Ruminal Biohydrogenation in Holstein Cows Fed Soybean Fatty Acids as Amides or Calcium Salts

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ABSTRACT

Fatty amides of high oleate fats and calcium salts of palm oil were reported to resist biohydrogenation by ruminal microorganisms. This study was conducted to determine whether converting polyunsaturated fat sources to amides and calcium salts had equal ability to resist biohydrogenation. A total mixed ration consisting of forage and concentrate contained (dry basis): 1) 2.45% soybean oil (SBO), 2) 2.75% calcium salt of SBO, 3) 2.75% amide of SBO, or 4) 2.75% of a mixture of the calcium salt and amide (80:20, wt/wt) of SBO. The 4 diets were fed ad libitum to 4 multiparous lactating Holstein cows fitted with ruminal cannulas in a 4 × 4 Latin square with 21-d periods. Omasal samples were taken to measure postruminal fatty acid content and determine the extent of ruminal biohydrogenation. Adding SBO to the diets as either calcium salts or amides increased omasal flow of C18:2 (*n*-6) from 25 to 39 g/d. Omasal flow of C18:1 increased from 36 to 49 g/d when SBO was fed to cows as calcium salts, but increased to 86 g/d when SBO was fed as amides. Adding the soybean amide to the diet more than doubled the delivery of C18:1 (*n*-9) to the omasum of lactating cows, but it also increased *trans* fatty acid production in the rumen accompanied by milk fat depression. In this study, calcium salts and amide derivatives of fatty acids were both effective in enhancing omasal flow of unsaturated fatty acids in lactating dairy cows. Amides were more effective than calcium salts for increasing the postruminal flow of oleic acid.

(Key words: biohydrogenation, calcium salt, fatty acyl amide, omasal sample)

Abbreviation key: AMD = amide of SBO added at 2.75% of DM, CAS = calcium salt of SBO added at 2.75% of DM, MIX = mixture of the calcium salt and

amide (80:20, wt/wt) of SBO added at 2.75% of DM, SBO = soybean oil.

INTRODUCTION

Jenkins et al. (1996) examined the ability of a fatty acyl amide, made by reacting butylsoyamide with soybean oil (SBO), to resist biohydrogenation and increase linoleic acid content in milk fat without disrupting ruminal fermentation. Results of this trial showed that amide of SBO increased linoleic acid percentage in milk when compared with SBO alone (6.28% vs. 4.77%). The same trial by Jenkins et al. (1996) also showed that SBO disrupted ruminal fermentation as shown by a depression in acetate:propionate, which was avoided when the SBO was converted to an amide. Later research conducted with fatty acyl amides (Jenkins, 1998) focused on protecting oleic acid. By feeding oleamide to Holstein cows, Jenkins (1998) was able to increase the percentage of oleic acid in milk fat compared with feeding canola oil or the control diet (48.2, 35.1, and 23.2% of total fatty acids), respectively.

Calcium salts of palm fatty acids also were reported to resist biohydrogenation (Klusmeyer and Clark, 1991; Wu et al., 1991), which subsequently led to the feeding of a variety of fat sources as calcium salts to increase milk concentration of specific fatty acids such as CLA (Giesy et al., 2002) or oleic acid (Chouinard et al., 1997). However, feeding calcium salts of soybean and linseed oils to lactating cows failed to enhance milk concentrations of their most abundant fatty acids, namely linoleic and linolenic acids, respectively (Chouinard et al., 1998). Therefore, protection from ruminal biohydrogenation has not been established for calcium salts of unsaturated fat sources as it has for calcium salts of palm fatty acids.

The objective of this study was to compare the relative effectiveness of fatty acyl amides vs. calcium salts of SBO in enhancing the delivery of unsaturated fatty acids to the omasum of lactating Holstein cows. Because calcium salts may protect unsaturated fatty acids by encapsulating them within a water-insoluble matrix (Fotouhi and Jenkins, 1992), protection from biohydro-

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Table 1. Fatty acid composition of fat supplements used in diets fed to Holstein cows.

	Fat supplement ¹			
	SBO	MIX	CAS	AMD
	(g/100 g)			
C16:0	11.1	10.8	10.6	11.0
C18:0	3.85	4.36	4.43	4.16
C18:1 (<i>n</i> -9)	20.2	21.8	21.7	22.2
C18:2 (<i>n</i> -6)	54.7	52.2	52.5	51.1
C18:3 (<i>n</i> -3)	6.21	4.65	4.75	4.13
C22:0	0.45	0.33	0.32	0.36
Other	3.45	5.86	5.73	7.07

¹SBO = soybean oil, MIX = calcium salt and fatty acyl amide (80:20, wt/wt) of SBO, CAS = calcium salt of SBO, and AMD = fatty acyl amide of SBO.

generation also was tested for a mixture (20:80) of amide and calcium salts. Amides of SBO were added at the time of calcium salt formation to determine whether the calcium salts might shield the amide bond from microbial attack, thus enhancing protection of fatty amides from biohydrogenation.

