.22	0.17	0.85
*trans*9-16:1	2.2	1.4	2.4	1.9	0.4	0.37	0.62	0.70
18:0	132	120	151	117	18	0.27	0.43	0.39
Cis 18:1								
9	101	91	112	91	12	0.55	0.53	0.63
11	4.5	4.0	5.0	3.8	0.5	0.26	0.28	0.45
12	4.1	3.3	4.9	3.9	0.5	0.13	0.68	0.82
13	0.9	0.6	1.1	0.7	0.1	0.01	0.05	0.16
15	0.8	0.8	1.1	0.8	0.1	0.03	0.52	0.09
Trans 18:1								
6,7,8	0.3	0.3	0.4	0.6	0.2	0.49	0.22	0.60
9	0.3	0.3	0.4	0.4	0.1	0.48	0.35	0.77
10	0.4	0.6	0.9	1.3	0.2	0.02	0.01	0.58
11	3.1	3.2	4.4	5.2	0.4	0.04	0.02	0.46
12	1.1	1.0	1.4	1.2	0.2	0.37	0.85	0.70
13,14	1.5	1.4	2.1	1.8	0.2	0.15	0.29	0.68
16	0.7	0.7	1.1	0.8	0.2	0.05	0.25	0.16
Isolated 18:2								
*c*9,*c*12	652	540	686	543	90.1	0.32	0.26	0.81
*t*9,*t*12	0.2	0.1	0.2	0.2	0.1	0.40	0.70	0.47
*t*9,*c*12	1.5	1.8	1.3	1.7	0.3	0.59	0.59	0.79
*t*11,*c*15	1.5	1.0	1.5	0.9	0.1	0.01	0.01	0.36
Conjugated 18:2								
*c*9,*t*11	1.5	1.2	2.0	2.0	0.3	0.23	0.25	0.61
*t*10,*c*12	0.0	0.2	0.5	0.7	0.02	0.01	0.01	0.92
18:3n3	59	48	62	47	7.3	0.31	0.23	0.77
20:3n3	18	15	21	15	4.7	0.17	0.31	0.49
20:4n6	32	26	35	26	7.0	0.35	0.31	0.79
20:5n3	16	13	18	14	4.3	0.38	0.50	0.98
Total	1205	1016	1287	1021	164	0.32	0.30	0.75

[1] Values are the average of means obtained at the end of 48 h of infusion.
[2] Effects due to CLA and linear (L) or quadratic (Q) effects of CLA dose.

Table 3.5. Milk fatty acid yields by cows infused continuously into the rumen for 2 d with 0, 45, 90, or 180 g/d of a conjugated linoleic acid (CLA) mixture [1].

	CLA (g/d)					Effect [2]		
	0	45	90	180	SE	CLA	L	Q
	g/d							
4:0	48.2	49.4	46.8	43.0	2.0	0.01	0.01	0.02
6:0	23.6	23.4	23.6	19.0	0.6	0.01	0.01	0.01
8:0	11.6	11.2	11.6	8.6	0.2	0.01	0.01	0.01
10:0	21.6	20.0	21.4	15.0	0.6	0.01	0.01	0.01
12:0	22.6	21.0	22.4	16.2	0.6	0.01	0.01	0.01
14:0	86.6	83.0	82.8	65.6	1.1	0.01	0.01	0.01
*cis*9-14:1	7.2	6.4	6.6	5.2	0.2	0.01	0.01	0.04
16:0	234.2	228.8	222.4	185.6	5.2	0.01	0.01	0.01
6:0 to 16:0	399.4	385.2	384.6	312.4	8.4	0.01	0.01	0.01
*cis*9-16:1	18.4	18.2	17.4	14.4	0.5	0.01	0.01	0.01
*trans*9-16:1	4.2	4.4	4.2	3.8	0.2	0.03	0.07	0.02
18:0	113.6	127.8	120.6	120.6	4.1	0.01	0.04	0.01
Cis 18:1								
9	266.2	276.2	271.2	243.4	6.2	0.01	0.01	0.01
11	10.0	10.2	10.0	8.8	0.3	0.01	0.01	0.01
12	6.6	6.2	6.2	6.2	0.2	0.67	0.28	0.61
13	4.2	4.2	3.8	3.4	0.3	0.17	0.07	0.43
15	2.2	2.4	2.2	2.2	0.2	0.65	0.52	0.72
Trans 18:1								
6,7,8	2.2	2.4	2.4	2.4	0.04	0.01	0.01	0.14
9	1.6	1.8	1.8	1.8	0.05	0.05	0.01	0.37
10	2.2	2.6	2.8	3.8	0.1	0.01	0.01	0.01
11	8.2	8.6	8.8	10.2	0.1	0.01	0.01	0.07
12	2.0	2.2	2.4	2.8	0.04	0.01	0.01	0.26
13,14	4.0	4.8	5.4	5.6	0.1	0.01	0.01	0.27
16	2.8	2.8	3.0	3.0	0.1	0.21	0.08	0.70
Isolated 18:2								
*c*9,*c*12	25.8	26.8	25.2	23.0	0.3	0.01	0.01	0.01
*t*9,*t*12	0.2	0.2	0.2	0.2	0.02	0.16	0.34	0.79
*t*11,*c*15	1.2	1.2	1.2	0.8	0.02	0.01	0.13	0.02
Conjugated 18:2								
*c*9,*t*11	6.2	6.0	6.4	6.6	0.1	0.04	0.04	0.31
*t*10,*c*12	0.0	0.2	0.6	1.0	0.02	0.01	0.01	0.13
18:3n3	4.4	4.6	4.4	3.8	0.04	0.01	0.01	0.01
20:3n3	0.6	0.8	0.8	0.6	0.03	0.28	0.69	0.06
20:4n6	1.4	1.4	1.6	1.2	0.03	0.04	0.38	0.01
20:5n3	0.1	0.1	0.1	0.1	0.02	0.16	0.03	0.97
Total	947.0	964.4	940.0	825.0	15.0	0.01	0.01	0.01

[1] Values are the average of means obtained from 12 through 96 h after the start of infusion.
[2] Effects due to CLA and linear (L) or quadratic (Q) effects of CLA dose were calculated on means obtained from 12 through 96 h after infusion.

Table 3.6. Normalized ratios [1] of fatty acids in milk fat from cows infused continuously into the rumen for 2 d with 0, 45, 90, or 180 g/d of a conjugated linoleic acid (CLA) mixture [2].

	CLA (g/d)					Effect [3]		
	0	45	90	180	SE	CLA	L	Q
Ratio								
14:0 ⇒ 14:1	0.079	0.072	0.074	0.073	0.002	0.04	0.03	0.12
16:0 ⇒ 16:1	0.072	0.072	0.072	0.072	0.004	0.96	0.74	0.79
18:0 ⇒ 18:1	0.70	0.68	0.69	0.66	0.003	0.01	0.01	0.90
18:1;*t*11 ⇒ 18:2;*c*9,*t*11	0.43	0.41	0.42	0.39	0.004	0.01	0.01	0.94
18:2 ⇒ 20:4n6	0.049	0.053	0.058	0.049	0.003	0.11	0.83	0.03
18:3 ⇒ 20:5n3	0.059	0.054	0.054	0.046	0.004	0.38	0.10	0.79

[1] Normalized ratio = mg/g product/[mg/g substrate + mg/g product].
[2] Values are the average of means obtained from 12 through 96 h after the start of the infusion
[3] Overall effect due to CLA and linear (L) or quadratic (Q) effects of CLA dose were calculated on means obtained from 12 through 96 h after infusion.

CHAPTER 4

Exogenous *trans*10,*cis*12-18:2 Reduces *De Novo* Synthesis and Desaturation of Milk Fatty Acids in Cows Fed Diets Containing High-Oleic or High-Linoleic Oil.

ABSTRACT

*Cis*9,*trans*11-18:2 (9/11CLA) and *trans*10,*cis*12-18:2 (10/12CLA) are produced in the rumen during hydrogenation of dietary polyunsaturated fatty acids, and could affect lipid metabolism in the mammary gland. To determine the effects of an elevated supply of 9/11CLA or 10/12CLA on *de novo* synthesis and desaturation of long-chain fatty acids, four Holstein cows fed (2.5% of DM) high-oleic sunflower (HO) or high-linoleic safflower oil (HL) were infused (0.625 g/h) with 9/11CLA or 10/12CLA for 48 h via the abomasum. Treatments were assigned in a 2 $\times$ 2 factorial design. The assigned diets were fed for 11 d prior to each 48-h infusion period. Milk samples were obtained at -12 and 0 h before infusion, at 12, 24, 36, and 48 h during infusion and at 60, 72, 84, and 96 h after infusion. Milk yield and DMI were not affected by treatment. Percentages and yields of protein, lactose, and SNF in milk also were not affected by treatment. Milk fat percentage and yield, however, decreased 25% from 0 to 96 h in response to infusion of 10/12CLA compared with 9/11CLA regardless of diet. Exogenous 9/11CLA and 10/12CLA were incorporated primarily into blood plasma phospholipid, triglyceride, and free fatty acid fractions. Arterial concentrations of 9/11CLA or 10/12CLA in triglycerides plus free fatty acids at 48 h, averaged 8 mg/g of total fatty acids. Approximately 65% of arterial 9/11CLA was extracted by the mammary gland, whereas 80% of the 10/12CLA was extracted. Yields of *trans*11-18:1, 9/11CLA, and 18:2n6 in milk fat before infusion were higher when HL was fed compared with HO. Infusion of 9/11CLA, regardless of diet, increased 9/11CLA in milk fat by 44%. Although 10/12CLA was not detectable in milk fat before infusion, it averaged 6 mg/g of total fatty acids and 2 g/d after 48 h. Regardless of diet, yields of saturated fatty acids with 16 carbons or less, 9/11CLA, and 20:4n6 decreased due to 10/12CLA infusion. Due to a 40% increase in 18:0 concentration by 48 h of 10/12CLA

infusion, however, yield of 18:0 was not affected. In contrast, infusion of 10/12CLA increased yields of *trans*11-18:1 and 18:2n6 in milk fat. Normalized ratios of 18:0 to *cis*9-18:1, *trans*11-18:1 to 9/11CLA, and 18:2n6 to 20:4 in milk fat decreased in response to infusion of 10/12CLA, regardless of diet. Results indicated 10/12CLA affects lipid metabolism in the bovine mammary gland by simultaneously reducing *de novo* synthesis of saturated fatty acids and reducing desaturation of 18:0, *trans*11-18:1, and 18:2n6.

