

Effect of Alfalfa Forage Preservation Method and Particle Length on Performance of Dairy Cows Fed Corn Silage-Based Diets and Tallow

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ABSTRACT

A study was conducted to evaluate the effect of including alfalfa preserved either as silage or long-stem or chopped hay on DMI and milk fat production of dairy cows fed corn silage-based diets with supplemental tallow (T). Fifteen Holstein cows that averaged 117 DIM were used in a replicated 5 × 5 Latin square design with 21-d periods. Treatments (DM basis) were: 1) 50% corn silage:50% concentrate without T (CS); 2) 50% corn silage:50% concentrate with 2% T (CST); 3) 25% corn silage:25% short-cut alfalfa hay:50% concentrate with 2% T SAHT); 4) 25% corn silage:25% long-cut alfalfa hay:50% concentrate with 2% T (LAHT); and 5) 25% corn silage:25% alfalfa silage:50% concentrate with 2% T (AST). Cows were allowed ad libitum consumption of a TMR fed 4 times daily. Diets averaged 16.4% CP and 30.3% NDF. Including 2% T in diets with corn silage as the sole forage source decreased DMI and milk fat percentage and yield. Replacing part of corn silage with alfalfa in diets with 2% T increased milk fat percentage and yield. The milk fat of cows fed CST was higher in *trans*-10 C18:1 than that of cows fed diets with alfalfa. No effect of alfalfa preservation method or hay particle length was observed on DMI and milk production. The milk fat percentage and yield were lower, and the proportion of *trans*-10 C18:1 in milk fat was higher for cows fed LAHT than for cows fed SAHT. Alfalfa preservation method had no effect on milk fat yield. Ruminal pH was higher for cows fed alfalfa in the diets, and it was higher for cows fed LAHT than SAHT. Feeding alfalfa silage or chopped hay appears to be more beneficial than long hay in sustaining milk fat production when 2% T is fed with diets high in corn silage. These results support the role of *trans* fatty acids in milk fat depression.

(Key words: tallow, milk fat, alfalfa, particle length)

Abbreviation key: A:P = ruminal acetate to propionate ratio; AST = 25% corn silage:25% alfalfa si-

lage:50% concentrate with 2% T; BH = biohydrogenation; CLA = conjugated linoleic acid; CS = 50% corn silage:50% concentrate without T; CST = 50% corn silage:50% concentrate with 2% T; FA = fatty acid; LAHT = 25% corn silage:25% long-cut alfalfa hay:50% concentrate with 2% T; SAHT = 25% corn silage:25% short-cut alfalfa hay:50% concentrate with 2% T; T = tallow.

INTRODUCTION

The differential responses to dietary fats observed when feeding different basal diets to dairy cows have been attributed to diet × fat interactions. Smith et al. (1993) showed that the depression in milk production and fat percentage observed when cottonseed and tallow (T) were added to corn silage diets was overcome by replacing 25 or 50% of the corn silage with alfalfa hay. Onetti et al. (2001) indicated that supplementing 2 and 4% T or choice white grease to diets containing processed high grain hybrid corn silage as the sole forage source had negative effects on rumen fermentation, DMI, and milk fat percentage. A study designed to evaluate whether replacing 25 or 50% of corn silage with alfalfa silage, typical forage fed in the Midwest, would reverse this negative impact (Onetti et al., 2002) showed no beneficial effect. We hypothesized that the reason for the differences in our findings compared with the study by Smith et al. (1993) was the use of alfalfa silage vs. alfalfa hay. Grant and Weidner (1992) observed that replacing approximately 15% of alfalfa silage with alfalfa hay, when 11.6% whole soybeans (DM basis) were fed, increased milk fat percentage and had no effect on 4% FCM. Total chewing time in that study was greatest for diets with alfalfa hay; this was mainly due to increased rumination. Cows consuming diets consisting of 55% alfalfa hay spent more time ruminating and chewing per kilogram of NDF than cows consuming the same amount of alfalfa silage (Nelson and Satter, 1990). Rumination and total chewing activities have been associated with increased saliva output, which plays an essential role in buffering acids produced during rumen fermentation and stabilizing rumen pH (Beauchemin and Buchanan-Smith, 1989; Al-

Received April 18, 2003.

Accepted July 28, 2003.

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len, 1997). Replacing alfalfa silage with coarsely chopped alfalfa hay increased rumination activity and numerically increased mean ruminal pH (Allen and Grant, 2000). Most of the studies that observed increased rumination and rumen pH when replacing alfalfa silage by alfalfa hay also observed increased particle size in the diets.

Milk fat depression is a consequence of shifts in biohydrogenation (BH) pathways, accumulation of *trans* fatty acids (FA) in the rumen, and subsequent inhibition of milk fat synthesis in the mammary gland (Bauman and Griinari, 2001). Kalscheur et al. (1997) observed reduced duodenal *trans* FA flow, decreased *trans* FA content in milk, and increased milk fat percentage when buffer was added to high-concentrate diets, implying a direct effect of pH on ruminal BH. Therefore, we hypothesized that alfalfa hay, particularly long-stem alfalfa hay, but not alfalfa silage would alleviate T-induced milk fat depression of dairy cows fed corn silage-based diets by decreasing *trans* FA formation in the rumen.

The objective of this study was to evaluate the effects of including alfalfa preserved either as silage or chopped or long-stem hay on chewing behavior, ruminal pH, *trans* FA formation in the rumen, and milk fat production of dairy cows fed corn silage-based diets with supplemental T. A second objective was to investigate whether the milk fat depression observed when T is fed with diets with corn silage as the only forage source is related to incomplete BH of polyunsaturated FA.

MATERIALS AND METHODS

Animals

Fifteen multiparous Holstein cows that averaged (\pm SD) 117 ± 28 DIM and 688 ± 7 kg of BW were used in a replicated 5×5 Latin square design with 21-d periods. Two squares consisted of cows with rumen fistulas and one of cows without rumen fistulas. Cows within a square were assigned randomly to dietary treatments. Treatment sequences were ordered to minimize residual or carryover effects of any treatment in the succeeding period. Cows were housed individually in a tie-stall and stanchion barn and had free-choice access to water. All cows were injected with 250 mg of bST (Posilac, Monsanto Company, St. Louis, MO) on the same day every week. Animals were handled according to the recommendations and procedures approved by the Animal Care and Use Committee of the College of Agriculture and Life Science of the University of Wisconsin-Madison. Two cows were removed from the study due to teat injuries. One cow was removed during the first experimental period, and a replacement cow was used for the rest of the experiment. The second cow was

removed from the experiment during the last period; therefore, information of the preceding 4 periods was used for this particular cow.

