Postruminal flow, digestibility, and utilization of fatty acylamides or conjugated linoleic acid for milk fat synthesis by lactating Holstein cows

by

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(ABSTRACT)

Four Holstein cows with ruminal and duodenal cannulas were used to evaluate the effects of dietary fatty acylamides (canolamide) or abomasally infused conjugated linoleic acid on milk production and composition. In the first experiment, cows were fed diets with no supplemental fat (control), or the control diet supplemented at 3.3% of DM with canola oil, canolamide, or a mixture of equal amounts of canola oil and canolamide in a 4 x 4 Latin square. DMI and milk yield were decreased when cows were fed canolamide. Intake and duodenal flow of diet components and fatty acids were decreased by canolamide, but their apparent digestibilities were not affected by treatment. Fat supplementation decreased concentrations of fatty acids with 8 to 16 carbons and increased the concentration of oleic acid in milk. In the second experiment, cows were infused abomasally with 200 g linoleic acid (LA) or a mixture of 100 g LA plus 100 g conjugated linoleic acid (LA-CLA) for 24 h in a single crossover design. Infused CLA was a mixture of 70% 18:2; Δ^{9}*cis*, Δ^{11}*trans* and 30% 18:2; Δ^{10}*trans*, Δ^{12}*cis*. Milk yield and DMI were not affected by treatment. Milk fat percentage and yield were decreased by LA-CLA. Concentration and yield of oleic and arachidonic acid and fatty acids with 6 to 16 carbons in milk were reduced by LA-CLA. Stearic acid and CLA concentrations in milk, however, were higher in response to LA-CLA. Infusion of LA-CLA led to increased (from 23 to 45%)

concentration of unsaturated fatty acids with a concomitant decrease (from 70 to 42%) in saturated fatty acid concentration in milk fat. Feeding canolamide at 3.3% significantly decreased DMI and milk yield compared with canola oil or the mixture of canola oil and canolamide. However, results indicated that oleic acid and CLA concentration in milk fat can be increased proportionally to their flow into the small intestine. Utilization of these fatty acids for milk fat synthesis may cause a reduction in the amount of medium and short chain fatty acids synthesized de novo within the mammary gland. Furthermore, CLA appears to be a potent inhibitor of milk fat synthesis and desaturation of stearic and linoleic acid.

DEDICATION

I would like to dedicate this thesis to my parents, Jorge and Julia Loor. They were always supportive of my decisions, and constantly voiced their opinions about education and personal development throughout my life. This thesis is also dedicated to my brothers, Miguel and Wilfrido, my sisters, Sonya, Sofia, and Silvia, my grandmother, Rosathe, and my extended family in Ecuador. They have been an influence in all aspects of life within these formative years. I also want to dedicate this thesis to my beloved Carmen, the most beautiful and sweet woman I have met. Her support, love, and attention are always in my presence.

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Dr. Tom Jenkins (Clemson University) synthesized the canolamide supplement used in the first study and conducted amide analysis. Dr. Clement Ip (Roswell Park Cancer Institute) donated the CLA mixture used in the infusion trial. I sincerely thank them for their contribution and cooperation.

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INTRODUCTION

Human health concerns regarding intake of saturated fatty acids from ruminant milk and meat products have prompted a desire to decrease saturated and increase unsaturated fatty acid content of foods derived from ruminant animals. The hypercholesterolemic effects of saturated fatty acids have been positively correlated with the incidence of coronary heart disease. In particular, the saturated fatty acids 14:0 and 16:0 increase plasma cholesterol in humans (Denke and Grundy, 1992). Over one half of the saturated fatty acids in milk are 14:0 (15%) or 16:0 (41%) (Grummer, 1991).

The intent of ongoing research is to alter the fatty acid composition of milk and milk based products from a highly saturated (10:0, 12:0, 14:0, and 16:0) to a more unsaturated (18:1; Δ^9 *cis*, 18:2, and 18:3) profile. Biohydrogenation of dietary unsaturated fatty acids in the rumen minimizes their flow from the rumen to the small intestine for absorption; thus, addition of plant oils rich in unsaturated fatty acids to ruminant diets leads to only minimal increases in the unsaturated fatty acid content of body tissues or milk. Production of highly unsaturated milk fat apparently can only be accomplished by sources of unsaturated fatty acids that are protected in a manner that prevents their biohydrogenation in the rumen and increases their flow to the small intestine for absorption

Abomasal infusions of plant oils containing 52 to 86% 18:1; Δ^9 *cis* indicated that the amount of oleic acid absorbed in the intestine is highly correlated with the amount present in the oil source (Bandara et al., 1996; La Count et al., 1994). The amount absorbed in the intestine

then was proportional to its content in blood plasma and milk fat. Thus abomasal infusion of unsaturated fatty acids indicated cows have the capacity to transfer substantial quantities of these fatty acids to milk, as long as they escape hydrogenation in the rumen.

The reaction of unsaturated fatty acids with primary amines produces fatty acylamides that resist biohydrogenation and cause less interference in ruminal fermentation (Fotouhi and Jenkins, 1992; Jenkins et al., 1996). Concentration of 18:2 in blood plasma of sheep and milk fat of dairy cows was increased when fatty acylamides were added to a basal diet at 3.5 to 5% of the DM (Jenkins, 1995; Jenkins et al., 1996).

The need for better understanding of lipid metabolism in the dairy cow is important not only for efficient implementation of protected fat supplements into the feeding scheme, but for manipulating the concentration of specific fatty acids in milk that may play an important role in the prevention of human disease. For example, conjugated linoleic acid (CLA) is a potent anticarcinogen found in high concentrations in products derived from ruminant tissues or milk (Belury, 1995). The acronym CLA refers to a mixture of positional and geometrical isomers of linoleic acid with conjugated double bonds (Ip et al., 1994) formed during the enzymatic hydrogenation of linoleic acid by rumen microorganisms (Fujimoto et al., 1993). CLA, primarily 18:2; Δ^9 *cis,* Δ^{11} *trans* , is the major isomer in milk but its concentration can vary significantly due to season, pasture quality, or level of unsaturated oil supplementation in the diet (Parodi 1977; Kelly et al., 1997).

Our laboratory recently reported that bovine mammary cells incorporated CLA and oleic acid into cell lipids in proportion to their content in the culture media (Dawson and Herbein, 1996). In view of the anticarcinogenic effects of CLA, enrichment of the CLA content in milk

fat is desirable. Data suggested that increased supply of 18:1; Δ^9 *cis* and CLA to the mammary gland will lead to greater recovery of these unsaturates in milk fat.

The objectives of the present study were twofold. First , to evaluate the effects of feeding a fatty acylamide synthesized from a source of oleic acid (high-oleic canola oil) on :

1. extent of fatty acylamide and unsaturated fatty acid biohydrogenation in the rumen,
2. DM, ADF, N, OM, and fatty acid flow to the small intestine,
3. apparent digestibility of the above nutrients in the gastrointestinal tract,
4. fatty acid concentration in blood plasma,
5. milk production and milk component yields, and
6. fatty acid concentration and yield in milk fat,

Second, to evaluate the effects of abomasal infusions of 18:2 or a mixture of 18:2 and conjugated linoleic acid for 24 h on:

1. DM, OM, N, and fatty acid flow to the small intestine,
2. apparent digestibility of the above nutrients in the gastrointestinal tract,
3. fatty acid distribution in blood plasma lipid fractions,
4. milk production and composition, and
5. fatty acid concentration and yield in milk fat.

CHAPTER 2

Review of literature

Lipid metabolism in the rumen

The microorganisms within the rumen of cattle, sheep, and goats represent a major point at which dietary lipids entering the gastrointestinal tract are metabolized. Microbial metabolic activity in the rumen is responsible for hydrolysis of esterified plant lipids and for the subsequent hydrogenation of the released unsaturated fatty acids. Furthermore, the microorganisms are capable of lipid synthesis *de novo*, using short chain fatty acids resulting from microbial carbohydrate and amino acid metabolism (Harfoot, 1981). Hydrolysis, hydrogenation and synthesis *de novo* of microbial lipid in the rumen contribute significantly to the characteristic composition of ruminant tissue lipids. Extensive biohydrogenation of 18:2 in the rumen leads to the formation of 18:2; Δ^9 *cis*, Δ^{11} *trans*, 18:1; Δ^{11} *trans*, and 18:0 (Fujimoto et al., 1993).

Ruminants derive their dietary lipids from forages. Lipids comprise 6 to 8% of the dry weight of leaf tissue, and are characterized by their high content of glycolipids and phospholipids (Harfoot, 1981). The fatty acid composition of leaf lipids is dominated by the presence of a high proportion of unsaturated fatty acids, especially 18:2 and 18:3, along with smaller amounts of 18:1; Δ^9 *cis*.

Ruminant diets are commonly supplemented with feedstuffs of high caloric value (concentrates) with the intent to increase the dietary intake of fatty acids and triglyceride (Coppock and Wilks, 1991). Whereas forages contain a high proportion of 18:2 exclusively, the

fatty acids in many of the oil seeds commonly used in concentrates contain high proportions of 18:2 (30 to 60%) and 18:1; Δ^9 *cis* (25 to 35%) (Kennelly, 1996).

Dawson and Kemp (1970) first reviewed the literature regarding biohydrogenation of dietary fats. The hydrogenation of polyunsaturated fatty acids after ruminal lipolysis of triglycerides in the rumen of sheep occurred fairly rapidly, and a substantial amount of *trans* and conjugated isomers of 18:2 were produced. Furthermore, it was determined that rumen bacteria were capable of synthesizing esterases and lipases, which are responsible for hydrolyzing ester linkages of dietary acyl lipids.

Polan et al. (1964) showed that *Butyrivibrio fibrisolvens* was capable of hydrogenating 18:2 to an octadecenoic acid but not 18:0. Furthermore they determined that biohydrogenation of 18:2 by mixed rumen bacteria obtained from a fistulated steer grazed on summer pasture was 4-fold greater compared with mixed rumen bacteria obtained from the steer grazing on winter pasture. They hypothesized that the seasonal variation may have been due to changes in the microbial population or their metabolic activity. Fujimoto et al. (1993) demonstrated that 57 strains of *B.fibrisolvens* isolated from the rumen of sheep could hydrogenate 18:2 and 18:3 to 18:2; Δ^9 *cis*, Δ^{11} *trans*, and 18:1; Δ^{11}*trans.* There were only two unidentified cellulose digesting bacterial strains which could hydrogenate 18:1; Δ^{11}*trans* to 18:0. Earlier, it had been determined that there was an absolute requirement for a 18:2; Δ^9*cis*, Δ^{12}*cis* configuration along with a free carboxyl group at carbon one as specific substrates of the Δ^9*cis*, Δ^{11}*trans* isomerase isolated from *B. fibrisolvens* (Harfoot, 1981). The subsequent pathways by which these conjugated isomers of 18:2 and 18:3 are hydrogenated to stearic acid by rumen microorganisms are not so clear. Most workers have assumed that, if partially hydrogenated fatty acids are produced in large amounts,

then these are major intermediates in the metabolic pathway. On this basis, the sequence of events in the biohydrogenation of linoleic acid can be summarized as illustrated in Figure 1.

The situation with respect to 18:3 is complicated by the production of a wide range of geometric and positional isomers of the 18 carbon fatty acids (Dawson and Kemp, 1970). Using information from this source, the simplest metabolic pathways that can be constructed at the present time are illustrated in Figure 2.

It is not known by what means monoenoic acids are hydrogenated to 18:0. On the basis of the accumulation of 18:1; Δ^{11}*trans* in the rumen it was suggested that, in the mixed population of bacteria in rumen contents, there are two distinct populations of hydrogenating organisms (Dawson and Kemp, 1970; Fujimoto et al., 1993). One of those converting 18:2; Δ^{9} *cis*, Δ^{11} *trans* to 18:1; Δ^{11}*trans*, the second converting the 18:1; Δ^{11}*trans* to 18:0. Table 1 shows bacterial strains isolated from the rumen along with the substrates used by the organism and the major end products of biohydrogenation.

The physiological reason for biohydrogenation in the anaerobic environment of the rumen are still unknown. It has been reported that polyunsaturated fatty acids are more toxic to anerobic bacteria than the products of hydrogenation (Keweloh and Heipieper, 1996). Therefore one function of biohydrogenation may be to destroy growth inhibiting substances (Verhulst et al., 1986). Another suggestion is that the main function of biohydrogenation was disposal of excess reducing power (metabolic hydrogen) in the rumen (Mackie et al., 1991). Another possible role for hydrogenation concerns the protection of ruminant tissues from the effects of high intakes of polyunsaturated fatty acids. High intakes of polyunsaturated fatty acids, coupled with a low intake of vitamin E, can lead to myopathic conditions in ruminants (Dawson and Kemp, 1970).

Figure 1. Probable sequence of reactions in the biohydrogenation of linoleic acid.

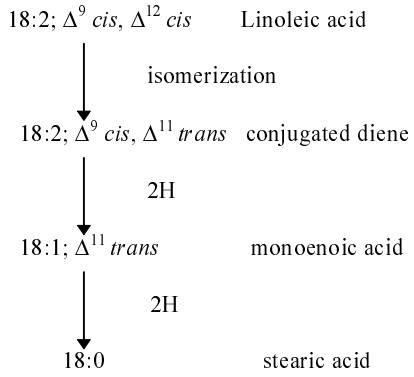
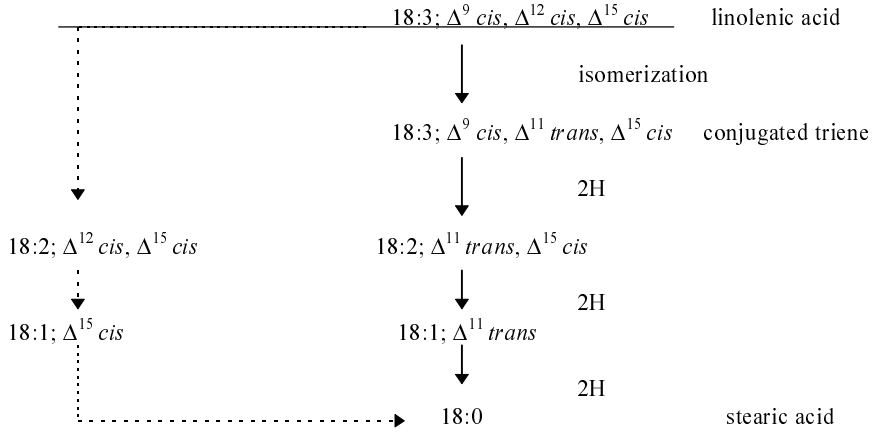


Figure 2. Probable metabolic pathways in the biohydrogenation of Linolenic acid.



(figures 1 and 2 adapted from Harfoot, 1981)

Table 1. Ability of bacteria isolated from the rumen to hydrogenate linolenic, linoleic and oleic acids.

Organism	Linolenic acid	Linoleic acid	Oleic acid
	Metabolic products of Hydrogenation		
Butirivibrio fibrisolvens A38	18:3; Δ^9 *cis*, Δ^{11} *trans*, Δ^{15} *cis*	18:2; Δ^9 *cis*, Δ^{11} *trans*	
Micrococcus sp.	isomerized, then hydrogenated	18:1; Δ^{11} *trans* major intermediate	
Ruminococcus albus	*18:3; Δ^9 *cis*, Δ^{11} *trans*, Δ^{15} *cis* *18:2; Δ^{11} *trans*, Δ^{15} *cis* 18:1; Δ^{11} *trans* (95%) 18:1; Δ^{11} *cis* (5%)	*18:2; Δ^9 *cis*, Δ^{11} *trans* 18:1; Δ^{11} *trans* (95%) 18:1; Δ^{11} *cis* (5%)	not hydrogenated
Fusocillus T344	*18:3; Δ^9 *cis*, Δ^{11} *trans*, Δ^{15} *cis* *18:2; Δ^9 *cis*, Δ^{11} *trans* 18:1; Δ^{15} *cis* (85%)	*18:2; Δ^9 *cis*, Δ^{11} *trans* 18:1; Δ^{11} *trans* (65%) 18:0 (35%)	18:1; Δ^{11} *trans* (5%) 18:1; Δ^9 *cis* (5%) 18:0 (90%)
Fusocillus babrahamensis P2/2	*18:3; Δ^9 *cis*, Δ^{11} *trans*, Δ^{15} *cis* 18:2; Δ^{15} *cis*, Δ^{11} *trans* (15%) 18:1; Δ^{15} *cis* (85%)	*18:2; Δ^9 *cis*, Δ^{11} *trans* 18:1; Δ^{11} *trans* (70%) 18:0 (30%)	18:0 (80%) 18:0-OH (20%)
R8/5 Gram-negative rod	18:2; Δ^{15} *cis*, Δ^{11} *trans* (25%) 18:1; Δ^{15} *cis* (50%) 18:1; Δ^{15} *trans* (25%)	18:1; Δ^{11} *trans* (50%) 18:0 (40%)	18:2; Δ^9 *cis* (60%) 18:0-OH (40%)
EC7/2 Gram-negative rod	18:2; Δ^{15} *cis*, Δ^{11} *trans* (70%) 18:1; Δ^{11} *trans* (30%)	18:1; Δ^{11} *trans* (100%)	not hydrogenated

* transient appearance as intermediate.
Source: Harfoot (1981)

Fatty acids of bacterial lipids are largely saturated and it could be postulated that biohydrogenation serves to convert forage fatty acids into a suitable form for incorporation into the bacterial cell without the large energy expenditure required for synthesis of saturated fatty acids *de novo* (Harfoot, 1981).

Lipid composition of rumen microorganisms

The lipid composition of mixed rumen bacteria consists of 30% phospholipids and 70% non-phospholipids by weight (Harfoot, 1981). Unesterified fatty acids composed more than 40% of the non-phospholipid fraction. However, the most obvious feature of the lipid composition of rumen bacteria compared with that of the forage entering the rumen was the high proportion of saturated fatty acids present, mainly 16:0 and 18:0.

Mixed rumen protozoa contained 16:0 (48%) as the major fatty acid in all lipid classes, along with smaller proportions of 18:0, 18:1; Δ^9 *cis*, 18:2, and 18:3 (10, 21, 10 and 2.3%, respectively) (Harfoot, 1981). Cellulolytic bacteria also were found to require straight and branched fatty acids with 4 or 6 carbons for incorporation into bacterial long chain fatty acids.

Distribution of lipids in the rumen

Information concerning the distribution of lipids between the different components of rumen digesta is relatively scarce. Harfoot (1981) reported 80% of lipid in the rumen is associated with the food particles. The proportion of lipid associated with protozoal and bacterial fractions were 16 and 4% , respectively. Analysis of the distribution of dietary lipids in the rumen is cumbersome, partly because of the heterogeneity of the rumen environment and considerable variation in lipid composition of ingredients in ruminant diets.

More recently, Legay-Carmier and Bauchart (1989) determined that bacterial populations may be divided into three main compartments, i. e. bacteria associated with the liquid phase and bacteria either loosely or firmly attached to feed particles. In particular, lipid and fatty acid concentrations were twice as high in solid-adherent bacteria than in liquid-associated bacteria. Bauchart et al. (1990) showed that the fatty acid content in total lipids of solid-adherent bacteria was 2.5 to 3.1 higher than that of liquid-adherent bacteria and increased for both populations to the same extent in response to lipid supplemented diets.

Effect of dietary fat on DM intake and digestibility

Fat supplements added to dairy rations have helped to provide a dietary strategy to offset the negative body energy balance that occurs in early lactation (Grummer et al., 1991). Investigators over the past few decades have conducted research to characterize the effects of supplementing diets of lactating cows with various fats or oils that might lead to improvements in milk fat yield, or to modifications of the milk fatty acid composition that may benefit the consumer.

Studies investigating the relationship between dietary fat supplementation, as oilseeds , and DM intake have been equivocal. Weisen et al. (1989) reported that whole and extruded canola seed (3% of diet DM as added fat) did not influence DM intake. In contrast, Johnson et al. (1988) and Kennelly (1987) observed a significant depression in DM intake as a result of including raw peanut hearts (3.8% of diet DM as added fat) and ground canola seed (5 or 7.5% of diet DM as added fat) in the diet of lactating cows. Jenkins and Jenny (1992) fed canola oil alone or in combination with prilled fat at 5.7% of the diet DM and reported lower fatty acid digestibility but no change in DM intake, DM digestibility or fiber digestibility. With respect to

DM intake, Firkins and Eastridge (1994) found that feeding supplemental fat above 3.5% of the DM generally led to reduced feed consumption. The decrease was more apparent as the degree of unsaturation of the fat supplement increased. Apparent fatty acid digestibility, however, was greater when unsaturated fats were fed compared with tallow.

Fats and oils added to ruminant diets often depress ruminal fermentation and fiber digestibility (Palmquist, 1988) but the degree of inhibition varies with fat source. Jenkins (1987) showed that corn oil and oleic acid (*in vitro*) decreased fiber digestibility. Increasing the saturation and(or) esterification of the component fatty acids, however, are two factors that lessen the inhibitory effects of dietary lipid supplementation on ruminal fermentation (Palmquist and Jenkins, 1980; Chalupa et al., 1984). The mode of action whereby fats and oils in the rumen cause a reduction of fiber digestibility is not understood entirely. Devendra and Lewis (1974) proposed that fatty acids act directly on ruminal microbes to inhibit their growth and metabolism or that they coat fiber particles, thus blocking the action of bacterial cellulases.

Effect of feeding protected fat supplements on DM intake and digestibility

Biohydrogenation of unsaturated fatty acids in the rumen poses a challenge to efforts aimed at altering the fatty acid composition of tissues or milk in cattle. In essence, the unsaturated fatty acids must be fed in a form that resist biohydrogenation in the rumen. The most common approaches are to feed protected lipids, which have been chemically (formaldehyde treatment, Ca-salts, fatty acylamides) or physically (heat) treated to resist microbial saturation in the rumen.

Mattos and Palmquist (1974) found that protecting full-fat soyflour with formaldehyde treatment did not affect DM intake or apparent digestibility of dietary components. More

recently, Ashes et al. (1992) fed canola seeds protected in a matrix of aldehyde-treated protein at 6.5% of the diet DM without deleterious effects on DM intake.

