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*Trans*18:1 and 18:2 Isomers in Blood Plasma and Milk Fat of Grazing Cows Fed a Grain Supplement Containing Solvent-Extracted or Mechanically Extracted Soybean Meal

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ABSTRACT

Thirty Holstein cows grazing mixed clover-grass pastures for 12 wk from May through July were fed a grain supplement containing solvent-extracted soybean meal (SES), or mechanically extracted soybean meal (MES) to determine whether differences in supplemental 18:2n6 fatty acid intake altered secretion of unsaturated fatty acids. Groups of 10 cows each were fed in two equal feedings a supplement (7.3 kg/d) containing ground corn plus either 1.8 kg of SES, 2.2 kg of MES, or 2.2 kg of MES plus 30 g of methionine hydroxy analog (Alimet; MESM). Fatty acid content (% of DM) of grass and clover in pastures averaged 1.9 and 1.5%, respectively. Concentration of 18:3n3 was higher in grass compared with clover (532 vs. 454 mg/g of total fatty acids). Yield of milk (32 kg/d average) and milk components did not differ by supplements. Total blood plasma fatty acids (mg/ml) during wk 4 were higher due to MESM (1.0) compared with MES (0.6) or SES (0.6). Cows fed MESM or MES had greater concentrations of 18:2n6, *trans*11-18:1, and *cis*9,*trans*11-18:2 in plasma compared with cows fed SES. The additional *trans*11-18:1 was found exclusively in plasma triglycerides, whereas the additional *cis*9,*trans*11-18:2 was found in plasma phospholipids and free fatty acids. Daily yields of 18:2n6, *trans*11-18:1 and *cis*9,*trans*11-18:2 in milk fat were greater for cows fed MES or MESM compared with SES. Results indicate yields of *trans*11-18:1, *cis*9,*trans*11-18:2, 18:2n6, and 18:3n3 in milk fat of pasture-fed cows were enhanced by feeding a grain supplement containing mechanically extracted, rather than solvent-extracted, soybean meal.
(**Key words:** *trans*-vaccenic acid, *cis*9,*trans*11-18:2, *trans*11,*cis*15-18:2, soybean meal)

Abbreviation key: MES = mechanically extracted soybean meal, **MESM** = mechanically extracted soybean meal plus methionine hydroxy analog, **SES** = solvent-extracted soybean meal.

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INTRODUCTION

Milk fat is the richest natural dietary source of the *cis*9,*trans*11 isomer of conjugated linoleic acid, which is considered a potent anticarcinogen (Ip et al., 1999). In the rumen, *cis*9,*trans*11-18:2 results primarily from isomerization of dietary 18:2n6 during the first step of the biohydrogenation process (Kepler and Tove, 1967). Subsequent reductions of the double bonds at carbons 9 and 11 yield *trans*11-18:1 and 18:0, respectively, as major products (Polan et al., 1964). Dietary 18:3n3 also undergoes biohydrogenation by being first isomerized to a conjugated triene (*cis*9,*trans*11,*cis*15-18:3), followed by reductions of the double bonds at carbons 9, 15, and 11 to yield *trans*11,*cis*15-18:2, *trans*11-18:1, and 18:0, respectively (Wilde and Dawson, 1966). Biohydrogenation of 18:2n6 or 18:3n3 also can result in variable production of *trans*-18:1 isomers (with double bonds at positions 9, 10, and 12) and *cis*-18:1 isomers (with double bonds at positions 9, 12, and 15) (Kemp et al., 1975).

In pasture grasses and legumes, 18:2n6 or 18:3n3 account for 17 and 60% of total fatty acids (Hawke, 1973). Milk from grazing cows contains more *trans*-fatty acids than milk from cows fed a TMR. *Trans*11-18:1 accounted for 55% of total *trans*-18:1 isomers in milk fat from grazing cows compared with 33% in milk from cows fed a TMR (Precht and Molkentin, 1997). Concentrations of *trans*18:1 isomers with double bonds at other positions (4 through 10 and 12 through 16), however, did not differ due to diet. Concentration of *cis*9,*trans*11-18:2 in milk from grazing cows also was greater compared with milk from cows fed a TMR (Kelly et al., 1998).