Diets

Experimental treatments, as a percentage of total diet DM, were: 1) 50% corn silage:50% concentrate without T (CS); 2) 50% corn silage:50% concentrate with 2% T (CST); 3) 25% corn silage:25% short alfalfa hay:50% concentrate with 2% T (SAHT); 4) 25% corn silage:25% long alfalfa hay:50% concentrate with 2% T (LAHT); and 5) 25% corn silage:25% alfalfa silage:50% concentrate with 2% T (AST). Diets were formulated to be isonitrogenous and to meet or exceed the National Research Council (NRC, 2001) nutrient allowances for a lactating cow (623 kg of BW and 150 DIM) producing 36.2 kg/d of milk with 3.3% fat and eating 24.7 kg of DM. Ingredient composition of experimental diets is shown in Table 1. When replacing corn silage with alfalfa in diets containing T, the amount of corn grain was increased and soybean meal was reduced to make diets isonitrogenous and isoenergetic. The corn hybrid used was Cargill 3677 (Cargill Inc., Minneapolis, MN) and was selected for high grain yield to minimize treatment differences in NDF. Corn silage was processed (2-mm roll clearance) and chopped at a theoretical length of 12 mm. Second crop alfalfa (Voyager II, Lemke Seeds, Mequon, WI) was harvested as hay in bales or as silage in an upright silo, on the same day. Theoretical length of chop of alfalfa silage was 10 mm. Short hay was obtained by processing the long hay through a chopper (AgriMetal Inc., Wickham, QC, Canada). Average (\pm SD) DM, NDF, and CP concentration of corn silage was 36.0 ± 3.3 , 38.1 ± 5.4 , and 8.5 ± 0.3 ; of alfalfa silage was 52.4 ± 0.9 , 42.3 ± 1.7 , and 18.7 ± 0.7 ; of long hay was 89.0 ± 1.8 , 47.1 ± 0.4 , and 18.1 ± 0.7 ; and of short hay was 89.0 ± 1.8 , 45.9 ± 1.8 , and $15.8 \pm 0.2\%$, respectively. Tallow was incorporated into concentrates and then added to TMR. Fatty acid composition (g/100 g of FA) of T (Packerland Packing Co., Inc, Green Bay, WI) was 2.6% C14:0, 24.2% C16:0, 3.4% C16:1, 19.2% C18:0, 44.2% C18:1, 3.4% C18:2, 0.3% C18:3, and 2.7% other FA. Cows were fed the diets 4 times daily (0900, 1500, 2100, and 0300 h) as a TMR to allow 10% feed refusal on an as-fed basis. For LAHT, long-stem hay was hand-mixed in the manger with the rest of the feed ingredients that were fed as a TMR.

Nutrient composition of experimental diets is shown in Table 2. Supplemental T increased NE_L content of diets by 0.07 Mcal/kg DM. Neutral detergent fiber concentration was 1.6 percentage units lower for CST compared with CS, likely due to less inclusion of soybean hulls in this diet. Content of NFC was similar for CS

Table 1. Ingredient composition (% of DM) of experimental diets.¹

	CS	CST	SAHT	LAHT	AST
Corn silage	50.0	50.0	25.0	25.0	25.0
Alfalfa hay			25.0	25.0	
Alfalfa silage					25.0
Corn grain	18.0	18.0	22.8	23.0	23.0
Soybean hulls	14.4	11.2	9.6	9.6	9.6
Soybean meal, 48%	6.4	7.4	5.0	5.0	5.0
Distiller's grains, dry	5.0	5.25	5.25	5.25	5.25
Meat and bone meal	3.0	3.0	3.0	3.0	3.0
Blood meal	1.0	1.0	1.0	1.0	1.0
Urea	0.4	0.4	0.2		
Limestone	0.9	0.9	0.4	0.4	0.4
Magnesium oxide	0.05	0.05			
Trace mineral salt ²	0.5	0.5	0.45	0.45	0.45
Vitamin mix ³	0.3	0.3	0.3	0.3	0.3
Tallow		2.0	2.0	2.0	2.0

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²Contained 0.55% Mn, 0.55% Zn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, and 0.002% Co.

³Contained 3304 IU/g of vitamin A, 1101 IU/g of vitamin D, and 11 IU/g of vitamin E.

and CST, and it was slightly lower for alfalfa diets with T compared with CST. Fatty acid concentration of diets with 2% T was similar for the different alfalfa treatments and was slightly higher for CST, likely due to higher contribution of FA from corn silage. Mean particle size and particle size distribution of experimental diets are presented in Table 3. Mean particle size and distribution were similar for both diets, with corn silage as the only forage source. Mean particle sizes of SAHT and AST were similar and averaged 3.5 mm. Particle size distribution of these 2 treatments was also similar,

with SAHT having slightly higher proportion of particles in the pan, probably due to the breakdown of particles during chopping. Including 25% long-stem alfalfa hay in diets increased mean particle size of the diet and the proportion of longer particles.

Sampling and Laboratory Analysis

Dry matter content of forages and concentrates was determined weekly using a 60°C forced-air oven; results were used to adjust as-fed ratios in the TMR. The TMR

Table 2. Chemical composition of experimental diets.¹

	CS	CST	SAHT	LAHT	AST
DM, %	51.5	51.6	65.5	65.6	57.5
NE _L , ² Mcal/kg DM	1.50	1.57	1.56	1.56	1.56
CP, % DM	16.5	16.6	16.9	16.5	16.6
NDF, % DM	30.8	29.2	30.7	30.7	29.5
NFC, ³ % DM	41.3	41.2	39.3	39.9	40.5
Fatty acids, % DM	4.1	5.2	4.9	4.8	4.9
	(g/100 g of Fatty acids)				
C16:0	16.8	18.7	19.9	18.6	18.8
C16:1	0.6	1.4	1.5	1.2	1.3
C18:0	4.9	8.9	10.3	8.5	9.1
<i>trans</i> -C18:1	0.5	1.3	1.7	1.3	1.2
<i>cis</i> -C18:1	26.2	30.0	28.5	28.9	29.1
C18:2	38.3	29.1	27.8	30.2	29.7
C18:3	4.3	3.2	3.7	4.0	4.2
Other	8.5	7.4	6.5	6.2	6.5

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²Calculated (NRC, 2001).

³Nonfibrous carbohydrates.

Table 3. Particle size distribution (\pm SD) expressed as percentage of wet sample on screen and mean particle size (\pm SD) of experimental diets¹ offered to cows.