INTRODUCTION

Dairy products are the primary source of conjugated linoleic acid (CLA) isomers in the food chain. The CLA isomers originate from partial hydrogenation of 18:2n6 in the rumen (Kepler and Tove, 1967). Under most dietary conditions, *cis*9,*trans*11-18:2 is the primary CLA produced. Isomers of CLA, however, are transient intermediates of the hydrogenation process leading to preferential accumulation of *trans*11-18:1 and 18:0 (Kemp et al., 1975). *Trans*11-18:1 can serve as a substrate for endogenous synthesis of *cis*9,*trans*11-18:2, via Δ^9 desaturase, in the mammary gland of the cow (Griinari et al., 2000) and human tissues (Adlof et al., 2000). The concentration of *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat is enhanced by feeding unsaturated oils (Kelly et al., 1998a). In contrast, feeding greater amounts of grain plus supplemental oil in place of forage leads to production of milk fat with lower concentration of saturated fatty acids and greater concentrations of *trans*10-18:1 and *trans*10,*cis*12-18:2 relative to *trans*11-18:1 and *cis*9,*trans*11-18:2 (Griinari et al., 1998; Piperova et al., 2000).

Administration of CLA mixtures to lactating cows decreased fatty acid synthesis in the mammary gland, leading to reduced milk fat secretion (Loor and Herbein, 1998; Chouinard et al., 1999a). As indicated by their concentrations in milk fat, desaturation of long-chain fatty acids in the above tissues also appeared to be decreased. When pure sources of CLA isomers became available, Baumgard et al. (2000b) determined that *trans*10,*cis*12-18:2, but not *cis*9,*trans*11-18:2, was responsible for depressed milk fat percentage and concentrations of saturated medium-chain fatty acids in milk fat. The concentration of 18:0 and the ratio of 18:0 to *cis*9-18:1 in milk fat were increased by infusion of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 into the abomasum (Baumgard et al., 2000b), indicating both isomers might potentially inhibit Δ^9 desaturase. In rat liver homogenates *trans*10,*cis*12-18:2 decreased Δ^9 desaturase activity, whereas *cis*9,*trans*11-18:2 decreased Δ^6 desaturase activity (Bretillion et al., 1999).

During milk fat synthesis, a portion of 18:0, *trans*11-18:1, 18:2n6, and 18:3n3 derived from the diet or the rumen is substrate for endogenous synthesis of *cis*9-18:1, *cis*9,*trans*11-18:2, 20:4n6, and 20:5n3, respectively, via Δ^9, Δ^5, or Δ^6 desaturase

(Hermansen et al., 1995; Griinari et al., 2000; Enjalbert al. 2000). Activity of Δ^9 desaturase in lactating mammary tissue is substantially higher relative to the dry period, when activity of adipose tissue Δ^9 desaturase predominates (Ward et al., 1998).

Initial studies using one or both of the CLA mixtures containing *cis*9,*trans*11-18:2 plus *trans*10,*cis*12-18:2, indicated CLA isomers may be effective as anticarcinogenic, antidiabetic, and antilipogenic agents in the diet of laboratory animals (Pariza, 1999). Later studies, however, demonstrated that dietary *trans*10,*cis*12-18:2 was the isomer responsible for reduced lipogenesis in the rodent carcass (Park et al., 1999b). In contrast, milk fat-derived *cis*9,*trans*11-18:2 prevented growth of human mammary cancer cells more effectively than synthetic *trans*10,*cis*12-18:2 (O'Shea et al., 2000). Thus, metabolic responses to *cis*9,*trans*11-18:2 and *trans*10,*cis*12-18:2 may differ substantially, but both isomers may have implications in human health (Parodi, 1999).

The objectives of this study were to evaluate *de novo* synthesis and desaturation of fatty acids in lactating cows when the supply of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 for uptake by the mammary gland was enhanced by infusing the isomers via the abomasum. Diets contained either high-oleic or high-linoleic oil to vary the basal amounts of oleic acid, linoleic acid, and their hydrogenation intermediates flowing from the rumen to the mammary gland during infusion of each CLA isomer. Changes in the concentrations and yields of milk fatty acids were measured to assess relative changes in lipogenesis and desaturation during oil feeding and CLA infusions.

MATERIALS AND METHODS

Animals and Diets

Four mid-lactation primiparous Holstein cows (between 126 and 138 DIM) were used in a 2 × 2 factorial design with four 15 d periods to evaluate responses to feeding diets (Table 4.1) supplemented (2.5% of DM) with high-oleic sunflower oil or high-linoleic safflower oil (Table 4.2), and infusion of *cis*9,*trans*11-18:2 (9/11CLA) or *trans*10,*cis*12-18:2 (10/12CLA) into the abomasum. Diets were formulated using Dair4 (Stallings et al., 1985) to meet nutrient requirements of cows producing 30 kg milk and

consuming 19 kg of DM daily. During a 4-d adaptation before the study and between each of the periods, incremental portions of concentrate containing equal amounts of high-oleic sunflower and high-linoleic safflower oil (2.5% of DM) were substituted (25, 50, 75, and 100%) for a basal concentrate and fed with forage as a TMR. Subsequently, the assigned diets were fed for 11-d prior to infusion of CLA isomers during each experimental period. Cows were housed in a tie-stall barn during the experiment. Diets were prepared daily as a TMR, and offered in equal amounts at 1400 and 0200 h. Feed refusals were weighed at 1400 h. Daily feed allotment was calculated to allow 5 to 10% feed refusals. Cows were milked at 0100 and 1300 h. Milk was collected in a stainless steel bucket, weighed, and thoroughly mixed prior to obtaining samples at each milking from -12 h to 96 h relative to the start of infusion. The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

Infusion Procedures

During abomasal infusions, 9/11CLA or 10/12CLA (Natural Lipids, Norway) was suspended in skim milk and infused for 48 h (Table 4.2). The CLA isomers were emulsified in skim milk to ensure a uniform supply of CLA during daily infusions. Emulsions were prepared the day prior to infusion by combining 15 g of CLA with 3.5 g glycerol (Eastman Kodak Co., Rochester, NY) and 1.8 g soy lecithin powder (Refined, Alfa®, Ward Hill, MA) in 975.2 mL of skim milk at room temperature. The mixture was homogenized at 12,000 rpm for 2 min with a Polytron® PT 10/35 homogenizer (Brinkmann Instruments, Westbury, NY), and checked for the presence of clumps before stirring at medium-to-high speed for 30 min at room temperature. Emulsions were dispensed into 1 L Viaflex® plastic bags (Baxter Corporation, Deerfield, IL) and stored at 4 °C until infusion. Abomasal infusion of 9/11CLA and 10/12CLA began at 1400 h.

During infusion, bags containing CLA emulsions were attached to a flat platform on a wrist-action shaker (Burrell Corporation, Pittsburgh, PA) set at low speed. Emulsions were infused via Tygon® tubing (1.6 mm i.d., 0.8 mm wall; Fisher Scientific Co., Pittsburgh, PA) that passed through a Harvard Peristaltic pump (55-1762; Harvard

Apparatus, South Natick, MA). Flow from the pump was via Tygon® tubing (3.2 mm i.d., 1.6 mm wall) that passed through the rumen cannula, rumen and omasum, and into the abomasum. A perforated Nalgene® plastic bottle (60 mL) was attached to the end of the tubing to secure it in the abomasum. The tubing was primed with 15 mL infusate at the start of infusion, and flow rate was set at 41.7 mL/h.

Sampling, Measurements, and Analysis

Forages and concentrates were sampled during the last day of each experimental period. Samples were dried in a forced-air oven at 60 °C, and stored in a sealed container at room temperature until analyzed. Equal amounts of samples from each period were combined to determine chemical composition. In preparation for analyses, dried forages and concentrates were ground first through a 2 mm screen (Thomas-Wiley Laboratory Mill), then through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Forages and concentrates were analyzed for ADF and NDF (Van Soest et al., 1991) and total N (AOAC, 1975).

Two 50 mL aliquots of milk were collected at -12 and 0 h before infusion, 12, 24, 36, and 48 h during infusion, and 60, 72, 84, and 96 h after infusion. One aliquot containing Bronopol (D & F Control Systems, San Ramon, CA) was stored at 4 °C until analyzed for fat, protein, SNF, and lactose by infrared analysis with a 4-channel spectrophotometer (Virginia Dairy Herd Improvement Association). The second aliquot was stored at -20 °C until the end of the experiment, then thawed and centrifuged at 10,000 $\times$ *g* for 1 h to harvest milk fat for fatty acid analysis.

For plasma total fatty acid analysis, blood samples (10 mL) were obtained from the coccygeal artery immediately after the collection of milk samples. For analysis of fatty acids in blood plasma lipid fractions, blood samples (10 mL) were obtained every 2-h from -12 to 0 h before infusion, and 36 to 48h during infusion from the coccygeal artery and mammary subcutaneous abdominal vein. Blood was transferred to tubes containing 286 IU heparin in 100 µL of sterile saline and centrifuged at 3,000 × *g* for 15 min for harvesting plasma. An equal volume of plasma from each of 6 arterial and venous samples was pooled and used for analysis. Plasma was stored at -20 °C until lipid extraction and fatty acid analysis.

Plasma total lipids were extracted with chloroform/methanol (2:1, vol/vol). Subsequently, blood plasma lipid fractions (free fatty acids, phospholipids, cholesterol esters, and triglycerides) were isolated (Agren et al., 1992) using Bond Elut® aminopropyl disposable columns (500 mg) in a Vac Elut® system (Analytichem International, Harbor City, CA). Fatty acids in forages, concentrates, milk fat, and blood plasma were methylated by in situ transesterification with 0.5N methanolic NaOH as described by Park and Goins (1994). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters from all samples were separated on a 100 m $\times$ 0.25 mm i.d. fused silica capillary column (CP-Sil 88,Chrompack, Middelburg, The Netherlands).

