**The biohydrogenation of linoleamide in vitro and its effects on linoleic acid
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The biohydrogenation of linoleamide in vitro and its effects on linoleic acid concentration in duodenal contents of sheep[1]

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ABSTRACT: Previous studies showed that oleamide was protected from ruminal biohydrogenation and increased $18:1(n–9)$ concentration in milk when fed to lactating dairy cows. To appraise whether this protection extended to linoleamide, a rumen in vitro experiment was conducted to determine biohydrogenation of linoleamide followed by two sheep experiments to evaluate whether linoleamide could increase $18:2(n–6)$ concentration in duodenal contents. Treatments for the in vitro and sheep studies consisted of three diets containing no added lipid (control), linoleic acid, or linoleamide. Lipids were added at 10% (DM basis) of the in vitro substrate (ground grass hay). The three substrates were incubated with mixed ruminal microbes in triplicate, and 5 mL of culture contents was taken at 0, 24, and 48 h for analysis of $18:2(n–6)$ concentration by gas chromatography. The concentrations of $18:2(n–6)$ (corrected for $18:2(n–6)$ in the control cultures) at 0, 24, and 48 h were 2.51, 0.38, and 0.11 mg/5 mL for the linoleic acid cultures compared to 2.10, 1.35, and 1.08 mg/5 mL for the linoleamide cultures. Compared to linoleic acid, the cultures containing linoleamide had higher $18:1(n–9)$ and lower concentrations of biohydrogenation products including *trans*-18:1 and 18:0. Three sheep with duodenal cannulas were fed the three diets in two separate 3×3 Latin squares each with 2-wk periods. The two squares only differed in the amount of added lipid (1.5 vs 5% of the ration DM). When the lipids were added at 1.5% of the ration DM, they had little effect on duodenal $18:2(n–6)$ concentration (2.8, 3.6, and 4.3 mg/g DM for the control, linoleic acid, and linoleamide treatments, respectively). At 5% of the ration DM, both lipid supplements increased duodenal $18:2(n–6)$ concentration over the control diet with a greater response observed for linoleamide (2.5, 12.2, and 16.8 mg/g DM for the control, linoleic acid, and linoleamide treatments, respectively). This study demonstrates reduced biohydrogenation of linoleamide based on its ability to maintain a higher concentration of $18:2(n–6)$ in ruminal cultures and in duodenal contents of sheep compared to free linoleic acid.

Key Words: Duodenum, Fatty Acids, In Vitro, Linoleic Acid, Rumen, Sheep

Introduction

The conversion of unsaturated fatty acids (R-COOH) to simple amides ($R-CONH_2$) reduces their biohydrogenation by ruminal microbes (Reeves et al., 1998) and potentially has application to increase postruminal flow of selected unsaturated fatty acids. Thus far, only oleamide has been studied in detail to determine the extent of its protection from biohydrogenation and intestinal absorption in ruminants. The rate of disappearance of $18:1(n–9)$ from ruminal in vitro cultures was reduced 60% when added as oleamide vs the free acid, and feeding oleamide to sheep did not increase fecal excretion of energy or fatty acids (Reeves et al., 1998). A potential application of oleamide may be to decrease the $16:0:18:1(n–9)$ ratio in animal food products, which should be beneficial to human health (Chilliard et al., 2000). When dairy cows were fed oleamide at 3.5% of the ration DM, milk $18:1(n–9)$ increased from 23 to 48% of total fatty acids concomitantly with a decline in milk 16:0 from 34 to 20% (Jenkins, 1998).

Protecting polyunsaturated fatty acids (**PUFA**) from biohydrogenation by their conversion to amides has not been investigated. Increasing the absorption of PUFA in ruminants may be useful in altering the physical properties of milk (Chilliard et al., 2000) or regulating tissue synthesis of the metabolically active eicosenoids and prostaglandins (Gorlin, 1988; Kinsella et al., 1990). Although feeding oleamide to dairy cows did not increase milk $18:2(n–6)$ (Jenkins, 1998), this was not a

Table 1. Fatty acid content and composition of the linoleic acid and linoleamide supplements used in the ruminal in vitro cultures and sheep studies

Fatty acids	Linoleic acid	Linoleamide
Total, g/100 g fat	93.0	84.7
Composition, g/100 g total		
16:0	2.6	2.5
18:0	1.0	0.9
18:1 (n–9)	28.9	28.9
18:2 (n–6)	61.4	61.8
18:3 (n–3)	5.4	5.3
Other	0.7	0.6

Table 2. Ingredients and composition of diets containing 5% added linoleic acid or linoleamide (sheep Exp. 1)

Item	Control	Linoleic acid	Linoleamide
Ingredient, % of DM			
Alfalfa meal	30.0	30.0	30.0
Cracked corn	34.0	28.0	28.0
Soybean meal (48% CP)	5.0	6.3	6.3
Soybean hulls	30.0	30.0	30.0
Linoleic acid	0.0	5.0	0.0
Linoleamide	0.0	0.0	5.0
Salt[a]	0.5	0.5	0.5
Ammonium chloride	0.5	0.5	0.5
Composition, % of DM			
CP	16.7	17.5	19.1
NDF	45.4	43.4	42.8
ADF	27.8	28.0	27.9
Fatty acids	2.2	5.9	5.2

[a]Morton 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.

fair evaluation due to the low PUFA concentration of oleamide (5 g 18:2 (n–6)/100 g total fatty acids).