Intact oil seeds also provide a degree of protection from biohydrogenation by microbial enzymes. The extent of protection varies depending on the physical and chemical nature of the oilseed hull and the seed size. Casper et al. (1988) replaced 20% of a concentrate mixture fed to lactating cows with high-oleic sunflower seeds (80% 18:1; Δ^9 *cis*) and did not observe changes in DM intake. Another method is treatment of whole canola seeds with alkaline hydrogen peroxide to weaken the seed coat while simultaneously protecting long chain polyunsaturated fatty acids from ruminal biohydrogenation (Hussein et al., 1996). This supplement added at 5% of dietary DM increased amounts of 18:1; Δ^9 *cis*, 18:2, and 18:3 flowing to the duodenum of steers and had no effects on DM intake.

Complexing fatty acids with metal cations to form insoluble salts (Jenkins and Palmquist, 1984) led to the development of Ca-salts of palm oils (Megalac®), partially hydrogenated tallow (Alifet®) and partially hydrogenated non-esterified long chain fatty acids (Booster fat®) (Rode, 1992). Fatty acid profile of these commercial protected fat supplements is highly saturated and may be relatively insoluble in the rumen. Table 2.

The reaction of unsaturated fatty acids with primary amines (butylamine, ethanolamine) produces fatty acylamides that resist biohydrogenation and cause less disruption of ruminal fermentation. Fatty acylamides resisted breakdown and loss of double bonds *in vitro* (Fotouhi and Jenkins, 1992) and *in vivo* (Jenkins, 1995; Jenkins et al., 1996). The rate of disappearance of

Table 2. Fatty acid composition of selected fat sources.

Source	Fatty acid (% of total fatty acids)						
	16:0	18:0	18:1; Δ^9 *cis*	18:2	18:3	SAT [a]	UNS [b]
Tallow	26	19	40	5	1	48	52
Alifet [c]	27	37	31	1	-	67	33
Booster fat [c]	25	22	45	2	-	50	50
Megalac [c]	51	4	35	8	-	57	43
Oilseeds							
Canola oil	4	2	52	25	13	6	94
Cottonseed	25	3	17	54	-	29	71
Linseed oil	5	3	20	16	55	9	91
Safflower oil	7	2	9	80	<1	10	90
Soybean oil	8	3	24	58	8	10	90
Sunflower oil	6	4	20	66	<1	12	88

[a] SAT = saturated, [b] UNS = unsaturated, [c] Alifet®, Booster fat®, and Megalac® are commercially prepared lipid sources which are marketed for use in dairy cattle diets.

Source: Kennelly (1996)

linoleic acid from culture media was lower for the fatty acylamide than the free linoleic acid treatment when incubated in vitro with rumen contents (Fotouhi and Jenkins, 1992).

When butylsoyamide, made by reacting butylamine with soybean oil, was fed to sheep at 5% of dietary DM, DM intake and total tract ADF digestibility were not affected (Jenkins, 1995). Similarly, DM intake remained unchanged when Holstein cows were offered diets supplemented with butylsoyamide at 3.5% of the dietary DM (Jenkins et al., 1996).

Effect of dietary fat supplements on rumen VFA production and pH

Volatile fatty acids (VFA), principally acetate, propionate, butyrate, valerate, caproate, isobutyrate, isovalerate, and 2-methyl-butyrate are produced in the rumen as end products of microbial fermentation. Dietary carbohydrate in the form of cellulose, hemicellulose, pectin, starch and soluble sugars are the primary fermentable substrates. The contribution from lipid is very small, because lipids normally represent a small proportion of the diet and only the carbohydrate moiety (glycerol or galactose arising from lipid hydrolysis) of lipid is fermented (Cotta and Hespell, 1986). The fermentation pattern in the rumen is determined to a great extent by the type of dietary carbohydrate in the fiber fraction and its digestibility.

Lipids added to cattle diets can reduce microbial capacity for fermentation in the rumen, causing reduced digestibility of structural carbohydrates and VFA production. Igwuegbu and Sutton (1982) reported a 65% decrease in ADF digestibility, and a 50% decrease in OM digestibility with increasing amounts of linseed oil (up to 10% of the diet DM) in sheep diets. Additionally, there were marked decreases in the molar proportions of acetate (from 64.0 to 52.8 m*M*) and butyrate (from 12.2 to 4.7 m*M*), and a linear increase in the proportion of propionate (from 18.2 to 36.7 m*M*). In addition, ruminal pH was slightly reduced (from 6.62 to 6.48) by

addition of linseed oil. Jenkins (1987) found that increasing the concentration of corn oil or tallow fatty acids in medium for rumen bacterial cultures from 0 to 10% increased propionic acid production, thus causing the ratio of acetic to propionic acid (A:P) to decrease. However, supplementing sheep diets with corn oil at 2.4% of the diet DM did not significantly affect total VFA (64.7m*M* and 62.0m*M*) , pH (6.19 and 6.24) or A:P (3.35 and 3.25) (Jenkins and Fotouhi, 1990).

Chalupa et al. (1984) reported a decrease in VFA production when free long chain fatty acids were incubated with rumen contents. Lauric acid (14:0) decreased VFA production by 69% and induced a high A:P (40:1). Palmitic (16:0) and oleic acid (18:1; Δ^9 *cis*) lowered A:P by 23 and 54% respectively. However, when in the form of Ca-salts or triglyceride, oleic and palmitic acid did not cause changes in VFA production. Casper et al. (1988) fed intact high-oleic or regular sunflower seeds to dairy cows at 4.9% of the diet DM without affecting production of total VFA or rumen pH; although A:P was higher in response to high oleic sunflower seeds (2.56 versus 1.94) due to higher acetate production (59.7 m*M* versus 54.3 m*M*). Thus, intact oil seeds provide protection of their lipid content, and have minimal influence on microbial fermentation (Murphy et al., 1990).

More recently, Fotouhi and Jenkins (1992) found that lynoleoyl methionine ethyl ester (fatty acylamide) when incubated with rumen microorganisms did not inhibit bacterial fermentation. Higher acetate production (64.4 m*M*) in response to the fatty acylamide treatment compared with free linoleic acid (59 m*M*), and lower propionate (25.5 m*M* versus 33.1 m*M*) suggested lesser inhibition by the former due to the inert nature of the fatty acylamide in the rumen. Subsequently, Jenkins (1995) fed diets containing 5% soybean oil or butylsoyamide to sheep and reported no effects on total VFA (54.4 m*M*) or A:P (2.96) when the butylsoyamide

supplement was fed; whereas feeding soybean oil reduced total VFA (38.7m*M*) and A:P (2.56). When lactating cows were offered diets supplemented with either soybean oil or butylsoyamide at 3.5% of the diet DM, total VFA (118.9 m*M* versus 106.6 m*M*) and A:P (3.19 versus 2.93) were higher in response to butylsoyamide compared with soybean oil (Jenkins et al., 1996).

It appears that free long chain saturated and unsaturated fatty acids cause changes in the normal rumen fermentation pattern by shifting microbial metabolism from acetate to propionate production. However, diets with a lipid supplement partially protected from ruminal microbial action apparently do not influence carbohydrate metabolism of rumen microflora.

Effects of dietary fat on rumen ammonia

Palmquist and Conrad (1978) reported increased rumen ammonia concentrations (from 8.9 mg/dL to 13.1 mg/dL) in lactating cows when hydrogenated fat was increased from 5.7 to 10.8% of the diet DM. Higher rumen ammonia concentrations were also observed by Casper et al. (1988) when feeding high-oleic sunflower (13.9 mg/dL) and regular sunflower seeds (11.9 mg/dL) at 4.9% and 5.8% of the DM, respectively, to dairy cows; whereas Mielke and Shingoethe (1981) found that feeding heat treated or untreated soybeans (5.4% of the diet DM) decreased rumen ammonia. Jenkins and Fotouhi (1990) also reported a reduction in rumen ammonia concentration (14.0 mg/dL) when sheep diets were supplemented with corn oil (2.4% of the diet DM) compared with a control concentrate (19.7 mg/dL). Ikwuegbu and Sutton (1982) reported that increasing linseed oil in the diet of sheep (0 to 40 mL/d) decreased rumen ammonia from 16.7 mg/dL to 6.2 mg/dL. In contrast, Doreau et al. (1993) found no difference in rumen ammonia concentration when rapeseed oil or Ca-salts of rapeseed oil were fed at 7.7% and 8.5% of the DM.

Lower ammonia concentration in the rumen may indicate protection of dietary CP from microbial degradation, or lower rumen protozoal numbers. The recycling of nitrogen in the rumen is expected to decrease with a decreased population of protozoa, thus creating a situation where the microbes could utilize ammonia nitrogen efficiently for microbial protein synthesis (Tesfa et al., 1992).

Effect of supplemental fat on dietary N digestibility and microbial protein synthesis

Protein metabolism in the rumen may be altered if fat supplements interfere with fermentation. Mattos and Palmquist (1974) reported lower N digestibility when a formaldehyde-treated soyflour supplement (3.6% of diet DM) was fed to dairy cows; whereas, Wu et al. (1994) observed increased N digestibility for diets containing whole cottonseed (12% of diet DM) plus two levels of prilled tallow fatty acids (2.2 and 4.4%) or safflower oil (2.2%); although whole cottonseed alone depressed N digestibility compared with the diets containing tallow fatty acids or safflower oil. Palmquist and Conrad (1978) also reported higher N digestibility (68 versus 71%) due to increasing the amount of hydrogenated fat from 5.7 to 10.8% of diet DM in the diets of Jersey and Holstein cows.

Hussein et al.(1996) observed lower N digestibility (59.8 and 61.1%) when alkaline peroxide-treated canola seeds and crushed canola seeds were fed to steers at 10% of diet DM. Also, these authors found that the efficiency of bacterial protein synthesis (170.8 g/d versus 125.3 g/d) and N flow (102.1 g/d versus 97.1 g/d) to the duodenum were enhanced by treated canola seeds compared with crushed canola seeds. Ikwuegbu and Sutton (1982) reported a higher rate of bacterial protein synthesis (7.5 g/d versus 9.7 g/d) with decreased N digestibility

(77% versus 68%) when linseed oil was added to sheep diets, at 0 or 26 mL/d. The flow of N to the duodenum also was slightly increased by linseed oil supplementation.

Jenkins and Jenny (1992) noted increased N digestibility when 5% of the diet DM was replaced by prilled fat, canola oil or any combination of the two. However, no significant difference in N digestibility was found when Ca-salts of palm oil fatty acids, prilled fat, tallow, saturated fatty acids, hydrogenated animal fat, animal-vegetable blend or Ca-salts were supplemented to dairy diets (Grummer, 1988; Palmquist, 1991).

The type and amount of dietary fat appears to influence N digestibility, but responses are highly variable. Unsaturated fatty acids in oil seeds or free oils apparently increase the synthesis of bacterial protein and duodenal flow of N, possibly due to decreased number of protozoa and greater utilization of ammonia N for bacterial protein synthesis in the rumen (Tesfa et al., 1992).

Effect of dietary fat on long chain fatty acid flow to the duodenum

A marked divergence of opinions exist as to the relative amount of lipid that passes into the duodenum in comparison to that ingested in the diet. It was demonstrated in sheep on a high-roughage diet that the amount of fatty acids entering the duodenum may exceed that ingested by 40%; whereas by feeding a high proportion of concentrate, this difference can rise to 104% (Sutton et al. 1970). Steele (1983) found that 88 to 97% of dietary long chain fatty acids flowed to the duodenum when sheep were fed ryegrass hay or ryegrass silage supplemented with soybean oil at 8% of the diet DM. Klusmeyer and Clark (1991) reported that the flow of total 18 carbon fatty acids to the duodenum corresponded to the amount of Ca-salts of long chain fatty acids fed to dairy cows, and increased by 300 g/d. The total recovery of 18 carbon fatty acids at the duodenum, however, tended to increase when the control diet was fed (+ 34%) compared

with Ca-salt supplemented diets (+ 21%). Bauchart et al. (1987) also found a net gain (17.3%) in the amount of long chain fatty acids reaching the abomasum when cows were given diets with 60% hay, 7% soybean and canola meal, and 33% concentrate; whereas net losses (-22.2%) of total dietary long chain fatty acids were observed when diets were supplemented with milk at 33% of the DM.

More recently, Hussein et al. (1996) reported a decrease (5.5%) in total flow of fatty acids when alkaline hydrogen peroxide-treated canola seeds were fed to steers (5% of diet DM). Similarly, the data of Ferlay et al. (1993) indicated flows of long chain fatty acids to the duodenum were 41.2 and 27.8% lower than long chain fatty acid intakes when cows were fed diets containing 8.4% Ca-salts of rapeseed oil or 7.6% pure rapeseed oil.

From reviewing the literature, Ferlay et al. (1993) noted that a large number of trials, in which Ca-salts of rapeseed oil or rapeseed oil were fed, reported lower flows of fatty acids to the duodenum relative to the amount of long chain fatty acid intake. Based on these findings, the authors suggested that catabolism of long chain fatty acids might take place in the ruminal epithelium, as noted earlier by Jackson et al. (1964) and demonstrated *in vitro* by Cook et al. (1968). Previously, Wu et al. (1991) also indicated that the loss of long chain fatty acids in the stomach might be attributed to absorption, degradation to shorter chains, or underestimation of duodenal flow associated with sampling or applications of digesta markers.

Long chain fatty acid flow to the duodenum increases proportionally to the amount in the diet, provided that DM intake is not compromised. It also appears that when conventional diets are fed, rumen microbes can synthesize long chain fatty acids from substrates other than dietary fatty acids. Some of the literature, however, indicates that protected or unprotected oils at > 5% of diet DM may decrease long chain fatty acid flow, primarily due to ruminal catabolism to

shorter carbon chains and(or) absorption from the rumen. In addition, factors associated with measurements of duodenal flow may complicate accurate estimation of the recovery of long chain fatty acids at the duodenum in response to feeding supplemental fat.

Lipid digestion and absorption

In adult ruminants, lipid digestion begins in the enlarged forestomach (reticulorumen). The initial stages of ruminant digestion are characterized by intense lipolysis, fatty acid hydrogenation, and *de novo* lipid synthesis by microorganisms. As indicated in the previous section, some degradation of long chain fatty acids may be expected but very little absorption of saturated or insoluble (calcium-bound) long chain fatty acids takes place in the forestomach and abomasum (Noble, 1981).

Lipids in postruminal digesta are mainly saturated non-esterified fatty acids (from dietary and microbial origin; 70%) and small and variable amounts of microbial phospholipids (10 to 20%) absorbed predominantly on particulate matter (Leat and Harrison, 1975). Similarly, triglycerides of protected fat diets also may be present in postruminal digesta associated with solid material. In the acidic conditions of abomasal and duodenal digesta (pH 2.0 to 2.5), non-esterified fatty acids are fully protonated, thus enhancing lipid absorption onto the surface of particulate matter (Noble, 1981).

Lipid digestion occurs in a biphasic medium that consists of an insoluble particulate phase to which free fatty acids and phospholipids are attached and a soluble micellar phase containing dissolved fatty acids. Transfer of free fatty acids to the micellar phase occurs gradually as chyme flows 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.

When conventional diets are fed, 15 to 25% of total fatty acids are absorbed readily in the upper jejunum (pH 2.8 to 4.2); 55 to 65% of fatty acids are absorbed in the middle and the lower jejunum (pH 4.2 to 7.6). In diets containing protected lipids, lipid digestion occurs as it does in monogastric animals, in a biphasic system consisting of an oil phase and micellar phase. 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 (7.5) for lipase activity, triglyceride hydrolysis and fatty acid absorption do not take place before the mid jejunum (Leat and Harrison, 1975).

Generally, the intestinal absorption coefficient of individual fatty acids ranges from 80% (for saturated fatty acids) to 92% (for polyunsaturated fatty acids) when conventional diets contain from 2 to 3% added fat (Bauchart, 1993). 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 content generally results in higher apparent intestinal fatty acid digestibility because biliary secretions in the small intestine contribute additional fatty acids, and production of fatty acids in the large intestine is minimal. 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 diet DM) and 1000 g/d (6% of diet DM) to dairy cows. This decrease 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 saturated fatty acids with 12 to 16 carbons and unsaturated fatty acids with 18 carbons in the total tract increased, whereas apparent digestibility of 18:0 decreased, by feeding diets that contained Ca-salts of long chain fatty acids compared with a basal diet. Apparent intestinal digestibility of unsaturated fatty acids also was higher when Ca-salts were fed compared with the control diet. Steele and Moore (1968) observed a lower apparent digestibility for 18:0 (53%) than 16:0 (82%) or 14:0 (100%) when fed individually to sheep, and suggested that saturated fatty acids with a higher melting point would decrease formation of micelles in the small intestine.

McLeod and Buchanan-Smith (1971) compared the digestibility of different sources and forms of dietary lipid when fed to sheep at 3 or 4.8% of diet DM. Hydrogenated tallow (dry flaked form; 66% 18:0 and 30% 16:0), soybean oil (20.1% 18:1; Δ^9 *cis* and 59% 18:2), blended hydrogenated tallow (melted prior to mixing), and blended fatty acids (melted prior to mixing; 47% 16:0 and 46% 18:0) were used as the supplemental lipids. They found that tallow had a lower digestibility (38%) compared with soybean oil (98%) when fed at 3% of diet DM. In addition, melting and mixing the tallow resulted in a significant improvement in digestibility over dry flaked tallow (40% versus 30%), but saturated, blended fatty acids were better digested (73%) than either form of tallow. The authors concluded that the hydrogenation process resulted in tallow with a physical form that resisted dispersion and hydrolysis in the rumen and solubilization in the small intestine.

Buttery et al. (1977) suggested that fatty acylamides may be dissociated by one or more hydrolytic enzymes in pancreatic fluid. When sheep were fed 10 g linoleoyl methionine (a fatty acylamide) per day, duodenal flow and absorption of 18:2 increased without altering fecal linoleic acid content (Fotouhi and Jenkins, 1992). However, when sheep diets were supplemented with butylsoyamide (a faty acylamide made by reacting butylamine with soybean oil, which contains primarily 18:2) or soybean oil (47.2 g/d) apparent digestibility of the amide was lower (39.6%) due to excretion of amide (28.6 g/d) in feces (Jenkins, 1995). In contrast, digestibility of fatty acids in soybean oil was 80.8%.

Effect of dietary fat on plasma fatty acids

There is appreciable selectivity in incorporation of absorbed fatty acids from the intestinal lumen into the various lipid classes synthesized within the mucosal cells of the ruminant small intestine, and this selectivity is reflected in the fatty acid composition of lymph lipids. Christie and Hunter (1978) reported the fatty acid content of lipid fractions in intestinal lymph of sheep given a basal diet. The triglyceride fraction (79% of lymph lipids) contained only 7% polyunsaturated fatty acids (18:2 and 18:3) compared with 27% in phosphatidyl choline (11.8% of lymph lipids) and 24.5% in the cholesterol ester fraction (6.1% of lymph lipids).

In ruminants, lipid absorption and lymphatic transport to plasma are essentially a continuous processes subject to increases only when large amounts of dietary lipids are consumed (Noble, 1981). As previously discussed, unprotected dietary unsaturated fatty acids are subject to biohydrogenation in the rumen, yielding primarily saturated fatty acids flowing to the small intestine. If flow of unsaturated fatty acids to the small intestine increases, the increase should be reflected in the composition of lymphatic and blood plasma.

Moore et al. (1968) found that in sheep fed dried grass (57% 18:3) 18:3 content of blood plasma cholesterol esters was 10.2%, and phospholipids contained 5.4% 18:3. In contrast, hay (18% 18:3) induced a more modest response (5.1% 18:3 in cholesterol ester and 2.5% 18:3 in phospholipid). Noble et al. (1969) infused 40 g of maize oil triglycerides (64.4% 18:2, and 23% 18:1; Δ^9 *cis*) into the rumen of sheep for one hour and detected an increase in the proportion of 18:2 and 18:1; Δ^9 *cis* in the triglyceride fraction of plasma from 3.9 and 30.4% at 0 h to 15.1 and 35.7% at 6 h , respectively. Similarly, the proportion of 18:2 in the free fatty acid fraction at 6 h (11%) was greater than that at 0 h (2.5%). The concentration of 18:2 in cholesterol esters and phospholipids was 22.4 and 11.9% at 0 h. However, peak values (29.1% and 25.8%) were not observed until after 24 h post infusion. In the triglyceride fraction, a linear increase in 18:0 concentration was observed from 0 h (28.2%) to 24 h (45%).

La Count et al. (1994) infused long chain fatty acids from canola oil (62.5% 18:1; Δ^9 *cis*) or high-oleic sunflower oil (86% 18:1; Δ^9 *cis*) into the abomasum sequentially at 0, 133, 267, 400, 267, 133, and 0 g/d for 3 d at each rate for a total of 21 d. They reported higher 18:1; Δ^9 *cis* and lower 16:0 and 18:0 proportions in plasma triglycerides in response to the infusion sequence. Abomasal infusions of 40 g linseed oil (21.3% 18:2 and 71.1% 18:3) or maize oil (22.9% 18:1; Δ^9 *cis* and 64.4% 18:2) in sheep for 1 h also resulted in rapid incorporation of 18:2 and 18:3 into plasma triglycerides (Moore et al., 1968). The concentration of 18:2 and 18:3 began to rise from 5 and 4%, respectively, at 1.5 h after infusion, peaked at 4 h at 12.4 and 34%, respectively and remained elevated up to 10 h after infusion of linseed oil stopped. Similarly, infusion of maize oil caused 18:2 to increase in the triglyceride fraction from 5.6% at 1 h to 41.4% at 6 h. The proportions of 16:0 and 18:0 varied inversely with concentrations of 18:2 and 18:3, while that of 18:1; Δ^9 *cis* remained unchanged.

Jenkins et al. (1996) fed diets supplemented with soybean oil or butylsoyamide at 3.5% of diet DM, and found higher 18:2 concentration in plasma of cows fed butylsoyamide (59% of total fatty acid) compared with soybean oil (52% of total fatty acids). An increased in plasma 18:2 concentration also was observed when sheep were fed butylsoyamide compared with soybean oil at 5% of diet DM (Jenkins, 1995).

Thus dietary unsaturates escaping ruminal biohydrogenation can be effectively incorporated into lipid fractions within the intestinal mucosa, released into lymph, and transported to blood plasma for transport to all tissues of the animal. Apparently, 18:2 is preferentially incorporated in cholesterol esters and phospholipids, whereas 18:1; Δ^9 *cis* is primarily found in triglycerides. However, concentration of long chain fatty acids in all lipid fractions may be expected to increase in proportion to their flow to the small intestine.