For forages, concentrates, and milk fatty acid analysis (0.5 μL methyl esters in hexane injected at an 80:1 split ratio), the injector temperature was maintained at 250 °C and the detector temperature was maintained at 255 °C. The initial oven temperature was held at 70 °C for 1 min, increased 5 °C/min to 100 °C (held for 2 min), then increased at 10 °C/min to 175 °C (held for 40 min), and 5 °C/min to a final temperature of 225 °C (held for 15 min). Hydrogen was the carrier gas.

Analysis of fatty acids in total plasma or individual plasma lipid fractions required injection of 0.5 or 2.5 μL methyl esters in hexane (splitless). The injector temperature was maintained at 150 °C and the detector temperature at 275 °C. The purge valve on the GC was closed for 0.8 or 1.5 min after injection of methyl esters from total plasma or lipid fractions. The initial column temperature was 40 °C (held for 1.5 min), then increased at 40 °C/min to 100 °C (held for 10 min), 20 °C/min to 175 °C (held for 45 min), and 10 °C/min to a final temperature of 220 °C (held for 25 min). Ultra pure helium was the carrier gas.

Extraction of individual fatty acids by the mammary gland was calculated by adding the amount of each fatty acid in the triglyceride fraction to the amount in the free fatty acid fraction of arterial plasma for comparison with venous plasma. This was necessary because plasma triglycerides are hydrolyzed in the capillaries prior to

uptake of free fatty acids, and a portion of the free fatty acids released appear in venous plasma resulting in negative arterio-venous differences if only free fatty acids are considered in the calculation (Enjalbert et al., 1998). We also calculated extraction ratios of total fatty acids in cholesterol esters and phospholipids, but substantial variation precluded attempts to calculate extraction of individual fatty acids. Extraction (%) of fatty acids from arterial plasma by the mammary gland was calculated as [(arterial - venous concentration)/arterial concentration] $\times$ 100.

Statistical Analysis

Data for DMI, milk production and composition, fatty acid intake, fatty acids in plasma, fatty acids in plasma lipid fractions, fatty acids in milk fat, and normalized ratios of milk fatty acids are reported as Least squares means $\pm$ SEM. Data for DMI, milk production, milk composition, fatty acid intake, milk fatty acids, and normalized ratios of milk fatty acids were analyzed as a factorial design with repeated measures using the MIXED procedure of SAS (2000). Main effects in the model included cow, period, oil supplement, CLA isomer, time, oil by isomer interaction, oil by isomer by time interaction, and residual error. Data for plasma fatty acid profiles and fatty acid extraction were analyzed as a factorial design without repeated measures using the MIXED procedure of SAS (2000). Main effects in the model included cow, period, oil supplement, CLA isomer, oil by isomer interaction, and residual error. One cow in the high-oleic oil group receiving 9/11CLA was omitted from all statistical analyses, because the infusion line inadvertently was dislodged from the abomasum into the rumen. Overall differences between treatment means were considered to be significant when $P \leq 0.05$. However, all P values are presented in tables.

RESULTS

DMI and Milk Production and Composition

Overall, DMI and milk production throughout the 96 h sampling period did not differ in response to diet or CLA isomer (Table 4.3). Percentages and yields of protein, lactose, and SNF in milk also did not differ. Milk fat percentage from 24 to 96 h,

however, was substantially reduced by infusion of 10/12CLA, regardless of diet (Figure 4.1). The lower overall fat concentration in response to 10/12CLA reduced overall milk fat yield by 25% compared with 9/11CLA (Table 4.3).

Fatty Acid Intake and Total Plasma Fatty Acid Concentrations

Estimated total fatty acid intake (g/d) from oil-supplemented diets was similar for all treatments and averaged 1,071 g/d (Table 4.4). Intakes of 14:0, 16:0, *cis*9-16:1, and 18:0 were slightly, but significantly, higher when HL was fed. As expected, the primary fatty acid in the DMI was *cis*9-18:1 when HO was fed and 18:2n6 when HL was fed. Concentration of total fatty acids in blood plasma at the end of the 48-h infusion period (1,957 μg/mL) was similar for all treatments (Table 4.5). When cows were fed HO, concentrations of *cis*9-18:1, *trans*6/7/8-18:1, *trans*9-18:1, and 18:3n3 were greater compared with feeding HL. When cows were fed HL, concentrations of 18:2n6 and the primary biohydrogenation intermediates, *cis*9,*trans*11-18:2 and *trans*11-18:1 were elevated in plasma. In addition, concentrations of *cis*12-18:1, *trans*12-18:1, and *trans*16-18:1 were elevated when HL was fed. Concentrations of *cis*9,*trans*11-18:2 in plasma increased in response to 9/11CLA infusion, whereas *trans*10-18:1 and *trans*10,*cis*12-18:2 were elevated in response to 10/12CLA infusion.

Fatty Acid Distribution in Blood Plasma Lipid Fractions

Samples obtained between 36 and 48 h were used to determine the distribution of *cis*9-18:1, 18:2n6, *trans*11-18:1, and CLA isomers in blood plasma lipid fractions for transport to the mammary gland. The lipid fractions included free fatty acids, phospholipids, cholesterol esters, and triglycerides (Figure 4.2). Concentrations (mg/g total fatty acids) of *cis*9-18:1 and 18:2n6 in all lipid fractions reflected the amount of each fatty acid contained in HO or HL. Oleic acid concentration in phospholipids, cholesterol esters, triglycerides, and free fatty acids when HO was fed averaged 100, 32, 97, and 96 mg/g, respectively, compared with 53, 19, 50, and 53 mg/g when HL was fed. In contrast, 18:2n6 concentration in phospholipids, cholesterol esters, triglycerides, and free fatty acids due to feeding HL averaged 450, 880, 100, and 100 mg/g, respectively, compared with 370, 850, 50, and 50 mg/g when HO was fed.

The concentration of *trans*11-18:1 in phospholipids, triglycerides, and free fatty acids was elevated when cows were fed HL compared with HO regardless of isomer (Figure 4.2). Overall, the concentrations of individual CLA isomers in triglycerides and free fatty acids increased in proportion to the amount of isomer infused. *Trans*10,*cis*12-18:2 was only detectable when 10/12CLA was infused, and averaged 2, 11, and 6 mg/g, respectively, in phospholipids, triglycerides, and free fatty acids. The elevated concentrations of CLA isomers in blood plasma lipids at 36 to 48 h corresponded with the peak in their concentrations in milk fat (data not shown).

Fatty Acids in Arterial Plasma Triglycerides plus Free Fatty Acids

To estimate the primary pool of fatty acids available to the mammary gland for uptake and incorporation into milk fat, the sum of individual fatty acids in plasma triglycerides plus those in free fatty acids (TG+FA) was determined (Annison et al., 1967). Arterial concentration of total TG+FA at 48 h averaged 99 μg/mL and did not differ across treatments (Table 4.6). When cows were fed HO concentrations of *cis*9-18:1 and 18:3n3 in TG+FA were 76 and 38% greater compared with feeding HL. In contrast, feeding HL resulted in elevated concentrations of *cis*12-, *cis*15-, *trans*10-, *trans*11-, *trans*12-, *trans*13/14-, *trans*16-18:1, and 18:2n6. Infusing 10/12CLA regardless of diet, elevated concentrations of *cis*11-, *cis*12-, *cis*13-, *trans*6/7/8-, *trans*9-, *trans*10-, and *trans*12-18:1. Concentrations of 14:0 and 18:3n3 in TG+FA also were elevated by 10/12CLA infusion. Greater concentrations of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in TG+FA was expected in response to infusion of either CLA isomer, but alterations in concentrations of the *cis*- and *trans*- isomers of 18:1, 14:0, or 18:3n3 were not. The cause(s) of the response may be due to the effects of the CLA isomers on tissues other than the mammary gland.

Extraction Ratios of Fatty Acids by the Mammary Gland

Mammary gland extraction of total fatty acids from TG+FA did not differ due to treatments and averaged 39% (Table 4.7). Despite greater concentrations of several fatty acids in TG+FA in response to HO or HL, extraction ratios for most fatty acids did not differ due to treatment. The exception was extraction of 18:2n6, which was greater

when cows were fed HL compared with HO. Infusion of 10/12CLA resulted in lower extraction of 18:0 and higher extraction of *cis*9,*trans*11-18:2, regardless of diet. Extraction of *trans*10,*cis*12-18:2 during 10/12CLA infusion averaged 81%, which was numerically higher than the extraction ratios for all other fatty acids flowing to the mammary gland in blood plasma.

Milk Fatty Acid Yields

Concentration of saturated fatty acids with 6 to 16 carbons (Figure 4.1), regardless of diet, decreased 19% from 0 to 60 h in response to 10/12CLA then remained low until 96 h. Overall yields of fatty acids with 4 to 16 carbons were reduced when 10/12CLA was infused (Table 4.8). Reductions in the yields of the short- and medium-chain fatty acids were the primary cause of the 19% reduction in the yield of total fatty acids in response to 10/12CLA.

*Trans*10,*cis*12-18:2 was not detectable in milk fat before infusion, but its concentration peaked (6 mg/g total fatty acids) at 48 h then remained elevated in milk fat when cows were infused with 10/12CLA (data not shown). Yield of *trans*10,*cis*12-18:2 averaged 2 g/d from 0 to 96 h. The basal concentration before infusion of *cis*9,*trans*11-18:2 (10 mg/g) was greater due to feeding HL compared with HO, and accounted for the effect of diet on its yield in milk fat (Table 4.8). Similarly, the basal concentration of *trans*11-18:1 was greater when HL was fed compared with HO (Figure 4.3). During 10/12CLA infusion, concentration of *cis*9,*trans*11-18:2 in milk fat was reduced by 40% when cows were fed HL. As a result, yield of *cis*9,*trans*11-18:2 decreased with 10/12CLA infusion.

The basal concentration of 18:0 in milk fat did not differ due to diet (144 mg/g), but the 18:0 concentration increased by approximately 40% during 10/12CLA infusion (Figure 4.3). As a result of the lower yield of milk fat in response to 10/12CLA infusion, however, the yield of 18:0 from 0 to 60 h did not differ due to type of infusion (Table 4.8). The concentration of *cis*9-18:1 in milk fat did not change during infusion of 9/11CLA when cows were fed HO or HL. However, when HL was fed and 10/12CLA was infused, *cis*9-18:1 concentration was 12% lower from 24 to 60 h (significant diet by isomer by time interaction). The greater availability of dietary *cis*9-18:1 when HO was

fed, compared with HL, probably masked the potential effects of 10/12CLA infusion on desaturation of 18:0. Indicators of inhibition of desaturase activity are discussed in the "normalized ratios" section below.