This study was conducted to determine whether linoleamide maintains higher concentrations of 18:2 (n–6) in ruminal cultures after 24 and 48 h of incubation compared to an equal quantity of free acid. The linoleamide supplement also was fed to sheep to determine whether the amide resisted biohydrogenation in vivo and enhanced 18:2 (n–6) concentration in duodenal contents.

Materials and Methods

Lipid Supplements

Linoleic acid was purchased from Henkel Corporation (Emersol 315, Cinncinati, OH) and added to the in vitro substrate and sheep diets as received, or it was used to synthesize linoleamide (Table 1). The linoleamide was synthesized by combining linoleic acid with urea (1:2 molar ratio). Butylated hydroxytoluene was added to the fatty acid at 0.05% to protect against oxidative rancidity. The fatty acid-urea mixture was placed in a household pressure cooker and flushed with nitrogen gas, and the lid was sealed. The container was heated to 190°C on a hot plate for 4 h. The cooker was then removed from the hot plate and cooled to < 100°C. Contents of the cooker were strained through four layers of cheesecloth to remove solids. The product passing through the cheesecloth was allowed to cool until it solidified to a hard, waxy consistency. No attempt was made to remove unreacted fatty acids or urea. The product was melted prior to being mixed with the other concentrate ingredients.

Rumen In Vitro

In vitro incubations followed the procedure of Goering and Van Soest (1970) except that flasks were gassed with CO_2 before incubation and during each sample collection rather than continuously. Also, culture volumes were increased as described by Beam et al. (2000) to allow sufficient volume for subsampling. Inoculum was taken from a nonlactating, ruminally fistulated Holstein cow fed 1 kg of concentrate and with ad libitum

access to bermudagrass hay as described in exp. 1 to 4 of Beam et al. (2000).

To determine the initial and final 18:2 (n–6) concentration after sufficient incubation time to assess extent of biohydrogenation, samples were taken from each culture flask at 0, 24, and 48 h while being stirred with a magnetic stir bar. Two 5-mL samples were immediately placed in an ice bath until all samples were taken and could be stored at –5°C. They were later analyzed for fatty acids by gas/liquid chromatography following methylation in methanolic HCl for 16 h to allow for the complete conversion of fatty amides to methyl esters. An additional 5-mL sample was taken at 24 and 48 h and analyzed for VFA (Jenkins et al., 2000). Substrates consisted of bermudagrass hay (ground in a Wiley mill through a 2-mm screen) containing no added lipid (control), 10% linoleic acid, or 10% linoleamide. Lipids were added at levels higher than normally encountered in vivo to ensure that linoleic acid caused a drop in the acetate:propionate ratio, with the objective of determining whether the drop could be reversed with linoleamide. Because purities of the fat sources were not examined in detail prior to starting the experiment, the linoleic acid and linoleamide supplements were added to the hay substrate on an equal weight basis. In vitro cultures were run in triplicate at 39°C under anaerobic conditions.

Corrected linoleic and oleic acid concentrations were calculated by subtracting fatty acids in the control cultures from fatty acids in the cultures with added lipid. The corrected fatty acids at each sample time were divided by the corrected fatty acids at 0 h to determine the fraction remaining (**FR**).

Sheep Studies

Sheep experiment 1 evaluated three diets (Table 2) that contained either no added fat (control), 5% added

linoleic acid, or 5% linoleamide (DM basis). Lipids were added in place of corn with additional soybean meal added to keep the diets isonitrogenous. The complete diets met or exceeded CP and mineral requirements (NRC, 1985) of the sheep, which averaged 36 kg BW at the start of the study.

Prior to feeding the experimental diets, the sheep (Polypay × Border Leicester × Romney) were castrated, wormed, and surgically fitted with a simple T-cannula (ANKOM Technology, Fairport, NY) in the duodenum anterior to the bile and pancreatic ducts. Surgery was done under general anesthesia with a minimum of 10 d of recovery before feeding the experimental diets. Procedures for surgery and postsurgical animal care were approved by The Institutional Animal Care and Use Committee (Protocol No. 00-010).

