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The Role of Alfalfa in Alleviating Milk Fat Depression When Tallow is Supplemented to Corn Silage-Based Diets

Final Report

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Scientific 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 x 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); 5) 25% corn silage: 25% alfalfa silage: 50% concentrate with 2% T (AST). Cows were allowed ad libitum consumption of a TMR fed four 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 % and yield. Replacing part of corn silage with alfalfa in diets with 2% T increased milk fat % and yield. 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. Milk fat % and yield were lower, and 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 higher for cows fed LAHT than SAHT. Feeding alfalfa silage or chopped hav 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; BH = biohydrogenation; CLA = conjugated linoleic acid.

Introduction:

The differential responses to dietary fats observed when feeding different basal diets to dairy cows have been attributed to diet by fat interactions. Smith et al. (1993) showed that the depression in milk production and fat percentage observed when cottonseed and tallow were added to corn silage diets was overcome by replacing 25 or 50% of the corn silage with alfalfa hay. Onetti et al. (2001a) indicated that supplementing 2 and 4% tallow 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 if 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 to 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 this study was greatest for diets with alfalfa hay, and this was mainly due to increased rumination. Cows consuming diets consisting of 55% alfalfa hay spent more time ruminating and chewing per kg 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; Allen, 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 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*-fatty acid flow, decreased *trans* fatty acid 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 tallow induced milk fat depression of cows fed corn silage-based diets by decreasing *trans* fatty acid 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* fatty acid formation in the rumen, and milk fat production of dairy cows fed corn silage-based diets with supplemental tallow. A second objective was to investigate if the milk fat depression observed when tallow is fed with diets with corn silage as the only forage source is related to incomplete BH of polyunsaturated fatty acids.

Experimental procedure:

Fifteen multiparous Holstein cows that averaged (\pm SD) 117 \pm 28 DIM and 688 \pm 7 kg BW were used in a replicated 5 x 5 Latin square design with 21 d periods. Two squares consisted of cows with rumen fistulas and one of cows without rumen fistulas. 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 bovine somatotropin (Posilac®, Monsanto Company, St. Louis, MO) on the same day every week.

Experimental treatments, as a % of total diet DM, were: 1) 50% corn silage:50% concentrate without tallow (CS); 2) 50% corn silage: 50% concentrate with 2% tallow (CST); 3) 25% corn silage: 25% short alfalfa hay: 50% concentrate with 2% tallow (SAHT); 4) 25% corn silage:25% long alfalfa hay:50% concentrate with 2% tallow (LAHT); and 5) 25% corn silage:25% alfalfa silage:50% concentrate with 2% tallow (AST). Diets were formulated to be isonitrogenous, and to meet or exceed NRC (2001) nutrient allowances. Ingredient and chemical composition of experimental diets are presented in Tables 1 and 2, respectively. Corn silage was processed (2 mm roll clearance) and chopped at a theoretical length of 12 mm. Second crop alfalfa 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, Que., Canada). Mean particle size and particle size distribution of experimental diets are presented in Table 3. Average DM, NDF and CP concentration of corn silage was 36.0, 38.1 and 8.5%, of alfalfa silage was 52.4, 42.3 and 18.7, of long hay was 89.0, 47.1 and 18.1, and of short hay was 89.0, 45.9 and 15.8%, respectively. Tallow was incorporated into concentrates and then added to TMR. Cows were fed the diets four times daily (0900, 1500, 2100 and 0300 h) as a TMR to allow 10 % feed refusal on an asfed basis.

The TMR amount offered and refused was measured daily. Orts were collected on d 17 to 19 of each experimental period for DMI and particle size and distribution determination. Particle size and distribution of the offered TMR was also determined. Forage and concentrate samples were collected weekly and analyzed for DM, OM, CP, NDF, and fatty acid concentrations. Milk production was recorded during the last 5 d of each period. Milk samples from the a.m. and the p.m. milking were taken on d 17 to 21 of each period and were analyzed

for fat, protein and solids-not-fat. Additional milk samples were collected on d 17 and 18 of each period, composited and analyzed for fatty acid composition of milk fat. Rumen samples from fistulated cows were taken at 0 (0900h), 2, 4, 6, 8, and 10 h postfeeding for pH, VFA and ammonia determination. Digesta flow at the omasal canal was determined by continuous marker infusion. Spot omasal canal samples were taken four times daily at 1-h intervals on d 18 to 20 so that the composited 12 samples represented sampling every h over a 12 h period (0900 to 2100h). Chewing behavior was monitored visually on d 16 of each experimental period during 24 h.