Supplementing daily pasture intake with full-fat soybeans or rapeseed increased *cis*9,*trans*11-18:2 in milk fat (Lawless et al., 1998), indicating the addition of 18:2n6 in the diet will yield more *cis*9,*trans*11-18:2 in milk fat. Oil seeds are typically processed to remove the oil, but the method used to process oilseeds for lactating cows may influence *cis*9,*trans*11-18:2 content in milk fat. For example, feeding extruded cottonseed or soybeans increased *cis*9,*trans*11-18:2 concentration in milk fat 100% compared with controls (Dhiman et al., 1999b). Lipid content of mechanically extracted soybean meal (**MES**; SoyPlus, West Central Cooperative, Ralston, IA) is nearly threefold greater than that of solvent-extracted soybean meal (**SES**), thus it can provide more 18:2n6 for biohydrogenation in the rumen and may alter fatty acid composition of milk fat.

Methionine and lysine were considered the most limiting amino acids for milk production in dairy cows grazing high quality pastures, because extensive degradation of ingested pasture protein may limit the supply of protein and amino acids to the small intestine. When cows were fed equal amounts of ryegrass and white clover pasture, amino acid-N flow to the duodenum was higher than with feeding fresh ryegrass alone (Kolver et al., 1999). However, methionine flow was only 71% of its absorbable requirement and may have limited milk production. In addition to serving as a methyl donor for methylation and synthesis of DNA, methionine is required for synthesis of membrane phospholipids and plasma lipoproteins (Gruffat et al., 1996). Methionine also influences lipid metabolism in the mammary gland, as indicated by higher milk fat percentage (Polan et al., 1970; Huber et al., 1984) and concentrations of *cis*9-16:1, 18:0, or *cis*9-18:1 in milk fat (Canale et al., 1990) when lactating cows were fed rumen-protected methionine.

A supplemental source of dietary energy and RUP may be necessary to meet metabolizable energy and protein requirements of high producing grazing cows. Hypothetically, a supplement containing corn grain combined with MES (RUP = 60% of CP) should meet the dietary requirements of a grazing cow more closely than a supplement containing corn grain plus SES (RUP = 43% of CP). The methionine content of soybean meal may, however, be limiting. Thus, the addition of rumen-protected methionine to soybean meal may benefit the high producing grazing cow. The objectives of this study were to evaluate the above hypothesis with respect to 1) milk production and component yields, 2) changes in concentrations of *cis* and *trans* isomers of 18:1 and *cis,trans* isomers of 18:2 in blood plasma and their yields in milk fat.

MATERIALS AND METHODS

Grazing Management and Experimental Design

An 11-ha clover-grass pasture at the Virginia Tech Dairy Center was subdivided into four paddocks of approximately 2.7 ha each, and further subdivided daily to provide intensive rotational grazing. The predominant species in swards were orchardgrass (*Dactylis glomerata*), white clover (*Trifolium repens*), and red clover (*Trifolium pratense*). The proportions of Kentucky bluegrass (*Poa pratensis*) were small compared with the above species. The relative proportions of major species varied across paddocks and throughout the grazing season. During grazing, an electrified nylon string separated grazing areas within a paddock. Water was always available near the cows. Cows grazed between 0400 and 1030 h and 1500 and 2200 h. At other times, cows were kept in a dirt lot with ad libitum access to orchardgrass hay and water. Stubble remaining in pasture fields after a grazing session was either clipped or mob-grazed by dry cows.

Thirty lactating Holstein cows between 17 and 119 DIM were used for a 12-wk study from May through July 1998. Cows were initially fed a TMR for 2 wk. To obtain an estimate of group-fed DMI before grazing, the amounts of feed offered and refused during the last 4 d were recorded. Cows then were adapted to grazing for 7 d (wk 0) by allowing them equal time each day to pasture or to TMR. At the end of the adjustment period, cows were ranked by milk production, blocked by yield, then assigned to one of three groups randomly within blocks. Each group was offered 6.7 kg/d (DM basis) of a supplement (Table 1) containing corn grain combined with SES, MES (SoyPlus), or MES plus 30 g of liquid methionine hydroxy analog (Alimet, Novus Intl., Inc., St. Louis, MO) (**MESM**). Cows were group-fed their assigned supplement in equal proportions after milking at 0100 and 1300 h. Supplement refusals were negligible. Body weight was determined at 0, 6, and 12 wk.

Sample Collection and Analyses

Milk production was recorded electronically at each milking throughout the study. At 2-wk intervals from 0 through 12 wk, a 30-ml aliquot of milk was collected in a vial containing bronopol (D & F Control Systems, San Ramon, CA) at 1300 h. Milk was analyzed (United D.H.I. Lab, Blacksburg, VA) for fat, protein, lactose, and SNF content by infrared analysis with a four-channel spectrophotometer (Foss Electric, Hillerød, Denmark). At 4-wk intervals (wk 0, 4, 8, and 12), an additional aliquot of milk was collected without bronopol, then stored at −20°C. Subsequently, samples were thawed at room temperature and centrifuged at 10,000 × *g* for 1 h to isolate milk fat for fatty acid analysis.