Three sheep were fed the three diets in a 3 × 3 Latin square with 2-wk periods. The sheep were housed individually in stainless steel pens in an environmentally controlled room. All animals had ad libitum access to feed and water. Feed was provided twice daily at 0900 and 1600. Feed refusals were removed and weighed each day prior to the morning feeding. Feed samples (mixed diet) were taken daily over the last 4 d of each period. Duodenal contents (approximately 20 mL) were collected twice daily over the last 4 d of each period starting with samples at 0900 and 1300 on d 11. Duodenal samples were taken 1 h later than the previous day during d 12, 13, and 14. Samples of duodenal contents were composited for each sheep in each period and freeze-dried.

Samples of feed, feed refusals, and dried duodenal contents were ground in a centrifugal mill through a 0.5-mm screen. Ground samples were analyzed for DM (100°C), Kjeldahl N (AOAC, 1990), ADF, and NDF (Procedure A, Van Soest et al., 1991). Fatty acids in ground feed, refusal, and duodenal samples were methylated in acetyl chloride and methanol (1:10, vol/vol) at 70°C for 16 h to ensure complete hydrolysis of the amide bond (Jenkins et al., 2001). Heptadecanoic acid was added to all samples as an internal standard.

Methyl esters of fatty acids were analyzed by gas/liquid chromatography on a 30-m × 0.25-mm i.d. polyamino glycol capillary column (Supleco, Bellefonte, PA). The column oven was programmed from an initial temperature of 150°C held for 2 min, increased at a rate of 2 C°/min, and then held at a final temperature of 220°C for 10 min. Injector and detector temperatures were maintained at 250°C. Helium was used as the carrier gas.

Sheep Exp. 2 also was a 3 × 3 Latin square using the same three duodenally cannulated sheep. It was run 2 mo after sheep Exp. 1 to evaluate the effects of a lower dose of added lipid. All methods were the same as sheep Exp. 1 except that diets contained only 1.5% added linoleic acid or linoleamide (Table 3). Adjustments were made in corn and soybean meal percentages to accommodate the lower fat percentage.

Table 3. Ingredients and composition of diets containing 1.5% added linoleic acid or linoleamide (sheep Exp. 2)

Item	Control	Linoleic acid	Linoleamide
Ingredient, % of DM			
Alfalfa meal	30.0	30.0	30.0
Cracked corn	34.0	32.0	32.0
Soybean meal (48% CP)	5.0	5.4	5.4
Soybean hulls	30.0	30.0	30.0
Linoleic acid	0.0	1.5	0.0
Linoleamide	0.0	0.0	1.5
Salt[a]	0.5	0.5	0.5
Ammonium chloride	0.5	0.5	0.5
Composition, % of DM			
CP	15.6	15.9	17.0
NDF	44.2	43.3	43.5
ADF	29.3	28.0	28.8
Fatty acids	1.92	2.88	2.89

[a]Morton 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.

Statistics

Data from the in vitro and sheep experiments were analyzed by ANOVA using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC). Data from the vitro study were analyzed as a split-plot design accounting for variation due to treatment, replication (treatment), hour, and the treatment × hour interaction. Treatment effects were tested against an error term of replication (treatment). Because the treatment × hour interaction was not significant for any VFA, only the treatment main effect is shown in Table 4.

Each sheep experiment was analyzed as a 3 × 3 Latin square design. The analysis for the in vitro and sheep experiments included single degree of freedom contrasts for two planned comparisons: 1) the control vs linoleic acid treatments and 2) the linoleic acid vs linoleamide treatments. Exact probability values (<0.15 are given for each contrast.

Results

Rumen In Vitro

Corrected 18:2 (n–6) concentration in the linoleic acid cultures declined from 2.51 to 0.11 mg/5 mL over 48 h of incubation (Figure 1). Compared to the linoleic acid cultures, the corrected 18:2 (n–6) concentrations in the linoleamide cultures were lower ($P < 0.01$) at 0 h but higher ($P < 0.01$) at 24 and 48 h. Differences in 0 h 18:2 (n–6) concentrations between the two lipid treatments were eliminated by expressing results as FR (Figure 1). The FR of 18:2 (n–6) was higher ($P < 0.01$) for linoleamide than for the linoleic acid treatment at both 24 and 48 h of incubation.