	CS	CST	SAHT	LAHT	AST
Screen size, ² mm					
26.9	0.3 \pm 0.1	0.3 \pm 0.1	1.4 \pm 0.5	12.0 \pm 0.9	0.8 \pm 0.1
18.0	1.4 \pm 0.3	1.2 \pm 0.3	4.9 \pm 1.0	3.0 \pm 0.5	4.7 \pm 0.4
8.98	27.2 \pm 1.1	27.7 \pm 1.1	19.9 \pm 1.5	15.9 \pm 0.6	22.0 \pm 0.8
5.61	12.0 \pm 0.7	12.1 \pm 0.4	10.8 \pm 0.6	9.9 \pm 0.3	13.2 \pm 0.5
1.65	31.3 \pm 1.7	31.1 \pm 0.7	30.4 \pm 1.4	28.4 \pm 0.4	30.9 \pm 1.2
Pan	27.8 \pm 2.3	27.7 \pm 1.5	32.5 \pm 2.5	30.8 \pm 0.7	28.4 \pm 1.4
MPS, ³ mm	3.6 \pm 0.2	3.6 \pm 0.1	3.4 \pm 0.3	4.1 \pm 0.1	3.6 \pm 0.1

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²Square hole diagonal.

³MPS = geometric mean particle size.

amount offered and refused was measured daily. Orts were collected on d 17 to 19 of each period and dried 48 h in a 60°C forced-air oven for DMI determination. Forage and concentrate samples were collected weekly, dried 48 h in a 60°C forced-air oven, and ground to pass a 2-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Organic matter was determined by oven drying at 550°C for 720 min. Samples were analyzed for NDF (Mertens, 1999), CP (AOAC, 1990), and FA (Sukhija and Palmquist, 1988). The nonfibrous carbohydrate component was calculated as 100 - (NDF + ether extract + CP + ash). Ether extract content was estimated from FA analysis as FA + 1 (NRC, 2001). Diets and orts particle size and distribution were determined using an oscillating screen particle separator according to the American Society of Agricultural Engineers standard S424 (American National Standards Institute, 1988). The separator has 5 screens with nominal diagonal openings of 26.90, 18.00, 8.98, 5.61, and 1.65 mm and a pan.

Cows were milked twice daily, and milk production was recorded at each milking during the final 5 d of each period. Milk samples from the a.m. and p.m. milking were collected on 5 consecutive days (d 17 to 21 of each period). They were analyzed for fat, CP, and solids-not-fat by infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI). Milk samples from d 17 and 18 were composited for FA analysis. Milk fat was isolated as described by Stine et al. (1954). Fatty acid transesterification was performed according to the method described by Chouinard et al. (1999). Fatty acids methyl esters were injected into a gas chromatograph (Perkin Elmer Autosystem, Norwalk, CT) equipped with a 100-m (i.d., 0.25 mm) WCOT-fused silica capillary column coated with CP-Sil 88 (Chrompack #CP7489, Varian Inc., Walnut Creek, CA). Helium was utilized as the carrier gas. Three different runs

were performed for each sample. The FA profile was determined using a temperature gradient run (50 to 190°C at 4°C/min). An isothermal run (160°C) was used to separate most *trans*-octadecenoic FA. A second isothermal run (180°C) was used to separate *trans*-15, and *trans*-13/14 from *cis*-9 C18:1 that coelute as one peak during the isothermal run at 160°C. Peaks were identified utilizing individual FA from Supelco Inc. (Bellefonte, PA), Sigma Chemical Co. (St. Louis, Mo), and Matreya Inc. (Pleasant Gap, PA). To convert area percentages to weight percentages, response correction factors for each FA methyl ester were calculated utilizing a certified butter oil (CRM 164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium).

Rumen fluid was sampled from fistulated cows before (0 h) and at 2, 4, 6, 8, and 10 h after the 0900 h feeding on d 21 of the experimental period. Samples were taken from 5 different locations in the rumen with a metal filter probe. Rumen pH was determined immediately after the samples were collected (Twin pH-meter model B-213, Spectrum Technologies Inc., Plainfield, IL). One milliliter of rumen fluid was acidified with 20 μ L of 50% H₂SO₄ and frozen until analysis for VFA by GLC (Perkin Elmer Autosystem, Norwalk, CT) as described by Bal et al. (2000), and 1 mL was mixed with 20 μ L of 50% TCA and frozen until analysis for NH₃-N (Chaney, 1962).

Digesta flow at the omasal canal in rumen-cannulated cows (n = 5) was determined by continuous marker infusion (France and Siddons, 1986). Indigestible NDF (Huhtanen et al., 1994), YbCl₃ (Siddons et al., 1985), and Co-EDTA (Uden et al., 1980) were used as markers for large particle phase, small particle phase, and fluid phase, respectively. Ytterbium chloride and Co-EDTA were continuously infused into the rumen at a constant rate of 2.9 g of Co/d and 4.2 g of Yb/d from d 15 to 20.

Digesta flowing out of the rumen was collected using an alternating vacuum and pressure system developed by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000). Spot samples (200 ml) were collected from the omasal canal every 4 h during d 18 to 20 of the experimental periods. Time of sampling was advanced each day such that the composited samples represented sampling from every hour over a period of 12 h (0900 to 2000 h). Omasal composites (2.4 L) were separated into 3 digesta phases as follows. Samples were squeezed through one layer of cheesecloth, and solids retained were defined as the large particle phase. The filtrate was centrifuged at $10,000 \times g$ at 4°C for 15 min. The supernatant was defined as the fluid phase, and the pellet as the small particle phase. Digesta phases were frozen, freeze-dried, and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA) and analyzed for NDF (Mertens, 1999) and FA (Sukhija and Palmquist, 1988). Concentrations of Co and Yb in omasal phases were determined by direct current plasma emission spectroscopy (Spectra Metrics, Inc., subsidiary of Beckman Instruments, Inc., Andover, MA; Combs and Satter, 1992).

Chewing behavior was monitored visually on d 16 of each experimental period during 24 h. Eating and rumination activities were noted every 5 min and were assumed to persist for the entire 5-min interval. Chewing activities per unit of DM and NDF intake were calculated for each cow using mean intakes for each period.

Calculations

Concentrations of Co, Yb, and indigestible NDF in large and small particles phases, and of Co and Yb in fluid phase of omasal digesta composites, were used to reconstitute omasal true digesta flowing out of the rumen based on the triple-marker method (France and Siddons, 1986). Fatty acid flow was calculated by multiplying DM flow by the concentration of FA in the reconstituted omasal true digesta.

Apparent ruminal BH of individual C18 unsaturated FA was calculated as described by Wu et al. (1991):

$$\text{BH, \%} = 100 - [100 \times (\text{FUFA}/\text{FTFA})/(\text{IUFA}/\text{ITFA})]$$

where FUFA = omasal true digesta flow of individual unsaturated C18 fatty acid, FTFA = omasal true digesta flow of total C18 FA, IUFA = intake of individual unsaturated C18 fatty acid, and ITFA = intake of total C18 FA.