Blood samples (10 ml) were obtained during wk 0 and 4 from the jugular vein. After collection, blood was transferred to tubes containing 286 IU of heparin in 100 μl of sterile saline and centrifuged at 3000 × *g* for 15 min for harvesting plasma. Plasma was stored at

Table 1. Composition of supplements.[1]

	SES	MES	MESM
Ingredients		% of DM	
Ground corn[2]	66.9	60.8	60.5
Soybean meal, 48% CP[3]	25.0	0.0	0.0
SoyPlus[4]	0.0	31.1	31.1
Mineral/vitamin mix[5]	6.6	6.7	6.7
$NaHCO_3$	1.5	1.4	1.2
Alimet[6]	0.0	0.0	0.5
Chemical composition			
CP, % of DM	19.2	21.1	21.4
RUP, % of CP	43.1	60.2	60.8
NE_L, Mcal/kg DM	1.95	2.01	2.01
Total fatty acids, % of DM	2.5	3.6	3.6
		mg/g of Total fatty acids	
16:0	105	109	109
18:0	47	28	28
*cis*9-18:1	214	159	159
18:2n6	612	660	660
18:3n3	22	43	43

[1]Twelve samples (collected weekly) of each supplement were composited and analyzed in duplicate. SES = Solvent-extracted soybean meal; MES = mechanically extracted soybean meal; MESM = MES plus methionine hydroxy analog.

[2]Ground corn: CP = 100 g/kg, RUP = 600 g/kg CP, fatty acids = 34 g/kg, and NE_L = 1.96 Mcal/kg.

[3]SES: CP = 500 g/kg, RUP = 340 g/kg CP, fatty acids = 13 g/kg, and NE_L = 1.93 Mcal/kg.

[4]SoyPlus (West Central Cooperative, Ralston, IA): CP = 483 g/kg, RUP = 600 g/kg CP, fatty acids = 49 g/kg, and NE_L = 2.10 Mcal/kg.

[5]Mineral/vitamin mix (Southern States Cooperative, Richmond, VA): salt (38 to 48 g/kg), $NaHCO_3$ (180 g/kg), Ca (145 to 174 g/kg), P (65 g/kg), Cl (58 g/kg), S (32 g/kg), Mg (22 g/kg), K (35 g/kg), Mn (1 g/kg), Zn (1 g/kg), Fe (0.3 g/kg), Cu (0.1 g/kg), I (0.02 g/kg), Co (0.003 g/kg), Se (0.005 g/kg), F (0.65 g/kg), retinyl acetate (0.36 g/kg), cholecalciferol (0.01 g/kg), DL-α-tocopherol acetate (0.59 g/kg).

[6]Alimet (Novus Intl., Inc., St. Louis, MO): 890 g DL-Methionine hydroxy analog/kg DM.

–20°C until analyzed for total fatty acids and fatty acids in plasma lipid fractions.

Throughout the study, a minimum of six samples of grass and clover from a paddock were collected along a diagonal transect across the area before grazing. Quadrants (0.25 m^2 each) were used to obtain the samples, leaving a stubble of ~5 cm. Grass and clover were separated, weighed, and dried in a forced-air oven at 55°C until constant weight. A sample of each supplement mixture was collected after each weekly mixing, then oven-dried at 55°C. Dried samples were stored in sealed plastic containers. Equal amounts of dried grass or clover were combined for each paddock to determine chemical composition and fatty acid profiles. This resulted in five samples of each forage from May, June, and July for analyses (Table 2). Dried forages and supplements were ground through a 2-mm screen (Thomas-Wiley Laboratory Mill), then through a 1-mm screen (Cyclotec mill, Tecator 1093, Hoganas, Sweden) before analyses for ADF and NDF (Van Soest et al., 1991), total N (AOAC, 1990), and fatty acids. Net energy (NE_L) and RUP content of each supplement (Table 1) was estimated using individual values for ground corn and SES (NRC, 1989). For MES, RUP values used were those specified by the manufacturer (SoyPlus).

Lipids were extracted from plasma (2 ml), herbage, and supplements with chloroform/methanol (2:1, vol/vol). Blood plasma lipid fractions (cholesterol esters, phospholipids, triglycerides, and FFA) were isolated (Kaluzny et al., 1985) from lipid extracts using Bond Elut aminopropyl disposable columns (Analytichem International, Harbor City, CA).