Cows fed HL had higher basal concentrations of 18:2n6 (Figure 4.3) and 20:4n6 (16 vs 13 mg/g) (data not shown). Similar to the response in 18:0 concentration, infusion of 10/12CLA increased the concentration of 18:2n6 and decreased 20:4n6 concentration (data not shown). As noted above, however, the depressed yield of nearly all fatty acids (Table 4.8) masked the divergent changes in concentrations of substrate/product fatty acid pairs for desaturase reactions.

Normalized Ratios of Milk Fatty Acids

Normalized ratios (mg/g product/[mg/g substrate + mg/g product]) were estimated to assess the extent of desaturation of specific fatty acids during milk fat synthesis (Palmquist and Santora, 1999). The basal ratios of 14:0 to *cis*9-14:1, 16:0 to *cis*9-16:1, 18:0 to *cis*9-18:1, *trans*11-18:1 to *cis*9,*trans*11-18:2, and 18:2n6 to 20:4n6 were higher when cows were fed HO compared with HL (Table 4.9). Higher ratios indicated cows desaturated more of the substrate fatty acids when they were fed HO compared with HL. Compared with 9/11CLA, however, infusion of 10/12CLA decreased the above ratios, regardless of diet. Lower ratios were evident after only 24 h of 10/12CLA infusion, regardless of diet, and were maintained until 72 h (data not shown). The decline in the ratios suggested exogenous *trans*10,*cis*12-18:2 reduced the amount and(or) activity of Δ^6, Δ^5, and Δ^9 desaturases in the mammary gland.

DISCUSSION

As reported in previous studies in which CLA mixtures (0 to 200 g/d) (Loor and Herbein, 1998; Chouinard et al., 1999a) or purified isomer preparations (10 g/d) were infused into the abomasum of cows fed basal diets (Baumgard et al., 2000b), DMI and milk production in the present study were not affected by infusion of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2. The amount of CLA infused was substantially lower than amounts of 18:2n6 or *trans*-18:1 isomers previously shown to depress DMI when

infused into the abomasum of lactating cows (Drackley et al., 1992; Gaynor et al., 1994). Greater flow of CLA into the small intestine, however, could potentially increase vagal afferent activity and might depress food intake (Randich et al., 2000). *Trans*10,*cis*12-18:2 may be primarily responsible for this effect, as indicated by a 4.3 kg/d decrease in DMI during its infusion into the abomasum of dairy cows (Baumgard et al., 2000b).

Regardless of CLA isomer infused, feeding high-oleic or high-linoleic oil did not affect total fatty acid intake but more than doubled intakes of *cis*9-18:1 or 18:2n6 (Table 4.4). Differences in intakes of *cis*9-18:1 and 18:2n6 due to type of oil fed, led to major changes in the profiles of most fatty acids in blood plasma lipids. *Cis*9-18:1 content of plasma was 66% greater when feeding high-oleic oil compared with high-linoleic oil (Table 4.5). In contrast, 18:2n6 concentration in blood plasma was 23% greater when high-linoleic oil was fed compared with high-oleic oil. Oleic acid accounted for 10% of total fatty acids in triglycerides, free fatty acids, and phospholipids when high-oleic oil was fed (Figure 4.2). Linoleic acid also accounted for 10% of triglycerides and free fatty acids, but was 42% of total fatty acids in phospholipids when high-linoleic oil was fed. These three lipid fractions contained 53% of total fatty acids in blood plasma. Plasma cholesterol esters also contained more *cis*9-18:1 or 18:2n6 in response to intake of high-oleic or high-linoleic oil, but 18:2n6 averaged 85% of total fatty acids regardless of oil type.

Concentrations of isomers derived from isomerization and hydrogenation of dietary *cis*9-18:1 or 18:2n6 also were proportional to intake of both fatty acids from the diet. For example, feeding high-oleic oil increased concentrations of *trans*6/7/8-18:1 and *trans*9-18:1 by 86 and 57% in blood plasma compared with feeding high-linoleic oil. Concentrations of *cis*12-18:1 and *trans*10-, *trans*11, *trans*12-, or *trans*16-18:1, however, were 69, 31, 82, 42, and 60%, respectively, greater in response to feeding high-linoleic oil compared with high-oleic oil. *Trans*11-18:1 was the primary *trans*-18:1 isomer in blood plasma, and it accounted for 42 or 55% of total *trans*-18:1 when cows were fed high-oleic or high-linoleic oil, respectively.

Among lipid fractions, triglycerides and free fatty acids contained 2 to 4% *trans*11-18:1, whereas phospholipids contained 0.8% *trans*11-18:1 (Figure 4.2).

Overall, *trans* isomers of 18:1 were primarily found in triglycerides (7% of total fatty acids), free fatty acids (5%), and phospholipids (2%). Greater amounts of fatty acids in the major plasma lipid fractions were an obligatory response to accommodate transport of fatty acids absorbed from the small intestine. However, some selectivity exists regarding the distribution of absorbed fatty acids in the major blood plasma lipid fractions of cows fed oil. Linoleic acid, the preferred substrate for lecithin:cholesterol acyl transferase, in bovine plasma is preferentially incorporated into phospholipids and cholesterol esters but extensive incorporation into triglycerides also can occur (Christie, 1981). Oleic acid is primarily esterified to plasma triglycerides, and it increases in proportion with the amount available for absorption (La Count et al., 1994). Greater flow of *trans*-18:1 isomers to the duodenum during hydrogenation of supplemental 18:2n6 in the rumen increased absorption and incorporation of 18:1 and 18:2 isomers into blood plasma triglycerides and phospholipids (Bickerstaffe et al., 1972). Thus, upon hydrolysis of triglycerides and phospholipids the plasma free fatty acid pool also contains more *trans*-18:1 isomers. Our results confirmed previous *in vivo* and *in vitro* observations indicating ruminal hydrogenation of *cis*9-18:1 and 18:2n6 gives rise to geometrical isomers of 18:1 with double bonds at positions 6 through 16 of the carbon chain (Bickerstaffe et al., 1972; Kemp et al., 1975). Under normal circumstances, however, *trans*11-18:1 is by far the primary *trans*-18:1 isomer resulting from hydrogenation of 18:2n6 in the rumen.

Whereas *cis*9,*trans*11-18:2 in plasma was proportional to dietary 18:2n6 intake, *trans*10,*cis*12-18:2 was detectable only after 10/12CLA was infused (Table 4.5). Infused 9/11CLA was primarily incorporated into plasma free fatty acids and triglycerides where it averaged 7 to 13 mg/g of total fatty acids. During infusion of 10/12CLA, regardless of diet, concentration of *trans*10,*cis*12-18:2 increased from non-detectable levels to 15 mg/g. Its concentration in triglycerides and free fatty acids averaged 11 and 6 mg/g after infusion of 10/12CLA (Figure 4.2). The triglyceride and free fatty acid fractions contained approximately 3% of total fatty acids in plasma, whereas the phospholipid fraction contained approximately 48% of total plasma fatty acids. However, absolute amounts of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in the three fractions during infusion of 9/11CLA or 10/12CLA was similar. Exogenous

*cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 during abomasal infusion in cows fed a basal diet previously were detected in plasma phospholipids and triglycerides only (Baumgard et al., 2000a). The increase in the proportions of *cis*9,*trans*11-18:2 or *trans*10,*cis*12-18:2 in triglycerides, free fatty acids, and phospholipids indicated they were readily available to the mammary gland.

Triglycerides, phospholipids, and cholesterol esters account for 57, 16, and 22% of total lipid fractions in very low-density lipoproteins (VLDL = 1% of total blood plasma lipid) of arterial plasma in lactating cows (Palmquist, 1976). During passage of blood through the udder, however, 70% of arterial triglycerides in VLDL and chylomicrons were hydrolyzed by lipoprotein lipase and their fatty acids made available for milk fat synthesis (Glascock and Welch, 1974). Lipoprotein lipase also hydrolyzed phosphatidylcholine in VLDL, but not cholesterol esters, derived from sheep lymph *in vitro* (Christie et al., 1986). Although the extraction of phospholipids is questionable, at high arterial concentrations there was net extraction by the lactating mammary gland (Thivierge et al., 1998). Therefore, it seems reasonable that the mammary gland could utilize non-esterified fatty acids derived from triglycerides and phospholipids. In our study, extraction ratios for total fatty acids in phospholipids ranged from -6 to 7% and for fatty acids in cholesterol esters from -0.1 to 7% (data not shown), but did not differ for treatments due to high variation. Despite the lack of uniform responses, our range of values was consistent with previous data (Thivierge et al., 1998). Blood plasma triglycerides, however, provide the majority of the fatty acids taken up by the mammary gland. Furthermore, calculating extraction ratios for fatty acids in triglycerides plus free fatty acids (TG+FA) accounts for the greater concentrations of free fatty acids in venous plasma which originate from hydrolysis of arterial triglycerides prior to uptake (Enjalbert et al., 1998).

With few exceptions, concentrations of individual fatty acids in plasma TG+FA (Table 4.6) reflected changes observed in total plasma fatty acids due to dietary *cis*9-18:1 or 18:2n6 intake plus hydrogenation (Table 4.5). Availability of *cis*9-18:1, *trans*9-18:1, and 18:3n3 for extraction was greater when high-oleic oil was fed compared with high-linoleic oil. In contrast, feeding high-linoleic oil improved the availability of *cis*12-, *cis*15-, *trans*10- through *trans*16-18:1, and 18:2n6. There were minor but significant

increases in the concentrations of 14:0 and some *cis*- or *trans*-isomers of 18:1 and 18:3n3 in response to 10/12CLA infusion compared with 9/11CLA (Table 4.6). This response to exogenous 10/12CLA was associated with an overall 30% increase in total fatty acids in the free fatty acid fraction (data not shown). Higher concentrations of total plasma free fatty acids were found previously during abomasal infusions of CLA mixtures (Loor and Herbein, 1998) or various doses (2 to 10 g/d) of *trans*10,*cis*12-18:2 (Baumgard et al., 2000a; Viswanadha et al., 2000).