MATERIALS AND METHODS

Fat Supplements

Fatty acid composition of the fat supplements is shown in Table 1. Commercial SBO (North Arkansas Wholesale Co. Inc., Bentonville, AR) used for the SBO diet contained 55% linoleic acid, 20% oleic acid, and 11% palmitic acid as determined by GLC. The fatty acyl amide, calcium salt, and a mixture of the calcium salt and fatty acyl amide (80:20, wt/wt) of SBO were supplied by Church and Dwight Co., Inc. (Princeton, NJ). The amide and calcium salt mixture was made by adding amides of SBO to the reactor at the terminal stage of calcium salt formation, at a point where both fat sources were plastic and could associate. The decision to include 20% amide in the amide and calcium salt mix was arbitrary, but was based on having a larger mass of calcium salts to surround and protect the fatty amide. All fat supplements were added to the concentrate ingredients without further processing.

Cows and Diets

Four multiparous Holstein cows (103 ± 19 DIM) fitted with ruminal cannulas were used in a 4 × 4 Latin square design. Trial periods consisted of 21 d with sample collection on the last 4 d of each period. Cows were housed in a tie-stall barn and had free access to water and assigned diets. Feed was offered each day at 0800 and 1600 h. Refusals were removed and recorded daily at 0700 h. Diets were fed to allow 5% orts. Cows were

milked daily at 0700 and 1900 h. Ten grams of chromic oxide in gelatin capsules were dosed twice daily (over the last 10 d of each period) via ruminal cannula just prior to feeding as an indigestible feed marker.

Diets were fed as a TMR consisting of forage and concentrate (1:1; DM basis). The forage portion contained corn silage and alfalfa hay in a 9:1 ratio (DM basis). Diets consisted of TMR plus 1) SBO added at 2.45% of DM, 2) calcium salt of SBO added at 2.75% of DM (CAS), 3) amide of SBO added at 2.75% of DM (AMD), and 4) a mixture of the calcium salt and amide (80:20, wt/wt) of SBO added at 2.75% of DM (MIX). Urea was added to SBO to balance the small amount of N supplied by the amide. Urea was left out of the MIX and CAS diets to determine any possible advantage of the amide over CAS, regardless of whether that advantage was caused by the N or fatty acid moieties. Fat sources were added to the TMR at concentrations needed to equalize added linoleic acid across all treatments. Ingredients, chemical composition, and fatty acid concentrations of the TMR are shown in Table 2.

Sampling and Analysis

Corn silage was analyzed weekly for DM, and adjustments were made to the ration to maintain a 50:50 (DM basis) forage:concentrate ratio. Forage (corn silage + alfalfa hay), concentrate, and ort samples were taken weekly, composited by cow and by period, dried at 55°C, and then ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 2-mm sieve. Ground samples were analyzed for DM (100°C), Kjeldahl N (AOAC, 1990), NDF (Van Soest et al., 1991), and fatty acids. Fatty acids were converted to methyl esters by incubation in 5% methanolic HCl for 16 h at 70°C as described by Jenkins et al. (2001). One milliliter of heptadecanoic acid (Cat. No. N-17-A; Nu-Check-Prep, Inc., Elysian, MN) was added as an internal standard (2 mg/mL). Fatty acid methyl esters were analyzed via GLC on a Shimadzu GC-14A equipped with a 100-m × 0.25-mm i.d. WCOT-fused silica column coated (0.20-μm film thickness) with CP-Sil 88 (Cat. No. 7489; Chrompack, Inc., Raritan, NJ). Initial oven temperature was 70°C (4 min), which was raised 13°C/min to 175°C (held for 27 min) and then increased again at 4°C/min to a final temperature of 215°C (held for 11 min). Temperatures of the injector and detector were 250°C. Hydrogen was used as the carrier gas at a linear velocity of 40 cm/s. Nutrient composition of the diets (Table 2) was calculated based on forage and grain analyses and their contribution to the diet.

Omasal samples were taken at 0800, 1100, and 1400 h on d 19 of each period. Sample times were delayed 1 h on d 20 and 2 h on d 21, yielding a sample taken

Table 2. Ingredients and composition of the TMR fed to Holstein cows.