Fatty acids in milk fat and lipid extracts from blood plasma, plasma lipid fractions, herbage, and supplements were directly methylated with 0.5 *N* NaOH in methanol (Park and Goins, 1994) and 14% boron trifluoride in methanol. Undecenoate (Nu-Check Prep, Elysian, MN) was used as the internal standard. Samples were injected by auto-sampler into a Hewlett-Packard 5890A gas chromatograph equipped with a flame-ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters of fatty acids in blood plasma lipid fractions were separated using a 30-m × 0.25-mm i.d. fused silica capillary column (SP-2380, Supelco, Inc., Bellefonte, PA). Methyl esters in the remaining samples were separated using a 100-m × 0.25-mm i.d. fused silica capillary column (CP-Sil 88, Chrompack, Middelburg, The Netherlands).

For fatty acid analysis of milk, herbage, and supplement (0.5 μl of methyl esters in hexane injected at a 70:1 split ratio), the injector and detector temperatures were maintained at 255°C. The initial oven temperature was held at 70°C for 1 min, increased 5°C/min to 100°C (held for 2 min), then increased at 10°C/min to 175°C (held for 40 min), and 5°C/min to a final temperature of 225°C (held for 15 min). Ultra-pure hydrogen was the carrier gas.

Analysis of total fatty acids in blood plasma required injection of 2 μl of methyl esters (splitless). The injector temperature was maintained at 150°C and the detector temperature at 255°C. The purge valve on the GC was closed for 1.5 min after sample injection. The initial column temperature was 40°C (held for 1.5 min), then increased at 40°C/min to 100°C (held for 10 min), 25°C/min to 175°C (held for 70 min), and 10°C/min to a final temperature of 220°C (held for 20 min). Ultra-pure hydrogen was the carrier gas.

Analysis of fatty acids in plasma lipid fractions required injection of 2 μl of methyl esters in hexane (30:1 split ratio). The injector and detector temperatures were maintained at 225°C. The initial column temperature was 60°C (held for 3 min), then increased at 5°C/min to 205°C (held for 12 min) and 2°C/min to a final

Table 2. Composition of clover and grass in mixed pastures.[1]

Item	Grass			Clover			SEM	Effects		
	May	June	July	May	June	July		Species	Month	Species by month
	% of DM									
NDF	65.5	64.9	63.8	25.5	27.5	28.4	1.1	0.01	0.78	0.15
ADF	29.9	29.6	29.0	19.7	22.4	22.9	0.8	0.01	0.24	0.04
CP	21.1	21.9	21.6	25.5	25.2	24.5	0.7	0.01	0.80	0.58
Total fatty acids	2.2	1.9	1.7	1.6	1.5	1.5	0.1	0.01	0.01	0.01
	mg/g of Total fatty acids									
14:0	5.3	7.1	6.5	5.1	5.5	5.7	0.3	0.01	0.01	0.01
16:0	192.1	211.3	224.4	229.5	244.1	252.3	13.1	0.01	0.01	0.23
*cis*9-16:1	1.9	3.8	4.3	3.3	3.6	3.5	0.7	0.93	0.04	0.17
18:0	16.3	17.7	19.8	33.8	35.5	35.7	1.7	0.01	0.03	0.42
*cis*9-18:1	22.1	17.6	22.1	35.8	37.7	38.6	3.7	0.01	0.09	0.23
18:2n6	203.9	205.5	223.6	211.1	226.1	233.6	7.3	0.01	0.01	0.26
18:3n3	558.5	536.9	501.1	481.6	448.2	431.9	16.1	0.01	0.01	0.39

[1]Samples were collected throughout May, June, and July from each of four fields before being grazed. After compositing by field, 15 samples of clover and grass were used for chemical analyses. Values shown in the table are the average of five observations for May, June, or July.

temperature of 220°C (held for 2 min). Helium was the carrier gas.

Statistical Analysis

Data are reported as least squares means ± SEM. Data were analyzed using the MIXED procedure of SAS with or without repeated measures (SAS, 2000). Observations at wk 0 served as covariate for statistical analyses, with or without repeated measures, of all data except forage composition. The model for statistical analysis of BW, milk yield, milk component percentages and yields, and milk fatty acid yields included: covariate adjustment, supplement (SES, MES, or MESM), week, supplement × week interaction, cow within supplement, and residual error. The model for statistical analysis of blood plasma fatty acids included: covariate adjustment, supplement (SES, MES, or MESM), cow within supplement, and residual error. The model for statistical analysis of forage chemical composition included: forage species, month of collection, species × month interaction, paddock, and residual error. For all data except forage chemical composition, orthogonal contrasts were used to determine differences due to supplements. Differences were designated as significant at $P \leqslant 0.05$. However, all P values are presented in tables. The orthogonal contrast presented in tables and figures are MES + MESM versus SES, and MES versus MESM.