All data were analyzed as a Latin square design using the mixed procedure of SAS (SAS User's Guide, 1998). Pre-planned statistical contrasts were used to test the effect of tallow supplementation when corn silage was the only forage source (CS vs. CST); the effect replacing 50% of corn silage with alfalfa in diets containing tallow (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 tallow. Least square means are reported. Unless otherwise stated, significance was declared at P < 0.05. Trends towards significance were considered at $0.05 \le P < 0.15$.

Results and discussion:

Intakes of Nutrients and Milk Yield and Composition. Dry matter intake of cows fed CST was 1.6 kg/d lower (P < 0.01) than that of cows fed CS (Table 4). This is in agreement with our 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% tallow was fed. Similarly, Onetti et al. (2002) and Ruppert et al. (2003) reported increased DMI as alfalfa silage:corn silage ratio of diets containing tallow increased. Intake of calculated NE₁ did not differ between diets and averaged 41.3 Mcal/d (data not shown). 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. Cows fed SAHT tended (P < 0.11) to have higher NDF intake than cows fed LAHT. This was likely due to selective sorting against long particles in the manger. Intake of particles retained on the top screen (> 26.9 mm) of the separator was 72 % of predicted intake for cows fed LAHT (data not shown). 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 as compared to AST might be partly explained by the slightly lower NDF content of AST.

Milk production was not affected by dietary treatments, and averaged 44.4 kg/d (Table 4). Milk fat percentage and yield significantly (P < 0.002) decreased when 2% tallow was added to diets with corn silage as the sole forage source. These results agree with our previous results (Onetti et al., 2001 and 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. 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, probably related to extensive sorting against long particles in the manger. There was a trend (P < 0.10) for reduced milk fat percentage when cows were fed AST than 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 tallow 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 tallow 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% tallow did not affect milk protein percentage or yield of cows. When 2% tallow 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. No effect of dietary treatments was observed for solids-not-fat yield.

Fatty Acid Composition of Milk Fat. Proportion of short chain fatty acids (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 (Table 5). 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 fatty acids (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 fatty acid profile observed during dietary induced milk fat depression, and with previous experiments (Onetti et al., 2001 and 2002) that reported milk fat depression when 2% supplemental tallow 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.

Replacing 50% of the corn silage with alfalfa in diets containing supplemental tallow did not affect the content of C4 to C14 fatty acids, 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 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 to cows fed corn silage as the only forage source (Table 4).

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 fatty acids were increased when LAHT was fed relative to SAHT. The increase in the proportion of trans-10 in milk fat for cows fed LAHT is in agreement with the lower milk fat percentage and yield for this dietary treatment compared to SAHT.

Milk fat content of C16:0 tended (P < 0.06) to be lower for cows fed AST relative to cows fed SAHT. Milk fat concentrations of C18:0, C18:2 and C18:3 were significantly higher in milk fat of cows fed SAHT as compared to 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 fatty acids.

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 d, regardless of tallow content of the diet. However, cows fed CST tended (P < 0.13) to spend more time eating/kg DMI, and spent more (P < 0.01) time ruminating/kg NDF intake and chewing/kg DMI as compared to cows fed CS. These results suggest that cows fed supplemental tallow required

more time for eating and ruminating as intakes of DM and NDF decreased compared to cows fed the same diet without tallow. The reason for greater chewing activity for cows fed supplemental tallow is not clear.

Replacing 50% of corn silage with alfalfa in diets with 2% tallow increased (P < 0.001) total time spent eating/d and eating time per kg/DMI, and decreased (P < 0.03) total time spent ruminating and rumination time/kg NDF. 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 to all corn silage when diets contained tallow. 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 d and more time eating/kg DMI than cows fed SAHT (P < 0.001). Contrary to our expectations, total time spent ruminating, as well as time spent ruminating/kg NDF intake was lower for cows fed LAHT than for cows fed SAHT (P < 0.001 and P < 0.07, respectively). 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. Total time spent chewing of cows was similar for both treatments. Therefore, decreasing particle size of hay in this study did not alter the physical effectiveness of forage NDF. 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 as compared to chopped hay.