Statistical Analysis

All data were analyzed using the mixed procedure of SAS (SAS User's Guide, 2001). Dry matter and NDF

intakes, milk production and composition, and chewing behavior were analyzed as a replicated 5×5 Latin square. The model included the fixed effects of square, period, treatment, and the interactions square \times period, square \times treatment, and period \times treatment. Cow within square was the specified term for the random statement. Ruminal pH, $\text{NH}_3\text{-N}$, and VFA from fistulated cows were analyzed by time as repeated measures in a replicated 5×5 Latin square design. The final model included the effects of square, period, treatment, time, and the interaction of treatment \times time. The specified term for the random statement was cow within square and period \times treatment \times cow within square. The covariance structure used to fit the model was selected based on the Akaike's information criterion of the mixed models of SAS (SAS User's Guide, 2001). Intake and flow of FA were analyzed in a single Latin square, and the model used included the fixed effects of period and treatment and the random effect of cow.

Preplanned statistical contrasts were used to test the effect of T supplementation when corn silage was the only forage source (CS vs. CST); the effect of replacing 50% of corn silage with alfalfa in diets containing T (CST vs. SAHT + LAHT + AST); and the effect of hay particle length (SAHT vs. LAHT) and of alfalfa preservation method (SAHT vs. AST) in diets with 2% supplemental T. Least square means are reported. Unless otherwise stated, significance was declared at $P < 0.05$. Trends towards significance were considered at $0.05 \leq P < 0.15$.

RESULTS AND DISCUSSION

Intakes of Nutrients and Milk Yield and Composition

Table 4 shows intakes of nutrients and milk yield and composition as affected by experimental diets. Dry matter intake of cows fed CST was 1.6 kg/d lower ($P < 0.01$) than that of cows fed CS. A similar response to supplementing 2% T with diets high in corn silage was observed in a previous study (Onetti et al., 2001). Replacing 50% of the corn silage with alfalfa tended ($P < 0.12$) to increase DMI of cows when 2% T was fed. Similarly, Onetti et al. (2002) and Ruppert et al. (2003) reported increased DMI as the alfalfa silage:corn silage ratio of diets containing T increased. Smith et al. (1993) observed similar DMI of cows fed 2.5% T when 25 or 50% of the corn silage was replaced with alfalfa hay in the diets. There was a trend ($P < 0.07$) for a period \times treatment interaction for DMI. The reasons for this trend are unknown, as no consistent pattern was observed in the interaction. However, no period \times treatment interaction was detected for energy and fiber intakes. Intake of calculated NE_L did not differ between diets and averaged 41.3 Mcal/d (data not shown). Intake

Table 4. Effects of dietary treatments on nutrient intakes, milk production, and milk composition.

	Treatment ¹					SE	Statistical contrasts (<i>P</i> <) ²			
	CS	CST	SAHT	LAHT	AST		A	B	C	D
DMI, kg/d	27.6	25.9	26.7	26.6	26.5	0.6	0.01	0.12	NS	NS
NDFI, ³ kg/d	8.3	7.7	8.1	7.7	7.7	0.2	0.01	NS	0.11	0.10
Milk, kg/d	44.9	44.3	44.8	44.3	43.6	1.8	NS	NS	NS	NS
Fat, %	3.12	2.68	3.17	2.96	3.32	0.12	0.001	0.001	0.03	0.10
Fat, kg/d	1.38	1.17	1.39	1.31	1.45	0.06	0.002	0.001	0.10	NS
Protein, %	3.03	2.96	2.93	2.94	2.84	0.04	0.02	0.01	NS	0.001
Protein, kg/d	1.36	1.31	1.30	1.30	1.23	0.05	NS	NS	NS	0.05
SNF, kg/d	3.9	3.8	3.8	3.8	3.7	0.16	NS	NS	NS	NS

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²A = CS vs. CST; B = CST vs. SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS = not significant.

³Neutral detergent fiber intake.

of NDF was lower ($P < 0.01$) for cows fed CST than for cows fed CS, probably due to a combination of lower DMI and a slightly lower NDF concentration in the diet. Replacing part of the corn silage with alfalfa had no effect on NDF intake. Neutral detergent fiber intake tended ($P < 0.11$) to be higher for cows fed SAHT than LAHT. This was likely due to selective sorting against long particles in the manger. Sorting was calculated according to Leonardi et al. (2003) as actual intake of particles retained on the top screen (>26.9 mm) expressed as a percentage of their predicted intake. Intake of particles retained on the top screen was 72% of predicted intake for cows fed LAHT. Because long particles usually are higher in NDF concentration than the TMR, sorting against them would likely result in lower NDF intake. The trend ($P < 0.10$) for higher NDF intake for cows fed SAHT compared with AST might be partly explained by the slightly lower NDF content of AST (30.7 and 29.5 for LAHT and AST, respectively, Table 2).

Milk production was not affected by dietary treatments and averaged 44.4 kg/d (Table 4). Supplementing 2% T to diets with corn silage as the only forage source had different effects on milk yield response in previous studies, with decreased (Onetti et al., 2001), increased (Onetti et al., 2002), or no change (Onetti et al., 2003) in milk production. Different responses were likely due to differences in the effects of T on energy intake. No significant effect of forage combinations on milk yield was observed in the present study. Similarly, milk yield was not affected when different combinations of alfalfa silage or hay and corn silage were fed (Smith et al., 1993; Onetti et al., 2002; Ruppert et al., 2003).

Milk fat percentage and yield significantly ($P < 0.002$) decreased when 2% T was added to diets with corn silage as the sole forage source. These results agree

with our previous results (Onetti et al., 2001, 2002) and with data from Ruppert et al. (2003). Replacing 50% of the corn silage with alfalfa in the diets resulted in increased ($P < 0.001$) milk fat percentage and yield. In a previous experiment (Onetti et al., 2002), replacing 25 or 50% of the corn silage with alfalfa silage did not alleviate the negative effect of 2% supplemental T on milk fat production; however, milk fat yield increased linearly as the proportion of alfalfa silage in the diets increased independently of the fat content of the diet. Ruppert et al. (2003) showed that T tended to decrease milk fat content to a greater extent when the corn silage to alfalfa silage ratio in the basal diet was 40:10 than when it was 10:40 (DM basis). Similarly, Smith et al. (1993) reported that the depression in milk fat percentage observed when 2.5% T was fed with corn silage diets was overcome by replacing 25 or 50% of the corn silage with alfalfa hay. It is not possible to discern in the present study if the effects of including alfalfa on milk fat production are strictly due to a forage effect or if a T × forage interaction occurred. Contrary to our expectations, cows fed LAHT had lower ($P < 0.03$) milk fat content and tended ($P < 0.10$) to produce less milk fat than cows fed SAHT. This effect appears to be related to selective sorting against long particles. Mean particle size of the consumed LAHT diet (calculated from actual intakes of material on individual screens and pan) was 3.7 mm and was lower than that of LAHT offered (Table 3); however, it was slightly higher than mean particle size of consumed SAHT, which remained identical to that of SAHT offered. When cow 4611 was excluded from the analysis because she consumed only 21% of long particles (mean particle size of consumed diet was 3.1 mm), the trend towards a significant difference between LAHT and SAHT for milk fat yield disappeared. These results suggest that feeding long-stem hay might

be less effective than chopped hay in sustaining milk fat production if sorting occurs in the manger. The extent of sorting by individual animals fed TMR has been shown to be extremely variable (Leonardi and Armentano, 2003). There was a trend ($P < 0.10$) for increased milk fat percentage when cows were fed AST compared with SAHT, and it was likely due to the slightly lower milk production, as milk fat yield was not affected.