Corrected 18:1(n–9) concentration in the linoleic acid cultures also declined with incubation time, but the decline in 18:1(n–9) over time was much lower for the

Table 4. Volatile fatty acids in cultures of mixed ruminal microbes containing substrates supplemented with either 10% linoleic acid or 10% linoleamide

| Volatile fatty acid	Diet[a]			SEM	P-value[b]	
	Control	Linoleic acid	Linoleamide		1	2
Total, mmol/L	73.2	64.6	62.2	2.2	0.03	NS
Acetate, mol/100 mol	61.2	58.6	60.6	0.4	<0.01	0.02
Propionate, mol/100 mol	17.9	20.4	16.7	0.07	<0.01	<0.01
Butyrate, mol/100 mol	9.7	9.0	10.0	0.1	0.02	<0.01
Isobutyrate, mol/100 mol	2.6	2.8	2.9	0.05	0.05	0.10
Valerate, mol/100 mol	4.8	5.1	5.5	0.1	0.14	0.05
Isovalerate, mol/100 mol	3.8	4.1	4.3	0.1	0.13	NS
Acetate:propionate ratio	3.43	2.88	3.63	0.03	<0.01	<0.01

[a]Means were averaged over all incubation times.
[b]Probabilties that 1) the control and linoleic acid diets differed and 2) the linoleic acid and linoleamide diets differed. NS = contrasts with $P > 0.15$.

linoleamide cultures (Figure 2). Similar to 18:2 (n–6), the FR of 18:1(n–9) at 24 and 48 h were lower ($P <$ 0.01) for the linoleic acid cultures compared with the linoleamide cultures.

The 0-h concentration of *trans*-18:1 and 18:0 were equal in both the linoleic acid and linoleamide cultures (Figure 3). By 24 h of incubation, *trans*-18:1 concentra-

tion was higher ($P < 0.05$) for linoleic acid compared to linoleamide. Trans 18:1 concentration stabilized from 24 to 48 h for both lipid supplements. The concentration of 18:0 also was higher ($P < 0.05$) for linoleic acid compared with linoleamide at 24 and 48 h of incubation. Unlike *trans* 18:1, the concentration of 18:0 continued to increase from 24 to 48 h.
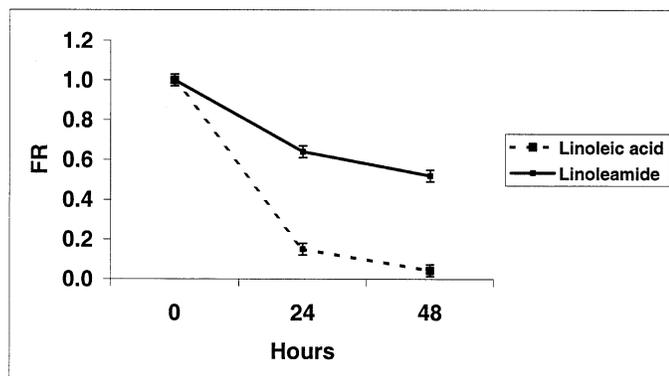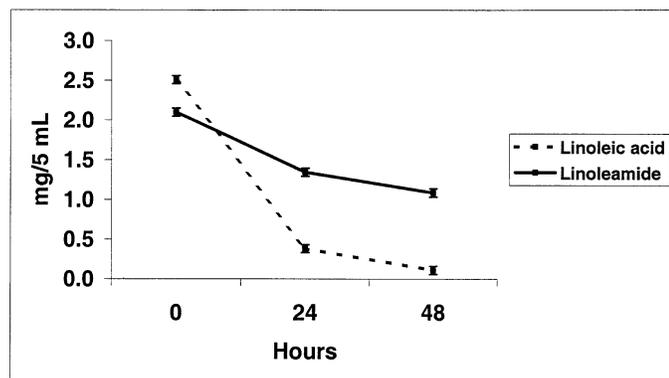
Figure 1. The effect of incubation time on corrected (treatment minus control values at the same incubation time) 18:2 (n–6) concentration (mg/5 mL culture) and fraction remaining (FR) in cultures of mixed ruminal microbes containing substrate supplemented with either 10% linoleic acid or 10% linoleamide. Each point is the mean of three observations with bars for the pooled SEM.
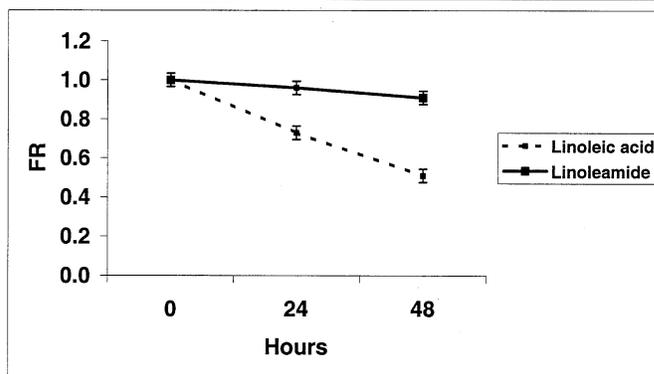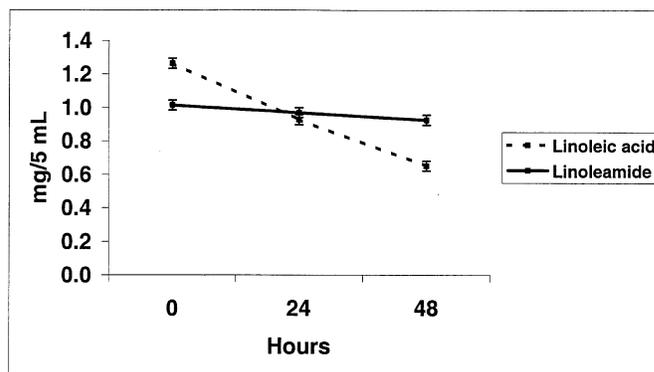
Figure 2. The effect of incubation time on corrected (treatment minus control values at the same incubation time) 18:1 (n–9) concentration (mg/5 mL culture) and fraction remaining (FR) in cultures of mixed ruminal microbes containing substrate supplemented with either 10% linoleic acid or 10% linoleamide. Each point is the mean of three observations with bars for the pooled SEM.
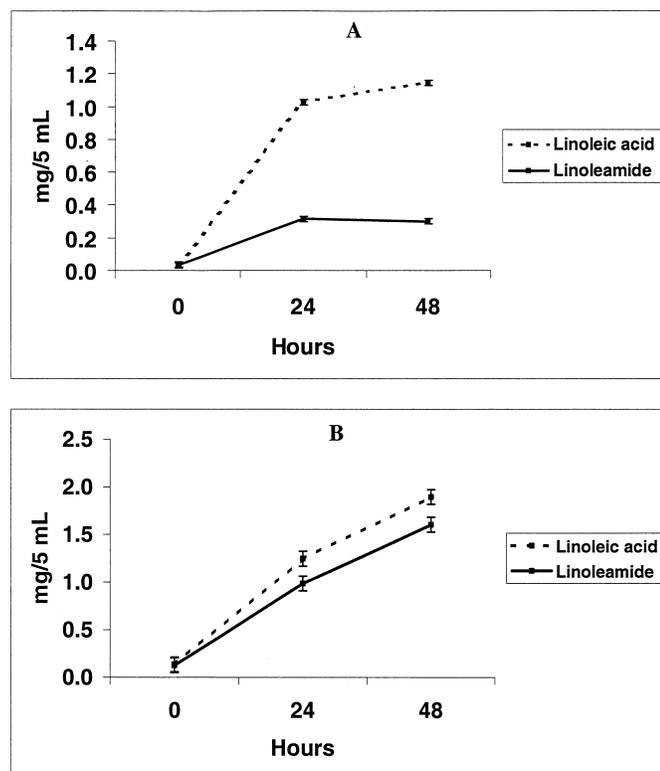
Figure 3. The effect of incubation time on (A) corrected (treatment minus control values at the same incubation time) *trans*-18:1 and (B) corrected 18:0 concentration in cultures of mixed ruminal microbes containing substrate supplemented with either 10% linoleic acid or 10% linoleamide. Each point is the mean of three observations with bars for the pooled SEM.