	Diet ¹			
	SBO	MIX	CAS	AMD
Ingredient (% of DM)				
Corn silage	45.0	45.0	45.0	45.0
Alfalfa hay	5.00	5.00	5.00	5.00
Cracked corn	16.9	16.9	16.9	16.9
Whole barley	11.7	11.7	11.7	11.7
Soybean meal	15.6	15.6	15.6	15.6
Soybean oil	2.45			
Ca salt/amide (80:20) of soybean oil		2.75		
Ca salts of soybean oil			2.75	
Amide of soybean oil				2.75
Defluorinated phosphate	1.10	1.10	1.10	1.10
Limestone	0.55	0.55	0.55	0.55
Sodium bicarbonate	0.65	0.65	0.65	0.65
Trace mineral salt ²	0.50	0.50	0.50	0.50
K, Mg, S ³	0.27	0.27	0.27	0.27
Urea	0.27	0.00	0.00	0.00
Zn methionine ⁴	0.07	0.07	0.07	0.07
Vitamin A, D, & E premix ⁵	0.025	0.025	0.025	0.025
CP	174	169	167	176
NDF	261	263	260	266
Fatty acids	52.2	53.6	57.1	61.1
C12:0	0.20	0.20	0.19	0.20
C14:0	0.06	0.07	0.09	0.07
C16:0	7.55	7.55	8.19	8.49
C18:0	1.65	1.79	2.09	2.05
C18:1(<i>n</i> -9)	9.83	10.3	11.3	12.1
C18:2(<i>n</i> -6)	25.0	25.6	26.0	29.4
C18:3(<i>n</i> -3)	3.30	3.09	3.10	3.29
C20:0	0.25	0.22	0.24	0.24
Other	4.36	4.78	5.90	5.26

¹Diets contained either 2.45% soybean oil (SBO), 2.75% calcium salts of SBO (CAS), 2.75% amides of SBO (AMD), or 2.75% blended fat (MIX) consisting of calcium salts and amides of SBO (80:20, wt/wt).

²Morten Salt Division (Chicago, IL) specified to contain: 93 to 98% NaCl, not less than 0.35% Zn, 0.28% Mn, 0.175% Fe, 0.035% Cu, 0.007% I, and 0.007% Co.

³Dynamate (Eastern Minerals Inc., Henderson, NC) contained not less than 18% K, 11% Mg, and 22% S.

⁴Zinpro Corporation (Chaska, MN).

⁵Lutavit (BASF Co., Mt. Olive, NJ) supplied 22,000 IU/g of vitamin D₃, 17,600 IU/g of vitamin A, and 66 IU/g of vitamin E.

at each hour between feedings. Omasal samples were taken using a modified procedure of Huhtanen et al. (1997). Briefly, omasal samples were aspirated through tygon tubing (16 mm i.d.) that was passed through the ruminal cannula and inserted into the reticulo-omasal opening. A hand-operated vacuum pump (36 cc per stroke) was attached to a filter flask, and 150 mL of omasal contents were retrieved. Omasal samples were composited over sampling times and immediately frozen. Frozen samples were later thawed and a subsample was lyophilized. Lyophilized samples were ground in a centrifugal mill through a 0.5-mm sieve and analyzed for DM (100°C), Kjeldahl N (AOAC, 1990), NDF (Van Soest et al., 1991), Cr (atomic absorption, Brookside Labs, Inc., New Knoxville, OH), and fatty acid content as previously described. To separate individual *trans*-C18:1 isomers in omasal contents, the omasal

samples were re-analyzed on the previously mentioned GLC according to Griinari et al. (1998) with minor modifications to the column temperature. Initial column temperature was 160°C for 45 min then ramped 4°C/min to a final temperature of 215°C (held for 11 min). Chromatograms of the milk fatty acids were similar to the chromatographs reported by Griinari et al. (1998), which provided a basis for peak identification. Further verification of peak identity was established by comparison of peak retention times to commercially purchased *trans* monoene standards (Supelco, Bellefonte, PA).

Daily milk production was recorded electronically throughout the duration of the experiment. Three consecutive morning and evening milk samples (6 samples) were taken at the end of each period. The samples were composited by cow, and a subsample containing isobronopol was sent to DHIA (Virginia Dairy Herd Improve-

Table 3. Intake, omasal flow, and apparent ruminal digestibilities of DM and fiber in Holstein cows fed diets containing protected and unprotected soybean oil (SBO).

	Diet ¹				SEM	Contrast ²		
	SBO	MIX	CAS	AMD		1	2	3
Intake (kg/d)								
DM	20.1	20.1	20.0	17.2	0.61	NS	0.08	0.02
NDF	5.25	5.30	5.21	4.57	0.16	NS	0.08	0.03
Flow to omasum (kg/d)								
DM	12.4	12.5	13.9	10.7	0.93	NS	NS	0.05
NDF	2.56	2.63	3.18	2.34	0.19	NS	NS	0.02
Rumen digestibility (%)								
DM	35.9	39.6	28.7	35.5	4.71	NS	NS	NS
NDF	49.2	51.2	36.9	46.0	4.19	NS	NS	NS

¹Diets contained either 2.45% SBO, 2.75% calcium salts of SBO (CAS), 2.75% amides of SBO (AMD), or 2.75% blended fat (MIX) consisting of calcium salts and amides of SBO (80:20, wt/wt).