Despite the increase in concentrations of the various *cis*- and *trans*-18:1 isomers in plasma TG+FA due to oils or 10/12CLA, extraction ratios for these fatty acids did not change. Assuming that mammary blood flow remained constant, the extent of the increases in concentration apparently was not large enough to influence extraction. Only the extraction of 18:2n6 was greater when high-linoleic oil was fed, regardless of isomer, because its concentration in TG+FA was 147% higher compared with feeding high-oleic oil and it accounted for 13% of total TG+FA. Although concentrations of 18:0 in TG+FA were constant across treatments, infusion of 10/12CLA decreased the extraction ratio for 18:0 by 6 percentage units regardless of diet. In contrast, the extraction ratio for *cis*9,*trans*11-18:2 increased due to 10/12CLA infusion. Our values are the first estimates of extraction of the major CLA isomers from blood plasma TG+FA by the mammary gland of lactating cows.

At high levels of dietary fat supplementation (>3.5% of DM) mammary blood flow rate decreased (Cant et al., 1993), but higher concentrations of fatty acids in plasma TG+FA apparently overcomes this effect and leads to greater extraction and uptake (Enjalbert et al., 1998). Differences in extraction and uptake of fatty acids also might be related to positional distribution in triglycerides. Stearic acid, *trans*-18:1 isomers, and 18:2n6 are primarily found in positions sn-1 and sn-3, which are readily cleaved by lipoprotein lipase prior to uptake by the mammary gland (Christie, 1981). Extraction of *trans*10,*cis*12-18:2 was 80% when abomasal infusion was used to increase its concentration in arterial TG+FA. Thus, CLA isomers may be preferentially esterified in the sn-1 or sn-3 position of triglycerides.

Compared with 9/11CLA, infusion of 10/12CLA decreased milk fat concentration from 3.5% before infusion to 2.1% at 72 h (Figure 4.1). This represented a 40%

reduction in concentration and led to an overall 25% reduction in yield (Table 4.3). Basal concentrations of saturated fatty acids with 6 to 16 carbons averaged 370 mg/g of total fatty acids, and were 34% lower than typically seen in milk fat from cows fed diets without supplemental oil (Palmquist et al., 1993). A large portion of the reduction in *de novo* synthesis due to feeding unsaturated oils occurs as a result of greater uptake and secretion of dietary and ruminally-derived fatty acids (Clapperton and Banks, 1985). Exogenous fatty acids compete for esterification with newly synthesized short-chain fatty acids in mammary cells, and could lead to feedback inhibition of lipogenic enzymes (Palmquist et al., 1993). Results from a recent study indicated that supplemental *cis*9-18:1 was preferentially incorporated into the sn-2 position of the milk fat triglyceride at the expense of 16:0 (DePeters et al., 2001). The net effect was lower concentration of 16:0 but higher *cis*9-18:1 concentration in milk fat.

Because of the small amount of *trans*10,*cis*12-18:2 required to reduce milk fat percentage, CLA isomers appear to depress *de novo* fatty acid synthesis in a manner distinct from that caused by dietary *cis*9-18:1 or 18:2n6. Despite lower concentrations of 16:0 in milk fat when 18:2n6 was infused into the abomasum, only the infusion of a CLA mixture reduced 16:0 and milk fat concentrations (Loor and Herbein, 1998). It seems that a *trans*-10 double bond in the CLA is required for inhibition of lipogenesis. For example, infusions of *trans*10,*cis*12-18:2 or *cis*8,*trans*10-18:2 in combination with *cis*9,*trans*11-18:2 reduced milk fat synthesis to a similar extent (Loor and Herbein, 1998; Chouinard et al., 1999b).

In the present study, the extent of the decrease in milk fat yield observed with 10/12CLA infusion was unexpected because the transfer efficiency for supplemental dietary lipid to milk fat is high (Palmquist et al., 1993). Oil supplementation had the potential to overcome reductions in *de novo* synthesis caused by either CLA. However, lower concentrations (Figure 4.1) and yields of saturated 6:0 to 16:0 (Table 4.8) corresponded with the gradual increase in concentration of *trans*10,*cis*12-18:2 in milk fat from 0 to 48 h during 10/12CLA infusion (data not shown), and accounted for the overall reduction in milk fat yield. The temporal nature of the decrease in lipogenesis observed with very small concentrations (6 mg/g) of *trans*10,*cis*12-18:2, were consistent with a sequence of events which may have begun with reductions in

synthesis of mRNA for acetyl-CoA carboxylase and fatty acid synthase. Lower mRNA abundance for both enzymes were found in mammary tissue of lactating mice fed *trans*10,*cis*12-18:2 (Lin et al., 2000). The present study confirmed initial evidence indicating *trans*10,*cis*12-18:2 at very small concentrations is extremely effective in reducing milk fat percentage and *de novo* fatty acid synthesis in dairy cows.

Stearic acid in plasma TG+FA across treatments accounted for 40% of total fatty acids available for extraction (Table 4.6). After uptake by the mammary gland, 18:0 becomes the primary substrate for Δ^9 desaturase (Enjalbert et al., 1998, 2000). *Trans*11-18:1 derived from the rumen also is a substrate for Δ^9 desaturase and leads to endogenous synthesis of *cis*9,*trans*11-18:2 in the mammary gland (Griinari et al., 2000). Because most of the 14:0 plus 16:0 found in milk fat is synthesized *de novo*, desaturation to *cis*9-14:1 and *cis*9-16:1 may be a good estimate of basal activity of the Δ^9 desaturase (Griinari et al., 2000).

As indicated by normalized ratios (Table 4.9), the extent of desaturation of 16:0, 18:0, and *trans*11-18:1 prior to infusion of any CLA was higher when high-oleic oil was fed compared with high-linoleic oil. However, the marked increase in milk fat 18:0 and *trans*11-18:1 concentrations from 24 through 48 h when 10/12CLA was infused (Figure 3), regardless of diet, suggested desaturation of fatty acids derived from plasma was severely decreased by exogenous *trans*10,*cis*12-18:2. Desaturation of *trans*11-18:1 to *cis*9,*trans*11-18:2 apparently was very sensitive to exogenous 10/12CLA, because yield of *cis*9,*trans*11-18:2 in milk fat was reduced by 50% regardless of diet. The depressed ratios of 14:0 to *cis*9-14:1 and 16:0 to *cis*9-16:1, despite their lower concentrations in milk fat, provide evidence that desaturation of endogenously synthesized fatty acids is also sensitive to 10/12CLA.

Reduced desaturation, resulting in accumulation of 18:0 in the mammary gland during infusion of 10/12CLA, might have lowered 18:0 extraction from plasma TG+FA. In contrast, the reduction in desaturation of *trans*11-18:1 to *cis*9,*trans*11-18:2 may have decreased endogenously synthesized *cis*9,*trans*11-18:2 concentration in the mammary gland. The lower amount of *cis*9,*trans*11-18:2 in the gland could have enhanced its extraction from TG+FA when 10/12CLA was infused (Table 4.7). The reduction in yield of *cis*9,*trans*11-18:2, despite greater extraction from blood, in combination with the

lower normalized ratio for *trans*11-18:1 ⇒ *cis*9,*trans*11-18:2 during 10/12CLA infusion suggests endogenous synthesis (via desaturation) may be the primary source of *cis*9,*trans*11-18:2 in milk fat.

Expression of Δ^9 desaturase activity is markedly reduced by *trans*10,*cis*12-18:2, but not *cis*9,*trans*11-18:2, in rodent adipose, liver, and mammary gland tissue (Choi et al., 2000; Park et al., 2000; Lin et al., 2000). The negative effect of *trans*10,*cis*12-18:2 on desaturation activity in bovine mammary gland might be mediated by reductions in the transcription of the Δ^9 desaturase gene (Lin, 2000).

Plasma-derived 18:2n6, through elongation and desaturation via Δ^5 and Δ^6 desaturases (Hermansen et al., 1995), is the major source of 20:3n6 and 20:4n6 in milk fat. Prior to infusion, feeding high-linoleic oil compared with high-oleic oil resulted in greater extraction of 18:2n6 from TG+FA (Table 4.7) and led to greater concentration (data not shown) and yield of 20:4n6 in milk fat (Table 4.8). However, from 60 through 84 h after infusion of 10/12CLA, the concentration of 18:2n6 in milk fat (Figure 4.3) increased but 20:4n6 decreased (data not shown) regardless of diet. Similar responses were not found when 18:2n6 was infused into the abomasum of lactating cows (Loor and Herbein, 1998), suggesting that the presence of a *trans*10 double bond in the CLA is associated with the reduction in 20:4n6 concentration and yield.

As mentioned earlier, the presence of the *trans*10 double bond, either in CLA or as *trans*10-18:1 (Piperova et al., 2000), may be required to induce lower milk fat synthesis in the mammary gland of dairy cows. It has been speculated that *trans*10,*cis*12-18:2 is an intermediate in the hydrogenation of 18:2n6, which accumulates when high-grain low-forage diets are fed (Griinari and Bauman, 1999). In response to a high-grain diet, *trans*10-18:1 accounted for 60% of all *trans*-18:1 isomers (16% of total milk fatty acids) but *trans*10,*cis*12-18:2 only represented 10% of total CLA isomers (1% of total milk fatty acids) (Piperova et al., 2000). Thus, the involvement of *trans*10,*cis*12-18:2 in diet-induced milk fat depression is questionable. Greater production of *trans*10-18:1 in the rumen depressed milk fat percentage and yield by inhibiting the activity and mRNA abundance for acetyl-CoA carboxylase and fatty acid synthase (Piperova et al., 2000). Desaturation of 18:0 did not seem to be affected, as the concentrations of 18:0 and *cis*9-18:1 in milk fat were similar compared with

controls. At least in mouse liver, *trans*10-18:1 did not inhibit Δ^9 desaturase activity compared with *trans*10,*cis*12-18:2 (Park et al., 2000). Thus, *trans*10,*cis*12-18:2 and *trans*10-18:1 seem to affect overall lipogenesis in the bovine mammary gland by different mechanisms.