Effect of dietary fat on milk production

It is generally assumed that conventional ruminant diets do not contain sufficient amounts of energy in the form of fat to support optimum production. Banks et al. (1976) showed that a low-fat (81 g fat/d) ration limited milk production. Mean milk yield (kg/d) was 10.4 for cows fed the low-fat ration compared with 14.1, 13.0 or 15.2 when fed isocaloric rations containing 555 g/d soybean oil, 517 g/d palm oil, or 539 g/d tallow, respectively. Clapperton and Steele (1985) also reported higher milk yields when tallow (400 g/d) was added to a barley-grass silage diet. Similarly, Harrison et al. (1995) observed higher consistency of milk production from wk 3 to 44 of lactation when cows were supplemented with whole cotton seed alone (12% of diet DM) or whole cottonseed plus Ca-salts of fatty acids (14.7% of diet DM). Schingoethe et al. (1996) also

found 8% higher milk yields when feeding extruded soybeans (17% of diet DM) or sunflower seeds (7.5% of diet DM).

Some studies also reported that supplemental fat did not increase milk production. Casper et al. (1988) found no benefit from feeding regular sunflower (65% 18:2) or high-oleic sunflower seeds (80% 18:1; Δ^9 *cis*) through wk 15 of lactation. Markus et al. (1996) did not obtain enhanced milk production from whole sunflower seeds (7.1% of DM) or tallow (2.7% of DM).

Rumen-protected fat supplements have been studied for a number of years to determine their potential to increase milk production in dairy cows. Chalupa (1991) compiled the results of 10 studies with Ca-salts of palm fatty acids and the average response for milk yield was + 2.40 kg/d. Yang et al. (1978) found that feeding formalin-treated sunflower seeds (69% 18:2) at 35% of diet DM increased cumulative milk yield and percentage of total milk yield (305 d lactation cycle) during the first 120 d of lactation. Goering et al. (1977) reported slight increases in milk yield when feeding formaldehyde-treated cottonseed oil (8.3% of DM). Jenkins et al. (1996) also observed higher FCM when feeding butylsoyamide (24.2 kg/d) compared with soybean oil (23.4 kg/d) at 3.5% of diet DM. Others found no significant differences in milk yield when feeding formaldehyde-treated canola seeds (Ashes et al., 1992), formaldehyde-treated safflower, coconut, or soybean oil (Astrup et al., 1976), formaldehyde-treated canola meal (Rae et al., 1983) or heat-treated soybeans (Mielke and Schingoethe, 1981).

Dietary fat can increase energetic efficiency in lactating cows by increasing total energy intake, direct incorporation of dietary fatty acids into milk, and promoting nutrient partitioning toward milk production (Palmquist, 1994). However, a lack of response to added fat could be attributed to factors such as decreased voluntary intake (especially if the amount of dietary fat

needed for milk fat synthesis is exceeded), stage of lactation, breed, season or interactions among these factors(Coppock and Wilks, 1991).

Effect of dietary fat on milk fat production and composition

Under normal dietary conditions 16:0, 18:0, and 18:1; Δ^9 *cis* are the principal fatty acids present in blood triglycerides of ruminant animals. Palmquist and Mattos (1978) using 1-[^{14}C]-18:2 reported that approximately 50% of fatty acids are synthesized *de novo* in the mammary gland from β-hydroxybutyrate and acetate. Dietary fat contributes an estimated 44% of total milk fatty acids, whereas endogenous sources provide 6%. However, Moore and Steele (1968) observed that increased mammary uptake of blood fatty acids resulted in decreased *de novo* synthesis of fatty acids from β-hydroxybutyrate and acetate. Similarly, feeding increased levels of tallow inhibited *de novo* synthesis of short chain fatty acids (Storry et al., 1973). It was suggested that acetyl CoA carboxylase was inhibited by increased amounts of long chain fatty acids from dietary origin.

Dietary fat can potentially increase milk fat production, especially during early to mid lactation. De Peters et al. (1985) showed that increasing whole cottonseed from 0 to 20% of diet DM increased milk fat from 3.19 to 3.61%. Dunkley et al. (1977) fed protected tallow at 0, 15 or 30% of diet DM, and also observed that milk fat production increased from 3.4 to 4.31%.

Chemically protected (formaldehyde-treated, fatty acylamides, or heat treated oil seeds) lipids also allowed selectivity in the modification of milk fatty acid composition when fed to cows. Mattos and Palmquist (1974) reported that yield of 18:2 (171.6 g/d) in milk fat was higher when formaldehyde-treated soyflour was compared with untreated soyflour (106.7 g 18:2/d) or a basal diet (70.9 g 18:2/d). Milk yield and milk fat yield were greater in response to both

soyflour supplements compared with control. Morrison and Hawke (1978) supplemented grazing cows with formaldehyde-treated sunflower seed (3.0 kg/d; 68.1% 18:2) or dried grass and reported higher milk fat yield (690 versus 560 g/d) and milk 18:2 content (15.5 versus 1.8% of total fatty acids) due to feeding protected sunflower seed. Mielke and Schingoethe (1981) found that heat-treated and untreated soybeans (fatty acid composition not reported) at 24.3% of diet DM increased concentrations of 18:1; Δ^9 *cis* (26.9 and 24.1% of milk fatty acids) and 18:2 (2.8 and 3.0% of milk fatty acids) acids and lowered concentrations of fatty acids with 6 to 14 carbons when compared to soybean meal (18:1; Δ^9 *cis* = 20.5% and 18:2 = 2.1%). Feeding butylsoyamide at 3.5% of DM resulted in higher linoleic acid content in milk fat (6.28% of total fatty acids) compared with soybean oil (4.77%) or a basal diet (3.60%) (Jenkins et al., 1996).

Ashes et al. (1992) indicated that formaldehyde-treated canola seeds (60% 18:1; Δ^9 *cis*, 20% 18:2, and 10% 18:3) at 6.5% of DM increased milk fat yield (0.93 versus 1.07 kg/d) without increasing milk yield. The concentrations of 18:1; Δ^9 *cis*, 18:2, and 18:3 (29.2, 4.9, and 2.6%, respectively in milk fat) increased in response to feeding the supplemented diet compared with the control (23.8, 2.2, and 1.6%, respectively). The concentrations of 12:0, 14:0, and 16:0 were lower (3.6, 9.5, and 19.9%) with the supplemented diets compared with control (4.3, 11.8, 26.7%). Khorasani et al. (1991) reported 18:1; Δ^9 *cis* concentration in milk fat increased from 18.7 to 31.3% in response to incremental amounts (0, 4.5, 9, and 13.2% of diet DM) of jet-sploded whole canola seed fed to dairy cows; whereas concentration of 16:0 decreased from 35.5 to 16.2% and 14:0 decreased from 11.5 to 9.1%.

Recently, Casper et al. (1988) also observed higher yields of oleic acid in milk fat when feeding regular sunflowers (20% 18:1; Δ^9 *cis* and 68% 18:2) or high-oleic sunflowers (80% 18:1;

Δ^9 *cis* and 10.7% 18:2) at 20% of DM compared with a basal diet. Both supplements decreased the percentages of fatty acids in milk with 6 to 16 carbons.

The concentration of short and medium chain fatty acids in milk are substantially reduced when protected or unprotected dietary unsaturated fatty acids are fed to dairy cows, whereas the concentrations of 18:1; Δ^9 *cis*, 18:2, and 18:3 are normally increased.

Effect of unsaturated fatty acids on de novo synthesis of fatty acids

As mentioned, feeding dietary fats containing large amounts of long chain unsaturated fatty acids led to reduced production of saturated fatty acids in milk fat. Abomasal infusions have been used as a tool for evaluating the potential to alter the content of specific fatty acids in milk fat. Rindsig and Schultz (1974) infused 250 or 500 mL of safflower oil (80% 18:2) over 4 h each day for 3 wk and observed decreased concentrations of 14:0, 16:0, 18:0, and 18:1; Δ^9 *cis* in milk fat; whereas 18:2 concentration increased. La Count et al. (1994) reported decreased proportions of fatty acids with 6 to 16 carbons in milk in response to sequential infusions (0, 133, 267, and 400 g/d, each for 3 d) of long chain fatty acids from canola oil (62.5% 18:1; Δ^9 *cis*) or high-oleic sunflower oil (86% 18:1; Δ^9 *cis*); whereas 18:1; Δ^9 *cis* and 18:2 concentrations increased. Chilliard et al. (1991) also found lower concentrations of 14:0, 16:0 and higher concentrations of 18:1; Δ^9 *cis*, 18:2, and 18:3 in milk fat when mid lactation cows were infused with 1100 g of rapeseed oil (61% 18:1; Δ^9 *cis*, 21% 18:2, and 8% 18:3) into the duodenum daily. Abomasal infusions of a mixture of unsaturated fatty acids (24% 18:1; Δ^9 *cis*, 55% 18:2, and 7% 18:3) at 438 g/d for 14 d also resulted in lower concentration of fatty acids with 10 to 16 carbons and higher oleic, linoleic, and linolenic acid in milk fat (Drackley et al., 1992).

The changes in milk fatty acid composition induced by feeding or infusing dietary lipids into the abomasum have been typical. Specifically, infused unsaturated long chain fatty acids have resulted in decreased proportions of short and medium chain fatty acids and increased proportions of long chain unsaturated fatty acids. One theory for this effect states that increasing unsaturation and chain length of dietary long chain fatty acids available to the mammary gland in blood plasma affects *de novo* synthesis in the gland by directly depressing acetyl-CoA carboxylase activity (Drackley et al., 1992). Direct evidence of the effects of unsaturated fatty acids on saturated fatty acid synthesis at the cellular level was reported recently. *In vitro* studies with bovine and mouse mammary cells indicated that mouse cells incorporated 18:1; Δ^9 *cis* and 18:2 to a greater extent than bovine cells; whereas, bovine cells incorporated 18:3 and CLA (18:2; Δ^9 *cis*, Δ^{11} *trans*) to a greater extent than mouse cells (Dawson and Herbein, 1996). Greater uptake of CLA was negatively correlated with *de novo* synthesis of 16:0 from acetate in bovine and mouse cells.

Effect of dietary fat on milk protein production

High fat diets often reduced milk protein production, particularly when protected lipids were fed. A compilation of results from 10 studies in which Ca-salts of palm fatty acids were fed indicated average milk protein percentage decreased by 0.16%(Chalupa, 1991). Protected tallow (0, 15, and 30% of DM) caused a linear decrease in milk protein production (3.18, 3.03, and 2.85%, respectively) (Dunkley et al., 1977). The authors indicated that the effect was specifically on the casein fraction. Heat-treated soybeans and whole cottonseeds plus Ca-salts of long chain fatty acids depressed milk protein production (-0.08 and -0.17%, respectively) (Mielke and Schingoethe, 1981; Harrison et al., 1995). In contrast, milk protein percentage was not depressed

by formaldehyde-treated canola seeds, hydrogenated soybean oil, coconut oil, canola meal or butylsoyamide (Ashes et al., 1992; Astrup et al., 1976; Jenkins et al., 1996).

The metabolic events that reduce milk protein synthesis in the mammary gland are not known. In a summary of seven trials in which whole soybeans were fed to lactating cows at 3.5, 4.4, or 5.3% of diet DM, Casper and Schingoethe (1989) reported reduced concentrations of several essential amino acids in arterial plasma and reduced mammary arterio-venous differences. Cant et al. (1993) determined mammary amino acid utilization by feeding a high (yellow grease at 4% of diet DM) or low (0% added fat) fat diet in combination with ruminal or abomasal casein infusions. Milk protein percentage was lowered by the high fat diets. However, there were reduced net losses of amino acid nitrogen and carbon into urea, ammonia, acetate, and glucose, indicating more efficient utilization of amino acid nitrogen and carbon by the mammary gland of a cow fed supplemental fat. In addition, elevated arterio-venous differences for essential amino acids were thought to have compensated for a reduction in mammary blood flow rate when cows were fed the high fat diet. In contrast, Wu and Huber (1994) concluded that the reduction in milk protein concentration when fat was fed to dairy cows was simply a reflection of lower amounts of amino acids flowing to the udder.

Few studies have reported increased milk protein percentage in response to feeding supplemental fat. Astrup et al. (1976) reported a significant increase (0.32 units) in milk protein percentage when cows were fed formaldehyde-treated soybean oil (400 g/d). Khorasani et al. (1991) found that feeding jet-sploded canola seeds at 4.5% of diet DM caused an increase (0.17 units) in milk protein percentage.

Conjugated linoleic acid in milk fat

Conjugated linoleic acid (CLA) is a newly recognized anticarcinogenic fatty acid that accounts for 0.24 to 2.8% of total fatty acids in milk fat (Parodi, 1997). The acronym CLA is a collective term which refers to a mixture of the conjugated positional and geometric isomers of linoleic acid (18:2; Δ^9 *cis*, Δ^{12} *cis*). Theoretically, the double bonds on the conjugated 18:2 molecule could be present at positions 9 and 11, 10 and 12, or 11 and 13, and can be in a *cis* or *trans* configuration at each position. However, it is 18:2; Δ^9 *cis*, Δ^{11} *trans* which is considered the biologically active isomer responsible for the anticarcinogenic properties of CLA (Ip et al., 1991). Rumen microorganisms preferentially isomerize linoleic acid to the Δ^9 *cis*, Δ^{11} *trans* configuration (Hughes et al. 1982), and it appears that this is the predominant isomer (90%) found in milk triglycerides (Parodi, 1977).

Variations in the proportions of CLA in milk fat due to effects of season and pasture quality have been reported previously. Booth et al. (1935) reported that the proportions of conjugated fatty acids in milk increased when cows were on summer pasture. Riel (1963) also found a two-fold increase in the content of conjugated fatty acids during summer months compared with winter months. More recently, a series of feeding trials were conducted in order to characterize the effects of feeding unsaturated vegetable oils on CLA concentration in milk (Kelly et al., 1997; McGuire et al., 1996). Overall, it appears that vegetable oils with a high content of polyunsaturated fatty acids (sunflower, soybean, and linseed oil) result in greater production of CLA in the rumen and transfer into milk. When sunflower oil (69% 18:2) was compared with peanut (52% 18:2) or linseed oil (51% 18:3), concentrations of CLA were 1.4, 2.4, and 1.7% of total fatty acids in milk fat (Kelly et al., 1997). Feeding corn oil (50% 18:2) at 3

to 7.2% of diet DM caused a relatively minor response in milk CLA concentration (0.69 versus 0.23% for the control).

McGuire et al., (1997) examined the presence of CLA in human milk and infant formula. Human milk contained 73% more CLA (0.22 to 0.54% of total fatty acids) than all infant formulas analyzed. The content of the biologically active isomer (18:2; Δ^9 *cis*, Δ^{11} *trans*) in human milk, ranged from 83 to 92% of all CLA isomers compared with 52 to 100% in infant formulas. Among infant formulas, those deriving their lipids from vegetable oils had lower concentration of CLA (0.033% of total fatty acids) compared with those containing animal fat (0.22% of total fatty acids) (McGuire et al., 1997).

The implications of these findings are that CLA production in the rumen and incorporation into milk fat increases when additional unsaturated oils are fed to dairy cows. Vegetable oils rich in linoleic acid could potentially raise CLA production to a greater extent than oils rich in monounsaturates or linolenic acid. Bovine milk contains higher levels of CLA than human milk or infant formula, but those formulas containing animal fat sources as an ingredient also contain moderate levels of CLA. Even though the relationship between dietary CLA intake and milk CLA content in lactating women has not been tested experimentally, previous research indicated that use of butter in food preparation resulted in higher (1.12% of total fatty acids) milk CLA compared with a conventional diet (0.58% of total fatty acids) (Fogerty et al., 1988).

CHAPTER 3

Modifying Oleic Acid Content of Milk Fat by Feeding Canolamide to Holstein Cows

ABSTRACT

This study assessed the potential for increasing oleic acid content of milk fat by feeding canolamide, made by reacting high-oleic canola oil with ethanolamine, as a means of protecting oleic acid from ruminal biohydrogenation. Four Holstein cows were fed a control diet, or the control diet supplemented at 3.3% of the DM with canola oil, canolamide , or a mixture of equal amounts of canola oil and canolamide in a 4 × 4 Latin square with four 21-d periods. DMI and milk yield were decreased by 25% and 13% when cows were fed canolamide exclusively. Percentages of fat and SNF in milk were not affected by treatment. However, protein percentage was lowest when cows were fed canolamide. Rumen pH was greater and rumen ammonia was lowest when canolamide was fed. Concentration of acetate in the rumen was lower when cows were fed canola oil or canolamide. In contrast, concentration of propionate was greater when cows were fed canola oil or canolamide. Fatty acid flow to the duodenum was proportional to intake, and thus lower in response to canolamide. Blood plasma total fatty acids and oleic acid increased by 13 and 84%, respectively, in response to supplemental fat. Concentration of milk fatty acids with 8 to 16 carbons was lower when cows were fed supplemental fat. Concentrations of oleic acid in milk fat ranked by treatment were canolamide > canola oil > canola oil plus canolamide > control. Inclusion of canolamide at 1.7% of diet DM was not detrimental to the parameters measured compared with canolamide at 3.3% of diet DM. Converting high-oleic canola oil to an amide provided partial protection against biohydrogenation of oleic acid as indicated by its elevated concentration in plasma and milk fat, but significantly reduced DMI and milk yield when fed at 3.3% of diet DM.

INTRODUCTION

Unsaturated fatty acids are extensively hydrogenated in the rumen of cattle and sheep, thus posing a major obstacle to increasing delivery of these fatty acids to the duodenum for absorption (Jenkins, 1993). As a result, fatty acid composition of adipose tissue and milk fat is highly saturated. Grummer (1991) indicated that milk fat contains approximately 70% saturated, 25% monounsaturated, and 5% polyunsaturated fatty acids. Evaluation of methods to manipulate the fatty acid composition of milk fat is receiving attention because saturated fatty acids, specially myristic and palmitic acid, can induce hypercholesterolemia in humans (Denke and Grundy, 1992).

Supplementing ruminant diets with unsaturated oils leads to minor increases in unsaturation of body tissues or milk. Fotouhi and Jenkins (1992) initially reported that fatty acylamides, made by reacting unsaturated fatty acids with primary amines, resist biohydrogenation. Feeding butylsoyamide, made by reacting soybean oil with butylamine, at 5% of DM to sheep increased plasma linoleic acid by 58% compared with a control diet (Jenkins, 1995). Similarly, when Holstein cows were fed butylsoyamide at 3.5% of DM, linoleic acid in plasma increased by 6% when compared to a control or soybean oil diet. Milk linoleic acid concentration also increased, averaging 3.6, 4.8, and 6.3% for the control, soybean oil, and butylsoyamide diets (Jenkins et al., 1996). A drawback identified in both studies was the low digestibility (39.6%) of butylsoyamide. However, in a recent study Jenkins and Thies (1997) determined that substituting ethanolamine for butylamine enhanced amide digestibility to 99.5%.

Linoleic acid-enriched dairy products may have decreased shelf life due to oxidation. However, this problem has been offset through the inclusion of oleic acid in ruminant diets. Ashes et al. (1992) reported that feeding canola seeds (60% oleic acid) encapsulated in a matrix

of aldehyde-treated protein significantly reduced proportions of lauric, myristic, and palmitic acid in milk fat while increasing oleic acid. Noakes et al. (1996) indicated that consumption of milk and dairy products from cows fed protected canola seeds resulted in a 4.5% reduction of total cholesterol as compared to conventional dairy products. The present study was conducted to evaluate the effects of canolamide, prepared by reacting high-oleic canola oil with ethanolamine, on DMI, ruminal parameters, fatty acid flow to the duodenum, apparent digestibility of diet components, plasma fatty acid profile, and milk production and composition.

MATERIALS AND METHODS

Animals and Diets

Four mid-lactation multiparous Holstein cows (averaging 135 DIM) previously cannulated in the rumen and proximal duodenum, were utilized in a 4 × 4 Latin square design with four 21-d periods to evaluate responses to four experimental diets. During a 3-d crossover between periods, the control diet without added fat was fed on d 1 and equal portions of the control plus the assigned diet for the next period were fed on d 2 and 3. Cows were housed individually in tie stalls and had free access to water and their assigned diet, which was offered daily at 1400 and 0200 h. Feed refusals were removed daily at 1130 h and weighed. Daily feed allotment was calculated to allow 5 to 10% feed refusal. Cows were milked each day at 1300 and 0100 h.

Experimental diets (Table 3) contained 17.5% alfalfa haylage, 30.7% corn silage, and 51.8% concentrate (DM basis). The control diet was supplemented at 3.3% of DM with canolamide (AMD), canola oil (OIL), or equal amounts of canola oil plus canolamide (OAM). Corn gluten meal was added to fat-supplemented diets to keep treatments isonitrogenous.

However, the amide diet contained higher CP due to the fatty acylamide. Dietary treatments were prepared individually each day as a total mixed ration. Concentrates were prepared in 800 lb batches, stored in sealed plastic containers, and removed as needed to mix with the forage on a daily basis. All diets were formulated to meet or exceed all nutrient requirements for lactating cows (NRC, 1989). The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

Lipid Supplements

High-oleic canola oil contained 77% oleic and 13% linoleic acid with minor amounts of palmitic, stearic, and linolenic acid. Canolamide was prepared at Clemson University (Dr. T. C. Jenkins; Animal, Dairy & Veterinary Sciences Department) by the procedure of Jenkins and Thies (1997) and shipped to the Virginia Tech Dairy Center in sealed metal drums. Briefly, 1.38 kg high-oleic canola oil per kg ethanolamine (BASF, Mt. Olive, NJ) were mixed in a tightly closed 114 L drum at 70 °C for at least 48-h (Jenkins et al., 1996). Canolamide contained 65% total fatty acids (DM basis) , indicating presence of unreacted ethanolamine. Fatty acid composition of the canolamide supplement resembled that of the high-oleic canola oil and contained 77% oleic and 12% linoleic acid.

Sampling, Measurements, and Analysis

Forages and concentrates were sampled on d 18 of each period and during batch mixing of concentrates. Samples were dried in a forced-air oven at 60 °C and stored in sealed plastic containers. An overall composite for determining chemical composition was obtained by combining samples from each period or concentrate batch. Feed refusals were collected on d 19

through 21, composited by period, dried in a forced-air oven at 60°C, and stored in sealed plastic containers until analyzed. In preparation for analyses, dried forages, concentrates, and feed refusals 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).