Milk protein percentage was lower ($P < 0.02$) for cows fed CST than for cows fed CS; however, no effect of T was observed for milk protein yield. Decreased milk protein percentage without changes in milk protein yield due to supplemental fats has been reported elsewhere (Wu and Huber, 1994). Replacing 50% of corn silage with alfalfa in diets containing T increased ($P < 0.01$) milk protein percentage; this was primarily due to AST treatment. No effect of replacing corn silage with alfalfa was observed for protein yield. Feeding alfalfa hay of different particle length in diets containing 2% T did not affect milk protein percentage or yield of cows. When 2% T was fed, alfalfa silage significantly decreased milk protein percentage ($P < 0.001$) and yield ($P < 0.05$) relative to alfalfa hay of similar particle length. Vagnoni and Broderick (1997) reported a greater increase in milk protein yield when RUP as fish meal was supplemented to cows fed alfalfa silage diets vs. alfalfa hay diets. These researchers concluded that protein status of cows fed alfalfa silage was poorer than that of cows fed alfalfa hay. No effect of dietary treatments was observed for solids-not-fat yield.

Fatty Acid Composition of Milk Fat

Fatty acid composition of milk fat is presented in Table 5. Fatty acid profile of milk fat was altered substantially when 2% T was included in diets, with corn silage as the only forage source. Proportion of short-chain FA (C4 to C14) in milk fat was decreased ($P < 0.001$), and proportions of C16:0 and C18:0 were similar for cows fed CS and CST. Cows fed CST had significant higher proportion of total C18:1 and of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) isomers. Content of *trans*-6/8, *trans*-9, and *trans*-10 C18:1, total *trans*-C18:1, and total *trans*-C18 FA (C18:1 isomers plus *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA) were increased in milk fat of cows fed CST vs. cows fed CS. These changes are consistent with changes in the FA profile observed during dietary induced milk fat depression and with previous experiments (Onetti et al., 2001, 2002) that reported milk fat depression when 2% supplemental T was fed with corn silage as the only forage source. Increased proportions of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA in milk fat support their role

as inhibitors of milk fat synthesis (Grinari et al., 1998; Baumgard et al., 2000).

Replacing 50% of the corn silage with alfalfa in diets containing supplemental T did not affect the content of C4 to C14 FA, tended ($P < 0.14$) to decrease content of C16:0, and significantly ($P < 0.02$) increased the proportion of C18:0, C18:2, and C18:3 in milk fat (Table 5). Proportion of the *cis*-9, *trans*-11 CLA isomer was not affected, and *trans*-10, *cis*-12 CLA isomer content was decreased ($P < 0.04$) when alfalfa replaced half of the corn silage in the diets. Milk fat of cows fed alfalfa in the diets had significant lower content of *trans*-6/8, *trans*-9, and *trans*-10 C18:1, and total *trans*-C18:1, and it had higher content of *trans*-12 C18:1. Decreased contents of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA in milk fat are in agreement with higher milk fat content and yield of cows fed 50% of forage from alfalfa compared with cows fed corn silage as the only forage source (Table 4). Total *trans*-C18 FA content in milk fat was lower ($P < 0.003$) when alfalfa replaced corn silage.

Feeding LAHT relative to SAHT significantly ($P < 0.05$) decreased C18:0, increased ($P < 0.02$) C18:2 and C18:3, and tended ($P < 0.07$) to increase *cis*-9, *trans*-11 CLA proportions in milk fat. Proportion of all *trans*-C18:1 isomers, except for *trans*-12, and the proportion of total *trans*-C18:1 and total *trans*-C18 FA were increased when LAHT was fed relative to SAHT. The increase in the proportion of *trans*-10 C18:1 in milk fat for cows fed LAHT is in agreement with the lower milk fat percentage and yield for this dietary treatment compared with SAHT. Selective sorting against long-stem hay by some animals fed LAHT likely resulted in favorable rumen conditions for the formation of this particular isomer and its successive incorporation into milk fat.

Milk fat content of C16:0 tended ($P < 0.06$) to be lower for cows fed AST relative to cows fed SAHT. Milk fat concentration of C18:0, C18:2, and C18:3 was higher ($P < 0.05$, $P < 0.004$, and $P < 0.02$, respectively) in milk fat of cows fed SAHT as compared with cows fed AST. Consistent with milk fat production data, alfalfa preservation method had minimal effects on *trans*-C18:1 isomer profile and did not affect milk fat content of total *trans*-C18 FA.

Chewing Behavior

Chewing activities are presented in Table 6. Cows fed corn silage as the sole forage source spent 196 min eating and 493 min ruminating per day, regardless of T content of the diet. However, cows fed CST tended ($P < 0.13$) to spend more time eating per kilogram of DMI, and spent more ($P < 0.01$) time ruminating per kilogram of NDF intake and chewing per kilogram of DMI compared with cows fed CS. These results suggest

Table 5. Fatty acid composition of milk fat as affected by dietary treatments.