CONCLUSIONS

High-oil feed ingredients increase the availability of unsaturated fatty acids and rumen-derived *trans*18:1 isomers in blood plasma for uptake and incorporation into milk fat. *Trans*11-18:1 and *cis*9,*trans*11-18:2 are the major intermediates produced during hydrogenation of dietary 18:2n6. Desaturation of *trans*11-18:1 in the mammary gland via Δ^9 desaturase, however, may be the major source of *cis*9,*trans*11-18:2 in milk fat. If conditions in the rumen were capable of enhancing the production of *trans*10,*cis*12-18:2, uptake of *trans*10,*cis*12-18:2 by the mammary gland could be detrimental to milk fat synthesis. Undesirable effects include reduced synthesis of 6:0 to 16:0, lower desaturation of 18:0 to *cis*9-18:1, and reduced milk fat yield.

Table 4.1. Composition of diets [1].

	HO	HL
Ingredients	% of DM	
Alfalfa silage	28.5	28.5
Corn silage	13.5	13.5
Orchardgrass hay	7.0	7.0
Ground corn	35.7	35.7
Soybean meal, 48%CP	7.6	7.6
SoyPlus [2]	3.5	3.5
Sunflower oil [3]	2.5	0.0
Safflower oil	0.0	2.5
Mineral/vitamin mix [4]	1.1	1.1
Limestone	0.4	0.4
Dicalcium phosphate	0.2	0.2
Chemical composition		
NDF	34.4	34.4
ADF	22.1	22.3
CP	16.0	16.0
Total fatty acids	5.3	5.3
	mg/g of total fatty acids	
14:0	1	2
16:0	91	117
*cis*9-16:1	2	2
18:0	29	34
*cis*9-18:1	555	242
18:2n6	292	573
18:3n3	30	30

[1] Four samples (collected in each period) of forages and supplements were composited and analyzed in duplicate.

[2] SoyPlus® (West Central Cooperative, Ralston, IA): CP = 483 g/kg, RUP = 600 g/kg CP, fatty acids = 49 g/kg, and NE_L = 2.10 Mcal/kg.

[3] High-oleic sunflower oil (HO) or high-linoleic (HL) safflower oil (Columbus Foods Co., North Albany, IL).

[4] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA): salt (38-48 g/kg), $NaHCO_3$ (180 g/kg), Ca (145-174 g/kg), P (65 g/kg), Cl (58 g/kg), S (32 g/kg), Mg (22 g/kg), K (35 g/kg), Mn (1 g/kg), Zn (1 g/kg), Fe (0.3 g/kg), Cu (0.1 g/kg), I (0.02 g/kg), Co (0.003 g/kg), Se (0.005 g/kg), F (0.65 g/kg), retinyl acetate (0.36 g/kg), cholecalciferol (0.01 g/kg), dl-α-tocopherol acetate (0.59 g/kg).

Table 4.2. Fatty acid composition of dietary oils and CLA emulsions for infusion.

	High-oleic sunflower	High-linoleic safflower	9/11CLA	10/12CLA
	mg/g of total fatty acids			
14:0	1	1	0	0
16:0	46	59	0	11
*cis*9-16:1	1	1	0	0
18:0	23	24	0	5
*cis*9-18:1	793	152	83	4
18:2 isomers				
*c*9,*c*12	132	760	0	0
*c*9,*t*11	0	0	907	18
*t*10,*c*12	0	0	10	962
18:3n3	4	3	0	0

Table 4.3. DMI, milk production, and milk component yields by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
	kg/d									
DMI	19.6	20.4	19.8	19.8	19.8	19.6	0.5	0.38	0.46	0.69
Milk	28.8	30.4	29.6	28.8	29.8	30.0	1.1	0.86	0.47	0.18
	%									
Fat	3.45	3.49	2.55	3.40	3.32	2.56	0.08	0.07	0.01	0.97
Protein	3.03	3.03	3.09	3.00	3.03	3.03	0.10	0.19	0.23	0.20
Lactose	4.63	4.61	4.61	4.65	4.64	4.65	0.10	0.10	0.58	0.84
SNF	8.38	8.39	8.46	8.47	8.54	8.43	0.10	0.10	0.45	0.05
	kg/d									
Fat	0.98	1.04	0.78	0.98	1.00	0.76	0.04	0.07	0.01	0.35
Protein	0.86	0.92	0.90	0.86	0.90	0.88	0.03	0.30	0.15	0.56
Lactose	1.34	1.40	1.36	1.34	1.38	1.40	0.20	0.77	0.52	0.19
SNF	2.40	2.54	2.50	2.42	2.54	2.52	0.20	0.60	0.26	0.59

[1] Values are the average of means obtained from 12 through 96 h after infusion.

[2] Effects due to diet (D), isomer (I), and their interaction (D × I) were calculated on means obtained every 12 h from 12 through 96 h after infusion.

[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.4. Estimated fatty acid intake by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d cis9,trans11-18:2 CLA (9/11) or trans10,cis12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
	g/d									
14:0	1.0	1.0	1.0	1.6	1.7	1.6	0.1	0.01	0.40	0.78
16:0	98.8	100.2	97.2	124.6	124.8	123.5	5.5	0.01	0.39	0.74
*cis*9-16:1	1.4	1.5	1.5	1.6	1.7	1.7	0.1	0.01	0.39	0.72
18:0	31.8	32.2	31.3	36.0	36.2	35.8	1.7	0.01	0.39	0.72
*cis*9-18:1	597.8	603.1	591.2	257.2	257.9	255.2	20.2	0.01	0.49	0.67
18:2n6	318.2	323.9	311.9	599.0	610.6	604.1	23.1	0.01	0.42	0.81
18:3n3	32.6	32.8	31.9	32.2	32.3	31.9	1.6	0.69	0.39	0.71
Total	1082.4	1095.6	1066.6	1062.6	1065.7	1054.3	52.0	0.37	0.39	0.70

[1] Values are the average of means obtained from 12 through 96 h after infusion.

[2] Effects due to diet (D), isomer (I), and their interaction (D × I) were calculated on means obtained every 12 h from 12 through 96 h after infusion.

[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.5. Concentrations of fatty acids in blood plasma from cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
	μg/mL									
14:0	5.3	4.9	6.8	5.7	5.8	6.6	0.1	0.55	0.06	0.41
*cis*9-14:1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.17	0.06	0.40
16:0	111	92	110	107	108	118	13	0.25	0.18	0.70
*cis*9-16:1	7.4	6.2	7.7	7.1	5.8	6.4	0.8	0.30	0.21	0.59
18:0	201	170	192	191	194	202	22	0.35	0.41	0.69
Cis 18:1										
9	113	95	106	62	59	63	6	0.01	0.16	0.46
11	2.8	2.8	3.4	2.6	2.5	3.0	0.3	0.22	0.06	0.76
12	6.7	4.5	6.1	9.5	8.7	9.2	1.2	0.02	0.35	0.66
13	0.7	0.4	0.5	0.8	0.3	0.4	0.1	0.24	0.11	0.93
15	0.8	0.7	0.7	0.7	0.9	1.0	0.1	0.10	0.60	0.54
Trans 18:1										
6,7,8	1.8	1.2	1.5	0.9	0.7	0.8	0.1	0.01	0.07	0.43
9	0.9	1.0	1.1	0.6	0.7	0.8	0.1	0.01	0.12	0.60
10	1.7	1.0	1.5	1.8	1.3	2.0	0.1	0.01	0.01	0.53
11	4.8	4.0	4.7	7.8	7.6	8.3	1.0	0.01	0.44	0.99
12	1.9	1.6	2.2	2.6	2.5	2.9	0.3	0.03	0.09	0.67
13,14	4.7	3.6	3.9	4.9	4.4	4.6	0.4	0.07	0.47	0.90
16	0.7	0.6	0.5	0.9	0.6	0.9	0.1	0.02	0.07	0.01
Isolated 18:2										
*c*9,*c*12	1006	844	972	1091	1089	1136	120	0.05	0.40	0.70
*t*9,*t*12	0.2	0.1	0.4	0.2	0.2	0.2	0.2	0.66	0.44	0.44
*c*9,*t*12	0	0.8	0.2	0.1	0.1	0.1	0.3	0.21	0.31	0.39
*t*9,*c*12	0.1	0.1	0	0.2	0.6	0	0.2	0.22	0.14	0.22
*t*11,*c*15	1.7	1.1	1.4	0.8	1.2	1.3	0.2	0.56	0.06	0.27
Conjugated 18:2										
*c*9,*t*11	1.6	2.7	1.5	1.9	3.8	2.5	0.7	0.05	0.03	0.91
*t*10,*c*12	0	0	3.0	0	0	3.0	0.3	0.98	0.01	0.61
18:3n3	51	44	53	33	32	35	5	0.01	0.18	0.53
20:3n3	27	25	29	26	26	29	4	0.72	0.18	0.81
20:4n6	30	28	33	31	29	35	6	0.51	0.05	0.91
20:5n3	8.2	8.1	11	9.8	8.2	11	2	0.90	0.06	0.62
Total	1870	1922	2169	2093	1920	1818	144	0.08	0.40	0.08

[1] Values are the average of means obtained at the end of the 48 h infusion.
[2] Effects due to diet (D), isomer (I), and their interaction (D × I) .
[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.6. Arterial concentrations of fatty acids in blood plasma triglycerides plus free fatty acids in cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
	μg/mL									
14:0	2.1	2.1	2.5	1.8	2.1	2.6	0.2	0.74	0.02	0.56
16:0	15.5	16.7	17.4	14.5	16.9	18.8	0.7	0.34	0.14	0.43
*cis*9-16:1	1.8	1.9	2.8	2.2	2.5	2.8	0.4	0.47	0.18	0.51
18:0	40.1	43.4	41.4	39.4	42.0	41.7	1.7	0.76	0.96	0.34
Cis 18:1										
9	8.1	8.3	8.9	6.4	4.6	5.1	0.5	0.01	0.32	0.86
11	0.5	0.5	0.6	0.4	0.5	0.6	0.02	0.26	0.01	0.49
12	1.1	0.8	0.9	1.0	1.3	1.6	0.1	0.01	0.03	0.30
13	1.2	0.9	1.2	0.9	0.9	1.3	0.1	0.34	0.01	0.27
15	0.4	0.4	0.5	0.4	0.6	0.6	0.02	0.01	0.22	0.71
Trans 18:1										
6,7,8	0.4	0.6	0.7	0.4	0.4	0.5	0.04	0.01	0.03	0.70
9	0.3	0.4	0.5	0.3	0.3	0.4	0.03	0.15	0.03	0.93
10	0.7	0.7	0.9	0.6	0.8	1.2	0.1	0.05	0.01	0.46
11	1.6	1.4	1.5	2.3	2.4	3.0	0.3	0.01	0.31	0.52
12	0.6	0.6	0.7	0.6	0.7	0.9	0.1	0.01	0.05	0.44
13,14	1.2	1.2	1.2	1.1	1.5	1.6	0.1	0.01	0.20	0.74
16	0.5	0.5	0.6	0.6	0.7	0.8	0.1	0.02	0.09	0.77
Isolated 18:2										
*c*9,*c*12	5.7	5.2	5.4	12.0	13.3	12.8	1.0	0.01	0.89	0.76
Conjugated 18:2										
*c*9,*t*11	0.3	0.7	0.3	0.3	0.7	0.4	0.1	0.18	0.01	0.66
*t*10,*c*12	0	0	0.8	0	0	0.7	0.04	0.36	0.01	0.19
18:3n3	0.7	1.0	1.2	0.7	0.7	0.9	0.1	0.01	0.01	0.75
Total	93.2	94.7	97.5	95.1	97.9	106.7	3.6	0.16	0.19	0.46

[1] Values are the average of means obtained from pooled samples collected every 2-h from 36 to 48 h of infusion.