Linoleic acid added to the cultures reduced total VFA concentration and acetate but increased propionate, causing a decline in the acetate:propionate ratio (Table 4). Linoleic acid also decreased butyrate but increased the remaining VFA. Linoleamide reversed the effects of linoleic acid on acetate, propionate, and the acetate:propionate ratio but still reduced total VFA concentration similar to linoleic acid.

Sheep Studies

Linoleic acid and linoleamide did not alter DMI when fed at either 5% (Table 5) or 1.5% (Table 6) of the ration DM. Adding 5% linoleic acid to the diet in sheep Exp. 1 increased the concentration of all fatty acids in the diet DM, but 18:2 (*n*–6) increased the most, followed by 18:1(*n*–9), as expected (Table 5). Compared to the linoleic acid diet, the concentrations of all fatty acids in the feed DM were lower for the linoleamide diet.

The concentrations of all fatty acids in the duodenal DM increased relative to their concentrations in the feed DM in sheep Exp. 1. Compared to the control diet, linoleic acid fed at 5% of the feed DM in sheep Exp. 1 increased the concentration of all unsaturated fatty acids in the duodenal DM. However, it had no effect on the concentrations of 16:0 or 18:0 in the duodenum. The concentration of *trans*-18:1 increased the most (increase of 26.8 mg/g) in the duodenal DM when 5% linoleic acid was fed to sheep, followed by 18:2 (*n*–6) and 18:1(*n*–9), which both increased by 10 mg/g.