RESULTS AND DISCUSSION

Chemical Composition of Pastures and Supplements

Supplements containing MES or MESM, compared with SES, had higher RUP concentration and total fatty acid content and provided more 18:2n6 and 18:3n3 (Table 1). Mechanical extraction (expeller extraction) of soybeans (SoyPlus) involves heating of cracked dried beans and extraction of oil inside expeller presses due to pressure created by a central revolving mechanism (Shaver, 1999). Solvent extraction also involves heating of soybeans but at lower temperatures for smaller periods of time, and the oil is removed by extraction with hexane. Thus, the resulting meal from the expeller process has a higher RUP and oil content than does solvent-extracted meals.

Chemical composition of grass and clover in mixed pastures differed (Table 2). Grass had greater concentrations of NDF, ADF, and total fatty acids compared with clover, but CP concentration was higher in clover. Values for NDF, ADF, and CP were in agreement with results obtained during previous grazing studies conducted by our laboratory. Although concentrations of NDF, ADF, and CP did not change between May and July, total fatty acid content of grass, but not clover, decreased. The primary fatty acid in grass and clover was 18:3n3, but the 18:3n3 concentration in total fatty acids was greater in grass than clover. Clover, however, contained more 16:0, 18:0, *cis*9-18:1, and 18:2n6 compared with grass. As grazing progressed from May through July, the concentrations of saturated fatty acids and 18:2n6 increased, but 18:3n3 concentration in grass and clover decreased. In previous studies, total concentration and profiles of fatty acids in grasses and clover during grazing were shown to change from spring through fall (Hawke, 1973; Bauchart et al., 1984; Dewhurst et al., 2001). At any stage of growth, however, grasses contained more fatty acids than clovers (Hawke, 1973).

Monogalactolipids are the major lipids in forages, and are primarily found in chloroplasts of leaf tissue (Hawke, 1973). Due to greater chloroplast concentration, green leaves from immature growing forage contain a higher lipid concentration than green leaves from mature forage (Hawke, 1973). Environmental temperature also affects forage lipid content and 18:3n3 concentration, both being higher at 15 to 20°C compared with 30°C (Hawke, 1973).

Overall, grain supplements were a primary source of *cis*9-18:1 and 18:2n6 during grazing, and supplement intake was constant throughout the 12-wk grazing period. In contrast, pasture was a primary source of 18:3n3, but the concentration of 18:3n3 declined from May through July, especially from the orchardgrass portion.

Body Weight, Milk Production, and Milk Component Yields

Although average BW of cows in SES (542 kg), MES (545 kg), and MESM (548 kg) were similar (SE = 9.7) during the 12-wk grazing period, the averages were numerically lower than the initial average weight (550 kg) of all cows at wk 0. Cows lost an average of 15 kg between wk 0 and 6, but gained an average of 8 kg between wk 6 and 12. Despite the similar energy content of good quality pasture and a typical TMR, grazing cows often do not consume enough forage DM to meet requirements for lactation plus physical activity (Kolver, 1997). In addition, weight loss associated with early lactation demands may have contributed to the declining BW averages between wk 0 and 6.

Cows averaged 47 kg of milk/d during the preliminary (TMR) period, but during grazing milk production declined steadily to approximately 30 kg/d by wk 8, then remained relatively constant until wk 12. Overall, there was a consistent tendency ($P = 0.08$) for cows fed MES or MESM to produce approximately 2 kg more milk per day than cows fed SES (Table 3). Percentages and yields of milk components, except SNF, did not differ due to treatments. The greater yield of SNF by cows fed MES or MESM was due to nonsignificant ($P = 0.07$) increases in protein and lactose yield.

Relative to published amino acid requirements, methionine and lysine (in this order) were identified as the most limiting amino acids for milk production by cows grazing mixed pastures (Kolver et al., 1999). Leucine, arginine, and histidine also were found to be colimiting. Methionine deficiency is not surprising, because its concentration in rumen microbial protein and in most feed proteins is low. According to the Cornell-Penn-Miner model (data not shown), the addition of methionine hydroxy analog to MES in this study should have alleviated the methionine deficiency of the diet, but lysine was limiting milk protein synthesis. Estimated average dietary N intake averaged 616 g/d for MES or MESM and 560 g/d for SES-supplemented cows. However, the N contribution from RUP, including methionine hydroxy analog in MESM, was expected to have been 50 g/d higher for cows fed MES or MESM compared with SES. The lack of response to supplemental methionine (MESM) suggests that lysine or other amino acids were limiting or affecting the physiological utilization of methionine for milk production. Also, energy intake was shown to be the limiting factor preventing greater milk production in high yielding grazing cows fed high versus low supplemental RUP (Hongerholt and Muller, 1998).