Time spent eating was similar for cows fed SAHT and AST. Cows fed SAHT spent more time ruminating per d and per kg NDF intake than cows fed AST (P < 0.05). Total chewing time, and time spent chewing/kg DMI was significantly higher for cows fed SAHT vs. cows fed AST. Alfalfa silage particles were probably reduced in size or were adequately wetted for swallowing in less time than alfalfa hay particles (Nelson and Satter, 1990). Increased rumination and chewing time of cows fed chopped alfalfa hay versus 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, NH₃-N, and total VFA concentrations, and molar proportion of VFA as affected by dietary treatments are shown in Table 7. No treatment x time interactions were detected for any of the variables measured. Ruminal pH was not affected by supplemental tallow when corn silage was the only forage source. Similar results were observed in our previous studies (Onetti et al., 2001 and 2003). Cows fed diets with supplemental tallow 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 NH₃-N concentration tended (P < 0.07) to decrease when tallow was added to corn silage diets, similar to our previous report (Onetti et al., 2001). Reduction in ruminal NH₃-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 NH₃-N concentration. Cows fed

LAHT had lower (P < 0.01) ruminal NH₃-N concentration than cows fed SAHT. Ruminal NH₃-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 tallow was included in diets with corn silage as the sole forage source, probably as a result of lower DMI. No effect of tallow 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% tallow significantly increased the molar proportion of acetate, decreased the molar proportion of propionate, and consequently, increased 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. Total VFA concentration was lower (P < 0.04) for cows fed AST as compared to SAHT. Effect of alfalfa preservation method and particle size on molar proportion of individual VFA was minor.

Intake, Omasal Flow and Biohydrogenation of Fatty Acids. 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 tallow was added to diets with corn silage as the only forage source (Table 8). These changes reflected changes in fatty acid composition of the diets and in DMI of cows. Despite the reduction in DMI due to tallow supplementation, intake of total C18 fatty acids tended (P < 0.08) to be higher for cows fed CST than for cows fed CS. Replacing corn silage with alfalfa in tallow-containing diets tended (P < 0.13) to decrease intake of C18:2, and significantly increased intake of C18:3. Total intake of C18 fatty acids 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 as compared to cows fed SAHT. Higher intakes of these polyunsaturated fatty acids 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 an 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 fatty acid 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 fatty acids 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. Replacing 50% of the corn silage with alfalfa 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 fatty acid flows. Feeding AST tended (P < 0.11) to decrease flow of trans-C18:1 relative to feeding SAHT. The reasons for this trend are not clear but might be related to slightly lower intake of this particular fatty acid.

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

BH, % = 100 - [100 x (FUFA/FTFA)/(IUFA/ITFA)]

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

Biohydrogenation of individual or total unsaturated C18 fatty acids was similar between CS and CST (Table 8). 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 fatty acids in the rumen, as 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), of which tallow contributed significant amounts. 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. Kalscheur et al. (1997) demonstrated the direct effect of ruminal pH on production 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 fatty acids compared to the same diet with buffer. In the present study, ruminal pH was lower for diets with corn silage as the only forage Alfalfa hay particle length or alfalfa preservation method did not affect BH of unsaturated fatty acids in the rumen.

Conclusions:

Milk fat percentage and yield of cows were decreased when tallow was fed at 2% of diet DM with diets with corn silage as the only forage source. Milk fat depression was associated with increased *trans*-C18:1 fatty acids flowing out of the rumen and their subsequent incorporation into milk fat. Accumulation of *trans*-C18:1 fatty acids in the rumen appears to be related to their formation as end products of ruminal biohydrogenation. We hypothesized that alfalfa hay, particularly long-stem alfalfa hay, but not alfalfa silage would alleviate tallow-induced milk fat depression of cows fed corn silage-based diets. However, the results suggest that alfalfa preserved either as silage or hay affects the response to supplemental tallow similarly when corn silage based-diets are fed. 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 fatty acids in the rumen, and increased milk fat percentage and yield. 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.

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Table 1. Ingredient composition (DM basis) of experimental diets¹.

	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			
Mineral-vitamin mix ²	0.8	0.8	0.75	0.75	0.75
Tallow ³		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, and 3304 IU/g of vitamin A, 1101 IU/g of vitamin D, and 11 IU/g of vitamin E.

³Fatty acid composition (g/100 g fatty acids) of tallow (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 fatty acids.

Table 2. Chemical composition of experimental diets¹.

	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/1	00 g of fatty ac	ids)	
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
trans C18:1	0.5	1.3	1.7	1.3	1.2
cis 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

 T CS = 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. 2 Calculated (NRC, 2001).