Compared to the linoleic acid diet, the linoleamide fed to sheep in Exp. 1 increased the concentration of all unsaturated fatty acids (except *trans*-18:1) in the duodenal DM at the expense of 18:0. The 18:2 (*n*–6) and 18:1(*n*–9) concentrations both increased 4.7 mg/g duodenal DM when linoleamide replaced linoleic acid in the diet. The increase in concentration of 18:3 (*n*–3) was only 0.3 mg/g duodenal DM. Replacing linoleic acid with linoleamide had no effect on total fatty acid concentration in the duodenal DM in sheep Exp. 1.

In sheep Exp. 2, the linoleic acid and linoleamide supplements were fed at 1.5% of the feed DM. Adding 1.5% linoleic acid increased the concentrations of 18:2 (*n*–6), 18:1 (*n*–9), and 16:0 in the feed, but it had no effect on the concentrations of 18:0 or 18:3 (*n*–3) (Table 6). Linoleic acid addition at 1.5% of the feed DM also increased total fatty acid concentration in feed from 19.2 to 28.8 mg/g. The only effects of replacing linoleic acid with linoleamide on fatty acid concentration in the feed DM were to reduce 18:1(*n*–9) and increase 18:3 (*n*–3).

The 1.5% linoleic acid supplement in sheep Exp. 2 increased total fatty acid concentration and the concentrations of both 18:2 (*n*–6) and 18:1(*n*–9) in the duodenal DM. No other fatty acids were affected. The only effect of replacing linoleic acid with linoleamide on duodenal fatty acids in sheep Exp. 2 was a numerical increase (*P* = 0.15) in 18:2 (*n*–6) concentration. The concentrations of 18:1(*n*–9) and 18:3(*n*–3) were not changed in duodenal DM by replacing linoleic acid with linoleamide in the feed.

Discussion

The concentration of 18:2 (*n*–6) in the cultures at 0 h and the concentration of 18:2 (*n*–6) in the sheep feed were lower for linoleamide than for linoleic acid. The lower fat content for linoleamide can be attributed to the presence of the ammonia moiety in the amide and also attributed to contamination from unreacted urea. Despite the lower supply of initial 18:2 (*n*–6) in the cultures and in the sheep feed supplemented with linoleamide, it still maintained higher 18:2 (*n*–6) in the cultures through 24 and 48 h and also higher concentrations of 18:2 (*n*–6) in duodenal contents compared to the linoleic acid supplement.

The higher 18:2 (*n*–6) concentrations maintained in vitro and in vivo by linoleamide demonstrated its protection from biohydrogenation. However, the protection was not complete. Loss of 18:2 (*n*–6) still occurred when linoleamide was exposed to fermentation by ruminal microbes, although the loss over time was markedly lower for linoleamide than for linoleic acid. Ruminal

Table 5. Feed intake and concentration of fatty acids in feed and duodenal contents of sheep fed 5% linoleic acid or 5% linoleamide (sheep Exp. 1)

| | Diet | | | | P-value[a] | |
Item	Control	Linoleic acid	Linoleamide	SEM	1	2
DMI, g/d	1,097	1,426	1,142	196	NS	NS
Fatty acid, mg/g feed DM						
16:0	3.7	4.5	4.2	0.06	0.01	0.07
18:0	0.8	1.2	1.1	< 0.01	< 0.01	< 0.01
18:1 (n−9)	4.9	16.8	13.6	0.3	< 0.01	0.02
18:2 (n−6)	10.1	32.1	28.1	0.4	< 0.01	0.02
18:3 (n−3)	1.2	3.1	2.9	0.06	< 0.01	0.14
Other	1.2	1.3	2.0	0.4	NS	NS
Total	22.0	59.0	51.9	0.7	< 0.01	0.02
Fatty acid, mg/g duodenal DM						
16:0	10.6	9.4	9.7	1.0	NS	NS
18:0	28.4	33.9	15.5	5.9	NS	0.15
Trans-18:1	5.0	31.8	48.6	7.8	0.14	NS
18:1 (n−9)	3.1	13.1	17.8	1.4	0.04	0.15
18:2 (n−6)	2.5	12.2	16.8	0.6	< 0.01	0.04
18:3 (n−3)	0.2	1.0	1.3	0.03	< 0.01	0.02
Other	3.5	4.4	4.5	0.5	NS	NS
Total	53.2	105.8	114.2	5.6	0.02	NS

[a]Probabilties that 1) the control and linoleic acid diets differed and 2) the linoleic acid and linoleamide diets differed. NS = contrasts with $P > 0.15$.

losses also were reported for oleamide. Reeves et al. (1998) showed that the disappearance rate of 18:1(n−9) from ruminal cultures declined from 6.4 to 2.5%/h when oleic acid was replaced with oleamide. However, this reduction in the rate of oleamide disappearance was still sufficient to increase milk 18:1(n−9) from 23.2 to 48.2% of total fatty acids when it was fed to lactating Holstein cows. Reeves et al. (1998) theorized that amides resist biohydrogenation only when the amide bond is intact because a free carboxyl group is required for

action of certain microbial enzymes associated with biohydrogenation.

