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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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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.

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 $0.05 \leq P < 0.15$.

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 NE_L 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 ($P <$

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 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 NH_3-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 NH_3-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 NH_3-N concentration. Cows fed LAHT had lower ($P < 0.01$) ruminal NH_3-N concentration than cows fed SAHT. Ruminal NH_3-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 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 source. 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 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
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

[1]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).

[3]Nonfibrous carbohydrates.

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

	Treatment[1]						Statistical contrasts ($P <$)[2]			
	CS	CST	SAHT	LAHT	AST	SE	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[4], kg/d	3.9	3.8	3.8	3.8	8.7	0.16	NS	NS	NS	NS

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

[3]Neutral detergent fiber intake.

[4]Solids-not- fat.

Other	9.5	9.9	9.6	9.2	8.2	0.7	NS	NS	NS	0.09
Total *trans*[4]	3.4	5.2	3.9	5.2	4.0	0.3	0.002	0.003	0.004	NS

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

[3]CLA = Conjugated linoleic acid.

[4]C18:1 isomers plus CLA c9t11 and t10c12.

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

	Treatment[1]						Statistical contrasts ($P <$)[2]			
	CS	CST	SAHT	LAHT	AST	SE	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

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

[3]Total VFA.

[4]Acetate to propionate ratio.

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

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