²Contrasts are designed as 1) SBO vs. other diets, 2) MIX vs. CAS and AMD, and 3) CAS vs. AMD.

ment Association, Blacksburg) for analysis of protein and SCC using near infrared analysis (AOAC, 1990). A separate sample from the composite (without isobronopol) was stored at -4°C . Samples without isobronopol were allowed to equilibrate to room temperature and were divided into two subsamples. The first of the subsamples were centrifuged at $21,000 \times g$ for 30 min at 4°C to separate the milk fat for fatty acid analysis. Fatty acids in milk fat were converted to methyl esters in sodium methoxide followed by methanolic HCl as described by Kramer et al. (1997). Fatty acid content was determined via GLC using the program previously described. The second subsample was analyzed for fat content using the Roesse-Gottlieb method (AOAC, 1990).

Data Presentation and Statistical Analysis

Although complete fatty acid profiles were obtained for omasal samples, only C18 fatty acids are presented to maintain focus on the main study objective, i.e., the ability of the fat supplements to resist ruminal biohydrogenation. The fatty acid designations of C18:0, C18:1 (*n*-9), C18:2 (*n*-6), and C18:3 (*n*-3) refer to stearic, oleic, linoleic, and linolenic acids, respectively. All data were analyzed as a 4×4 Latin square using the general linear model procedure of SAS (2000). The model included effects caused by diet, animal, and period. Planned comparisons of treatments included three orthogonal contrasts 1) SBO vs. other fat supplements, 2) MIX vs. AMD and CAS, and 3) AMD vs. CAS. Exact $P \leq 0.10$ are shown for each contrast.

RESULTS AND DISCUSSION

Intake and Ruminal Digestibilities

Dry matter ($P = 0.02$) and NDF intakes ($P = 0.03$) were lower for AMD than they were for CAS (Table 3). A previous study (Jenkins, 1999) reported a linear

decline in DMI as oleamide increased in the diet of cows, where DMI was depressed 1.7 kg/d for each percentage unit increase in dietary oleamide. When the fatty acyl amide was included in the MIX (0.55% of ration DM), DMI was not suppressed compared with CAS. Sheep responded differently to diets containing unsaturated amides. Dry matter intake did not change when sheep were fed 5% linoleamide compared with sheep fed a control diet (Jenkins and Adams, 2002). Because of the lower DMI for AMD in this study, flows of DM and NDF to the omasum were reduced ($P \leq 0.05$) for AMD compared with CAS (Table 3), although ruminal DM digestibilities were similar.

Flows of DM and NDF to the omasal canal were estimated in this study from a twice-daily pulse dose of a single marker (Cr). It has been suggested that a triple marker system is necessary to obtain accurate flow measurements from the omasal canal because of unrepresentative sampling of digesta particulate matter entering that site (Ahvenjarvi et al., 2000). In a later study (Ahvenjarvi et al., 2003), single markers (Yb, Cr, Co, or indigestible NDF) were shown to concentrate in different digesta fractions (liquid, small particulate, or large particulate phases) and resulted in large differences in flow measurements to the omasal canal. Despite Cr associating more with the large particulate fraction, there was good agreement between Cr alone and a triple marker (Co + Yb + Cr) system for determining flows of organic matter ($r = 0.88$) or NDF ($r = 0.98$) to the omasal canal (Ahvenjarvi et al., 2003).

Omasal sampling using Cr in this study yielded digestibilities of DM and fiber that were similar to digestibilities reported in previous investigations. For instance, ruminal digestibilities of DM and NDF averaged 37.7 and 44.9%, respectively, in a study using omasal sampling with three markers (Reynal and Broderick, 2003) compared with ruminal digestibilities of DM and NDF that averaged 34.9 and 45.8%, respectively, in

Table 4. Intake and omasal flow of total and C18 fatty acids and biohydrogenation of C18 fatty acids in Holstein cows fed diets containing protected and unprotected soybean oil (SBO).