Given that some microbial degradation of fatty amides occurs within the rumen, then the actual degree of protection is not a constant but instead would vary depending on ruminal turnover. Feeding conditions that promote a high degree of ruminal turnover, such as high concentrate rations fed at high levels of intake, would enhance the ruminal escape of linoleamide. Ruminal turnover was probably high for the sheep in Exp.

Table 6. Feed intake and concentration of fatty acids in feed and duodenal contents of sheep fed 1.5% linoleic acid or 1.5% linoleamide (sheep Exp. 2)

| | Diet | | | | P-value[a] | |
Item	Control	Linoleic acid	Linoleamide	SEM	1	2
DMI, g/d	1,842	1,778	1,779	30	NS	NS
Fatty acid, mg/g feed DM						
16:0	3.3	3.8	3.7	0.07	0.04	NS
18:0	0.8	1.1	1.2	0.1	NS	NS
18:1 (n−9)	4.1	7.7	6.6	0.2	< 0.01	0.07
18:2 (n−6)	8.4	13.6	13.7	0.4	0.01	NS
18:3 (n−3)	1.5	1.4	1.9	0.1	NS	0.14
Other	1.1	1.2	1.8	0.1	NS	NS
Total	19.2	28.8	28.9	0.4	<0.01	NS
Fatty acid, mg/g duodenal DM						
16:0	7.6	8.1	7.9	0.4	NS	NS
18:0	21.4	27.8	27.5	2.8	NS	NS
Trans-18:1	4.6	7.3	9.5	1.2	NS	NS
18:1 (n−9)	3.3	5.5	6.1	0.3	0.04	NS
18:2 (n−6)	2.8	3.6	4.3	0.2	0.14	0.15
18:3 (n−3)	0.6	0.6	0.8	0.05	NS	NS
Other	4.0	3.7	4.8	0.6	NS	NS
Total	44.3	56.5	60.9	3.1	0.11	NS

[a]Probabilities that 1) the control and linoleic acid diets differed and 2) the linoleic acid and linoleamide diets differed. NS = contrasts with $P > 0.15$.

1 and 2 because they were offered feed at ad libitum intake and particle size of the forage fraction (ground alfalfa hay and soybean hulls) was small.

Aside from 18:2 (n–6), changes in other fatty acids supported the resistance of the linoleamide supplement from biohydrogenation. The linoleamide cultures had a higher FR of 18:1(n–9) and lower concentrations of biohydrogenation products including *trans*-18:1 and 18:0. When sheep were fed 5% linoleamide, 18:0 also was lower in duodenal contents compared to linoleic acid. However, *trans*-18:1 concentrations in the duodenum were not different for the two lipid supplements. Fat supplements resistant to biohydrogenation usually also lessen or prevent negative effects of the unsaturated fatty acids on fermentation, signaled by normal total VFA concentrations and a normal ratio of acetate:propionate. Converting the linoleic acid to linoleamide reversed a drop in the ratio of acetate:propionate but had no effect on returning total VFA concentration to normal.

Duodenal 18:2 (n–6) concentration usually remains below 10 mg/g DM regardless of the type and amount of fat being fed. For example, duodenal 18:2 (n–6) concentrations calculated from previous studies were 1.3 mg/g DM for sheep fed 10.5% whole linseed (Wachira et al., 2000), 8.1 mg/g DM for dairy cows fed 3.7% high-linoleic sunflower oil (Kalscheur et al., 1997), 4.9 mg/g DM for dairy cows fed 3.6% tallow plus18% ground cottonseed (Pires et al., 1997), and 6.0 mg/g DM for dairy cows fed 5.9% calcium salts of palm fatty acids (Wu et al., 1991). Duodenal 18:2 (n–6) concentration reached 9.2 mg/g DM for sheep fed 6.7% calcium salts of soybean fatty acids (Enjalbert et al., 1994). However, in sheep Exp. 2, duodenal 18:2 (n–6) concentrations exceeded 12 and 16 mg/g DM for the 5% linoleic acid and linoleamide diets, respectively.

Compared to the control diet, feeding 5% linoleamide was effective at increasing duodenal 18:2 (n–6) concentration nearly seven-fold (from 2.5 to 16.8 mg/g). However, when linoleamide was compared to linoleic acid, duodenal 18:2 (n–6) concentrations were only increased 1.4-fold (12.2 to 16.8 mg/g DM). At the start of the study, the unprotected linoleic acid supplement was expected to increase duodenal 18:2 (n–6) concentration only slightly or not at all because of biohydrogenation. Because unprotected linoleic acid substantially increased duodenal 18:2 (n–6) concentration in this study, it became more difficult to establish a high degree of protection for the linoleamide.