Table 3. Particle size distribution (\pm SD) expressed as % 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 ± 0.1	0.3 ± 0.1	1.4 ± 0.5	12.0 ± 0.9	0.8 ± 0.1
18.0	1.4 ± 0.3	1.2 ± 0.3	4.9 ± 1.0	3.0 ± 0.5	4.7 ± 0.4
8.98	27.2 ± 1.1	27.7 ± 1.1	19.9 ± 1.5	15.9 ± 0.6	22.0 ± 0.8
5.61	12.0 ± 0.7	12.1 ± 0.4	10.8 ± 0.6	9.9 ± 0.3	13.2 ± 0.5
1.65	31.3 ± 1.7	31.1 ± 0.7	30.4 ± 1.4	28.4 ± 0.4	30.9 ± 1.2
Pan	27.8 ± 2.3	27.7 ± 1.5	32.5 ± 2.5	30.8 ± 0.7	28.4 ± 1.4
MPS ³ , mm	3.6 ± 0.2	3.6 ± 0.1	3.4 ± 0.3	4.1 ± 0.1	3.6 ± 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.

³Nonfibrous carbohydrates.

³MPS = Geometric mean particle size.

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

			Treatment 1				Sta	itistical cor	ntrasts (P	
	CS	CST	SAHT	LAHT	AST	SE	Ą	В	၁	D
DMI, kg/d	27.6	25.9	26.7	26.6	26.5	9.0	0.01	0.12	SN	NS
NDFI³, kg/d	8.3	7.7	8.1	7.7	7.7	0.2	0.01	SN	0.11	0.10
Milk, kg/d	44.9	44.3	44.8	44.3	43.6	1.8	SN	SN	NS	SN
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	90.0	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	SN	NS	NS	0.05
SNF ⁴ , kg/d	3.9	3.8	3.8	3.8	8.7	0.16	NS	NS	NS	NS
200	:	١.		11	100	I٠	1001	١.		١.

alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa 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 ²A = CS vs. CST; B = CST vs. SAHT + LAHT + AST; C = SAHT vs. LAHT; D = SAHT vs. AST; NS= not significant. ¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow;

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³Neutral detergent fiber intake. ⁴Solids-not- fat.

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

					,					
			Treatment	-			Stai	Statistical contrasts (P	itrasts ($P \cdot$	<) ₇
	CS	CST	SAHT	LAHT	AST	SE	A	В	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	90.0
C18:0	9.6	6.6	11.4	10.3	12.1	9.0	SN	0.02	0.05	NS
C18:1	22.6	27.2	26.2	26.5	27.1	0.8	0.001	SN	SN	NS
C18:1 isomers										
trans-6/8	0.30	09.0	0.49	0.62	0.44	0.04	0.001	0.04	0.01	NS
trans-9	0.35	0.47	0.37	0.48	0.37	0.04	0.04	0.11	0.04	NS
trans-10	0.75	2.15	1.00	1.71	0.78	0.22	0.001	0.001	0.01	NS
trans-11	0.86	0.89	0.89	1.03	1.06	0.05	NS	SN	0.11	0.05
trans-12	0.59	0.52	0.63	0.70	0.71	90.0	NS	0.02	NS	NS
Total trans-C18:1	2.84	4.62	3.35	4.53	3.36	0.27	0.001	0.01	0.01	SN
cis-9	16.9	20.6	20.4	19.6	20.5	9.0	0.001	SN	NS	NS
cis-11	0.22	0.12	0.23	0.30	0.24	0.05	NS	0.03	SN	NS
cis-12	0.51	99.0	0.52	0.57	0.48	0.07	0.15	0.08	SN	NS
CLA ³ c9t11	0.49	09.0	0.50	0.57	0.62	0.04	0.02	NS	0.07	0.01
CLA t10c12	0.01	0.04	0.05	0.03	0.03	0.002	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	0.05	0.02
Other	9.5	6.6	9.6	9.2	8.2	0.7	NS	NS	NS	0.0
Total trans ⁴	3.4	5.2	3.9	5.2	4.0	0.3	0.002	0.003	0.004	NS
									1	,

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

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³CLA = Conjugated linoleic acid.

⁴C18:1 isomers plus CLA c9t11 and t10c12.