	Diet ¹				SEM	Contrast ²		
	SBO	MIX	CAS	AMD		1	2	3
Intake, g/d								
Total FA	1052.1	1090.1	1111.8	1047.6	50.5	NS	NS	NS
C18:0	33.2	36.1	41.8	35.2	1.1	NS	NS	0.01
C18:1 (<i>n</i> -9)	197.7	207.5	226.8	208.0	6.0	0.06	NS	0.07
C18:2 (<i>n</i> -6)	502.3	516.4	520.5	506.0	14.4	NS	NS	NS
C18:3 (<i>n</i> -3)	66.0	62.3	61.8	56.4	2.0	0.04	NS	0.10
Total C18 ³	799.2	822.2	850.8	805.6	23.3	NS	NS	NS
Flow to omasum, g/d								
Total FA	1108.3	1170.1	1136.0	1050.9	115.4	NS	NS	NS
C18:0	519.9	433.3	490.2	410.8	39.6	NS	NS	NS
trans C18:1	156.7	229.8	140.3	198.7	40.0	NS	NS	NS
C18:1 (<i>n</i> -9)	36.1	41.3	48.5	85.5	9.5	0.09	0.07	0.03
C18:2 (<i>n</i> -6)	24.7	32.7	39.1	39.2	5.1	0.08	NS	NS
C18:3 (<i>n</i> -3)	5.2	6.1	6.9	5.5	0.7	NS	NS	NS
Total C18 ⁴	742.5	743.2	725.0	739.7	81.2	NS	NS	NS
Omasal concentration, g/kg of DM								
C18:0	42.0	35.9	35.4	38.5	2.76	NS	NS	NS
trans C18:1	12.6	16.9	10.3	18.5	2.44	NS	NS	0.05
C18:1 (<i>n</i> -9)	2.87	3.23	3.56	7.98	0.96	NS	0.08	0.02
C18:2 (<i>n</i> -6)	1.95	2.51	2.83	3.67	0.40	0.07	NS	NS
C18:3 (<i>n</i> -3)	0.42	0.46	0.50	0.52	0.04	NS	NS	NS
Biohydrogenation, %								
C18:1 (<i>n</i> -9) ⁵	81.1	80.7	77.9	61.4	4.71	NS	NS	0.05
C18:1 (<i>n</i> -9) ⁶	80.6	77.9	74.4	55.4	4.68	0.08	0.06	0.03
C18:1 (<i>n</i> -9) ⁷	70.3	68.1	67.9	33.0	8.10	NS	NS	0.02
C18:2(<i>n</i> -6) ⁵	94.8	93.9	92.2	92.4	1.05	NS	NS	NS
C18:2(<i>n</i> -6) ⁶	94.8	93.2	91.1	91.7	0.68	0.01	0.08	NS
C18:2(<i>n</i> -6) ⁷	92.2	90.2	89.1	87.5	1.40	0.10	NS	NS

¹Diets contained either 2.45% SBO, 2.75% calcium salts of SBO (CAS), 2.75% amides of SBO (AMD), or 2.75% blended fat (MIX) consisting of calcium salts and amides of SBO (80:20, wt/wt).

²Contrasts are designed as 1) SBO vs. other diets, 2) MIX vs. CAS and AMD, and 3) CAS vs. AMD.

³Consisting of C18:0, C18:1 (*n*-9), C18:2 (*n*-6), and C18:3 (*n*-3).

⁴Consisting of C18:0, *trans* C18:1, C18:1 (*n*-9), C18:2 (*n*-6), and C18:3 (*n*-3).

⁵Percentage biohydrogenation based on total grams of fatty acid loss across the rumen divided by the fatty acid intake.

⁶Percentage biohydrogenation as determined by Wu et al. (1991).

⁷Percentage biohydrogenation based on the concentration of fatty acid in feed minus omasum divided by feed.

the present study. Hristov and Ropp (2003) reported ruminal digestibilities of DM and NDF that averaged 36.3 and 38.9%, respectively, when calculated from duodenal samples.

Ruminal Biohydrogenation of Unsaturated Fatty Acids

Total fatty acid intakes were similar for all diets despite the lower DMI for AMD (Table 4). This can be attributed to the higher fatty acid concentration in the AMD diet (Table 2). The actual fatty acid concentration in the amide fat supplement was higher than expected, which led to overestimating the amount needed in the ration for balancing fatty acid intakes. Flows of fatty acid to the omasum were not affected by diet. Therefore, recoveries of total fatty acids at the omasum were the

same for all diets and averaged 103.8%. Fatty acid loss across the rumen is expected to be small or negligible based on studies showing minor microbial metabolism of long-chain fatty acids and insignificant absorption across the ruminal epithelium (Doreau and Chilliard, 1997). Fatty acids reaching the duodenum may exceed fatty acid intake because of contributions from microbial *de novo* synthesis of long-chain fatty acids (Doreau and Chilliard, 1997). However, in many previous studies, fatty acid loss across the rumen was observed. Doreau and Chilliard (1997) examined 113 observations from the literature on fatty acid balance across the rumen with an overall average of 80.1% recovery of ingested fatty acids at the duodenum.

Total C18 intakes were similar for all diets (Table 4). Also, intakes of C18:2 (*n*-6) were equal across treatments, which was predetermined by the selection of fat

levels added to the TMR. Intake of C18:2 (*n*-6) for AMD was similar to the other diets because its lower DMI was offset by a higher C18:2 (*n*-6) concentration (Table 2). Intakes of all remaining fatty acids were lower for AMD than for CAS.

The flow of C18:1 (*n*-9) to the omasum was lower ($P = 0.09$) for SBO than it was for the protected fat supplements. The AMD delivered 37 g more C18:1(*n*-9) to the omasum than CAS, even though C18:1(*n*-9) intake was lower for AMD (208 vs. 227 g/d). The flow of C18:2 (*n*-6) also was lower ($P = 0.08$) for SBO than it was for the other treatments, demonstrating some efficacy for the protected fat supplements to deliver unsaturated fatty acids post ruminally. Omasal flow of C18:2 (*n*-6) did not differ among the AMD, CAS, or MIX diets. Expressing omasal fatty acids in grams per kilogram of DM had similar diet effects as seen for flow (g/d). The only difference was that C18:1 (*n*-9) for SBO did not differ from the protected fats when expressed on a concentration basis.