Linoleic acid interferes with its own biohydrogenation when present in ruminal contents at high concentrations. The final step of biohydrogenation, in which *trans*-18:1 is converted to 18:0 seems more sensitive to 18:2 (n–6) concentration than the initial conversion of 18:2 (n–6) to *trans*-18:1, although both steps can be affected (Harfoot et al., 1973). The inhibitory effects of 18:2 (n–6) on biohydrogenation also appear more severe when it is supplied as the free acid rather than supplied as a triglyceride (Beam et al., 2000). This may explain why feeding 5% linoleic acid to sheep in Exp. 1 yielded duodenal 18:2 (n–6) concentrations that were considerably higher than when sheep were fed 5.7% soy oil (12.2 vs 2.6 mg/g DM, respectively) in the study of Enjalbert et al. (1994).

Because 18:2 (n–6) interferes with biohydrogenation more as the free acid, the resistance of linoleamide to biohydrogenation may appear lower when compared to unprotected linoleic acid rather than when compared to a control diet or a triglyceride supplement. If free linoleic acid appreciably interferes with biohydrogenation, as it did in this study, other fat supplements that equal or exceed linoleic acid in enhancing duodenal 18:2 (n–6) concentration could be argued as demonstrating good protection.

However, the difference between the free acid and triglyceride forms is concentration-dependent. At low levels of supplementation, the free acid may have little or no negative effect on biohydrogenation and yield unsaturated fatty acid flows to the intestine that are comparable to triglycerides or a control diet. As the level of supplementation is increased, greater intestinal flow of unsaturated fatty acid will likely occur for the free acid compared to the triglyceride.

It would be desirable to compare duodenal 18:2 (n–6) concentrations for ruminants fed linoleamide vs formaldehyde-treated fats because of the high protection shown for encapsulation of lipids by formaldehyde-treated proteins since the 1970s (Chilliard et al., 2000). Although information is abundant concerning the effects of formaldehyde-treated fats on fatty acid composition of tissue and milk lipids, data concerning its effects on duodenal fatty acid composition are scarce. In a study by Scott et al. (1971), goats were fed formaldehyde-treated linseed oil to examine the effects on fatty acid composition of abomasal contents. Although abomasal 18:2 (n–6) expressed in milligrams per gram of DM could not be determined from the Scott et al. (1971) study, their data showed that protected linseed oil increased abomasal 18:2 (n–6) from 3.1 to 11.5% of total fatty acids. In the present study, duodenal 18:2 (n–6) concentrations were 5.0, 12.5, and 14.0% of total fatty acids for the control, 5% linoleic acid, and 5% linoleamide treatments, respectively (data not shown). However, the intakes of 18:2 (n–6) were not identical for the two studies. Sutton et al. (1983) reported 94.7 mg 18:2 (n–6)/mg of protected linseed oil prepared according to the procedure of Scott et al. (1971). Using this concentration of 18:2 (n–6) for protected linseed oil, an estimate of the 18:2 (n–6) concentration in the feed used by Scott et al. (1971) is about 25 mg/g DM. Feed concentrations of 18:2 (n–6) in the present study were 32.1 and 28.2 mg/g DM for the linoleic acid and linoleamide treatments, respectively. Even though 18:2 (n–6) concentration in the feed was approximately 11% higher (28 vs 25 mg/g DM) for sheep fed 5% linoleamide (sheep Exp. 1) vs goats fed formaldehyde-protected linseed oil (Scott et al., 1971), the postruminal concentration of 18:2 (n–6) was 22% higher for linoleamide.

Oleamide added to the feed at less than 5% of the DM reduced feed intake in both cattle (Jenkins, 1998) and sheep (Reeves et al., 1998). Linoleamide had no effect on DMI in this study when fed at either 1.5 or 5% of the diet DM. Oleamide reduced feed intake when administered directly into the rumen of cattle, suggesting that the satiety response is physiological rather than palatability-related (Jenkins et al., 2000). However, in a study with beef cattle fed oleamide over a 9-wk period, the feed intake depression for oleamide lessened over time until nearly returning to normal by wk 9 (Jenkins et al., 2000).

Implications

The results of this study showed that linoleamide resists biohydrogenation by ruminal microbes consistent with the protection previously reported for oleamide. Although the protection of 18:2 (n–6) in amide form was not complete, it was sufficient to maintain higher concentrations of 18:2 (n–6) in ruminal cultures over 48 h of incubation and to increase the concentration of 18:2 (n–6) in duodenal contents of sheep. Linoleamide offers a mechanism, via dietary manipulation, to increase the intestinal absorption of 18:2 (n–6) in ruminant species when it is desired to manipulate the concentration of polyunsaturated fatty acids in milk or other tissue lipids.

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