Plasma Fatty Acid Profiles

Fatty acid concentrations in isolated blood plasma phospholipids on wk 4 of the study were greater for cows fed MES or MESM compared with SES (Table 4). Concentrations of fatty acids in other plasma lipid fractions and the sum of all fatty acids recovered in lipid fractions did not, however, differ due to treatment. Recovery of phospholipids and cholesterol esters from solid-phase extraction columns tends to decline as lipid content of plasma increases (Kaluzny et al., 1985). However, the distribution of fatty acids within a plasma lipid fraction is a valid method to compare treatment effects (Kaluzny et al., 1985). For example, the distribution of 18:2n6 and the intermediates in 18:2n6 biohydrogenation pathway in the rumen (*trans*11-18:1 and *cis*9,*trans*11-18:2) in each of the plasma lipid fractions are shown in Figure 1. Whereas 18:2n6 were higher in phospholipids, cholesterol esters, and triglycerides of cows fed MES or MESM, *trans*11-18:1 was elevated in the triglyceride fraction only. The *cis*9,*trans*11-18:2 isomer of conjugated linoleic acid was, however, elevated in the phospholipid and FFA fractions of cows fed MES or MESM compared with SES. Because the mammary gland extracts fatty acids primarily from the triglyceride and FFA fractions of plasma, greater concentrations of the above fatty acids should be available for milk synthesis in cows fed MES or MESM.

Under basal conditions, 18:2n6 and 18:3n3 in blood plasma of ruminants are selectively incorporated into plasma cholesterol esters and phospholipids because they are the major substrates for lecithin:cholesterol acyl transferase (Noble et al., 1972). Concentrations of dietary or ruminally derived fatty acids in the major plasma lipid fractions are directly proportional to the amounts of fatty acids absorbed from the small intestine (Noble et al., 1972). In this study, concentrations of *trans*11-18:1 and *cis*9,*trans*11-18:2 in lipid fractions

Table 3. Milk production, and milk component yields by grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM).[1]

		Supplements				Contrasts	
Item	TMR[2]	SES	MES	MESM	SEM	MES+MESM vs. SES	MESM vs. MES
Milk, kg/d	46.8	30.5	32.5	32.6	1.3	0.08	0.95
Composition			%				
Fat	3.66	3.29	3.28	3.30	0.11	0.96	0.86
Protein	2.86	2.74	2.73	2.72	0.05	0.79	0.91
Lactose	4.91	4.72	4.72	4.71	0.05	0.90	0.88
SNF	8.52	8.17	8.20	8.17	0.11	0.75	0.53
Yield			kg/d				
Fat	1.72	1.01	1.06	1.08	0.06	0.23	0.74
Protein	1.34	0.83	0.88	0.89	0.04	0.07	0.84
Lactose	2.30	1.43	1.54	1.54	0.07	0.07	0.97
SNF	3.98	2.48	2.50	2.51	0.11	0.05	0.94

[1]Values are the average of means obtained at wk 2, 4, 6, 8, 10, and 12 during grazing.

[2]TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

after 4 wk of grazing were substantially increased compared with concentrations during the preliminary period (TMR). The supplements were a primary source of 18:2n6, and the pasture was a primary source of 18:3n3 during grazing. Thus, fatty acid profiles in plasma lipid fractions provided evidence of enhanced flow of intermediates from incomplete hydrogenation of unsaturated fatty acids in the rumen of pasture-fed cows.

Direct analysis of fatty acids in plasma (without isolating lipid fractions) provides a valid quantification of individual fatty acids and the sum of all fatty acids in blood (Table 5). Supplemental methionine hydroxy analog apparently increased the amount of nearly all fatty acids in plasma. Methionine hydroxy analog (0.2% of concentrate DM) increased plasma triglyceride concentrations in lactating cows (Huber et al., 1984). When rats were fed a soybean protein-based diet supplemented with methionine (0 to 8% of diet DM), plasma very low-density lipoprotein, cholesterol ester, and phospholipid concentrations increased linearly (Sugiyama et al., 1998). Because methionine promotes the synthesis of phosphatidyl choline, which is essential for hepatic very low-density lipoprotein synthesis and secretion (Gruffat et al., 1996), it could be possible that such an effect led to higher total circulating plasma fatty acids in cows fed MESM.