Ruminal biohydrogenation in this study was calculated and compared using 3 different methods. The first measure of biohydrogenation was calculated based on the total grams of fatty acid loss across the rumen (consumed – omasal flow) as a percentage of fatty acid consumed. When examined this way, C18:2 (*n*-6) biohydrogenation ranged from 92 to 95% and did not differ among diets. Unlike C18:2 (*n*-6), the AMD diet had lower ($P = 0.05$) biohydrogenation of C18:1(*n*-9) compared with CAS (61.4% vs. 77.9%).

The extensive biohydrogenation of C18:2 (*n*-6) observed in this study is consistent with previous results where the majority of unsaturated fatty acids consumed by ruminants were lost prior to the duodenum. Losses of C18:2 (*n*-6) and C18:3 (*n*-3) from the mouth to duodenum in ruminant species averaged 80 and 92%, respectively, in a data set containing more than 100 observations from published studies (Doreau and Chilliard, 1997).

The second measure of biohydrogenation was calculated using the method described by Wu et al. (1991). This method corrects for losses in C18 flow to the omasum by calculating the ratio of individual 18-carbon fatty acids and total 18-carbon fatty acids ingested relative to the amount of individual 18-carbon fatty acids and total 18-carbon fatty acids reaching the omasum, respectively. Using this approach, C18:2 (*n*-6) biohydrogenation was higher ($P = 0.01$) for SBO than it was for the other fat supplements, and MIX was higher ($P = 0.08$) than CAS or AMD. For C18:1 (*n*-9) biohydrogenation, the SBO diet had higher biohydrogenation than the protected fats, and the AMD diet had lower ($P = 0.03$) biohydrogenation than CAS (55.4% vs. 74.4%).

The third measure of biohydrogenation was calculated based on the differences between fatty acid concentrations in the feed and omasal contents divided by their feed concentration. When examined this way, the only diet effects were higher ($P = 0.10$) ruminal biohydrogenation of C18:2 (*n*-6) for SBO than for the protected fats and lower ($P = 0.02$) biohydrogenation of C18:1(*n*-9) for AMD than for CAS. Determining ruminal biohydrogenation on a concentration basis avoids the need to use digestibility markers to determine digesta flow.

There were several common diet effects on biohydrogenation across all calculation methods. First, biohydrogenation of C18:1 (*n*-9) was less for AMD than for CAS, but AMD had no advantage over CAS for reducing biohydrogenation of C18:2 (*n*-6). The amide bond was proposed to reduce fatty acid loss in ruminal contents by tying up the carboxyl group, which must be unbound to promote the initial step in biohydrogenation (Doreau and Chilliard, 1997). This study suggests that the amide bond in simple fatty amides (made by reacting the carboxyl group with ammonia) is less stable when the fatty acyl chain is polyunsaturated vs. monounsaturated. Perhaps structural differences in the fatty acyl chain alter steric hindrances around the amide bond, affecting their stability to enzymatic attack.

A second common dietary effect on biohydrogenation across all 3 methods of calculation was that the MIX had little effect on biohydrogenation. The MIX attempted to use calcium salts as an encapsulating agent to enhance protection of amides by shielding the amide bond from microbial attack. If this were successful, biohydrogenation of unsaturated fatty acids in MIX would be lower than unsaturated fatty acids in AMD. There was no evidence of any advantage for MIX. Biohydrogenation was the same or higher for MIX than it was for CAS or AMD. It is possible that fatty acid profile of calcium salts may affect encapsulation properties. Calcium salts of saturated fatty acids are less dissociated than calcium salts of unsaturated fatty acids at any given ruminal pH (Sukhija and Palmquist, 1990). Because the calcium salts in this study were mostly unsaturated, the physical barrier surrounding the amide fatty acids might have been weakened by dissociation.

Calcium salts are rumen-inert and minimize disruption of fermentation, but they should undergo limited biohydrogenation if a free carboxyl group is required for hydrogenation as previously reported (Doreau and Chilliard, 1997). Fotouhi and Jenkins (1992) offered an explanation for biohydrogenation of calcium salts. They stated that, at normal ruminal pH, calcium linoleate is in equilibrium with a small amount of its corresponding free acid, i.e., linoleic acid. The linoleic acid fraction undergoes biohydrogenation because of its free carboxyl

Table 5. Concentration of individual and total C18:1 *trans* isomers in omasal contents of Holstein cows fed diets containing protected and unprotected soybean oil (SBO).