	Treatment ¹					SE	Statistical contrasts ($P <$) ²			
	CS	CST	SAHT	LAHT	AST		A	B	C	D
Profile	g/100 g of fatty acids									
C4 to C14	25.0	20.6	20.8	21.2	20.9	0.8	0.001	NS	NS	NS
C16:0	27.1	27.1	26.8	26.3	25.4	0.7	NS	0.14	NS	0.06
C18:0	9.6	9.9	11.4	10.3	12.1	0.6	NS	0.02	0.05	NS
C18:1	22.6	27.2	26.2	26.5	27.1	0.8	0.001	NS	NS	NS
C18:1 isomers										
<i>trans</i> -6/8	0.30	0.60	0.49	0.62	0.44	0.04	0.001	0.04	0.01	NS
<i>trans</i> -9	0.35	0.47	0.37	0.48	0.37	0.04	0.04	0.11	0.04	NS
<i>trans</i> -10	0.75	2.15	1.00	1.71	0.78	0.22	0.001	0.001	0.01	NS
<i>trans</i> -11	0.86	0.89	0.89	1.03	1.06	0.05	NS	NS	0.11	0.05
<i>trans</i> -12	0.59	0.52	0.63	0.70	0.71	0.06	NS	0.02	NS	NS
Total <i>trans</i> -C18:1	2.84	4.62	3.35	4.53	3.36	0.27	0.001	0.01	0.01	NS
<i>cis</i> -9	16.9	20.6	20.4	19.6	20.5	0.6	0.001	NS	NS	NS
<i>cis</i> -11	0.22	0.12	0.23	0.30	0.24	0.05	NS	0.03	NS	NS
<i>cis</i> -12	0.51	0.66	0.52	0.57	0.48	0.07	0.15	0.08	NS	NS
CLA ³ c9t11	0.49	0.60	0.50	0.57	0.62	0.04	0.02	NS	0.07	0.01
CLA t10c12	0.01	0.04	0.02	0.03	0.03	0.005	0.002	0.04	NS	NS
C18:2	5.2	4.2	4.4	5.4	5.2	0.2	0.001	0.003	0.001	0.004
C18:3	0.35	0.29	0.36	0.42	0.42	0.02	0.04	0.001	0.02	0.02
Other	9.5	9.9	9.6	9.2	8.2	0.7	NS	NS	NS	0.09
Total <i>trans</i> ⁴	3.4	5.2	3.9	5.2	4.0	0.3	0.002	0.003	0.004	NS

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²A = CS vs. CST; B = CST vs. SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS = not significant.

³CLA = Conjugated linoleic acid.

⁴C18:1 isomers plus CLA c9t11 and t10c12.

that cows fed supplemental T required more time for eating and ruminating as intakes of DM and NDF decreased compared with cows fed the same diet without T. The reason for greater chewing activity for cows fed supplemental T is not clear. Although all data is not consistent, fat supplementation has been shown to impair fiber digestion in the rumen in some studies (Palmquist and Jenkins, 1980). Decreased rate of ruminal

fermentation likely decreases the rate of particle size reduction during chewing by decreasing tissue fragility (Allen, 1996); therefore, more chewing time would be required. However, no effect of T on rumen fermentation (discussed later) was observed in the present study. The addition of 11.6% whole raw soybeans had no effect on the chewing activity of lactating cows (Grant and Weidner, 1992).

Table 6. Treatment effects on chewing behavior of cows.

	Treatment ¹					SE	Statistical contrasts ($P <$) ²			
	CS	CST	SAHT	LAHT	AST		A	B	C	D
Eating										
Time, min/d	194.9	196.9	250.2	295.3	242.6	9.5	NS	0.001	0.001	NS
Time/DMI, min/kg	6.9	7.6	9.3	11.2	9.2	0.4	0.13	0.001	0.001	NS
Ruminating										
Time, min/d	482.3	504.1	509.2	449.7	444.7	17.7	NS	0.02	0.002	0.002
Time/NDF intake, min/kg	58.1	65.6	64.2	58.9	57.8	3.1	0.01	0.03	0.07	0.04
Chewing										
Time, min/d	676.8	700.0	759.2	745.0	690.7	20.8	NS	0.08	NS	0.01
Time/DMI, min/kg	24.5	27.2	28.2	28.3	26.1	1.1	0.01	NS	NS	0.03

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²A = CS vs. CST; B = CST vs. SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS = not significant.

Replacing 50% of corn silage with alfalfa in diets with 2% T increased ($P < 0.001$) the total time spent eating per day and eating time per kilogram of DMI, and it decreased total time spent ruminating and rumination time/kg NDF ($P < 0.02$ and $P < 0.03$, respectively). Increased eating time suggests that cows needed more chews to achieve the critical particle size required for swallowing when alfalfa was included in the diets. Despite lower rumination activity, total time spent chewing tended ($P < 0.08$) to be higher for cows fed alfalfa and corn silage compared with all corn silage when diets contained T. This trend for an increase in chewing activity when alfalfa replaced half of the corn silage in tallow-containing diets was associated with higher milk fat percentage and yield.

Cows fed LAHT spent more time eating per day and more time eating per kilogram of DMI than cows fed SAHT ($P < 0.001$). Contrary to our expectations, total time spent ruminating, as well as time spent ruminating per kilogram of NDF intake was lower for cows fed LAHT than for cows fed SAHT ($P < 0.001$ and $P < 0.07$, respectively). The reason for this behavior is not obvious. Greater chewing prior to swallowing for cows fed LAHT probably reduced particle size to a greater extent than for cows fed SAHT, reducing the need for rumination. On the other hand, cows fed LAHT sorted against long particles rich in fiber, likely resulting in less need for rumination. Total time spent chewing by cows was similar for both treatments. Therefore, decreasing particle size of hay in this study did not alter the physical effectiveness of forage NDF. According to Mertens (1997), chewing activity is the animal response associated with physical effectiveness of fiber because it promotes salivary buffer secretion during eating and rumination. These results suggest that factors other than chewing activity might be responsible for reduced milk fat content when tallow is fed with long-stem hay compared with chopped hay.

Time spent eating was similar for cows fed SAHT and AST. Cows fed SAHT spent more time ruminating than cows fed AST ($P < 0.002$). This difference was not only due to less NDF intake of cows fed AST, because time spent ruminating per kilogram of NDF intake was also decreased ($P < 0.04$) by cows fed AST. Total chewing time and time spent chewing per kilogram of DMI was significantly ($P < 0.01$ and $P < 0.03$, respectively) higher for cows fed SAHT vs. cows fed AST. Nelson and Satter (1990) reported greater time spent ruminating and chewing per kilogram of NDF for cows consuming diets consisting of 55% alfalfa hay than cows consuming the same amount of alfalfa silage. These authors concluded that alfalfa silage particles were reduced in size or were adequately wetted for swallowing in less time. Increased rumination and chewing time of cows fed

chopped alfalfa hay vs. feeding alfalfa silage of similar particle length and distribution was not associated with increased milk fat yield in the present study.

Rumen Fermentation

Rumen pH, $\text{NH}_3\text{-N}$, and total VFA concentrations, as well as molar proportion of VFA as affected by dietary treatments, are shown in Table 7. No treatment \times time interactions were detected for any of the variables measured. Ruminal pH was not affected by supplemental T when corn silage was the only forage source. Similar results were observed in our previous studies (Onetti et al., 2001, 2003). Cows fed diets with supplemental T in which alfalfa replaced 50% of the corn silage had higher ruminal pH ($P < 0.02$). Lower ruminal pH of cows fed diets with corn silage as the only forage source might be partially explained by the high availability of rapidly fermentable starch due to corn silage processing, lower buffering capacity of corn silage relative to alfalfa, and decreased chewing activity. Ruminal pH was higher ($P < 0.03$) for cows fed LAHT than SAHT. However, this increase in pH was not related to chewing behavior, as time spent chewing was similar for LAHT and SAHT. There was no effect of alfalfa preservation method on ruminal pH, even though chewing activity was greater for cows fed SAHT than for cows fed AST.