Milk samples were obtained at each milking during d 19 and d 20 and used to determine milk composition. A 30 mL aliquot was collected in a 50 mL vial containing Bronopol (milk preservative; D & F Control Systems, San Ramon, CA) at 0200 and 1400 h, then stored at 4 °C for 4-d until analyzed for milk fat, protein, and SNF by the Virginia Dairy Herd Improvement Association. On d 21 of each period, another aliquot was collected without Bronopol, then frozen at -20 °C until the end of each period. Subsequently, samples were thawed at room temperature and centrifuged at 10,000 × g for 1 h to isolate milk fat. Harvested milk fat was stored at -20 °C until analyzed for fatty acid composition.

Six duodenal and fecal samples were collected at 1500, 0300, 1900, 0700, 2300, and 1100 h on d 18 through 21, to represent 4-h intervals within a 24-h period. Fecal grab-samples were collected and immediately dried in a forced-air oven at 60 °C, then composited at the end of each period. Duodenal digesta (200 mL) was collected into a plastic container and frozen. At the end of the experimental period, digesta samples were thawed at room temperature, composited, and homogenized with a Polytron© PT 10/35 homogenizer (Brinkmann Instruments, Westbury, NY). Aliquots of digesta were collected into two 100 mL plastic containers, frozen, and dried in a Dura-Top freeze dryer (FTS© systems, Inc., Stone Ridge, NY). Fecal and duodenal digesta composites were ground through a 2 mm screen (Thomas-Wiley Laboratory Mill), and a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) until analyzed for fatty acid and chemical composition. Feed components, feed refusals, duodenal

digesta, and feces were analyzed for ADF, NDF (Goering and Van Soest, 1970), total N, EE (AOAC, 1984), and OM (600°C for 5-h).

Blood samples (10 mL) were obtained on d 21 of each period at 1800 h from the coccygeal artery by venipuncture. Blood was transfered to tubes containing 286 IU heparin in 100 μL of sterile saline solution and centrifuged at 3000 × g for 15 min for harvesting the plasma. Plasma was stored at -20 °C until fatty acid analysis.

Ruminal fluid (600 mL) was collected each period on d 21 at 1600 h through the rumen cannula. One aliquot of rumen fluid (5 mL) was placed in tubes containing 1 mL 25% meta-phosphoric acid for determination of rumen NH3, and a second aliquot (5 mL) placed in tubes containing 1 mL 30 mM isocaproic acid (internal standard) for determination of rumen VFA. Aliquots were frozen at -20 °C. Subsequently, both aliquots were thawed, centrifuged at 2800 × g, filtered through a Metricel© GN-6 (0.45 m) filter (Gelman Sciences, Ann Arbor, MI), and stored in 5 mL vials at 4 °C until analyzed for ammonia and VFA content.

Ruminal pH was determined immediately after sampling using an Accumet pH Meter (Fisher Scientific, Raleigh, NC). Ruminal fluid was analyzed for ammonia-N (Weatherburn, 1967) (without use of urease) and VFA. Acetic, propionic, butyric, valeric, isovaleric and isobutyric acids were determined by flame ionization detection on a Varian Vista 4270 integrator (Varian Instruments, Palo Alto, CA). A VFA standard containing acetic (70 mol/mL), propionic (42 mol/mL), butyric (14 mol/mL), valeric (7 mol/mL), isovaleric (7 mol/mL), and isobutyric (7 mol/mL) acids was used to determine VFA concentrations in samples by integration. Five μL of sample were injected into a 18.3 m × 0.64 cm o.d. and 2 mm i.d. glass column (Supelco Inc., Belfonte, PA) packed with GP 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW.

Column, inlet, and detector temperatures were 115, 170, and 180 °C, respectively. Carrier gas (N2) flow was set at 80 mL/min with detector gases (hydrogen and air) set at 40 and 60 mL/min, respectively.

Chromium-mordanted fecal fibers were utilized as a digesta marker to determine flow rates at the duodenum and into feces. Chromium was attached to washed fecal fibers (6 g Cr/100 g fecal fibers) collected from two non-lactating cows fed orchardgrass hay by the procedure of Uden et al. (1980). Two 15 g doses of Cr-mordanted fecal fibers (1.29 g Cr/d) were placed into the rumen daily at 0600 h and 1800 h from d 11 to 21 of each period. Chromium was extracted from feces and duodenal digesta by wet ashing with HNO3 and HClO4 (Sandell, 1950). Chromium content was determined by atomic absorption spectrophotometry to calculate nutrient flow (Armentano and Russell, 1985).

Fatty acylamide content in neutral lipid fractions from concentrate, feed refusals, duodenal digesta, plasma, and milk fat was determined by HPLC (Rainin Instrument, Ridgefield, NJ) using a 100 mm × 4.6 i.d. silica column (Alltech Associates, Deerfield, IL). The isocratic mobile phase was 75% hexane, 20% isopropanol, and 5% propanol at a flow rate of 1.0 mL/min. The detector was a Varex MK III evaporative light scattering mass detector (Alltech Associates, Deerfield, IL). Quantitation of amide was by the external standard technique using stearamide (Jenkins and Thies, 1997).

Plasma total lipids were extracted with chloroform/methanol (2:1, vol/vol) (Folch et al., 1957). Fatty acids in forages, concentrates, fat supplements, feed refusals, duodenal digesta, milk fat, blood plasma, and feces were methylated by in situ transterification with 0.5N methanolic NaOH as described by Park and Goins (1994). Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Methyl esters of fatty acids were separated by

gas chromatography using a 30 m × 0.25 mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellefonte, PA). 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 per min to a final temperature of 220 °C (held for 2 min).

Statistical Analysis

Data are reported as Least squares means and SEM. All data were analyzed as a 4 × 4 Latin square using the GLM procedure of SAS (1988). Sources of variation in the model were cow, period, and treatment. Differences between treatment means were determined by non-orthogonal contrasts, and were designated as significantly different at $P < 0.05$. The non-orthogonal contrasts were: control versus all fat supplements (CON vs FAT), canola oil versus canolamide (OIL vs AMD), and oil/amide versus canolamide (OAM vs AMD). The following model was used for statistical analysis:

$$Yijk = \mu + Ci + Pj + Tk + E(ijk)$$

where

Yijk = DM, OM, CP, ADF, and individual fatty acid intake, apparent digestibility in the rumen and total tract, and duodenal flow, unsaturated fatty acid biohydrogenation, rumen parameters, plasma fatty acids, milk production and composition, and milk individual fatty acid profile,

μ = mean,

Ci = effect of cow (i = 1..4),

Pj = effect of period (j = 1..4),

Tk = effect of treatment (k = 1..4),

E(ijk) = residual error

RESULTS AND DISCUSSION

Intake and Duodenal Flow of Diet Components

Daily DMI was lower when fat was added to the diet (Table 4). Compared to control, feeding OIL or OAM decreased DMI by an average of 2.4 kg/d, whereas feeding AMD resulted in a decrease of 8 kg/d. Visual observation of feed refusals indicated considerable sorting of forage and concentrate when cows were fed AMD. Cows appeared to consume the forage fraction at the expense of the concentrate when AMD was fed, and although the degree of sorting was variable among cows, one cow in particular tended to sort both fractions regardless of diet fed.

Feeding fatty acylamides resulted in variable responses with regards to feed intake. Jenkins (1995) indicated that feeding butylsoyamide, made by reacting soybean oil with butylamine, to sheep resulted in increased DMI compared with soybean oil (740 and 581 g/d, respectively). When fed to lactating Holstein cows at 3.5% of DM butylsoyamide did not decrease DMI (Jenkins et al., 1996). However, a decrease in DMI was reported when hydroxyethylsoyamide (made by reacting soybean oil with ethanolamine) or oleamide (made by reacting oleic acid with ammonia) were fed to sheep or dairy cows at 5 or 3.5% of diet DM, respectively (Jenkins, 1997; Jenkins and Thies, 1997; Jenkins and Bertrand, 1997). As pointed out by Jenkins and Thies (1997), animal responses to fatty acylamides may vary with amount of unreacted amine (butylamine, ethanolamine) in the diet. Considering the molar ratio of amine to fatty acid during synthesis of canolamide (1.33:1), the purity of the canolamide supplement (76.2%), and the actual fatty acid content of canolamide (65% on a DM basis) (Dr. T. C. Jenkins, 1997, personal communication), unreacted ethanolamine in the AMD diet probably led to

reduced DMI. Unreacted ethanolamine in the rumen is not expected to be detrimental to microorganisms, because ruminal microbes can effectively utilize ethanolamine as a source for carbon and nitrogen (Shukla and Turner, 1980), or can incorporate it into membrane phospholipids (Jenkins, 1993). Thus, very little flow of free ethanolamine into the small intestine is expected. While canolamide had an intense ammonia odor caused by unreacted ethanolamine, inclusion of canolamide at 1.7% of DM (OAM) did not adversely affect feed intake when compared with canola oil (OIL). From the present study it is not clear whether intake depression was due to poor palatability or physiological effects potentiated by greater levels of canolamide in the diet (AMD).

Intake of amide averaged 137 g/d in response to OAM compared to 196 g/d when AMD was fed (Table 4). In spite of lower DMI due to AMD, cows still consumed a greater proportion of amide because of higher inclusion of this supplement in the concentrate. Flow of amide to the duodenum only averaged 7 and 6% of total amount consumed as OAM and AMD, respectively. Analysis of blood plasma by HPLC (Dr. T. C. Jenkins, 1997, personal communication) revealed no measurable amounts of amide. Previous studies have reported similar findings (Jenkins, 1995; Jenkins and Thies, 1997). This indicates that the amide bond in the fatty acylamide must be cleaved prior to absorption. Our data suggests that acidic conditions in the abomasum is a major factor involved in hydrolysis of amides.

As expected, reduced dry matter intake and duodenal flow in response to supplemental fat led to reduced intake and flow of ADF, OM, and CP (Table 4). Overall, similar responses with regards to intake and flow of ADF, OM, and CP were obtained when OIL or OAM were fed. However, feeding AMD resulted in lower flows of ADF, OM, and CP compared with OIL and OAM.

Digestion of Diet Components

Apparent digestibility of DM, OM, and ADF in the rumen did not differ among diets (Table 4), but the large SEM for this parameter indicates that there was considerable variation among cows. Apparent ruminal CP digestibility, however, was higher when supplemental fat was added to the diet. Feeding AMD also resulted in higher apparent ruminal CP digestibility (40%) compared with OAM (21%) or CON (16%). Addition of prilled fatty acids (2.5% of diet DM) to a high fat diet (whole cottonseeds at 7% of diet DM) also increased apparent CP digestibility in the rumen (Chan et al., 1997). Mir (1988) reported increased N disappearance when ground alfalfa hay supplemented with canola fatty acids at 3, 4, or 5% of diet DM was incubated in the rumen of cattle for 24-h. In the present study, lower DMI in response to supplemental fat may have led to longer retention time of feed constituents in the rumen, thus lengthening microbial fermentation time.

Apparent digestibility of DM, OM, CP, and ADF in the total tract were not affected by treatment. Similar results were reported by Jenkins (Jenkins, 1995, 1997) when butylsoyamide, hydroxyethylsoyamide or soybean oil were fed to sheep at 5% of DM. Ferlay and Doreau (1992), however, indicated that addition of canola oil at 5.5% of DM to the diet of lactating cows reduced apparent digestibilities of OM and ADF. They hypothesized that oil addition led to reduced microbial cellulolytic activity. Very often, addition of canola oil either in the form of seeds or as free oil in dairy rations has resulted in a decrease in DM, ADF, and OM digestibility in the total tract (Ferlay et al., 1994 ; Murphy et al., 1987). Most workers have suggested that vegetable oils with a high degree of unsaturation have greater antimicrobial effects in the rumen than saturated fat sources (Jenkins, 1993). Clearly, the level of fat supplementation in the present study was below published values reported to be detrimental to digestibility of diet components.

Rumen VFA

Concentration of total volatile fatty acids in rumen fluid was not affected by treatments (Table 5). Percentage of acetate, however, was lower in response to OIL or AMD compared with CON or OAM. Propionate percentage, in contrast, increased in response to OIL and AMD compared with CON and OAM. Ratio of acetate to propionate, therefore, decreased when OIL and AMD were fed compared with CON and OAM. Jenkins et al. (1996) indicated that fatty acylamides added to dairy diets at 3.5% of DM did not reduce acetate concentration or total VFA compared with soybean oil. Their findings were in agreement with those of Jenkins (1995) and lent support to the view that fatty acylamides substantially reduce negative effects of unsaturated oils on ruminal fermentation. The amide supplement used by Jenkins (1995) and Jenkins et al. (1996) was made with butylamine as opposed to ethanolamine used in the present study. Furthermore, DMI depression was not observed in either study.

More recently, Jenkins and Thies (1997) evaluated the potential of hydroxyethylsoyamide to resist biohydrogenation in vitro. They observed similar declines in linoleic acid concentration in culture media when substrates containing either 5% added linoleic acid or 10% added hydroxyethylsoyamide were incubated in vitro for 48-h with rumen inoculum from sheep. If lower DMI in our study led to longer retention time in the rumen it could be possible that microbial breakdown of the amide bond was only partially inhibited. Breakdown of the amide bond may have resulted in the availability of free carboxyl groups in unsaturated fatty acids, which Jenkins (1993) suggested were needed for expression of antimicrobial effects. As in the present study, Jenkins (1997) reported reduced DMI, lower acetate, higher propionate, and reduced ratio of acetate to propionate when sheep were fed hydroxyethylsoyamide at 5% of DM.

Butyrate concentration was lower in response to OIL compared with AMD. Tesfa et al. (1992) reported lower butyrate concentration when rapeseed oil was added at 5 or 10% of DM to diets of Holstein cows. Furthermore, addition of casein-formaldehyde treated rapeseed oil at 10% of DM also reduced butyrate concentrations compared with a control diet. These workers implicated lower protozoal numbers in response to rapeseed oil as the cause for lower butyrate.

Rumen pH and Ammonia-N

Rumen pH increased in response to OAM and AMD compared with CON or OIL (Table 5). Visual observation of ruminal contents when cows were fed AMD indicated considerable frothing. This effect, however, was not apparent when cows were fed OAM. It could be possible that unreacted ethanolamine contributed to the increase in pH. Similar observations were reported by Tesfa et al. (1992) when feeding casein-formaldehyde treated rapeseed oil to Holstein cows. Rumen ammonia nitrogen concentration (Table 5) decreased in response to supplemental fat, and was lower when AMD was compared with OIL. Other workers have reported decreased rumen ammonia nitrogen in response to linseed oil (Igwuegbu and Sutton, 1982), rapeseed oil (Ferlay and Doreau, 1992), corn oil (Jenkins and Fotouhi, 1990), or casein-formaldehyde-treated rapeseed oil (Tesfa et al., 1992) as well as formaldehyde-treated canola meal (Rae et al., 1983). Tesfa et al. (1992) speculated that unsaturated oils decrease rumen ammonia nitrogen mainly due to a decrease in rumen protozoa. They indicated that recycling of nitrogen in the rumen is expected to decrease with reduced protozoal numbers, thus creating a situation where microbes could utilize ammonia nitrogen more efficiently for microbial protein synthesis.

The level of plasma urea nitrogen (PUN) was significantly higher when cows were fed AMD compared with other treatments (Table 5). Based on higher digestibility of CP in the rumen and lower rumen ammonia nitrogen it is possible that feeding AMD increased transport of ammonia nitrogen from the rumen into blood plasma, and conversion of ammonia into urea within the liver. These processes may have reduced microbial protein synthesis in the rumen and CP flow to the small intestine (Table 4), and may have reduced availability of amino acids for milk protein synthesis by the mammary gland (Table 10).

Intake, Postruminal Flow, and Biohydrogenation of Fatty Acids

Total fatty acid intake (Table 6) was a reflection of DMI and the fatty acid content of the diet. Intake of fatty acids was greater in response to OIL and OAM compared with CON or AMD. Overall, intake of palmitic acid was equal to or lower than CON in response to fat-supplemented diets, because canola oil contained only 4% palmitic acid. Similarly, the canola oil contributed small amounts of stearic and linoleic acids (3 and 13%, respectively), and resulted in slightly greater intakes of these fatty acids when OIL or OAM were fed. The oleic acid content of canola oil, in contrast, provided greater oleic acid intake when all fat supplements were fed. For all other fatty acids, however, intake was always lowest when AMD was fed compared with OIL or OAM due to lower DMI.

Total fatty acid flow to the duodenum increased by approximately 272 g/d when OIL and OAM were fed, but decreased by 168 g/d when AMD was fed compared with CON. Oleic acid flow to the duodenum was higher, when cows were fed OIL, OAM, or AMD compared with CON. Feeding OIL or OAM decreased flow of palmitic and linoleic acid but increased stearic, and trans-vaccenic acid flow to the duodenum. Flow of stearic and trans-vaccenic acid were

lower in response to AMD compared with OIL, again due primarily to lower DMI. Positive net flow of fatty acids to the duodenum suggests appearance of fatty acids from endogenous sources or from microbial de novo fatty acid synthesis. However, in a recent trial Hussein et al. (1996) reported lower fatty acid flows when steers were fed diets containing canola seeds treated with alkaline hydrogen peroxide. From reviewing the literature, Ferlay et al. (1993) also found that a large number of trials with different fat supplements reported similar losses of fatty acids with 18-carbons. In the present study, recovery of 18 carbon fatty acids (Table 6) at the duodenum exceeded intake when CON, OIL, or OAM were fed compared with AMD. Recovery of unsaturated 18-carbon fatty acids (18:1, Δ9cis to 18:3) did not differ among treatments, indicating similar extents of biohydrogenation among treatments.

Apparent biohydrogenation of unsaturated fatty acids in the rumen was estimated by the equation of Wu et al. (1991) as follows:

Biohydrogenation (%) = 100 - [100 x (individual or total unsaturated C18 fatty acids total C18 fatty acids in duodenal digesta) / (individual or total unsaturated C18 fatty acids / total C18 fatty acid intake)]

The extent of apparent biohydrogenation of linoleic, linolenic, and total unsaturated fatty acids did not differ due to treatment (Table 7). Biohydrogenation of oleic acid, however, was higher when supplemental fat was fed but did not differ among fat supplements. Murphy et al. (1987) fed crushed canola seeds at 5.4% of DM to dairy cows and reported apparent biohydrogenation of 72.8, 86.7, and 88.5% for oleic, linoleic, and linoleic acid, respectively. When rapeseed oil was fed (5.7% of DM) to Holstein cows apparent biohydrogenation was 61.0, 92.7, and 97.5% for oleic, linoleic, and linolenic acid, respectively (Ferlay et al., 1993). Hussein et al. (1996), however, reported that feeding hydrogen peroxide-treated canola seeds to steers resulted in lower

biohydrogenation of oleic, linoleic, and linolenic acid (38.4, 64.7, and 48.4% respectively) compared with crushed canola seeds (62.3, 74.9, and 75% respectively). Data in Table 7 suggested that formation of the acylamide was not as succesful as anticipated in preventing biohydrogenation. Jenkins and Thies (1997) indicated that in vitro incubation of hydroxyethylsoyamide with rumen contents from sheep for 48-h resulted in similar losses of linoleic acid from culture medium compared with incubation of pure linoleic acid.

Digestion of Fatty Acids

Apparent intestinal digestibility of individual fatty acids (Table 8), with exception of stearic acid, did not differ among treatments. Stearic acid and total fatty acid digestibilities in the intestine were lower due to supplemental fat compared with CON. The response was due to the greater flow of stearic acid to the duodenum, primarily when OIL or OAM were fed. Similar responses were observed when oils or oilseeds were fed to lactating cows (Ferlay et al., 1993 ; Hussein et al., 1996). Intestinal digestion of total fatty acids (amount apparently absorbed) did not differ in response to added fat but was lower when AMD was fed compared with OIL or OAM. Amount of oleic acid apparently absorbed was higher when supplemental fat was fed; whereas, amount of palmitic acid apparently absorbed was lower (Table 8). Trans-vaccenic acid apparently absorbed was proportional to rate of flow to the duodenum and was higher in response to OIL compared with AMD. Apparent absorption of linolenate also was proportional to its flow to the duodenum and was higher in response to OAM compared with AMD. The amount of linoleic acid apparently absorbed was not different among treatments.

Blood Plasma Fatty Acids

Plasma total fatty acids (Table 9) were significantly increased by supplemental fat. The concentration of myristic, myristoleic, pentadecanoic, palmitoleic, heptadecenoic, and eicosatrienoic acid were reduced when supplemental oil was fed. Similar results were found when safflower or soybean oil were fed to ruminants, and it was suggested that oil supplementation reduced de novo lipid synthesis by ruminal microbes (O'Kelly and Spiers, 1993; Jenkins et al., 1994; Jenkins 1995). Plasma concentrations of stearic, oleic, trans-vaccenic, and conjugated linoleic acid were higher in response to supplemental fat compared to CON. Among fat supplements, concentration of oleic acid was higher in response to OIL compared with AMD, reflecting intake and duodenal flow of this fatty acid in response to each diet. However, oleic acid concentration in response to AMD or OAM was not different in spite of higher intake of oleic acid when OAM was fed. Higher concentrations of trans-vaccenic and conjugated linoleic acid, coupled with lower concentration of linoleic acid in response to OIL indicated considerable biohydrogenation in the rumen. Feeding AMD did not increase trans-vaccenic or conjugated linoleic acid as markedly as OIL did, indicating only partial protection of unsaturated fatty acids against biohydrogenation due to the amide bond.

Lactational Response and Milk Composition

Milk production did not differ due to supplemental fat (Table 10), but AMD reduced milk yield by approximately 5 kg/d compared with OIL or OAM. Atwal et al. (1991) reported a 3 kg/d reduction in milk yield when formaldehyde-treated canola oil was fed to dairy cows at 4% of the DM. Recently, Chouinard et al. (1997) reported a linear decrease in DMI with a quadratic response in milk yield when supplementing Ca-salts of canola oil from 0 to 4% of diet DM to

Holstein cows. In contrast, Jenkins and Bertrand (1997) did not see a depression in milk yield in spite of lower DMI when feeding oleamide at 3% of the diet DM. Usually, moderate increases in fatty acid intake increase efficiency of energy utilization by the mammary gland (Baldwin et al., 1980). Oxidation of fatty acids, instead of acetate, yields 10% more ATP . Also utilization of preformed fatty acids for fat synthesis prevents energy expenditure associated with de novo synthesis of fatty acids. Thus it was evident, as indicated in Table 4, that cows fed AMD did not consume sufficient energy to support milk synthesis at the rate observed for other treatments.