[2] Effects due to diet (D), isomer (I), and their interaction (D × I).

[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.7. Mammary gland extraction ratios of fatty acids in blood plasma triglycerides plus free fatty acids in cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
	%									
14:0	31	32	28	30	27	31	4	0.95	0.95	0.33
16:0	31	32	29	30	31	32	2	0.72	0.60	0.34
*cis*9-16:1	42	39	43	43	48	47	4	0.45	0.21	0.18
18:0	40	41	35	38	39	34	1	0.27	0.01	0.09
Cis 18:1										
9	42	44	43	41	44	40	3	0.70	0.42	0.55
11	39	36	39	41	43	40	4	0.43	0.96	0.49
12	34	35	47	33	37	43	5	0.87	0.17	0.63
13	30	48	55	48	51	67	5	0.62	0.06	0.37
15	23	22	23	31	32	30	4	0.08	0.92	0.60
Trans 18:1										
6,7,8	47	49	47	52	54	55	3	0.06	0.97	0.59
9	68	67	57	62	66	65	2	0.14	0.04	0.07
10	64	63	58	69	70	66	2	0.07	0.25	0.83
11	61	59	52	56	58	59	4	0.48	0.60	0.35
12	52	50	53	59	61	62	6	0.16	0.77	0.89
13,14	60	63	60	62	64	62	2	0.62	0.28	0.82
16	59	61	61	54	55	55	5	0.30	0.97	0.95
Isolated 18:2										
*c*9,*c*12	21	20	15	30	30	38	4	0.02	0.78	0.21
Conjugated 18:2										
*c*9,*t*11	56	62	84	62	67	71	3	0.23	0.01	0.02
*t*10,*c*12	. . .	. . .	81	. . .	. . .	80	6	0.72	0.01	0.79
18:3n3	45	43	40	41	44	43	4	0.54	0.11	0.07
Total	40	41	38	39	39	39	1	0.99	0.34	0.17

[1] Values are the average of means obtained from pooled samples collected every 2-h from 36 to 48 h of infusion.

[2] Effects due to diet (D), isomer (I), and their interaction (D × I).

[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.8. Milk fatty acid yields by cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [1].

		HO			HL			Effect [2]		
	Basal [3]	9/11	10/12	Basal	9/11	10/12	SEM	D	I	D × I
	g/d									
4:0	36.4	34.8	31.4	39.0	36.4	31.8	0.8	0.28	0.01	0.54
6:0	19.4	19.8	14.4	19.8	20.2	14.2	0.5	0.81	0.01	0.60
8:0	8.6	9.1	6.2	9.0	9.4	6.2	0.2	0.28	0.01	0.42
10:0	16.8	17.4	11.8	17.0	18.4	11.4	0.5	0.58	0.01	0.18
12:0	16.0	16.8	11.8	14.6	15.6	11.8	0.4	0.47	0.01	0.53
14:0	71.8	74.8	56.8	70.4	73.2	53.2	2.8	0.12	0.01	0.51
*cis*9-14:1	7.0	7.2	4.6	6.2	6.8	3.8	0.2	0.05	0.01	0.57
16:0	162.2	164.8	124.8	160.2	162.6	121.2	6.5	0.30	0.01	0.81
6:0-16:0	258.1	268.2	194.7	251.6	263.2	186.4	6.4	0.42	0.01	0.61
*cis*9-16:1	7.4	7.2	5.4	7.0	6.8	4.6	0.1	0.04	0.01	0.36
18:0	115.4	116.8	113.2	120.8	120.2	116.0	4.1	0.36	0.28	0.92
Cis 18:1										
9	277.4	277.8	231.1	244.8	248.4	194.8	7.5	0.01	0.01	0.44
11	3.0	2.8	2.8	2.8	2.8	2.4	0.1	0.21	0.32	0.27
12	0.6	0.6	0.4	1.2	1.2	1.0	0.02	0.01	0.02	0.68
13	1.0	1.0	0.8	0.8	0.8	0.6	0.02	0.08	0.02	0.33
15	1.6	1.6	1.4	1.6	1.8	1.4	0.1	0.37	0.04	0.38
Trans 18:1										
6,7,8	3.4	3.6	3.2	2.0	2.0	1.8	0.1	0.01	0.01	0.29
9	3.2	3.2	3.0	2.6	2.6	2.2	0.1	0.01	0.01	0.62
10	5.0	5.0	4.0	5.0	5.0	4.4	0.1	0.21	0.01	0.14
11	8.4	8.8	7.2	13.0	13.2	12.0	0.3	0.01	0.02	0.79
12	1.8	1.8	1.4	2.2	2.2	1.8	0.1	0.01	0.01	0.83
13,14	8.6	9.0	7.8	10.8	10.6	9.6	0.3	0.01	0.01	0.92
16	3.2	3.6	2.8	3.4	3.2	3.0	0.1	0.75	0.12	0.99
Isolated 18:2										
*c*9,*c*12	21.4	21.8	18.6	36.6	36.0	31.6	0.8	0.01	0.01	0.44
*t*9,*t*12	0.4	0.2	0.2	0.4	0.4	0.2	0.03	0.28	0.06	0.18
*c*9,*t*12	1.2	1.2	1.0	1.6	1.6	1.4	0.1	0.01	0.03	0.52
*t*9,*c*12	0.4	0.4	0.2	0.6	0.4	0.4	0.03	0.02	0.03	0.75
*t*11,*c*15	1.2	2.0	1.8	1.1	1.8	1.6	0.1	0.16	0.27	0.67
Conjugated 18:2										
*c*9,*t*11	5.2	7.4	3.8	7.0	10.2	5.4	0.2	0.01	0.01	0.09
*t*10,*c*12	0.0	0.0	1.8	0.0	0.0	2.0	0.1	0.20	0.01	0.34
18:3n3	2.2	2.0	2.0	2.4	2.4	2.0	0.2	0.08	0.04	0.36
20:3n3	0.8	0.8	0.6	1.0	1.0	0.6	0.01	0.02	0.01	0.23
20:4n6	1.2	1.2	0.8	1.4	1.4	1.0	0.04	0.01	0.01	0.46
20:5n3	0.6	0.6	0.6	0.8	0.6	0.6	0.03	0.11	0.10	0.19
Total	808.0	822.8	676.4	807.2	819.0	656.2	17.0	0.36	0.01	0.53

[1] Values are the average of means obtained from 12 through 96 h of infusion.

[2] Effects due to diet (D), isomer (I), and their interaction (D × I) were calculated on means obtained every 12 h from 0 through 96 h after infusion.

[3] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

Table 4.9. Normalized ratios[1] of fatty acids in milk fat from cows fed diets containing (2.5% of DM) high-oleic sunflower oil (HO) or high-linoleic safflower oil (HL) and infused into the abomasum for 2 d with 15 g/d *cis*9,*trans*11-18:2 CLA (9/11) or *trans*10,*cis*12-18:2 CLA (10/12) [2].

		HO			HL			Effect [3]		
	Basal [4]	9/11	10/12	Basal	9/11	10/12	SE	D	I	D × I
Ratio										
14:0 ⇒ 14:1	0.089	0.087	0.074	0.081	0.084	0.066	0.01	0.05	0.01	0.36
16:0 ⇒ 16:1	0.044	0.043	0.041	0.042	0.041	0.037	0.00	0.04	0.04	0.36
18:0 ⇒ 18:1	0.71	0.70	0.67	0.67	0.67	0.63	0.01	0.01	0.01	0.13
18:1;*t*11 ⇒ 18:2;*c*9,*t*11	0.39	0.46	0.34	0.35	0.43	0.31	0.01	0.01	0.01	0.86
18:2 ⇒ 20:4	0.052	0.048	0.040	0.034	0.035	0.028	0.00	0.01	0.01	0.60
18:3 ⇒ 20:5	0.13	0.14	0.15	0.17	0.16	0.14	0.01	0.40	0.79	0.16

[1] Normalized ratio = mg/g product/[mg/g substrate + mg/g product].
[2] Values are the average of means obtained from 12 through 96 h of infusion.
[3] Effects due to diet (D), isomer (I), and their interaction (D × I) were calculated on means obtained every 12 h from 0 through 96 h after infusion.
[4] Basal = The means of observations at -12 and 0 h before infusion are shown for comparison only.