Ruminal $\text{NH}_3\text{-N}$ concentration tended ($P < 0.07$) to decrease when T was added to corn silage diets, similar to our previous report (Onetti et al., 2001). Reduction in ruminal $\text{NH}_3\text{-N}$ concentration when fats are fed has been associated with decreased numbers of protozoa and decreased microbial nitrogen recycling (Ikwuegbu and Sutton, 1982; Onetti et al., 2001). No effect of replacing corn silage with alfalfa was observed on $\text{NH}_3\text{-N}$ concentration. Increasing the proportion of alfalfa silage in the diets increased ruminal $\text{NH}_3\text{-N}$ concentration at 4 h postfeeding when 2% T was fed in a previous study (Onetti et al., 2002). Cows fed LAHT had lower ($P < 0.01$) ruminal $\text{NH}_3\text{-N}$ concentration than cows fed SAHT. No effect of forage particle length on ruminal $\text{NH}_3\text{-N}$ was reported in the study by Yang et al. (2001). Ruminal $\text{NH}_3\text{-N}$ concentration was similar for cows fed alfalfa preserved either as silage or hay.

A trend ($P < 0.08$) for a decrease in total VFA concentration was observed when T was included in diets with corn silage as the sole forage source, probably as a result of lower DMI. No effect of T was observed on molar proportions of individual VFA or A:P when corn silage was the only forage source. Total VFA concentration did not differ when alfalfa replaced corn silage in diets containing tallow. Replacing corn silage with alfalfa in diets with 2% T increased ($P < 0.001$) the molar proportion of acetate, decreased ($P < 0.002$) the molar propor-

Table 7. Effects of dietary treatments on rumen pH, ammonia and VFA concentrations and molar proportion of VFA.

	Treatment ¹					SE	Statistical contrasts ($P <$) ²			
	CS	CST	SAHT	LAHT	AST		A	B	C	D
pH	6.23	6.26	6.32	6.40	6.31	0.05	NS	0.02	0.03	NS
NH ₃ -N, mg/dl	11.5	9.8	10.3	7.7	9.1	0.8	0.07	NS	0.01	NS
VFA, ³ mM	120.6	114.5	120.8	111.5	114.2	3.1	0.08	NS	0.01	0.04
	mol/100 mol									
Acetate	62.5	61.4	65.1	65.4	65.9	0.7	NS	0.001	NS	NS
Propionate	21.9	23.4	20.1	20.1	18.9	0.8	NS	0.002	NS	NS
Butyrate	11.9	11.4	11.0	10.9	11.5	0.4	NS	NS	NS	NS
Isobutyrate	0.7	0.6	0.8	0.7	0.8	0.04	NS	0.01	NS	NS
Isovalerate	1.4	1.5	1.3	1.3	1.5	0.08	NS	NS	NS	NS
Valerate	1.7	1.6	1.7	1.6	1.5	0.06	NS	NS	0.04	0.01
A:P ⁴	2.9	2.7	3.3	3.4	3.6	0.1	NS	0.001	NS	NS

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²A = CS vs. CST; B = CST SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS = not significant.

³Total VFA.

⁴Acetate-to-propionate ratio.

tion of propionate and, consequently, increased ($P < 0.001$) A:P in the rumen. Similar results were observed in our previous study (Onetti et al., 2002). Consistent with increased ruminal pH, cows fed LAHT had lower ($P < 0.01$) total VFA concentration in the rumen than cows fed SAHT. No effect of alfalfa hay particle size was observed on molar proportions of individual VFA, except for an increase ($P < 0.04$) in the proportion of valerate when SAHT was fed vs. LAHT. Total VFA concentration was lower ($P < 0.04$) for cows fed AST compared with SAHT. The effect of the alfalfa preservation method on molar proportions of individual VFA was minor; an increase ($P < 0.01$) in the proportion of valerate was observed for cows fed SAHT vs. AST. Changes in molar proportions valerate due to dietary treatments were small and difficult to interpret. Based on these results and those of our previous study (Onetti et al., 2002), the milk fat depression observed when 2% T was fed with diets with corn silage as the only forage source or with diets with long-stem alfalfa hay appears to be independent of rumen pH or shifts in rumen fermentation.

Intake, Omasal Flow, and Biohydrogenation of Fatty Acids

Dietary effects on intake and omasal flow of C18 FA, and the extent of biohydrogenation of unsaturated FA in the rumen are presented in Table 8. Intakes of C18:0, *trans*-C18:1, and *cis*-C18:1 were increased ($P < 0.02$), that of C18:2 was not affected, and intake of C18:3 tended to decrease ($P < 0.08$) when T was added to

diets with corn silage as the only forage source. These changes reflected changes in FA composition of the diets and in DMI of cows. Despite the reduction in DMI due to T supplementation, intake of total C18 FA tended ($P < 0.08$) to be higher for cows fed CST than for cows fed CS. Replacing corn silage with alfalfa in T containing diets tended ($P < 0.13$) to decrease intake of C18:2 and significantly ($P < 0.02$) increased intake of C18:3. Total intake of C18 FA was similar for tallow-containing diets, regardless of forage treatment. Intakes of C18:2 and C18:3 tended to be higher for cows fed LAHT compared with cows fed SAHT. Higher intakes of these polyunsaturated FA might be explained by the slightly higher concentration in the diet or proportionally higher concentrate and corn silage intakes relative to hay due to sorting against the long-stem hay. There was a trend ($P < 0.09$) for a decrease in *trans*-C18:1 intake of cows fed LAHT vs. SAHT, and of cows fed AST vs. SAHT, probably due to the lower concentration of this particular FA in the diets.

Supplemental tallow significantly increased ($P < 0.03$) omasal flow of *trans*-C18:1 and tended ($P < 0.12$) to increase flow of total C18 FA when corn silage was the only forage source. Increased flow of *trans*-C18:1 might be related to increased intake, incomplete BH or isomerization of *cis*-C18:1 in the rumen. Mosley et al. (2002) showed that BH of oleic acid by mixed rumen bacteria involves the formation of several positional *trans*-C18:1 isomers. It is not known whether the *trans*-C18:1 isomers derived from oleic acid are intermediates or end products of BH in the rumen (Mosley et al., 2002). Replacing 50% of the corn silage with alfalfa

Table 8. Effects of dietary treatments on fatty acid intake and omasal flow and extent of ruminal biohydrogenation.