Concentration of fat and SNF were not affected by any treatment. Therefore, 3.5% FCM reflected milk yield and was lower in response to AMD compared with other treatments. Milk protein decreased in response to AMD, but it was not affected by other treatments. As indicated earlier, feeding AMD increased CP digestibility in the rumen and resulted in higher PUN and lower CP flow to the duodenum for absorption and, thus, may have diminished the availability of amino acids for milk protein synthesis. A tendency ($P = 0.09$) for higher MUN was also apparent when AMD was fed compared with OIL (Table 5). Kirchgessner et al. (1986) indicated that reduced energy intake by cows led to higher PUN, MUN, and lower protein percentage in milk. Concentration of lactose in milk was higher when supplemental fat was fed. In contrast, other studies have reported lower concentrations of lactose when supplemental fat was added to the diet (Cant et al., 1991; DePeters et al., 1987). Lactose is considered the primary osmoregulator of milk volume (Linzell and Peaker, 1971) and its concentration fluctuates little. Milk component yields were a reflection of milk production and decreased significantly when AMD was fed compared with other treatments.

Milk Fatty Acid Composition

Milk fatty acid concentration (Table 11) was altered by inclusion of fat in the diet. Concentration of fatty acids with 8 to 16 carbons was lower in response to supplemental fat compared with CON. Decreased percentages of short and medium chain fatty acids in milk has been associated with reduction of acetyl CoA carboxylase activity as the mammary gland increases uptake and utilization of exogenous long chain fatty acids (Storry, 1988). Stearic acid concentration was increased in response to supplemental fat. The higher flow of stearic acid to the duodenum when fat was fed may have contributed to the greater percentage of this fatty acid in milk. Oleic acid concentration was elevated in response to supplemental fat, and among supplements, concentration was greater in response to AMD compared with OIL , or OAM. In contrast, concentration of linoleic acid did not differ due to fat supplementation, but was higher in response to AMD compared with OIL or OAM. Linolenic acid concentration was greater when supplemental fat was fed, especially in response to OIL or OAM compared with AMD. Trans-vaccenic and conjugated linoleic acid concentrations were increased by addition of supplemental fat. However, they increased more when cows were fed OIL or OAM compared with AMD.

The concentrations of pentadecanoic, heptadecanoic, and palmitoleic (Δ9trans) acid were decreased by supplemental fat. Ruminal bacteria synthesize odd chain fatty acids from odd-numbered VFA. However, dietary long chain fatty acids were previously reported to inhibit de novo synthesis of fatty acids by ruminal bacteria and protozoa (Palmquist and Jenkins, 1980). Feeding soybean oil also reduced pentadecanoic and heptadecanoic acid in plasma and sheep adipocytes (Jenkins, 1995; Jenkins et al., 1994). Based on the lower percentage of pentadecanoic acid in blood plasma when fat was fed, it could be possible that the lower percentage of

pentadecanoic acid in milk was a reflection of diminished de novo fatty acid synthesis in the rumen and adipose tissue. Lower concentrations of palmitoleic acid (Δ9 trans) in milk fat were previously reported by Chouinard et al. (1997) when Ca-salts of canola oil were fed to cows, but the significance of this effect was not discussed.

Eicosatrienoic and arachidonic acid percentages in milk were also lower in response to supplemental fat, but were lowest in response to OIL or OAM compared with AMD. Similar results were reported when dairy cows were fed soybean oil (Jenkins et al., 1996) or Ca-salts of canola oil (Chouinard et al., 1997), and may reflect inhibition of desaturation of polyunsaturated fatty acids as uptake of exogenous long chain unsaturated fatty acids by the mammary gland increased (Chang et al., 1992). Overall, our results regarding milk fatty acids were comparable with earlier reports when Ca-salts of rapeseed (Tesfa et al., 1992), formaldehyde-treated canola seeds (Ashes et al., 1992; Atwal et al., 1991), butylsoyamide (Jenkins et al., 1996), or oleamide (Jenkins and Bertrand, 1997) were fed to dairy cows.

Overall, a greater percentage of oleic with a concomitant decrease in percentages of trans-vaccenic and conjugated linoleic acid in milk fat when cows were fed AMD indicated that the amide bond in canolamide was partially protected from ruminal biohydrogenation.

SUMMARY AND IMPLICATIONS

In spite of lower DMI, milk production and component yields in response to AMD, percentages of lactose, fat, and solids-not-fat were not affected. However, milk protein was decreased. Supplemental fat increased total fatty acid and oleic acid concentration in plasma. As a result, milk oleic acid concentration was higher in response to added fat but was highest when AMD was fed.

Overall, feeding OIL or OAM resulted in similar responses with respect to DMI, milk production and composition, component yields, and duodenal flow and apparent digestibility of fatty acids and diet components. Ruminal concentration of acetate was markedly decreased by OIL but not by OAM. In contrast, propionate increased in response to OIL as compared with OAM. Total concentration of fatty acids in plasma was similar in response to OIL and OAM treatments but oleic acid was higher in response to OIL versus OAM. Milk oleic acid concentration also was higher when OIL was fed as compared with OAM. Inclusion of AMD at 1.7% of DM was, therefore, not detrimental to any of the parameters measured. However, OAM intake was not sufficient to increase plasma or milk oleic acid content to the extent OIL or AMD did.

The cause for reduced DMI in response to AMD is not clear. Visual observation of ruminal contents when cows were fed AMD indicated considerable frothing, whereas this effect was not apparent when cows were fed OAM. In addition, a potent ammonia odor was detected in rumen fluid from cows fed AMD. The presence of unreacted ethanolamine in the amide concentrate may have contributed to the ammonia odor and higher pH when cows were fed AMD.

Converting high-oleic canola oil to an amide provided partial protection against biohydrogenation of oleic acid, as indicated by its elevated concentration in plasma and milk fat. However, it appears that using ethanolamine, as opposed to butylamine (butylsoyamide), to synthesize fatty acylamides provided less protection against biohydrogenation in the rumen and lowered DMI by an unknown mechanism.

Table 3. Ingredient and chemical composition of diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

	CON	OIL	OAM	AMD
Ingredients, % of DM				
Alfalfa haylage	17.5	17.5	17.5	17.5
Corn silage	30.7	30.7	30.7	30.7
Corn grain	31.0	27.5	27.5	27.5
Supplemental fat	0	3.3	3.3	3.3
Soybean meal, 48% CP	10.5	10.5	10.5	10.5
Distiller's dried grains	7.2	7.2	7.2	7.2
Mineral/vitamin mix [1]	1.4	1.4	1.4	1.4
Vitamin E pre-mix [2]	1.7	1.7	1.7	1.7
Corn gluten meal	0	0.2	0.2	0.2
Chemical composition				
CP	15	15	15	16
ADF	16	17	16	16
NDF	25	24	25	25
EE	2	4	5	5

[1] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA) contained : 3.75 to 4.75% salt, 14.5 to 17.4% Ca, 6.5% P, 5.8% Cl, 3.2% S, 2.2% Mg, 3.5% K, 0.11% Mn, 0.13% Zn, 0.027% Fe, 0.013% Cu, 0.002% I, 0.0003% Co, 0.0005% Se, 0.065% F, 18% $NaHCO_3$, and 110,000 IU vitamin A, 44,000 IU vitamin D_3, and 550 IU vitamin E per kg.
[2] 33 IU vitamin E per kg.

Table 4. Intake, flow, and apparent digestibility of DM, OM, CP, ADF and amide in the digestive tract of Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Intake, kg/d								
DM	26.9	24.7	24.2	18.9	1.0	0.01	0.01	0.01
NE_L intake, Mcal/d [2]	41.3	42.9	42.2	32.9	1.1	0.17	0.01	0.01
OM	26.3	24.2	23.7	18.7	0.6	0.01	0.01	0.01
CP	4.5	3.6	3.6	2.8	0.1	0.01	0.01	0.01
ADF	4.2	4.1	4.0	3.2	0.1	0.05	0.05	0.06
Amide	0	0	0.14	0.20				
Flow to duodenum, kg/d								
DM	18.9	16.1	16.1	11.8	1.0	0.05	0.05	0.05
OM	18.8	16.0	16.1	11.7	1.3	0.05	0.05	0.05
CP	3.3	2.6	2.9	1.8	0.1	0.01	0.01	0.01
ADF	2.1	1.8	1.8	1.3	0.1	0.05	0.05	0.05
Amide	0	0	0.009	0.012				
Apparent digestibility in rumen, %								
DM	29	34	33	39	6.0	0.38	0.60	0.47
OM	28	34	32	39	6.0	0.36	0.56	0.45
CP	16	27	21	40	4.0	0.05	0.09	0.05
ADF	52	55	54	58	5.0	0.52	0.67	0.63
Apparent digestibility in total tract, %								
DM	71	71	68	71	2.0	0.64	0.95	0.36
OM	71	71	68	71	2.0	0.68	0.88	0.33
CP	70	70	69	73	2.0	0.85	0.36	0.17
ADF	53	50	45	45	2.0	0.11	0.34	0.87

[1] FAT = OIL + OAM + AMD
[2] NE_L intake = DMI × NE_L content

Table 5. Volatile fatty acid (VFA) concentration in rumen fluid, rumen pH and rumen ammonia-N, and plasma urea-N (PUN) from Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Total VFA, m*M*	197	209	189	184	10.0	0.78	0.12	0.76
VFA, %								
Acetate	62	59	63	58	1.0	0.31	0.86	0.05
Propionate	21	26	20	24	1.0	0.14	0.38	0.05
Butyrate	13	11	12	13	0.4	0.16	0.05	0.09
Valerate	1.6	1.6	1.7	1.8	0.1	0.59	0.09	0.16
Isobutyrate	1.0	0.8	0.9	0.9	0.02	0.01	0.19	0.17
Isovalerate	1.8	1.9	2.0	1.8	0.1	0.63	0.89	0.57
Acetate:propionate	2.9	2.3	3.1	2.5	0.2	0.21	0.60	0.05
Rumen pH	5.9	5.9	6.3	6.4	0.1	0.09	0.01	0.73
Rumen NH_3-N, mg/dL	12.1	11.1	9.5	8.7	0.6	0.05	0.05	0.47
PUN, mg/dL	13.3	13.1	13.9	15.6	0.5	0.20	0.02	0.06

[1] FAT = OIL + OAM + AMD

Table 6. Fatty acid intake and flow to the duodenum of Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Fatty acid intake, g/d								
Total	703	932	901	696	29	0.01	0.01	0.01
16:0	147	147	133	90	4	0.01	0.01	0.01
18:0	20	27	26	19	1	0.01	0.01	0.01
18:1; Δ^9 *cis*	128	327	315	267	21	0.01	0.09	0.16
18:2	335	350	345	263	8	0.17	0.01	0.01
18:3	71	78	74	55	2	0.49	0.01	0.01
Fatty acid flow to duodenum, g/d								
Total	754	1020	1031	586	59	0.05	0.01	0.01
16:0	132	124	129	80	6	0.05	0.01	0.01
18:0	398	573	609	327	28	0.01	0.01	0.01
18:1; Δ^9 *cis*	56	105	102	64	11	0.05	0.05	0.05
18:1; Δ^{11} *trans*	34	52	60	26	12	0.12	0.01	0.09
18:2	65	52	52	38	9	0.14	0.32	0.33
18:3	6	5	7	3	1	0.42	0.12	0.01
Fatty acid recovery at duodenum, %								
18:0 to 18:3	101	110	111	75	9	0.84	0.05	0.05
18:1; Δ^9 *cis* to 18:3	24	23	22	17	3	0.40	0.26	0.28

[1] FAT = OIL + OAM + AMD

Table 7. Apparent biohydrogenation[1] of unsaturated long chain fatty acids in the rumen of Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT [2]	OIL vs AMD	OAM vs AMD
						Probability		
Fatty acid, %								
18:1; Δ^9 *cis*	56	68	71	74	2	0.01	0.09	0.41
18:2	80	87	87	83	2	0.11	0.33	0.31
18:3	92	94	92	93	1	0.18	0.57	0.30
Total [3]	70	69	74	74	2	0.31	0.10	0.93

[1] Wu et al. (1991)
[2] FAT = OIL + OAM + AMD
[3] Total unsaturated 18 carbon fatty acids

Table 8. Apparent fatty acid absorption in the gastrointestinal tract of Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Intestinal digestibility, %								
Total[2]	79	65	68	70	4	0.05	0.33	0.64
16:0	79	71	73	71	3	0.08	0.98	0.67
18:0	82	59	64	71	4	0.01	0.10	0.29
18:1; Δ^9 *cis*	76	79	79	78	2	0.51	0.90	0.92
18:1; Δ^{11} *trans*	79	77	78	72	4	0.48	0.38	0.27
18:2	76	76	75	71	5	0.66	0.56	0.63
18:3	72	64	73	62	5	0.38	0.78	0.18
Apparent absorption, g/d								
Total[2]	597	661	698	415	66	0.94	0.05	0.05
16:0	104	89	94	58	6	0.01	0.01	0.01
18:0	327	338	393	233	38	0.89	0.10	0.05
18:1; Δ^9 *cis*	42	84	82	53	10	0.05	0.06	0.07
18:1; Δ^{11} *trans*	27	73	47	19	10	0.17	0.01	0.10
18:2	51	41	39	28	9	0.21	0.36	0.43
18:3	4	3	5	2	1	0.45	0.29	0.05

[1] FAT = OIL + OAM + AMD
[2] 16:0 to 18:3

Table 9. Fatty acid concentration in blood plasma of Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Total, μg/mL	935	1099	1025	1095	43.0	0.05	0.95	0.29
Fatty acid, g/100 g								
14:0	0.2	0.1	0.1	0.1	0.01	0.05	0.95	0.29
14:1; Δ^9 *cis*	0.8	0.7	0.7	0.7	0.02	0.01	1.00	0.93
15:0	0.3	0.2	0.2	0.2	0.02	0.01	0.76	0.34
15:1	0.3	0.3	0.2	0.3	0.06	0.70	0.97	0.54
16:0	8.1	7.2	7.1	7.3	0.10	0.01	0.61	0.28
16:1; Δ^9 *cis*	0.7	0.6	0.7	0.6	0.02	0.05	0.91	0.45
17:0	2.2	2.5	2.6	2.6	0.10	0.08	0.62	0.82
17:1	0.2	0.1	0.1	0.1	0.03	0.05	0.95	0.76
18:0	13.7	14.2	14.3	14.2	0.20	0.05	0.89	0.81
18:1; Δ^9 *cis*	5.7	9.9	8.8	8.8	0.20	0.01	0.01	0.82
18:1; Δ^{11} *trans*	0.7	1.5	1.3	1.0	0.10	0.01	0.05	0.16
18:2	55.8	52.0	53.2	54.0	0.70	0.01	0.11	0.56
18:2; Δ^9 *cis*, Δ^{11} *trans*	0.02	0.16	0.12	0.03	0.02	0.01	0.01	0.05
18:3	4.4	4.7	4.5	4.4	0.10	0.42	0.15	0.65
20:3	3.8	2.8	3.0	2.6	0.10	0.01	0.50	0.24
20:4	2.1	2.1	1.9	2.2	0.10	0.84	0.18	0.06
20:5	0.6	0.6	0.6	0.7	0.01	0.33	0.05	0.05

[1] FAT = OIL + OAM + AMD

Table 10. Milk production, composition, and component yields from Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Milk, kg/d	38.0	38.8	37.8	33.1	0.9	0.19	0.01	0.01
3.5% FCM, kg/d	38.4	37.6	38.0	31.0	1.0	0.10	0.01	0.01
MUN, mg/dL	12.2	11.8	12.7	13.0	0.4	0.56	0.09	0.64
MUN, g/d	4.7	4.6	4.8	4.3	0.2	0.76	0.22	0.07
Milk composition, %								
Fat	3.6	3.3	3.6	3.2	0.2	0.30	0.63	0.16
Protein	3.1	3.0	3.1	2.9	0.1	0.21	0.22	0.05
Lactose	4.9	5.0	4.9	5.0	0.03	0.01	1.0	0.08
SNF	8.6	8.8	8.8	8.7	0.10	0.31	0.30	0.40
Component yields, kg/d								
Fat	1.4	1.3	1.3	1.0	0.10	0.15	0.05	0.05
Protein	1.2	1.2	1.2	0.9	0.04	0.21	0.01	0.01
Lactose	1.8	2.0	1.9	1.6	0.10	0.84	0.05	0.05
SNF	3.3	3.4	3.3	2.8	0.10	0.44	0.01	0.05

[1] FAT = OIL + OAM + AMD

Table 11. Fatty acid concentration in milk fat from Holstein cows fed diets containing no supplemental fat (CON) or supplemented at 3.3% of DM with canola oil (OIL), equal amounts of canola oil and canolamide (OAM), or canolamide (AMD).

						Contrast		
	CON	OIL	OAM	AMD	SEM	CON vs FAT[1]	OIL vs AMD	OAM vs AMD
						Probability		
Fatty acid, g/100 g								
4:0	4.9	5.4	6.8	5.3	0.60	0.30	0.88	0.14
6:0	3.6	3.0	3.6	3.2	0.10	0.09	0.42	0.06
8:0	1.8	1.3	1.5	1.4	0.03	0.01	0.21	0.06
10:0	5.1	3.3	3.8	3.4	0.10	0.01	0.87	0.05
12:0	4.6	2.9	3.1	2.8	0.01	0.01	0.36	0.05
14:0	13.6	11.0	11.1	10.4	0.30	0.01	0.18	0.13
14:1; Δ^9 *cis*	1.9	1.7	1.5	1.3	0.20	0.07	0.13	0.43
15:0	1.8	1.2	1.2	1.3	0.10	0.01	0.79	0.73
16:0	31.2	20.9	21.4	21.0	0.70	0.01	0.96	0.62
16:1; Δ^9 *cis*	0.4	0.4	0.4	0.4	0.02	0.19	0.25	0.22
16:1; Δ^9 *trans*	1.8	1.3	1.1	1.2	0.10	0.01	0.51	0.62
17:0	0.6	0.5	0.5	0.6	0.02	0.01	0.05	0.05
17:1	0.2	0.2	0.2	0.2	0.02	0.43	0.30	0.30
18:0	7.7	11.6	12.7	13.6	0.70	0.01	0.10	0.41
18:1; Δ^9 *cis*	15.4	25.9	23.4	26.9	0.30	0.01	0.05	0.01
18:1; Δ^{11} *trans*	1.4	4.4	3.2	2.8	0.60	0.05	0.09	0.66
18:2	2.5	2.4	2.3	2.7	0.04	0.49	0.01	0.01
18:2; Δ^9 *cis*, Δ^{11} *trans*	0.5	1.1	0.9	0.6	0.10	0.05	0.05	0.09
18:3	0.5	0.7	0.7	0.6	0.01	0.01	0.01	0.05
20:0	0.2	0.3	0.3	0.3	0.02	0.01	0.36	0.15
20:3	0.2	0.1	0.1	0.1	0.01	0.01	0.35	0.18
20:4	0.15	0.12	0.12	0.13	0.003	0.01	0.05	0.05

[1] FAT = OIL + OAM + AMD

CHAPTER 4

Secretion of 18:2; Δ^9 *cis*, Δ^{11} *trans* Into Milk Fat in Response to Abomasal Infusions of Conjugated Linoleic Acid in Holstein Cows

ABSTRACT

Conjugated linoleic acid (CLA) is a potent anticarcinogen and it inhibits palmitic acid synthesis in cultured bovine mammary cells. The potential for increasing CLA content of milk fat and the effects of elevated CLA availability on milk fat secretion were investigated. Four Holstein cows were used in a single cross-over design with repeated measures to determine milk fatty acid concentration in response to a 24-h infusion of 200 g linoleic acid (LA) or a mixture of 100 g LA plus 100 g CLA (LA-CLA). Milk, blood, and digesta samples were obtained at 12 h before infusion and at 12-h intervals from 0 to 72-h. Duodenal flow of CLA increased within 24 h when LA-CLA was infused. Plasma linoleic acid was higher in response to LA at 24 and 36 h; whereas plasma CLA was higher in response to LA-CLA from 12 to 72 h. Milk fat percentages and fat yield from 24 to 72 h were lower in response to LA-CLA compared with LA. Concentration of CLA in milk increased from 0.5 g/100 g total fatty acids at 0 h to 3.3 g/100 g at 36 h in response to LA-CLA. Proportion of palmitic acid, however, was lower from 48 to 72 h in response to LA-CLA. In contrast, concentration of stearic acid at 36 h in response to LA-CLA was nearly doubled the concentration of stearic acid in response to LA. Oleic and arachidonic acid concentrations were lower from 12 to 48 h when LA-CLA was infused. Results indicated CLA content of milk fat reflects the amount available for absorption from the small intestine, and CLA appeared to be a potent inhibitor of desaturase activity and milk fat secretion.

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a mixture of positional and geometrical isomers of linoleic acid with conjugated double bonds (Ip et al. 1994). CLA is formed during the enzymatic hydrogenation of linoleic acid by rumen microorganisms. The initial step involves the isomerization of linoleic acid to the 18:2; Δ^9 *cis*, Δ^{11} *trans* conjugated dienoic form, followed by a preferential reduction of the Δ^9 *cis* double bond to form 18:1; Δ^{11} *trans* (Fujimoto et al. 1993). Parodi (1977) reported that the primary CLA (90%) in milk fat is the 18:2; Δ^9 *cis*, Δ^{11} *trans* isomer.

CLA, primarily 18:2; Δ^9 *cis*, Δ^{11} *trans* , has received much attention in recent years because research indicates it is effective as an anticarcinogenic agent (Belury 1995). CLA concentration in milk fat, however, can vary substantially. Griinari et al. (1995) indicated a range of 2.4 to 18.0 mg CLA/g of fat in milk samples from New York herds. Griinari et al. (1996) also observed that a dietary supply of unsaturated fatty acids was needed to increase concentrations of CLA in milk. McGuire et al. (1996) reported CLA concentration increased from 2 to 6.8 mg/g fat when corn oil supplementation increased from 3 to 7.2% of the diet dry matter.