Despite the larger amounts of fatty acids in plasma of cows fed MESM, comparison of responses to SES versus MES can be made on the basis of fatty acid proportions in blood plasma. The following discussion focuses on plasma fatty acid profiles in terms of proportions. Fatty acid proportions can be calculated from data in Table 5.

The profiles of biohydrogenation intermediates in blood plasma were affected by differences in the 18:2n6 or 18:3n3 content of the diets. Compared with SES,

Table 4. Concentrations of fatty acids in lipid fractions of plasma from grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM)[1].

		Supplements				Contrasts	
Lipid fractions	TMR[2]	SES	MES	MESM	SEM	MES+MESM vs. SES	MESM vs. MES
			μg/ml				
Phospholipid	303	268	283	306	13	0.05	0.26
Cholesterol ester	268	248	241	274	10	0.11	0.19
Free fatty acid	39	94	47	51	2	0.29	0.26
Triglyceride	34	32	17	19	1	0.15	0.24
Total	634	576	608	647	25	0.22	0.32

[1]Values are the means obtained during wk 4 of the study.

[2]TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

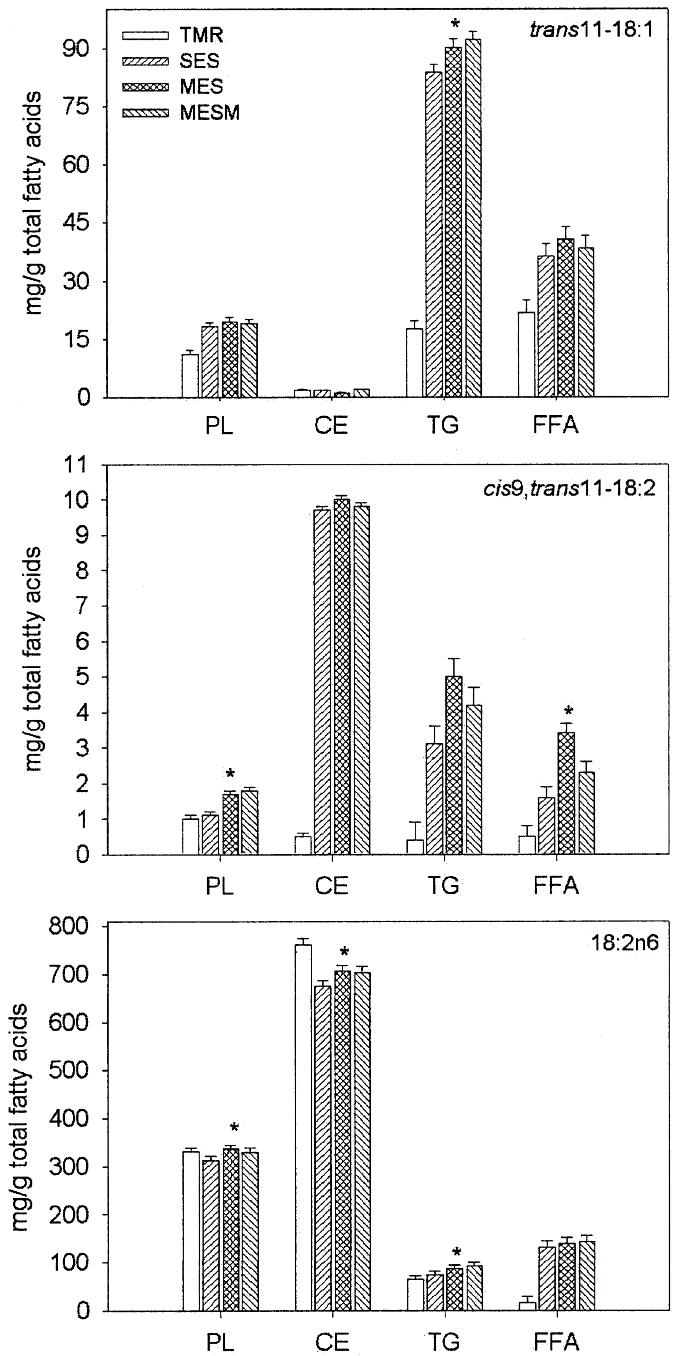

Figure 1. Distribution of *trans*11-18:1, *cis*9,*trans*11-18:2, and 18:2n6 in blood plasma phospholipids (PL), cholesterol esters (CE), triglycerides (TG), or FFA from grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM). Values for TMR are shown for comparison only. Asterisks denote differences ($P < 0.05$) due to feeding MES or MESM versus SES.

feeding MES or MESM increased the proportions of *trans*11-18:1 and *cis*9,*trans*11-18:2 in blood plasma by 60 and 45%, respectively. Increases in the concentrations of these hydrogenation intermediates in plasma fatty acids was most likely due to incomplete hydrogenation of supplemental 18:2n6 (MES or MESM) in the rumen. The fourfold greater concentration (Table 5) and proportion of *trans*11,*cis*15-18:2 in plasma during grazing, compared with the preliminary TMR, indicated incomplete hydrogenation of 18:3n3 in grass and clover. The proportion of *trans*11,*cis*15-18:2, in total plasma fatty acids did not, however, differ due to type of soybean meal contained in the supplement.