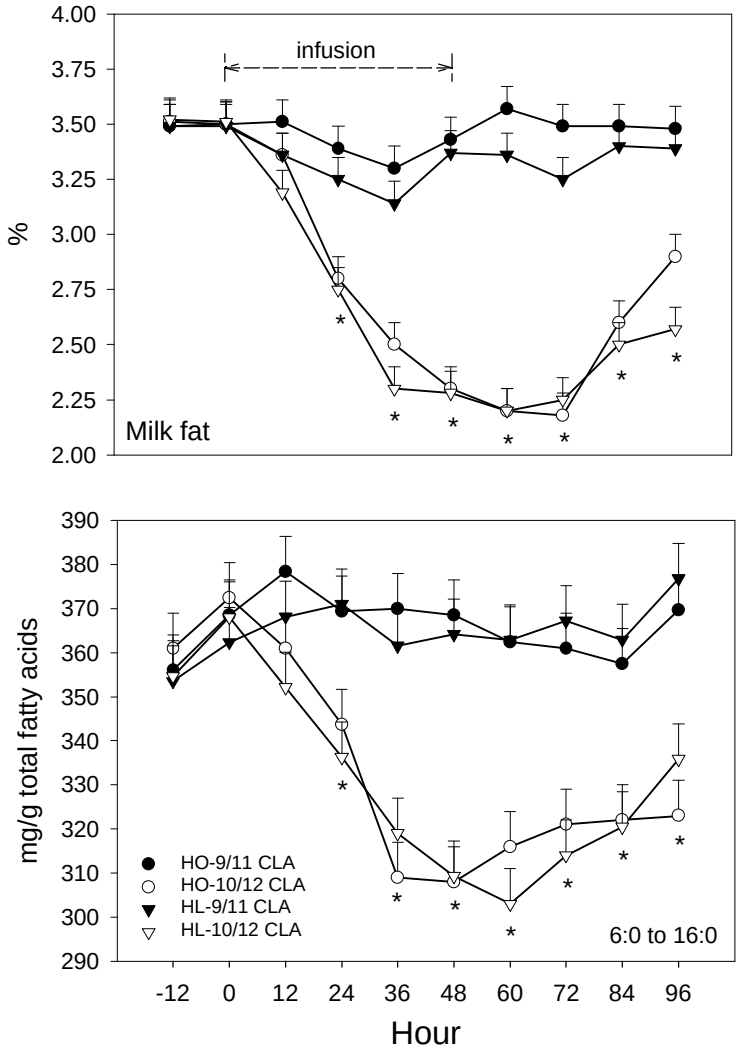


Figure 4.1. Milk fat percentage and concentration of 6:0 to 16:0 saturated fatty acids in milk fat from cows fed high-oleic (HO) or high-linoleic (HL) oil, and infused into the abomasum with *cis*9,*trans*11-18:2 (9/11 CLA) or *trans*10,*cis*12-18:2 (10/12 CLA) for 48 h. Values are means plus pooled SEM for four cows, except for HO-9/11CLA with three cows, at each 12-h interval. Asterisks indicate significant ($P < 0.05$) effect due to CLA isomers.

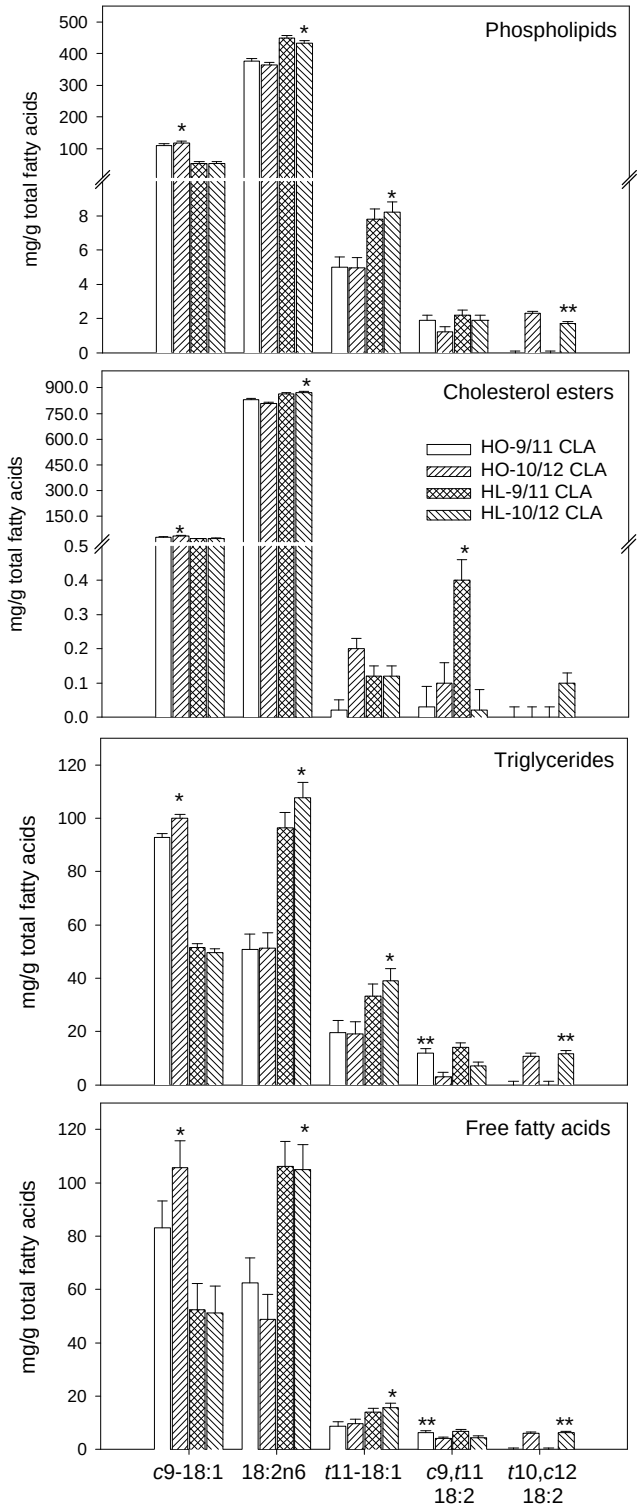


Figure 4.2. Distribution of *cis*9-18:1, 18:2n6, *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*10,*cis*12-18:2 at 48h in blood plasma phospholipids, cholesterol esters, triglycerides, or free fatty acids from cows fed high-oleic (HO) or high-linoleic (HL) oil, and infused into the abomasum with *cis*9,*trans*11-18:2 (9/11 CLA) or *trans*10,*cis*12-18:2 (10/12 CLA) for 48 h. Values are means plus pooled SEM for four cows, except for HO-9/11CLA with three cows. Asterisks denote differences ($P < 0.05$) due to diet (*) or isomer (**).

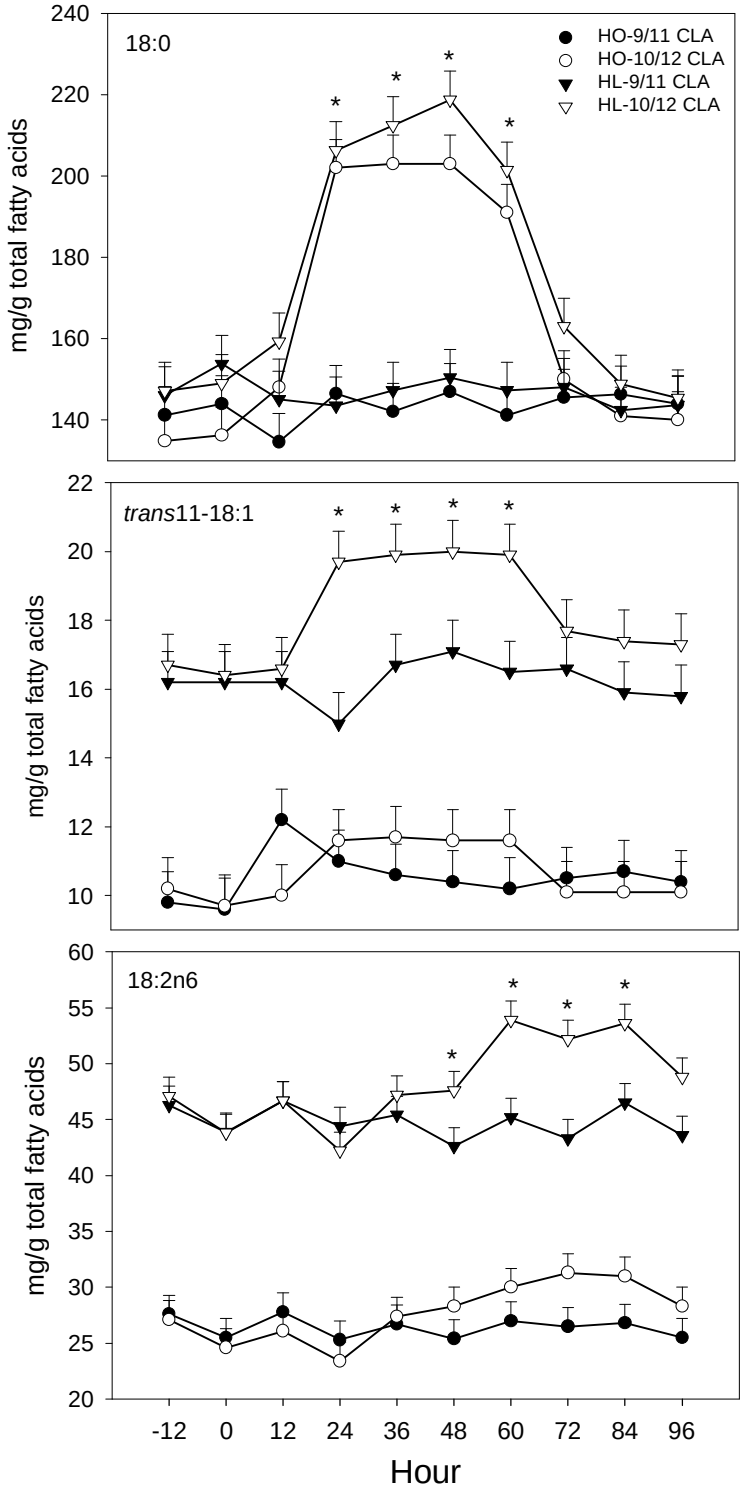


Figure 4.3. Concentrations of 18:0, *trans*11-18:1, and 18:2n6 in milk fat from cows fed high-oleic (HO) or high-linoleic (HL) oil, and infused into the abomasum with *cis*9,*trans*11-18:2 (9/11 CLA) or *trans*10,*cis*12-18:2 (10/12 CLA) for 48 h. Values are means plus pooled SEM for four cows, except for HO-9/11CLA with three cows, at each 12-h interval. Asterisks denote significant effect ($P < 0.05$) due to CLA isomer.

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