Trans isomers of unsaturated fatty acids, whether derived from the diet or from incomplete biohydrogenation of unsaturated fatty acids, depress milk fat percentage (Wonsil et al. 1994). Abomasal infusion of 18:1; Δ^{11} *trans* also decreased milk fat percentage from 4.1 to 3.1% and yield from 1.4 to 1.1 kg/d (Romo et al. 1996). Dawson and Herbein (1996) reported that bovine mammary cell cultures incorporated CLA in an amount proportional to the amount available in the medium. Furthermore, inhibition of de novo synthesis of palmitic acid and desaturation of stearic acid were proportional to the extent of CLA uptake by the mammary cells.

The objectives of this study were to evaluate the potential for increasing the concentration of CLA in milk fat and to establish the effects of increased availability of CLA on milk fat secretion and fatty acid composition.

MATERIALS AND METHODS

Animals and experimental design

Four multiparous Holstein cows (averaging 200 ± 32 d postpartum) previously cannulated in the rumen and proximal duodenum were used in a single cross-over design with repeated measures. The experimental protocol consisted of two 7-d periods without oil infusion prior to each 24-h infusion period. Milk, blood, digesta, and fecal samples were collected at -12 and 0 h before infusion, 12 and 24 h during infusion, and 36, 48, 60, and 72 h after infusion.

Treatments, diet, and animal management

Treatments consisted of 200 g of linoleic acid (LA) or a mixture of 100 g LA plus 100 g conjugated linoleic acid (LA-CLA). Fatty acid composition of infusates is listed in Table 12. The LA treatment (Table 12) contained mainly linoleic (62 g/100 g total fatty acids) and oleic acid (27 g/100 g) with minor amounts of linolenic, palmitic, and stearic acid. The CLA oil mixture contained exclusively 18:2; Δ^{9} *cis*, Δ^{11} *trans* (70 g/100 g) and 18:2; Δ^{10} *trans*, Δ^{12} *cis* (30 g/100 g). As a result, the LA-CLA treatment contained mostly 18:2; Δ^{9} *cis*, Δ^{11} *trans* (35 g/100 g) and linoleic acid (31 g/100 g), with similar amounts of 18:2; Δ^{10} *trans*, Δ^{12} *cis* (15 g/100 g) and oleic acid (14 g/100 g). Treatments (200 g) were dispensed into 500 mL Viaflex® containers (Baxter, Deerfield, IL) previously modified to contain separate inlet and outlet valves to allow

manual filling of the containers with oil via syringe. A one way stopcok with male Luer-Lock adapter (Baxter, Valencia, CA) was connected to the valve outlet and secured with double Bar-Lok cable ties (4 in). Infusions began immediately after 1400 h and continued for 24 h. Individual cows were housed in a tie-stall barn during the experiment and a basal diet without supplemental fat was prepared and offered in equal amounts at 1400 and 0200 h daily. Ingredients and chemical composition of the diet are listed in Table 13. The major fatty acids provided by the diet were palmitic (22 g/100 g), oleic (20 g/100 g), and linoleic (47 g/100 g) acid (Table 12). Cows were given free access to water throughout the study. Cows were milked in their individual tie-stalls during infusions. The experimental protocol was reviewed and approved by the Virginia Polytechnic Institute and State University Animal Care Committee.

Infusion procedures

Oil treatments were infused via Tygon® tubing (1/16 i.d., 1/32 wall; Fisher Scientific Co., Pittsburgh, PA) that was connected to the oil source and passed through a Harvard Peristaltic pump (55-1762; Harvard Apparatus, South Natick, MA). The above tubing was connected to the 16 ft of flexible Tygon® tubing (1/8 i.d., 1/16 wall) that passed through a small opening in the rumen cannula and was connected to a perforated Nalgene® plastic bottle (60 mL). Plastic bottles were inserted through the omasum into the abomasum, where they were secured with a plastisol flange cemented around the bottle neck. Bottles were placed into the abomasum 3 d prior to infusion and placement was confirmed before infusions. The tubing was primed with 15 mL of the infusate prior to start of infusions, and flow rate was set at 8.2 mL/h.

Digesta markers

Cr-mordanted to fecal fibers was used as digesta marker. Cr was attached to washed fecal fibers (6 g Cr/100 g fecal fibers) that were collected from two non-lactating cows fed orchardgrass hay by the Cr-mordant procedure of Uden et al. (1980). A 15 g dose of Cr-mordanted fecal fibers (1.29 g Cr/d) was placed in the rumen at 0600 h and 1800 h throughout the experimental period.

Measurements and sampling

Diet samples were collected during each infusion for chemical composition and fatty acid analysis. Samples were dried in a forced-air oven at 60 °C and stored in sealed plastic containers at room temperature. Daily feed refusals were recorded to calculate feed intake.

Milk production was measured daily (0200 h and 1400 h) throughout the study. Two 50 mL aliquots of milk were collected at -12 and 0 h before infusion, 12 and 24 h during infusion, and 36, 48, 60, and 72 h after infusion. One aliquot contained Bronopol (milk preservative; D & F Control Systems, San Ramon, CA) and was stored at 4 °C until analyzed for milk fat, protein, solids-not-fat, and lactose by the Virginia Dairy Herd Improvement Association . A second aliquot without preservative was initially frozen at -20 °C. After each period these samples were thawed at room temperature, and centrifuged at 10,000 × *g* for 1 h to harvest milk fat for fatty acid analysis.

Blood samples (10 mL) were obtained from the coccygeal artery by venipuncture after each milking. Blood was transferred to tubes containing 286 IU heparin in 100 μL of sterile saline and centrifuged at 3000 × *g* for 15 min for harvesting plasma. Plasma was stored at -20 °C until lipid extraction, and fatty acid analysis.

Duodenal digesta (200 mL) and fecal-grab samples were collected from each cow at -12 and 0 h before infusion, 12 and 24 h during infusion, and 36 and 48 h after infusion and stored at -20 °C. At the end of experimental period, digesta samples were thawed at room temperature and ground with a Polytron® PT 10/35 (Brinkmann Instruments, Westbury, NY) homogenizer. Aliquots of digesta were collected into two 100 mL plastic containers, frozen, and dried in a Dura-Top freeze dryer (FTS® sytems, Inc., Stone Ridge, NY). Fecal samples were dried at 60 °C.

Sample analysis

Lyophilized and oven-dried samples were ground through a 2 mm screen (Thomas-Wiley Laboratory Mill) then a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) prior to total nitrogen, ether extract, and organic matter analysis (AOAC 1984). Duodenal and fecal samples also were wet ashed with HNO_3 and $HClO_4$ as described by Sandell (1950). Chromium content then was determined by atomic absorption spectrophotometry to calculate nutrient flow to the duodenum and feces.

Lipids were extracted from plasma (2 mL) with chloroform/methanol (2:1, vol/vol) (Folch et al. 1957). Subsequently, blood plasma lipid fractions were isolated using Bond Elut® aminopropyl disposable columns (500 mg) with stainless steel frits, a Vac Elut® vacuum elution apparatus with adapters, and Vac Elut® sample collection racks (Analytichem International, Harbor City, CA) (Kaluzny et al. 1985).

Fatty acids in feed, duodenal digesta, feces, blood plasma, and milk fat were transmethylated by in situ transterification (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 by GLC on a 30 m × 0.25 mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellafonte, PA). 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 per min to a final temperature of 220 °C (held for 2 min). Peak identification was based on relative retention times of a commercial standard (Nu-Check Prep, Elysian, MN). Weight percentages of individual fatty acids were determined from response and recovery factors obtained from a multi-level calibration table via a HP 3396A integrator (Hewlett Packard, Avondale, PA) developed using mixtures of fatty acid methyl esters.

Statistical analysis

Data are reported as Least squares means and pooled SEM. Data for all variables (except those on Table 14) were analyzed by repeated measures analysis of covariance in a single cross-over design with the GLM procedure of SAS (SAS/STAT Version 6, SAS Institute, Cary, NC). Observations obtained at -12 and 0 h before infusion were averaged and served as covariate for observations at 12, 24, 36, 48, 60, and 72 h. Duodenal digesta was collected in the same sequence described, but up to 48 h only. Thus, fatty acid intake and flow to the duodenum are presented as averages over 48 h (Table 15). Dry matter intake values were collected on a 24 h basis, and were averaged daily for the 72 h period after infusion began (Table 14). Intake of dry matter at 24 h before infusion served as covariate for the average dry matter intake obtained during the 72 h period after infusion. These data were analyzed by analysis of covariance as outlined above. Fatty acid concentrations were expressed as a percentage of total fatty acids.

The regression of CLA in milk fat at 36 h on the CLA in plasma triglycerides at 24 h was used to estimate the apparent transfer of CLA from plasma triglycerides into milk fat. Similarly, milk fat percentage and palmitic acid in milk fat were regressed on the corresponding CLA in milk fat from 0 to 36 h. Treatment means were designated as significantly different at $P < 0.05$. The following model was used for statistical analysis :

$$Y_{ij} = \mu + P_i + T_j + \beta(X_{ij} - X) + E_{(ij)}$$

where,

Y_{ijk} = DM, OM, CP, and individual fatty acid intake, apparent digestibility in the rumen and total tract, and flow to the duodenum, plasma fatty acids, distribution of fatty acids in plasma lipid fractions, milk production and composition, and milk individual fatty acid profile,

μ = mean,

P_i = effect of period (i = 1, 2),

T_j = effect of treatment (j = 1, 2),

$\beta(X_{ij} - X)$ = covariate adjustment from the average of the same variables as in Y, obtained at -12 and 0 h before infusion,

$E_{(ijk)}$ = residual error

RESULTS

Food consumption

Intake of dry matter and diet components throughout the experiment were not affected by treatment (Table 14). Estimated dry matter and organic matter flow to the duodenum, however, was lower when LA-CLA was infused compared with LA. Crude protein and ether extract flow were not affected by treatments. Apparent digestibility of dry matter, crude protein, and organic matter in the rumen and total tract were not affected by oil infusion.

Fatty acid intake and flow to the duodenum

Total and individual fatty acid intakes were similar for both treatments (Table 15). Duodenal flow of total fatty acids was similar for both treatments, but palmitic acid flow was lower when cows were infused with LA-CLA. Whereas duodenal digesta obtained when cows were infused with LA-CLA contained measurable amounts of CLA at 12, 24, and 36 h after infusion, CLA in duodenal digesta when cows were infused with LA were not detectable. Apparent digestibility of total and individual fatty acids in the intestines and total tract did not differ between treatments (data not presented).

Plasma fatty acid concentration

Total concentration of fatty acids in blood plasma was similar for both treatments. (Table 16). Infusion of LA resulted in greater concentration (g/100 g total fatty acids) of linoleic acid from 12 to 36 h compared with LA-CLA infusion (Figure 3). Plasma CLA concentration, however, increased from 0 to 0.57 g/100 g within 24 h and then decreased linearly to 0.13 g/100 g by 72 h when LA-CLA was infused compared with LA (Figure 4). As a result of LA infusion,

plasma stearic, eicosatrienoic, and arachidonic acid concentration decreased from 0 to 36 h (Table 16); whereas, LA-CLA infusion raised their concentration within the same time period. This resulted in an overall time by treatment interaction ($P < 0.05$) for stearic and eicosatrienoic acid, whereas arachidonic acid approached significance ($P = 0.06$).

Fatty acid distribution in plasma lipid fractions

Infusion of either oil treatment for 24 h resulted in an increase (significant covariate effect) in the concentration of fatty acids in plasma free fatty acids, phospholipids, and cholesterols esters (Table 17). However, percentages of most individual fatty acids in plasma lipid fractions did not differ due to treatment. Exceptions were stearic acid in the free fatty acid fraction and oleic acid in the triglyceride fraction. The percentage of CLA in free fatty acids (Figure 5) after infusion of both treatments was similar with respect to pre-infusion levels, and was relatively higher than that seen in other fractions except triglycerides. Compared with LA, infusion of LA-CLA increased the proportion of CLA in phospholipids and triglycerides. Infusion of LA-CLA dramatically increased CLA in triglycerides from 0.2 to 3.6 g/100 g at 24 h. The high concentration of CLA in triglycerides at 24 h corresponded to its peak concentration in total plasma lipids (Figure 4), and it was positively related ($Y = 0.57 X + 0.83$; $r^2 = 0.70$; $P < 0.01$) with peak CLA concentration in milk fat.

Milk production and composition

Milk yield was not affected by treatments (Table 18). Whereas lactose concentration was similar for both oil infusions throughout the 72 h period, infusion with LA-CLA caused moderate, but significant, reduction of protein and solids-not-fat concentrations in milk

compared with the LA infusion. Concentration of fat was substantially reduced by infusion of LA-CLA from 0 to 48 h and remained lower by 72 h compared with LA (Table 7 and Figure 6). Lower fat concentration in response to LA-CLA reduced milk fat yield and 3.5% fat corrected milk (FCM) yield compared with LA. Yields of protein, lactose, and solids-not-fat were not significantly affected by treatment.

Fatty acid composition of milk fat

Infusion of LA-CLA increased CLA concentration (g/100 g total fatty acids) (Figure 7) in milk fat, but reduced total fatty acid yield (grams/12 h) (Table 19). The concentrations of caproic (Table 19) and palmitic acid (Figure 8) were also reduced when LA-CLA was infused compared with LA. A time by treatment effect was observed for the concentration of caproic, lauric, palmitoleic (Δ^9 *trans*), margaric, heptadecenoic, stearic (Figure 9), oleic (Figure 10), linoleic, and linolenic acid. When cows were infused with LA, stearic acid concentration decreased accompanied by an increase in oleic acid concentration (Figure 9, 10). The opposite response was observed when LA-CLA was infused. Arachidonic acid concentration (Figure 11) increased linearly from 0 to 36 h when LA was infused, such that it was nearly double the concentration of arachidonic acid in response to LA-CLA at 36 h. Infusion of LA-CLA led an increasing concentration of total unsaturated fatty acids with a concomitant decreasing concentration of total saturated fatty acid concentration in milk fat.

DISCUSSION

Mixtures of long chain unsaturated fatty acids (438 g/d) and *trans*-vaccenic acid (from 624 to 750 g/d) have depressed dry matter intake (DMI) when infused into the abomasum of lactating cows (Drackley et al. 1992, Gaynor et al. 1994, Romo et al. 1996). In the present study, infusion of 200 g of a relatively pure source of linoleic acid and CLA isomers for 24 h did not adversely affect DMI. In agreement with previous studies, digestibilities of dry matter, crude protein, and organic matter in the rumen and total tract were not affected by oil infusion (Drackley et al. 1992, Christensen et al. 1994). Estimated dry matter flow to the duodenum, however, did not correspond with DMI and was lower when cows were infused with the LA-CLA mixture. As a result, total fatty acid flow tended ($P = 0.1$) to be lower when LA-CLA was infused. A clear explanation for reduced dry matter flow to the duodenum in spite of similar dry matter consumption, is not apparent. Van Soest (1994) indicated that diurnal variation in chromium concentration in digesta and feces is a source of error when estimating nutrient flow and digestibility with chromium as an indigestible marker. Additional sources of error are also known to arise from difficulties in collecting representative samples of digesta from cannulated animals (Harmon and Richards 1997). This problem could be alleviated by continued sampling and compositing; however, this was not feasible for our experimental protocol.

Intake of total and individual fatty acids was similar for both treatments and averaged 559 g (Table 15). Data for flow of individual fatty acids to the duodenum indicated that recovery of fatty acids was, for the most part, a reflection of fatty acid profile of infusates. Oleic and linoleic acid were the predominant components of the LA treatment, and thus their flow to the duodenum tended ($P = 0.1$ and 0.2, respectively) to be higher when LA was infused. As expected, the concentration of CLA in duodenal chyme increased significantly from 0 to 24 h in response to

LA-CLA infusion. Overall, intestinal and total tract digestibilities of fatty acids were not affected by treatments.

Total fatty acid concentration in blood plasma was increased by 139 μg/mL in response to oil infusion. On average, infusion of LA and LA-CLA for 24 h elevated the proportion of fatty acids in the free fatty acid, phospholipid, cholesterol ester, and triglyceride fractions by 7.1, 34.3, 84.6, and 12.9 μg/mL, respectively (Table 17). Similar increases have been reported previously when plant oils were fed to lactating cows (Rindsig and Schultz 1974) or infused into the abomasum (Drackley et al. 1992). Apparently, a greater proportion of fatty acids in plasma lipid fractions is an expected physiological response by the animal to accommodate greater amounts of fatty acids absorbed from the small intestine (Bitman et al. 1973). Previous reports indicated that linoleic acid is preferentially incorporated into phospholipids and cholesterol esters (Christie 1980). However, when large amounts of long chain fatty acids are absorbed from the small intestine, the rate of synthesis of phospholipids may not be sufficient to accommodate the fatty acid input to blood and extensive incorporation into triglycerides can occur (Harrison et al. 1974). Our data indicate that infusion of LA increased linoleic acid in blood plasma (Figure 3) and that it was preferentially esterified in cholesterol esters, and triglycerides (Table 17). Eicosatrienoic, arachidonic and stearic acid were lower in total plasma lipids of cows infused with LA, whereas infusion of LA-CLA increased their concentration and resulted in a time by treatment interaction effect, with peak responses at 36 h (Table 16). Closer inspection of the fatty acid distribution in plasma lipid fractions revealed that infusion of LA reduced arachidonic and stearic acid in the free fatty acid fraction exclusively. However, lower proportions of eicosatrienoic acid were not apparent in any of the isolated fractions (Table 17). The activity of Δ^6 desaturase, which catalyzes the rate limiting step in the conversion of linoleic acid to

arachidonic acid, is inhibited by linoleic and oleic acid in non-ruminants (Kinsella et al. 1990). Given that the main site of fatty acid elongation and desaturation in ruminants is adipose tissue (St. John et al. 1991), reduced activity of this enzyme in response to infusion of LA could be responsible for the lower arachidonic acid concentration in blood plasma. Plasma oleic acid increased in a dose-dependent manner and was elevated in response to infusion of LA compared with LA-CLA. This effect was clearly seen in the triglyceride fraction (Table 17), and is in agreement with previous observations by La Count et al. (1994). In addition, a previous study demonstrated that the intestinal mucosa of steers fed unsaturated plant oils also possessed desaturase activity (Chang et al. 1992). Because the concentration of stearic acid in plasma from cows infused with LA was lower compared with LA-CLA infused cows, it is likely that at least some portion of the elevated plasma oleic acid was derived from desaturation of stearic acid in adipose or intestinal tissue. The greater proportion of stearic and arachidonic acid in plasma from cows infused with LA-CLA suggests that CLA, due to the conjugated double bond, may not be as detrimental to the activity of stearoyl-CoA (Δ^9) and Δ^6 desaturase in adipose tissue compared with linoleic acid.

Significant levels of CLA in total plasma lipids were only detected when LA-CLA was infused (Figure 4). However, we were able to determine the concentration of CLA in all plasma lipid fractions before and after infusion of either treatment, thus suggesting isolation of plasma lipid fractions is a more suitable method to determine CLA content of blood plasma. The bulk of CLA was found in free fatty acids and triglycerides, but the phospholipid fraction also contained elevated levels of CLA at 24 h after infusion (Figure 5). It is not clear why we could not detect CLA, except at 36 h, in total plasma fatty acids when LA was infused (Figure 5). The free fatty acid fraction accounted for approximately 3 % of total fatty acids at 0 and 24 h after infusion,

and it apparently contained higher proportions of CLA during non-infusion periods. The phospholipid fraction, however, contained 45 % of total fatty acids, but in terms of absolute amounts of CLA it was comparable to free fatty acids. The proportion of CLA in triglycerides dramatically increased from 0.2 to 3.6 g/100 g (0.08 to 2 μg/mL) by 24 h, which corresponded well with the peak concentration of CLA in milk fat (Figure 7).

Trans-vaccenic acid is preferentially concentrated in the sn-1 position of the plasma triglyceride (Christie and Moore 1971) where it can be rapidly hydrolyzed and extracted by tissues. Thompson and Christie (1990) determined that mammary extraction of *trans*-vaccenic acid from plasma triglycerides was approximately 74%. Therefore, it appears that during infusion of LA-CLA lipoprotein lipase was effective in releasing CLA bound to plasma triglycerides, and mammary cells actively absorbed and secreted this fatty acid into milk. It is important, however, to note that the concentration of CLA in blood at 72 h (Figure 4) was still well above that at 0 h, and it raises the possibility that circulating levels of this fatty remained above basal concentrations for an extended period of time. Based on the recovery of CLA in milk fat (22%) and its absence in fecal samples, it is probable that tissues other than the mammary gland also were extracting CLA from blood.

The production of milk was not affected by treatments (Table 18). Previous reports indicated lower milk yield when cows were infused with unsaturated fatty acids, but they concluded that depressed nutrient intake due to infusions was the primary reason for lower milk yield (Christensen et al. 1994). Milk concentration of lactose was similar among treatments; however, milk protein and solids-not-fat concentration were lower when LA-CLA was infused. Although previous studies reported similar findings when *cis*- or *trans*- octadecenoic acids were infused into the abomasum of Holstein cows (Gaynor et al. 1994, Romo et al. 1996), our data

suggests a rather consistent trend in milk protein and solids-not-fat concentration throughout the 72 h period, perhaps indicating inherent animal variation. Yields of protein, solids-not-fat, and lactose were not affected by treatments.

Compared with LA, infusion of LA-CLA linearly decreased milk fat concentration from 3.41% at 0 h to 2.34% at 48 h, which translated into a 32 and 34% reduction in the concentration and yield of milk fat, respectively (Figure 6 and Table 18). *Trans* fatty acids derived from rumen biohydrogenation of unsaturated fatty acids have been reported to inhibit mammary secretion of milk fat. The work of Wonsil et al. (1994) first demonstrated that there was a negative linear correlation between *trans*-vaccenic acid flow to the duodenum and milk fat percentage. Their data, along with that of Gaynor et al. (1994) and Romo et al. (1996), also confirmed that milk fat depression was proportional to the availability of *trans*-vaccenic acid for absorption in the small intestine. The mechanism by which *trans* fatty acids depress milk fat secretion, however, has not been elucidated.