	Treatment ¹					SE	Statistical contrasts ($P <$) ²			
	CS	CST	SAHT	LAHT	AST		A	B	C	D
Intake, g/d										
C18:0	59.0	117.9	125.7	120.5	115.2	6.9	0.002	NS	NS	NS
<i>trans</i> -C18:1	5.4	15.2	18.5	15.3	15.1	1.4	0.001	NS	0.09	0.09
<i>Cis</i> -C18:1	297.4	378.4	359.3	375.2	366.2	18.5	0.02	NS	NS	NS
C18:2	422.6	398.1	347.6	386.5	352.4	22.8	NS	0.13	0.12	NS
C18:3	47.5	41.0	44.7	50.9	49.8	2.9	0.08	0.02	0.06	NS
Total C18	832.9	948.3	895.8	948.4	899.7	43.8	0.08	NS	NS	NS
Omasal flow, g/d										
C18:0	484.4	547.9	586.7	518.0	528.1	41.6	NS	NS	NS	NS
<i>trans</i> -C18:1	96.3	137.9	127.9	120.6	104.7	11.6	0.03	0.14	NS	0.11
<i>cis</i> -C18:1	118.2	135.6	148.8	132.9	136.6	13.0	NS	NS	NS	NS
C18:2	60.3	56.6	62.3	59.0	66.1	6.3	NS	NS	NS	NS
C18:3	6.0	5.4	7.3	6.6	7.9	0.9	NS	0.12	NS	NS
Total C18	765.8	886.7	934.3	838.3	843.9	52.5	0.12	NS	NS	NS
Apparent biohydrogenation, %										
<i>cis</i> -C18:1	56.3	61.6	60.5	60.0	60.0	3.6	NS	NS	NS	NS
C18:2	83.6	85.0	82.2	82.4	79.1	2.2	NS	0.11	NS	NS
C18:3	85.7	86.1	83.7	85.4	82.3	2.0	NS	NS	NS	NS
Total C18 ³	73.1	74.4	72.1	71.8	70.0	2.4	NS	0.12	NS	NS

¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with 2% tallow.

²A = CS vs. CST; B = CST vs. SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS = not significant.

³Includes *cis*-18:1, C18:2, and C18:3.

when supplemental tallow was fed tended ($P < 0.14$) to decrease flow of *trans*-C18:1, suggesting a more complete BH. The trend ($P < 0.12$) for increased flow of C18:3 when alfalfa replaced corn silage was likely due to higher C18:3 intake. Alfalfa hay particle length resulted in no significant differences in individual or total FA flows. We hypothesized that feeding long hay would decrease *trans*-C18:1 FA flow to the lower gut relative to feeding chopped hay as a result of increased ruminal pH. However, the response to LAHT treatment was highly influenced by selective sorting against long-stem hay by some cows. Feeding AST tended ($P < 0.11$) to decrease flow of *trans*-C18:1 relative to feeding SAHT. The reason for this trend is not obvious as intake of *trans*-C18:1 was only 3.4 g/d higher for SAHT when compared with AST.

Similar to previous reports (Wu et al., 1991; Kalscheur et al. 1997), the extent of apparent ruminal BH increased as the degree of unsaturation increased. Biohydrogenation of individual or total unsaturated C18 FA was similar between CS and CST. By using this estimate of BH, incomplete BH of C18:2 and C18:3 would be reflected in lower apparent BH for *cis*-C18:1 and C18:2, respectively. In the present study, increased *trans*-10 C18:1 and *trans*-10, *cis*-12 C18:2 contents in milk fat, when tallow was supplemented with diets with corn silage as the only forage source, appears not to be caused by incomplete BH of polyunsaturated FA in the rumen. No difference in apparent BH was observed for

cows fed CS and CST. This is in agreement with the lack of effect of supplemental tallow on ruminal pH and fermentation. Consequently, the increased content of *trans*-C18:1 isomers in milk fat observed might be explained by accumulation of *trans*-C18:1 in the rumen from the BH of oleic acid (Mosley et al., 2002). Tallow contributed significant amounts of *cis*-C18:1 to the diets. When diets high in corn silage are fed, characteristics of the rumen environment probably cause shifts in the bacterial population favoring those species capable of converting *cis*-C18:1 to *trans*-C18:1 isomers. Increased proportion of *trans*-10, *cis*-12 CLA in milk fat was associated with increased flow of this particular FA out of the rumen (omasal flow of *trans*-10, *cis*-12 CLA was 0.7 and 1.5 g/d for CS and CST, respectively, $P < 0.06$, data not shown). It appears that *trans*-10, *cis*-12 CLA is formed from hydrogenation of linoleic acid when milk fat depressing diets are fed (Bauman and Griinari, 2001).

When half of the corn silage was replaced with alfalfa in diets containing tallow, apparent ruminal BH of C18:2 and of total C18 tended ($P < 0.12$) to decrease. These results are in disagreement with the trend for an increased flow of *trans*-C18:1 at the omasal canal observed for CST. Conversion of *trans*-C18:1 to C18:0 is the rate-limiting step in BH of polyunsaturated FA, leading to accumulation of *trans*-C18:1 in the rumen (Harfoot and Hazlewood, 1988). Kalscheur et al. (1997) demonstrated the direct effect of ruminal pH on produc-

tion of *trans* FA in the rumen. These researchers showed that feeding a high concentrate low forage diet without buffer increased the flow of *trans*-C18:1 FA compared with the same diet with buffer. Replacing corn silage with alfalfa decreased ($P < 0.02$) flow of *trans*-10, *cis*-12 CLA (data not shown). Decreasing dietary forage-to-concentrate ratio resulted in decreased ruminal pH and an increase in duodenal flow of *trans*-10, *cis*-12 CLA in sheep (Kucuk et al., 2001). In the present study, ruminal pH was lower for diets with corn silage as the only forage source. Alfalfa hay particle length or alfalfa preservation method did not affect BH of unsaturated FA in the rumen.

CONCLUSIONS

Milk fat percentage and yield of cows were decreased when tallow was fed at 2% of diet DM with diets of corn silage as the only forage source. Milk fat depression was associated with increased *trans*-C18:1 FA flowing out of the rumen and their subsequent incorporation into milk fat. *Trans*-C18:1 FA in the rumen appear to be formed during the biohydrogenation of oleic acid. Replacing 50% of corn silage with alfalfa in diets containing 2% tallow resulted in increased chewing activity, higher ruminal pH, lower formation of *trans*-C18:1 FA in the rumen, and increased milk fat percentage and yield. The results suggest that alfalfa, regardless of the preservation method, is more effective in sustaining milk fat production than processed corn silage when 2% supplemental tallow is fed. However, feeding long-stem hay might be less effective than chopped hay in maintaining milk fat production if selective sorting against long particles occurs in the manger.

ACKNOWLEDGMENTS

The authors thank the Fats and Proteins Research Foundation for partial funding of this study and Pack-erland Packing Co., Inc. (Green Bay, WI) for donating the tallow. Appreciation is expressed to the UW Dairy Cattle Research Center crew for animal care and feeding.

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