	Diet ¹				SEM	Contrast ²		
	SBO	MIX	CAS	AMD		1	2	3
	(g/kg of DM)							
<i>trans</i> 6-8	0.76	1.03	0.77	0.84	0.13	NS	NS	NS
<i>trans</i> 9	0.46	0.53	0.45	0.35	0.08	NS	NS	NS
<i>trans</i> 10	1.41	3.05	1.40	9.89	1.98	NS	NS	0.02
<i>trans</i> 11	8.80	10.6	6.43	6.16	0.94	NS	0.01	NS
<i>trans</i> 12	1.22	1.66	1.24	1.31	0.16	NS	0.10	NS
Total <i>trans</i>	12.6	16.9	10.3	18.5	2.44	NS	NS	0.05

¹Diets contained either 2.45% SBO, 2.75% calcium salts of SBO (CAS), 2.75% amides of SBO (AMD), or 2.75% blended fat (MIX) consisting of calcium salts and amides of SBO (80:20, wt/wt).

²Contrasts are designed as 1) SBO vs. other diets, 2) MIX vs. CAS and AMD, and 3) CAS vs. AMD.

group, forcing continual dissociation of calcium linoleate to maintain the linoleic acid-calcium linoleate equilibrium. Major end products from linoleic acid biohydrogenation, such as stearic acid, are then converted to the calcium salt to maintain the proper stearic acid-calcium stearate equilibrium. The end result is that most of the fatty acids remain as calcium salts and maintain ruminal inertness, but are converted from unsaturated to saturated.

Trans Fatty Acids in Omasal and Milk Samples

Total *trans* C18:1 concentrations (g/kg of DM) in the omasum were higher ($P = 0.05$) for AMD than they were for CAS (Table 5). Most the higher *trans* C18:1 concentration for AMD was due to a higher ($P = 0.02$) concentration of the *trans*-10 isomer. The MIX had higher concentrations of *trans*-11 ($P = 0.01$) and *trans*-12 C18:1 ($P = 0.10$) compared with the other protected fat supplements.

A marked increase in *trans* C18:1 in ruminal contents or milk was not seen in previous studies where fatty amides were fed to dairy cows at substantially higher concentrations than the 2.75% amide fed in this study. For instance, total *trans* C18:1 concentration was not elevated in ruminal contents of Holstein cows fed 4.2% oleamide (Jenkins et al., 2000). Conversely, Jenkins and Adams (2002) reported increased concentration of *trans* C18:1 in duodenal contents of sheep fed 5% linoleamide. Perhaps the amide-induced increase in ruminal *trans* C18:1 concentration may depend on the degree of amide unsaturation. In the present cow study and the previous sheep study (Jenkins and Adams, 2002), the amide supplement contained linoleic acid as the predominant fatty acid and also elevated ruminal *trans* C18:1 concentrations.

High concentrations of polyunsaturated fatty acids in ruminal contents can cause accumulation of *trans* C18:1 by inhibiting the conversion of *trans* C18:1 to

stearic acid, especially when they are present as the free acid (Harfoot and Hazlewood, 1988). Extensive cleavage of the amide bond by the ruminal microorganisms would release linoleic acid and perhaps inhibit the conversion of *trans* C18:1 to stearic acid. However, the release of linoleic acid from SBO should equal linoleic acid release from AMD, given that triacylglycerols undergo extensive lipolysis in the rumen (Harfoot and Hazlewood, 1988).

An alternative explanation is direct inhibition of biohydrogenation by intact amides. Kepler et al. (1970) demonstrated inhibition of linoleate isomerase by linoleamide and other derivatives of the carboxyl group. Inhibition of linoleate isomerase would cause accumulation of linoleic acid but not *trans* C18:1, unless the amide also inhibited reductases that convert *trans* C18:1 to stearic acid. Accounting for the *trans*-10 C18:1 accumulation is difficult, unless the amides of SBO redirected linoleic acid biohydrogenation from the *cis*-9, *trans*-11 C18:2 and *trans*-11 C18:1 pathway to a *trans*-10, *cis*-12 C18:2 and *trans*-10 C18:1 pathway (Griinari and Bauman, 1999).

Consistent with *trans* C18:1 changes in omasal contents, *trans* C18:1 in milk also was higher ($P = 0.08$) for AMD than it was for CAS (Table 6). When analyzed for milk fat percentage by the Roesse-Gottlieb procedure (AOAC, 1990), SBO had a higher ($P = 0.08$) percentage of milk fat. Milk fat percentage was lower ($P = 0.05$) for AMD than it was for CAS. The lower milk fat percentage for diets containing amide is believed to be due to the high concentration of *trans*-10 18:1 found in the omasum and milk. Baumgard et al. (2000) reported severe milk fat depression when CLA isomers containing a *trans*-10 double bond were infused abomasally. Griinari et al. (1998) specifically associated milk fat depression reported in their study to *trans*-octadecenoic acids produced by incomplete biohydrogenation.