Previous work in our laboratory indicated that bovine mammary cell cultures incorporated CLA from the medium in proportion to the amount present in the medium (Dawson and Herbein, 1996). Mammary cell synthesis of palmitic acid and desaturation of stearic acid, however, were negatively correlated with CLA uptake. Kelly et al. (1997) also found that supplementing dairy diets with plant oils rich in linoleic acid increased the formation of CLA in the rumen (Fujimoto et al. 1993) and incorporation into milk triglycerides (Kelly and Bauman 1996). Data from the present study indicated that infusion of LA-CLA for 24 h increased the percentage of CLA in milk fat almost seven-fold by 36 h (Figure 7). A concomitant decrease in palmitic acid concentration also was observed (Figure 8). Apparent transfer of CLA into milk fat was 22% in the present study and a negative relationship ($Y = -0.33 X + 3.6$; $r^2 = 0.70$; $P <$

0.01) between milk fat concentration and CLA content of milk fat was established. The committed step in de novo synthesis of fatty acids in animal tissues is catalyzed by acetyl CoA carboxylase (Wakil et al. 1983), and this appears to be the earliest unique point at which control can be exerted. In a classic study, Emken et al. (1987) reported that linoleic acid and its *trans* isomer (18:2; Δ^9 *trans*, Δ^{12} *trans*) dramatically reduced the activity of acetyl CoA carboxylase and other lipogenic enzymes in mouse liver. In contrast, incubating mouse adipocytes for 3 d with CLA did not reduce fatty acid synthetase activity, whose short-term regulation is not well known (Park et al. 1997). Our data suggests that both treatments may have decreased acetyl CoA carboxylase activity. However, the extent of inhibition of palmitic acid synthesis was greater when LA-CLA was infused. From our data we can deduce that CLA may have exerted an acute (short-term) type control on the activity of acetyl CoA carboxylase, which could have involved allosteric or metabolic regulation, and covalent modification of the enzyme (Wakil et al. 1983) by the activated fatty acyl CoA of CLA. This suggestion is supported by the negative relationship ($Y = -3.1 X + 35.8$; $r^2 = 0.71$; $P < 0.01$) between CLA and palmitic acid concentration in milk fat over the first 36 h after LA-CLA infusion was begun. Thereafter, palmitic acid concentration remained unchanged until 60 h, and it increased only slightly by 72 h compared with the relatively rapid return to normal when LA was infused. Previous work comparing *cis*- and *trans*-isomers of octadecenoic acid for their milk fat depressing properties could not establish a clear distinction between the isomers with respect to reduced palmitic acid concentration in milk fat (Gaynor et al. 1994, Romo et al. 1996). Our data strongly indicates that CLA may be a potent inhibitor of de novo synthesis of fatty acids in the mammary gland.

We observed a consistent increase in milk stearic acid concentration from 0 to 36 h (Figure 9) when LA-CLA was infused. Parallel to this increase was an initial reduction in oleic

acid concentration from 0 to 12 h, followed by a rather constant concentration from 12 to 36 h (Figure 10). Whereas a decline in the proportion of stearic acid was apparent from 36 to 72 h when LA-CLA was infused, oleic acid increased sharply during the same time frame. These effects were not reported previously, when linoleic (Drackley et al. 1992; Christensen et al., 1994) or *trans*-vaccenic acid (Romo et al. 1996) where infused into the abomasum of lactating cows. Kinsella (1970) demonstrated that mammary cells isolated from lactating bovine mammary glands actively desaturated absorbed stearic acid to oleic acid via Δ^9 stearoyl CoA desaturase, esterified both fatty acids into triglycerides, and secreted the triglycerides into culture media. Based on the linear increase in stearic acid and the lack of change in the proportion of oleic acid from 0 to 36 h, it appears that CLA may have drastically reduced the activity of Δ^9 stearoyl CoA desaturase activity in the mammary gland as was previously seen in adipose tissue of steers fed unsaturated oils (Chang et al. 1992). Infusion of LA apparently increased oleic acid in milk between 0 and 36 h. However, this increase could be ascribed to the higher concentration of oleic acid in the LA mixture (Table 12).

In a recent study, Belury and Kempa-Steczko (1997) demonstrated that mice fed increasing amounts of CLA in the diet (0 to 1.5% by weight) contained lower amounts of arachidonic acid, and greater CLA in hepatic neutral lipids. These authors suggested that CLA depressed Δ^6 desaturase activity, thus leading to reduced arachidonic acid synthesis. Although, α-linolenic acid is a substrate for arachidonic acid synthesis, the main route of eicosatrienoic and arachidonic acid synthesis in animal tissues is desaturation of linoleic acid to γ-linolenic acid via Δ^6 desaturase, elongation to eicosatrienoic acid, and a further desaturation to arachidonic acid via Δ^5 desaturase (Gurr 1984). Our data indicates that LA-CLA infusion reduced the concentration of eicosatrienoic (Table 19) and arachidonic acid (Figure 11) in milk fat significantly compared

with LA. Infusion of LA was associated with an increase in the concentration of both fatty acids from 0 to 36 h, thus indicating that linoleic acid was actively desaturated. Hermansen et al. (1995) showed that infusion of primrose oil (72% linoleic acid) into the abomasum (0 to 600 g/d) raised the proportions of eicosatrienoic and arachidonic acid in milk from Holstein cows. In contrast, feeding a high grain diet to lactating cows increased *trans*-vaccenic acid in milk fat globule membrane but did not affect arachidonic acid concentration (Palmquist and Schanbacher 1991). Similar results were observed in milk from sows fed partially hydrogenated fish oil (28% *trans*-vaccenic acid) (Pettersen and Opstvedt 1991). It appears that CLA may play an inhibitory role in the desaturation of unsaturated fatty acids by mammary gland Δ^6 and Δ^5 desaturase enzymes.

Overall, the data reported herein suggests that increasing delivery of CLA to the small intestine of lactating cows for absorption is not expected to affect DMI or milk yield. Increased flow of CLA to the duodenum could potentially raise the level of this fatty acid in blood plasma triglycerides, which then could be rapidly taken up by the mammary gland for milk triglyceride synthesis. Absorption of CLA by the mammary gland, however, may be detrimental for the synthesis of fatty acids with 16 carbons or less, stearic acid desaturation, and arachidonic acid synthesis; possibly due to direct inhibition of the enzymes involved in the process of de novo synthesis and desaturation of long chain fatty acids.

To our knowledge, this is the first study ascertaining the direct effects of elevated amounts of CLA available for absorption from the small intestine on milk fat secretion and fatty acid composition in lactating cows. We were driven to investigate the feasibility of enhancing CLA concentration in milk fat by the ever increasing amount of data indicating that CLA is a potent anticarcinogen, and is a natural component of milk. It is impressive that a modest amount

of CLA infused for 24 h, caused dramatic changes in milk fatty acid composition and yield after only 12 h, then maintained those changes until 72 h. The relationship between unsaturated fatty acid concentration and milk fat yield (Figure 12), in particular, suggests that CLA is a potent mediator of metabolic processes regulating aspects of lipid metabolism within the mammary gland. The role of CLA in lipid metabolism by the mammary gland of the cow is still uncertain, but has been extensively studied in tissues of laboratory animals. The paucity of data regarding this fatty acid and its effects on lipogenic enzymes during established lactation in the bovine warrants further investigation.

Table 12. Fatty acid composition of oil treatments and diet.

Treatment	16:0	18:0	18:1; Δ^9*cis*	18:2	18:2; Δ^9*cis*, Δ^{11}*trans*	18:2; Δ^{10}*trans*, Δ^{12}*cis*	18:3
			g/100 g total fatty acids				
LA	3	1	27	62	ND[2]	ND	7
LA-CLA	1	1	14	31	35	15	3
Diet	22	3	20	47	ND	ND	8

[1] Values are weight percentages of total (4:0 to 22:6) fatty acids.
[2] Not detected.

Table 13. Ingredient and chemical composition of the diet.

	% of dry matter
Ingredient	
Corn silage	30.8
Alfalfa haylage	17.7
Corn grain	30.7
Soybean meal, 48% CP	10.5
Distiller's dried grains	7.2
Mineral/vitamin mix [1]	1.4
Vitamin E pre-mix [2]	1.7
Chemical composition	
Crude protein	15
Acid detergent fiber	18
Neutral detergent fiber	29
Ether extract	3

[1] Mineral/vitamin mix (Southern States Cooperative, Richmond, VA) contained : 3.75 to 4.75% salt, 14.5 to 17.4% Ca, 6.5% P, 5.8% Cl, 3.2% S, 2.2% Mg, 3.5% K, 0.11% Mn, 0.13% Zn, 0.027% Fe, 0.013% Cu, 0.002% I, 0.0003% Co, 0.0005% Se, 0.065% F, 18% $NaHCO_3$, 110,000 IU vitamin A, 44,000 IU vitamin D_3, and 550 IU vitamin E per kg.
[2] 33 IU vitamin E per kg.

Table 14. Intake and digestibility of dry matter, organic matter, and crude protein of Holstein cows abomasally infused with linoleic acid or an equal mixture of linoleic and conjugated linoleic acid.

	Linoleic acid 200 g	100 g Linoleic acid + 100 g conjugated linoleic acid	SEM	$P <$ [1]
Intake, kg/d				
Dry matter	23	21	4	0.7
Organic matter	22	20	4	0.7
Crude protein	3	3	1	0.7
Flow to duodenum, kg/d				
Dry matter	13	9	1	0.02
Organic matter	12	8	1	0.02
Crude protein	2	2	0.2	0.5
Apparent digestibility in the rumen, %				
Dry matter	38	53	8	0.3
Organic matter	41	60	6	0.1
Crude protein	42	47	2	0.1
Apparent digestibility in the total tract, %				
Dry matter	71	68	3	0.5
Organic matter	71	68	3	0.5
Crude protein	69	67	4	0.7

[1] Probability of statistical difference due to treatment effects.

Table 15. Fatty acid intake and flow to the duodenum [1] of Holstein cows abomasally infused with linoleic acid or an equal mixture of linoleic and conjugated linoleic acid.

	Linoleic acid 200 g	100 g linoleic acid + 100 g conjugated linoleic acid	SEM	$P <$ [2]
Fatty acid intake, g/48 h				
Total	577	540	22	0.3
16:0	124	116	4	0.2
18:0	18	18	1	0.2
18:1; Δ^9 *cis*	115	107	5	0.3
18:2	275	256	11	0.3
18:3	46	45	2	0.6
Fatty acid flow to duodenum, g/48 h				
Total	723	601	45	0.1
16:0	124	103	5	0.05
18:0	343	307	24	0.3
18:1; Δ^9 *cis*	71	49	8	0.1
18:1; Δ^{11} *trans*	30	26	2	0.2
18:2	79	49	13	0.2
18:2; Δ^9*cis*, Δ^{11}*trans*	ND[3]	3	1	0.05
18:2; Δ^{10} *trans*, Δ^{12}*cis*	ND	1	0.4	0.2
18:3	6	4	1	0.3

[1] Duodenal digesta was collected at -12, 0, 12, 24, 36, and 48 h only. Thus fatty acid intake and flow to the duodenum are presented as averages over 48 h.
[2] Probability of statistical difference due to treatment effects.
[3] Not detected.

Table 16. Fatty acid concentration in blood plasma from Holstein cows abomasally infused with linoleic acid or an equal mixture of linoleic acid and conjugated linoleic acid.

	Linoleic acid 200 g							100 g Linoleic acid + 100 g conjugated linoleic acid								
	Hour															
Fatty acid	12	24	36	48	60	72	Mean	12	24	36	48	60	72	Mean	SEM	$P <$[1]
Total, µg/mL	908.4	947.0	951.5	898.2	876.8	893.2	912.5	1033.6	958.8	941.3	885.3	869.1	880.8	928.2	66.2	0.6
	g/100 g total fatty acids															
14:0	0.18	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.21	0.20	0.21	0.20	0.20	0.01	0.3
14:1; Δ^9 *cis*	0.81	0.74	0.74	0.73	0.75	0.74	0.75	0.77	0.76	0.73	0.75	0.75	0.74	0.75	0.04	0.9
15:0	0.34	0.25	0.25	0.26	0.26	0.26	0.27	0.33	0.26	0.27	0.26	0.26	0.27	0.28	0.1	0.9
15:1	0.46	0.25	0.24	0.37	0.34	0.48	0.36	0.22	0.38	0.23	0.36	0.29	0.37	0.31	0.1	0.4
16:0	8.20	8.25	8.23	8.23	8.38	8.19	8.25	8.15	8.29	8.21	8.05	8.15	8.44	8.22	0.1	0.5
16:1; Δ^9 *cis*	0.66	0.65	0.65	0.64	0.65	0.63	0.65	0.68	0.66	0.63	0.64	0.63	0.63	0.65	0.1	0.9
17:0	2.42	2.39	2.40	2.39	2.38	2.42	2.40	2.40	2.38	2.32	2.35	2.37	2.32	2.36	0.1	0.3
17:1	0.22	0.19	0.24	0.19	0.15	0.19	0.20	0.22	0.19	0.13	0.23	0.15	0.14	0.18	0.1	0.6
18:0 [2]	13.8	13.6	13.3	13.4	13.5	13.2	13.5	14.0	14.3	14.3	13.5	13.3	13.5	13.8	0.6	0.3
18:1; Δ^9 *cis*	5.49	5.29	4.84	4.86	4.71	4.64	4.97	5.45	5.19	4.97	4.80	4.86	4.84	5.02	0.16	0.6
18:1; Δ^{11} *trans*	0.65	0.63	0.62	0.68	0.66	0.63	0.65	0.66	0.69	0.70	0.62	0.67	0.67	0.67	0.05	0.5
18:3	4.64	4.42	4.25	4.31	4.46	4.54	4.44	4.65	4.41	4.29	4.46	4.56	4.58	4.49	0.14	0.4
20:3 [2]	3.53	3.36	3.31	3.42	3.45	3.60	3.45	3.58	3.51	3.64	3.51	3.42	3.36	3.50	0.12	0.3
20:4	2.28	2.19	2.17	2.20	2.24	2.29	2.23	2.26	2.23	2.30	2.32	2.36	2.37	2.31	0.1	0.05

[1] Probability of statistical difference due to treatment effects.
[2] Probability of statistical difference due to time by treatment interactions ($P < 0.05$).

Table 17. Fatty acid concentration in blood plasma lipid fractions of Holstein cows given abomasal infusions of linoleic acid or an equal mixture of linoleic acid and conjugated linoleic acid.

	Linoleic acid 200 g		100 g Linoleic acid + 100 g conjugated linoleic acid			
	Hour					
Fraction	0	24	0	24	SEM	$P <$ [1]
	g/100 g total fatty acids					
Free fatty acid						
Total, μg/mL [2]	30.1	32.6	29.8	41.4	2.0	0.03
14:0	2.7	2.7	3.0	2.5	0.1	0.3
14:1	2.2	1.2	1.6	1.3	0.2	0.8
15:1	2.5	2.7	2.7	2.6	0.3	0.8
16:0 [2]	22.6	21.9	23.0	20.4	0.6	0.1
16:1	2.7	2.5	4.7	2.5	0.3	0.9
17:0	1.4	1.8	1.6	2.0	0.2	0.6
17:1	2.3	2.4	2.6	2.2	0.3	0.7
18:0	36.6	35.4	36.6	38.8	0.5	0.01
18:1; Δ^{11} *trans*	3.2	1.7	5.7	2.1	0.2	0.2
18:1; Δ^{9} *cis*	8.5	9.3	9.0	7.8	0.6	0.1
18:2	10.6	14.3	10.9	12.0	1.2	0.3
18:3	0.6	0.7	0.5	0.7	0.2	0.9
20:3	0.7	1.1	0.7	1.4	0.3	0.5
20:4	0.9	0.5	1.0	0.9	0.1	0.08
Phospholipids						
Total, μg/mL [2]	523.8	549.1	538.4	581.8	25.2	0.4
16:0	14.7	14.6	14.2	15.8	1.0	0.5
17:0	2.8	1.5	1.0	1.5	0.6	0.9
18:0	31.2	31.2	32.3	30.6	0.6	0.6
18:1; Δ^{11} *trans*	0.7	0.5	0.6	0.5	0.04	0.5
18:1; Δ^{9} *cis* [2]	7.8	6.7	7.8	6.4	0.1	0.3
18:2	35.2	38.7	35.1	37.2	0.4	0.8
18:3	1.3	1.0	1.5	1.1	0.06	0.2
20:3	4.9	4.6	5.4	4.9	0.2	0.4
20:4	1.5	1.4	1.5	1.4	0.8	0.9

Table 17. Continued.

	Linoleic acid 200 g		100 g Linoleic acid + 100 g conjugated linoleic acid			
	Hour					
Fraction	0	24	0	24	SEM	$P <$ [1]
	g/100 g total fatty acids					
Cholesterol ester						
Total, μg/mL[2]	540.8	558.6	477.5	629.1	60.2	0.5
14:1	1.0	1.1	1.2	1.1	0.03	0.4
16:0	4.1	4.1	4.6	4.1	0.08	0.9
18:0	0.9	0.8	1.0	0.8	0.04	0.9
18:1; Δ^{11} *trans*	0.05	0.07	0.1	0.06	0.02	0.9
18:1; Δ^{9} *cis*	3.0	2.8	3.2	2.9	0.2	0.7
18:2	78.8	79.5	75.5	78.2	0.4	0.1
18:3	5.8	5.7	6.9	5.9	0.2	0.4
20:0	2.2	2.3	2.2	2.4	0.3	0.8
20:3	0.8	0.7	0.8	0.8	0.05	0.6
20:4	0.9	0.8	0.9	0.8	0.06	0.8
Triglyceride						
Total, μg/mL	41.3	49.0	36.0	54.1	5.8	0.6
14:0	1.9	1.6	1.7	1.8	0.1	0.2
14:1	1.4	1.3	1.5	1.4	0.05	0.9
15:0	1.4	1.3	1.4	1.4	0.09	0.8
16:0	18.6	16.2	18.2	16.6	0.7	0.7
17:0	5.2	4.3	5.1	4.7	0.4	0.5
18:0	36.6	32.1	39.7	36.3	2.6	0.3
18:1; Δ^{11} *trans*	2.0	1.5	1.9	1.7	0.5	0.8
18:1; Δ^{9} *cis*	6.0	8.6	5.7	6.7	0.3	0.01
18:2	20.3	29.1	18.5	21.5	3.3	0.2
18:3	3.8	4.4	3.7	3.7	0.4	0.3
20:4	1.1	1.0	1.0	0.9	0.1	0.6

[1] Probability of statistical difference due to treatment effects.
[2] Probability of statistical difference due to covariate effect ($P < 0.05$).

Table 18. Milk production, composition, and component yields of Holstein cows abomasally infused with linoleic acid or an equal mixture of linoleic acid and conjugated linoleic acid.

	Linoleic acid 200 g							100 g Linoleic acid + 100 g conjugated linoleic acid								
	Hour															
Item	12	24	36	48	60	72	Mean	12	24	36	48	60	72	Mean	SEM	$P <$[1]
Milk, kg	14	15	17	16	16	16	16	15	15	15	16	16	15	15	2	0.4
3.5% FCM	14	16	16	17	16	16	16	15	14	12	13	13	13	13	1	0.01
Composition, %																
Fat	3.51	3.47	3.15	3.22	3.16	3.35	3.31	3.29	2.96	2.42	2.34	2.41	2.66	2.68	0.2	0.01
Protein	3.11	3.06	3.14	3.27	3.17	3.14	3.15	2.98	3.02	2.97	2.98	2.98	3.02	2.99	0.1	0.05
Lactose	4.90	4.93	4.95	4.96	4.98	5.00	4.95	4.89	4.96	4.89	4.87	4.88	4.84	4.89	0.08	0.4
Solids-not-fat	8.75	8.74	8.83	8.97	8.91	8.93	8.86	8.64	8.73	8.61	8.63	8.60	8.58	8.63	0.1	0.05
Component yields, kg																
Fat	0.48	0.54	0.54	0.59	0.54	0.55	0.54	0.50	0.43	0.36	0.35	0.37	0.38	0.40	0.02	0.01
Protein	0.71	0.76	0.83	0.82	0.81	0.79	0.79	0.76	0.76	0.74	0.76	0.78	0.74	0.76	0.09	0.6
Lactose	0.43	0.47	0.53	0.54	0.52	0.50	0.50	0.46	0.46	0.45	0.46	0.47	0.45	0.46	0.05	0.3
Solids-not-fat	1.30	1.40	1.50	1.50	1.50	1.40	1.43	1.30	1.30	1.30	1.30	1.40	1.30	1.32	0.2	0.5

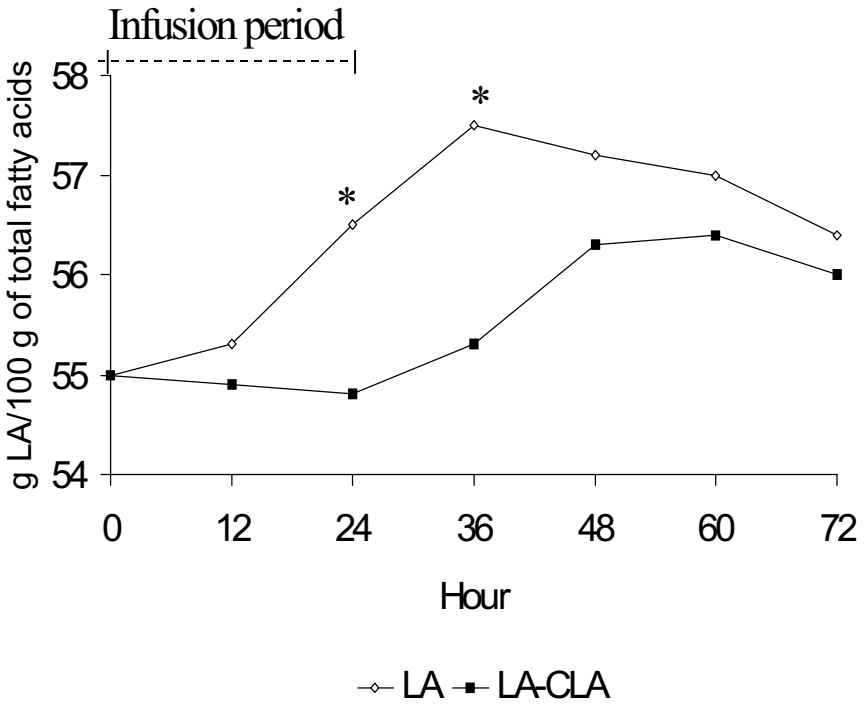
[1] Probability of statistical difference due to treatment effect ($P < 0.05$).