Table 6. Treatment effects on chewing behavior of cows.

a Branch and an annual transport to a comme										
			Treatment ¹				Sta	Statistical cor	contrasts $(P <)$) ²
	CS	CST	SAHT	LAHT	AST	SE	A	В	၁	D
Eating										
Time, min/d	194.9	196.9	250.2	295.3	242.6	9.5	NS	0.001	0.001	SN
Time/DMI, min/kg	6.9	7.6	9.3	11.2	9.2	0.4	0.13	0.001	0.001	SN
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	8.9/9	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
100-500		th 00%	- Tour tallound of the	Tr _ 500%	ممائه معمد	2002	O D D D D C D C D C D C D C D C D C D C	500 00 00 00 00 00 00 00 00 00 00 00 00	ollow. CAl	TT - 250%

CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; SAHI = 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.

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

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

			Trantment				7	orietical cor	tracte (D /	7
'			ricanneni			ļ	ว	ימרואנוזסו	(r) enemaria	/
•	CS	CST	SAHT	LAHT	AST	SE	A	В	C	D
Hd	6.23	6.26	6.32	6.40	6.31	0.05	SN	0.02	0.03	SN
NH3-N, mo/dl	11.5	8.6	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
		u	nol/100 mo	·						
Acetate	62.5	61.4	65.1	65.4	62.9	0.7	NS	0.001	NS	SN
Propionate	21.9	23.4	20.1	20.1	18.9	0.8	NS	0.002	NS	SN
Butyrate	11.9	11.4	11.0	10.9	11.5	0.4	NS	NS	NS	SN
Isobutyrate	0.7	9.0	0.8	0.7	0.8	0.04	SN	0.01	NS	SN
Isovalerate	1.4	1.5	1.3	1.3	1.5	0.08	SN	NS	NS	SN
Valerate	1.7	1.6	1.7	1.6	1.5	90.0	SN	NS	0.04	0.01
$\mathrm{A:P}^4$	2.9	2.7	3.3	3.4	3.6	0.1	NS	0.001	NS	NS

^TCS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow;

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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. 3T otal VFA. SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% long

⁴Acetate to propionate ratio.

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

					1					
		:	Treatmen				Sta	Statistical contrasts (ontrasts (F	, <) ²
	CS	CST	SAHT	LAHT	AST	SE	A	В	C	D
Intake, g/d										
C18:0	59.0	117.9	125.7	120.5	115.2	6.9	0.002	NS	NS	SN
trans C18:1	5.4	15.2	18.5	15.3	15.1	1.4	0.001	SN	0.09	0.0
cis C18:1	297.4	378.4	359.3	375.2	366.2	18.5	0.02	SN	NS	NS
C18:2	422.6	398.1	347.6	386.5	352.4	22.8	NS	0.13	0.12	SN
C18:3	47.5	41.0	44.7	50.9	49.8	2.9	0.08	0.02	90.0	NS
Total C18 832	832.9	948.3	895.8	948.4	7.668	43.8	0.08	SN	SN	SN
Omasal flow, g	p/									
C18:0	484.4	547.9	586.7	518.0	528.1	41.6	SN	NS	NS	NS
trans C18:1	96.3	137.9	127.9	120.6	104.7	11.6	0.03	0.14	SN	0.11
cis C18:1	118.2	135.6	148.8	132.9	136.6	13.0	NS	NS	SN	SN
C18:2	60.3	56.6	62.3	59.0	66.1	6.3	SN	NS	SN	NS
C18:3	6.0	5.4	7.3	9.9	7.9	6.0	SN	0.12	SN	SN
Total C18 765.8	765.8	886.7	934.3	838.3	843.9	52.5	0.12	SN	NS	NS
Apparent biohy	'drogenat	ion, %								
cis C18:1	56.3	61.6	60.5	60.0	60.0	3.6	SN	NS	SN	NS
C18:2	83.6	85.0	82.2	82.4	79.1	2.2	SN	0.11	NS	SN
C18:3	85.7	86.1	83.7	85.4	82.3	2.0	SN	SN	NS	NS
Total C18 ³	73.1	74.4	72.1	71.8	70.0	2.4	NS	0.12	NS	SN

long alfalfa hay + 50% concentrate with 2% tallow; AST = 25% corn silage + 25% alfalfa silage + 50% concentrate with SAHT = 25% corn silage + 25% short alfalfa hay + 50% concentrate with 2% tallow; LAHT = 25% corn silage + 25% ¹CS = 50% corn silage + 50% concentrate with 0% tallow; CST = 50% corn silage + 50% concentrate with 2% tallow; 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 16