In milk, C18:1 (*n*-9) was higher ($P < 0.01$) for AMD than for CAS, consistent with the lower biohydrogena-

Table 6. Yield, composition, and fatty acid profile of milk from Holstein cows fed diets containing protected and unprotected soybean oil (SBO).

	Diet ¹				SEM	Contrast ²		
	SBO	MIX	CAS	AMD		1	2	3
Milk yield and composition								
Yield, kg/d	34.6	32.5	32.0	32.4	0.99	0.09	NS	NS
Protein, %	2.82	2.84	2.77	2.81	0.05	NS	NS	NS
Fat, %	3.11	2.56	3.04	2.46	0.18	0.08	NS	0.05
Fatty acids, g/100 g								
C4:0	2.45	2.19	2.28	1.84	0.10	0.02	NS	0.02
C6:0	1.91	1.57	1.86	1.15	0.07	<0.01	NS	<0.01
C8:0	1.06	0.83	1.10	0.58	0.06	0.01	NS	<0.01
C10:0	2.27	1.83	2.53	1.30	0.15	0.07	NS	<0.01
C11:0	0.20	0.17	0.21	0.09	0.02	0.06	NS	<0.01
C12:0	2.61	2.32	2.99	1.81	0.16	NS	NS	<0.01
C14:0	9.39	9.19	10.4	7.49	0.52	NS	NS	0.01
C14:1	0.73	0.91	0.74	0.75	0.10	NS	NS	NS
C15:0	0.80	0.80	0.86	0.76	0.02	NS	NS	0.03
C16:0	23.8	23.6	25.1	22.3	0.66	NS	NS	0.03
C16:1	1.36	1.64	1.37	1.65	0.10	NS	NS	0.10
C18:0	13.8	12.0	12.0	12.9	0.50	0.04	NS	NS
<i>trans</i> C18:1	5.60	7.64	6.03	9.95	1.30	NS	NS	0.08
C18:1(<i>n</i> -9)	22.8	23.4	20.5	25.1	0.37	NS	NS	<0.01
C18:2(<i>n</i> -6)	3.56	2.68	3.89	3.86	0.18	NS	<0.01	NS
C18:3(<i>n</i> -3)	0.44	0.31	0.46	0.37	0.01	<0.01	<0.01	<0.01
<i>c</i> -9, <i>t</i> -11 CLA	1.01	1.13	0.88	0.60	0.07	NS	<0.01	0.03
C22:0	0.09	0.08	0.09	0.08	0.02	NS	NS	NS
C22:1	0.15	0.11	0.15	0.10	0.01	<0.01	NS	<0.01
Other	6.98	7.65	6.55	7.37	0.35	0.02	NS	NS

¹Diets contained either 2.45% SBO, 2.75% calcium salts of SBO (CAS), 2.75% amides of SBO (AMD), or 2.75% blended fat (MIX) consisting of calcium salts and amides of SBO (80:20, wt/wt).

²Contrasts are designed as 1) SBO vs. other diets, 2) MIX vs. CAS and AMD, and 3) CAS vs. AMD.

tion of C18:1 (*n*-9) from AMD (Table 4). The proportion of all fatty acids from C4:0 to C16:0 (except C14:1) were lower for AMD than they were for CAS. This result agrees with Jenkins (1998) in which 3.5% oleamide fed to Holstein cows increased 18-carbon unsaturated fatty acids in milk and decreased medium- to short-chain fatty acids compared with canola oil. Milk concentrations of C4:0, C6:0, and C8:0 were low for all diets, which has been attributed to high volatility of the methyl esters (Jensen et al., 1991). The percentage of milk C18:2 (*n*-6) was lower for MIX ($P < 0.01$) than it was for AMD or CAS. The MIX diet had the highest ($P < 0.01$) percentage of *cis*-9, *trans*-11 CLA compared with the other protected fat supplements. The SBO diet produced the highest percentage of C18:0 compared with the other fat supplements, consistent with higher ruminal biohydrogenation for SBO vs. the protected supplements.

Milk yields were reduced ($P = 0.09$) by the protected fats compared with SBO, but did not differ for AMD vs. CAS. Fatty acid concentration (Table 2) was higher for AMD than for CAS, which presumably supplied greater energy to maintain milk production despite the lower DMI for AMD. Milk protein concentrations were not affected by diet.

CONCLUSIONS

In this study, biohydrogenation of dietary C18:2 (*n*-6) in lactating cows decreased slightly when a SBO supplement was converted to amides or calcium salts. Biohydrogenation of dietary C18:1 (*n*-9) also decreased slightly when the SBO supplement was fed to cows as calcium salts. Biohydrogenation of C18:1 (*n*-9) in the rumen decreased extensively when the SBO was added to the ration as amides. The soybean amide more than doubled the delivery of C18:1 (*n*-9) to the omasum of lactating cows. However, the soybean amide in this study also increased *trans* fatty acid production in the rumen, leading to milk fat depression, which was not observed in earlier studies for amides of high oleic acid fat sources.

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