Table 19. Fatty acid concentration in milk fat from Holstein cows abomasally infused with linoleic acid or an equal mixture of linoleic and conjugated linoleic acid.

	Linoleic acid 200 g							100 g Linoleic acid + 100 g conjugated linoleic acid								
	Hour															
Fatty acid	12	24	36	48	60	72	Mean	12	24	36	48	60	72	Mean	SEM	$P <$[1]
Total, grams [2]	396.4	452.0	447.5	450.3	445.5	456.0	441.3	415.4	362.1	297.8	283.7	303.6	316.3	329.8	16.3	0.01
4:0	2.70	3.07	3.11	3.24	3.25	2.54	2.98	3.55	3.0	3.29	3.89	3.16	3.03	3.32	0.6	0.4
6:0	3.18	2.96	3.14	3.29	3.49	3.28	3.22	3.43	2.74	2.40	2.85	2.72	2.53	2.78	0.3	0.05
8:0	1.63	1.55	1.59	1.69	1.84	1.80	1.68	1.72	1.45	1.23	1.38	1.35	1.32	1.41	0.2	0.1
10:0 [2]	4.50	4.19	4.28	4.86	5.11	4.91	4.64	4.95	4.28	3.56	3.73	4.19	3.97	4.11	0.9	0.4
12:0 [2]	4.06	3.80	3.70	4.17	4.52	4.43	4.11	4.24	3.83	3.38	3.44	3.77	3.60	3.71	0.7	0.4
14:0	13.6	1.2	12.8	13.8	14.4	14.3	13.7	13.7	13.2	12.4	12.6	13.8	13.2	13.2	2.3	0.7
14:1; Δ^9 *cis*	2.0	1.93	1.81	1.87	1.90	1.95	1.91	1.71	1.40	1.24	1.30	1.48	1.52	1.44	0.6	0.2
15:0	1.56	1.50	1.54	1.62	1.52	1.56	1.55	1.42	1.53	1.53	1.58	1.58	1.61	1.54	0.1	0.8
16:1; Δ^9 *cis*	0.47	0.39	0.43	0.45	0.48	0.48	0.45	0.45	0.47	0.45	0.48	0.51	0.52	0.48	0.1	0.5
16:1; Δ^9 *trans* [2]	1.87	1.94	1.78	1.69	1.64	1.64	1.76	1.51	1.28	1.11	0.97	1.12	1.33	1.22	1.0	0.2
17:0 [2]	0.60	0.54	0.53	0.56	0.58	0.59	0.56	0.60	0.67	0.71	0.73	0.73	0.74	0.70	0.1	0.1
17:1 [2]	0.27	0.25	0.24	0.23	0.22	0.23	0.24	0.23	0.21	0.19	0.18	0.23	0.26	0.22	0.04	0.5
18:1; Δ^{11} *trans*	1.22	1.33	1.48	1.29	1.16	1.25	1.29	1.61	1.91	2.02	1.81	1.77	1.90	1.84	0.3	0.1
18:2 [2]	3.80	5.48	7.17	4.88	3.49	3.22	4.67	3.04	4.42	6.07	5.04	4.15	4.02	4.46	0.4	0.5
18:3 [2]	0.77	1.0	1.18	0.85	0.69	0.68	0.86	0.70	0.89	1.05	0.91	0.83	0.83	0.87	0.04	0.8
20:0 [2]	0.17	0.18	0.17	0.18	0.19	0.20	1.09	0.20	0.22	0.24	0.25	0.23	0.22	0.23	0.04	0.1
20:3 [2]	0.73	0.89	1.0	0.82	0.73	0.78	0.83	0.71	0.68	0.60	0.61	0.56	0.59	0.63	0.02	0.01

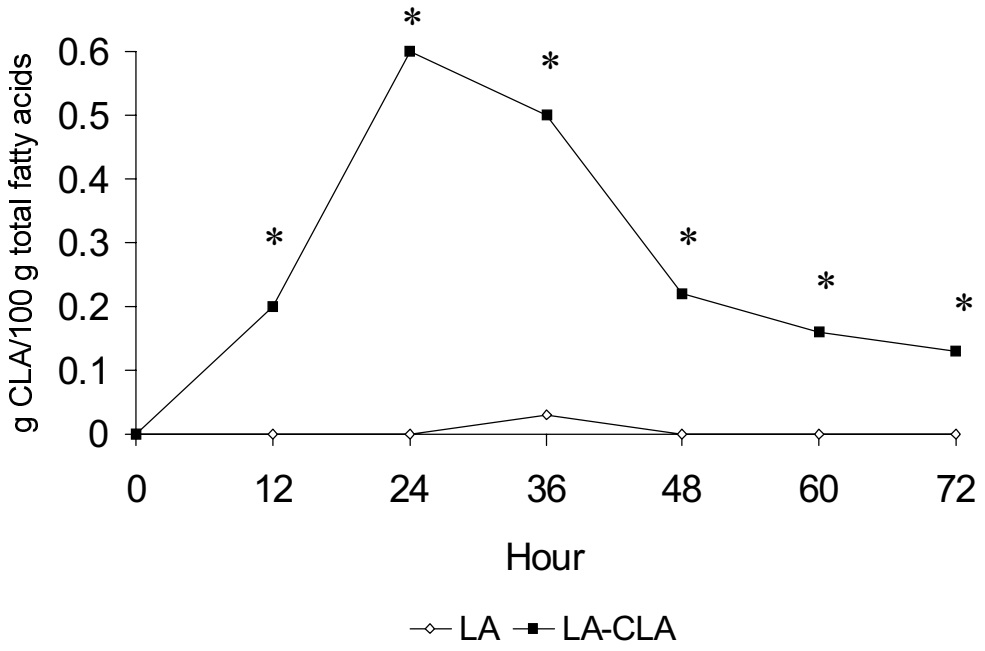
[1] Probability of statistical difference due to treatment effects.
[2] Probability of statistical difference due to time by treatment interaction effects ($P < 0.05$).

Figure 3. Linoleic acid concentration in blood plasma of Holstein cows during 72 h, after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



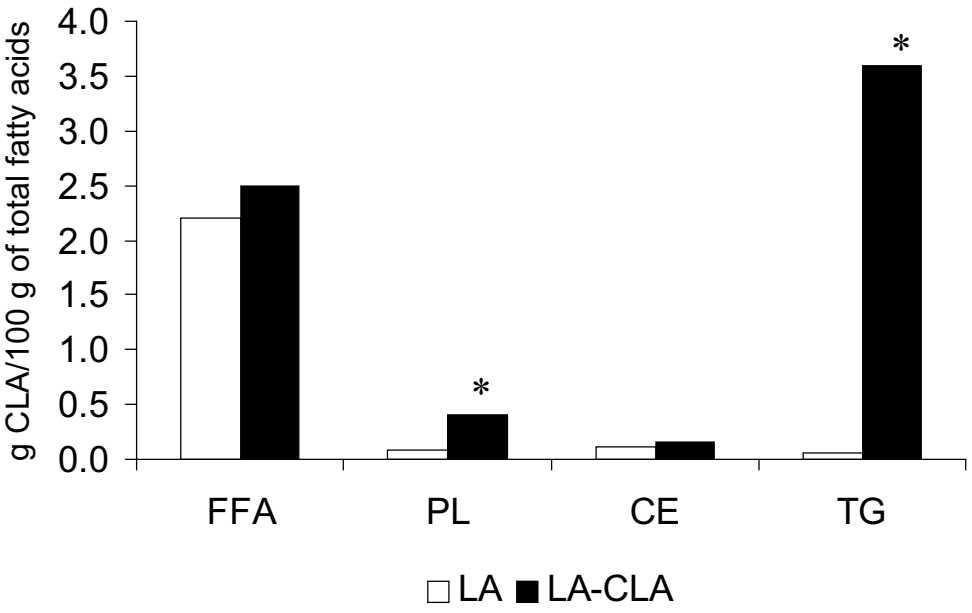
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.3, 0.2, 0.3, 0.4, 0.3, and 0.5, respectively)

Figure 4. Conjugated linoleic acid (18:2; Δ^{9}*cis*, Δ^{11}*trans* plus 18:2; Δ^{10}*trans*, Δ^{12}*cis*) concentration in blood plasma of Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



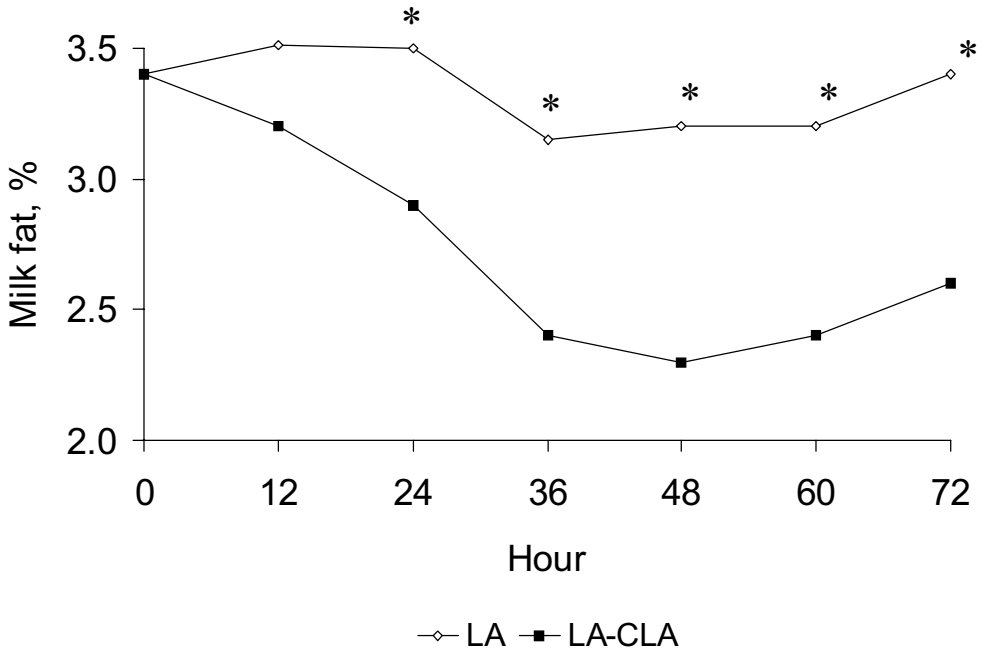
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.02, 0.03, 0.02, 0.05, 0.04, and 0.03 respectively)

Figure 5. Distribution of conjugated linoleic acid (18:2; Δ^{9}*cis*, Δ^{11}*trans* plus 18:2; Δ^{10}*trans*, Δ^{12}*cis*) in blood plasma free fatty acids (FFA), phospholipids (PL), cholesterol esters (CE), and triglycerides (TG) of Holstein cows at 24 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



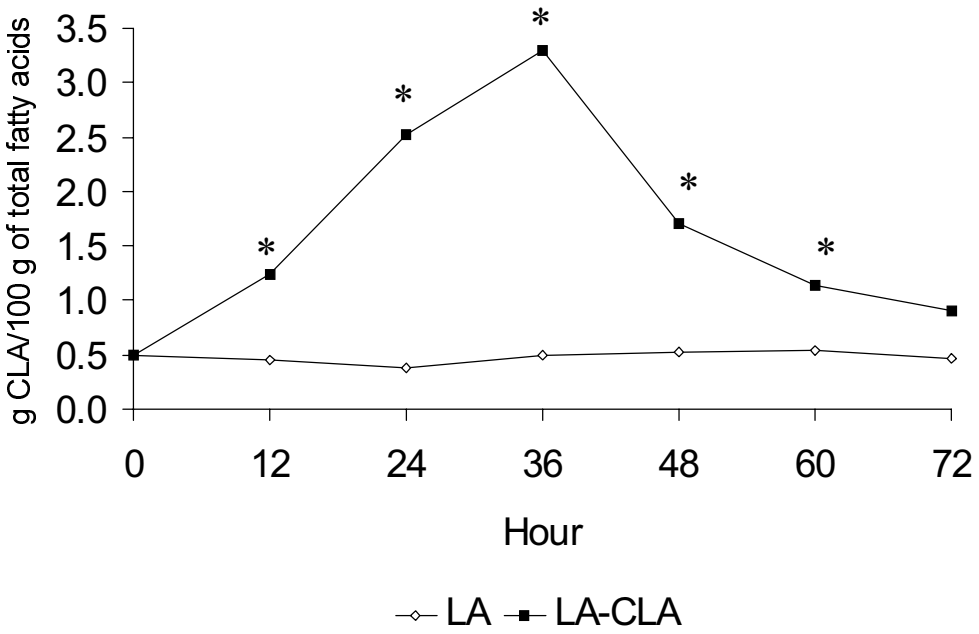
(The standard error for the treatment means at 24 h were 0.4, 0.02, 0.05, and 0.5 for FFA, PL, CE, and TG, respectively)

Figure 6. Concentration of fat in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic (LA-CLA) acid for 24 h.



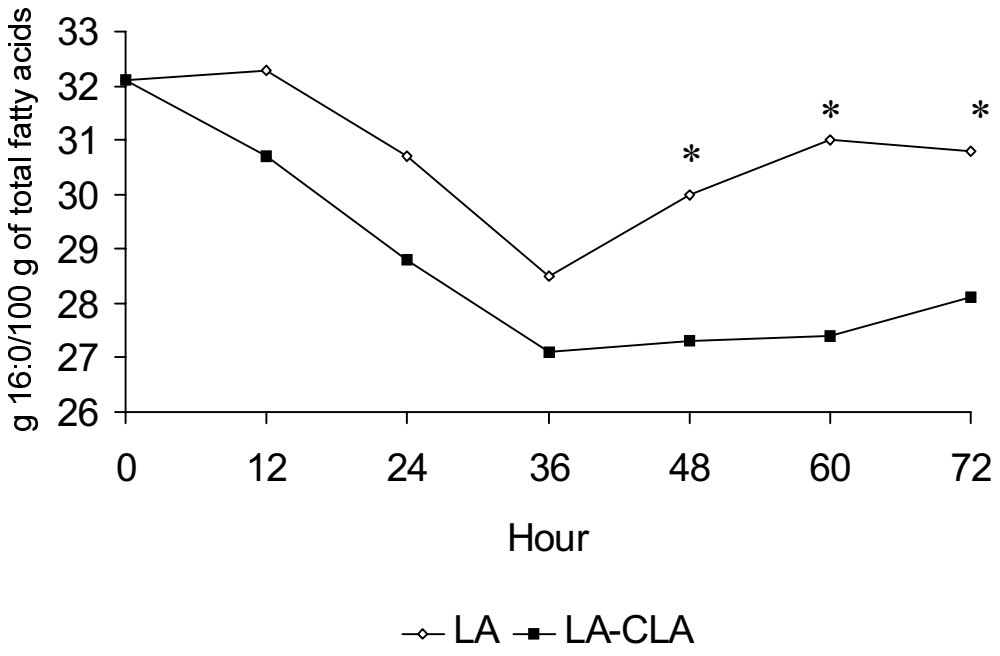
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.1, 0.1, 0.1, 0.2, 0.1, and 0.2, respectively)

Figure 7. Conjugated linoleic acid (18:2; Δ^{9}*cis*, Δ^{11}*trans* plus 18:2; Δ^{10}*trans*, Δ^{12}*cis*) concentration in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



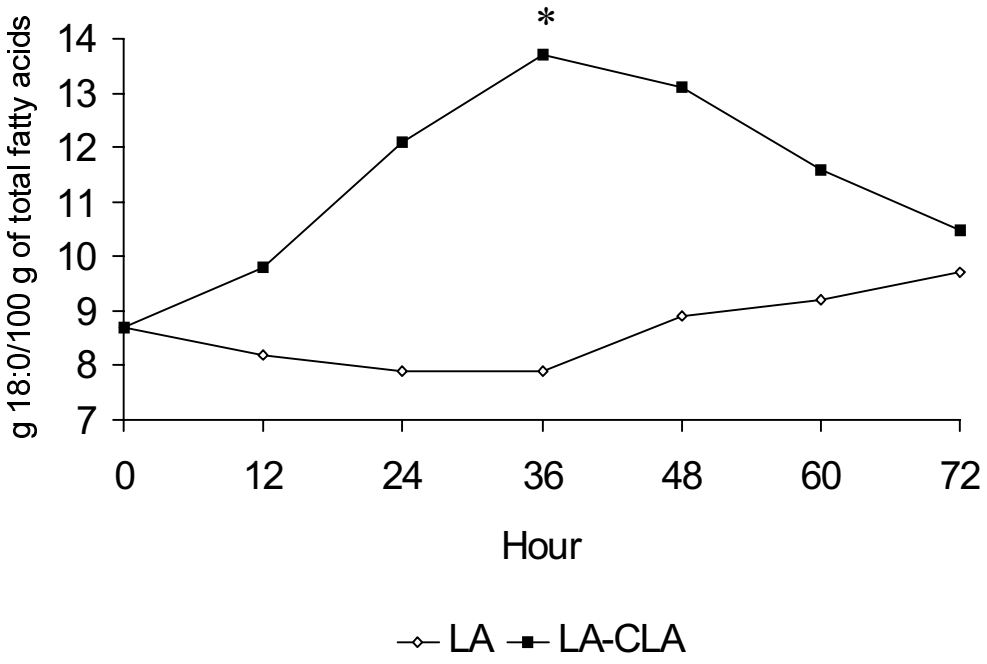
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.05, 0.17, 0.13, 0.18, 0.07, and 0.05, respectively)

Figure 8. Palmitic acid concentration in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



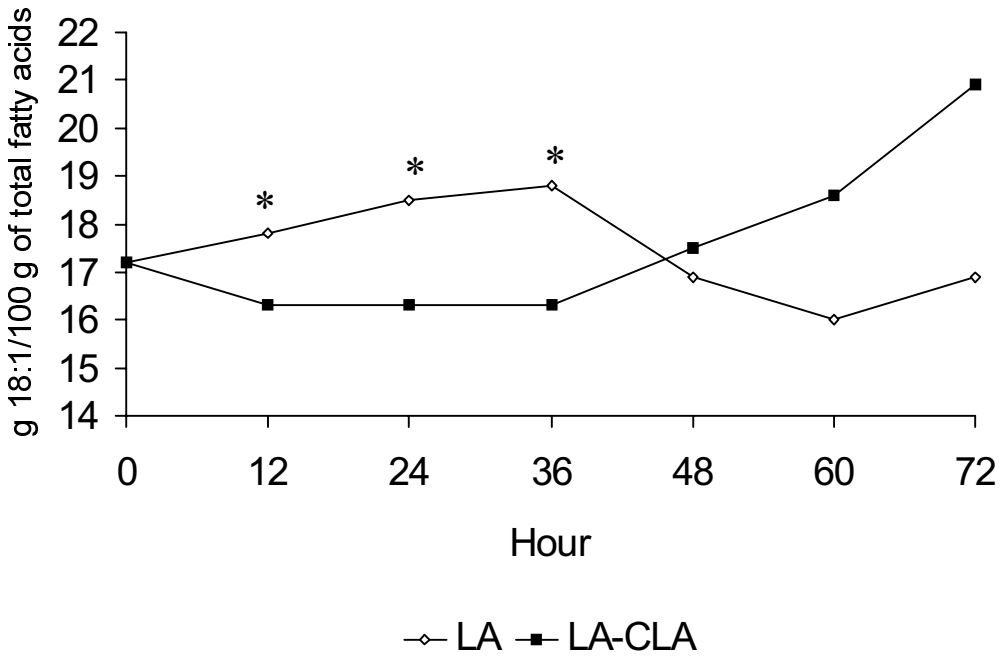
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.9, 1.1, 0.6, 0.7, 0.8, and 0.4, respectively)

Figure 9. Stearic acid concentration in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



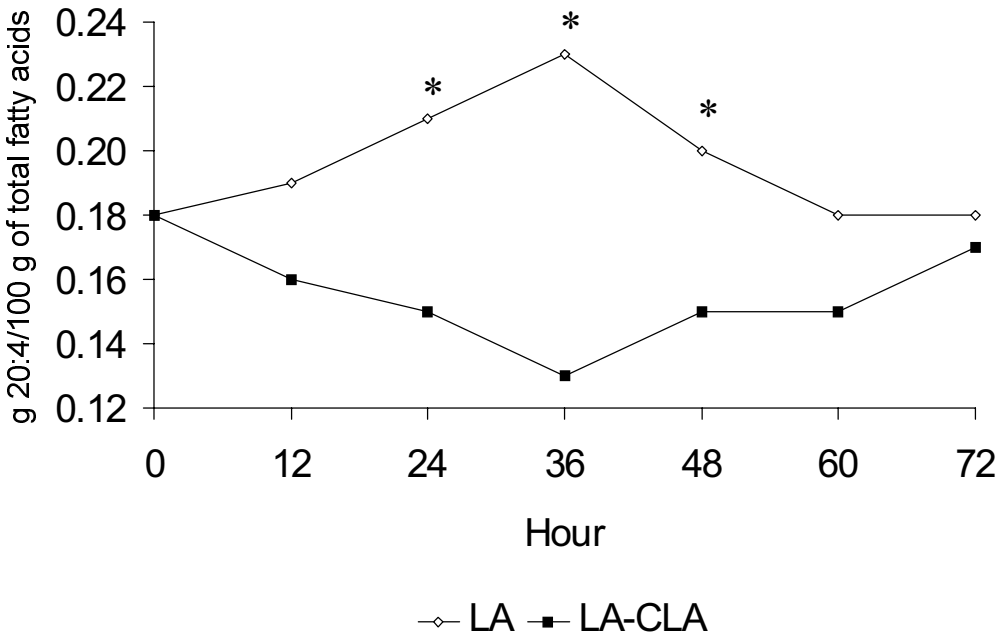
(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.8, 1.3, 1.5, 1.3, 1.4, and 1.2, respectively)

Figure 10. Oleic acid concentration in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.16, 0.17, 0.3, 1.2, 1.0, and 1.7, respectively)

Figure 11. Arachidonic acid concentration in milk from Holstein cows during 72 h after abomasal infusion of linoleic acid (LA) or linoleic-conjugated linoleic acid (LA-CLA) for 24 h.



(The standard error for the treatment means at 12, 24, 36, 48, 60, and 72 h were 0.007, 0.007, 0.01, 0.01, 0.01, and 0.007, respectively)

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