Milk Fatty Acid Yields

Feeding MES or MESM compared with SES did not affect the yield of total milk fatty acids, yields of individual saturated 6:0 to 16:0, or the sum of 6:0 to 16:0 (Table 6). Saturated fatty acids with 6 to 16 carbons can be synthesized de novo in the mammary gland, and accounted for approximately half of the total fatty acid yield even during the preliminary period (TMR). After the transition to pasture, the concentration or yield of 6- to 16-carbon fatty acids in milk fat did not differ for cows consuming pasture alone or pasture plus various supplement mixtures (Dhiman et al., 1999a). Grazing cows fed a supplement containing rapeseed (61% *cis*9-18:1) had a lower yield of 16:0 in milk fat (Lawless et al., 1998), but the lower yield was associated with increased amounts of *cis*9,*trans*11-18:2 and *trans*11-18:1 in milk fat.

Overall yields of 18:0, *cis*9-18:1, 18:2n6, and 18:3n3 during the 12-wk study were 16, 18, 25, and 20% greater, respectively, in response to MES or MESM compared with SES. Despite extensive desaturation of 18:0 to *cis*9-18:1 in the mammary gland, higher yields of 18:0 in milk fat during grazing can be expected because it is a major end product of hydrogenation of 18:2n6 or 18:3n3.

Yields of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in milk fat gradually increased from wk 0 through 8 (Figure 2). These fatty acids are the major intermediates in 18:2n6 and 18:3n3 hydrogenation in the rumen (Wilde and Dawson, 1966; Kepler and Tove, 1967), especially when fresh forage is fed. Their elevated concentrations in blood plasma (Table 5) and milk fat confirmed that pasture intake was a major factor driving this increase.

Greater concentrations of 18:2n6 in MES or MESM supplements were associated with a 23% greater yield of *trans*11-18:1 in milk fat compared with SES (Table 6). Cows fed MES or MESM also had greater yields of *trans*18:1 isomers with double bonds in the sixth, seventh, and eighth positions, but yields of *trans*13/14-18:1 were reduced by MES or MESM. Overall, the proportions of individual *trans*-18:1 isomers in milk fat

Table 5. Fatty acid concentrations in blood plasma of grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM).[1]

Fatty acid	TMR[2]	Supplements			SEM	Contrasts	
		SES	MES	MESM		MES+MESM vs. SES	MESM vs. MES
	μg/ml						
14:0	2.6	0.6	0.7	1.1	0.1	0.04	0.03
*cis*9-14:1	1.1	0.3	0.4	0.7	0.1	0.04	0.02
16:0	105	63	63	116	11.7	0.05	0.01
*cis*9-16:1	24	8.6	8.2	16	1.1	0.04	0.01
18:0	125	82	78	146	10.6	0.04	0.01
*Cis*18:1							
9	64	47	43	75.3	7.8	0.20	0.01
11	4.7	2.7	2.7	4.8	0.5	0.13	0.01
12	11	2.3	2.2	3.8	0.3	0.12	0.01
13	0.8	0.6	0.4	0.6	0.2	0.65	0.44
15	1.3	0.8	1.3	1.3	0.1	0.12	0.01
*Trans*18:1							
6,7,8	0.8	0.04	0.2	0.5	0.1	0.07	0.09
9	0.9	0.6	0.4	0.4	0.2	0.21	0.63
10	1.2	0.0	0.0	0.1	0.1	0.60	0.36
11	4.4	5.0	5.9	10	1.1	0.02	0.01
12	1.2	1.0	0.4	0.7	0.2	0.11	0.37
13,14	7.0	2.0	1.9	3.2	0.4	0.33	0.02
16	1.6	0.6	0.6	1.2	0.1	0.01	0.01
Isolated 18:2							
*c*9,*c*12	470	237	261	484	10	0.03	0.01
*t*9,*t*12	0.5	0.3	0.3	0.3	0.02	0.42	0.06
*c*9,*t*12	0.8	0.0	0.0	0.2	0.1	0.48	0.44
*t*9,*c*12	1.1	0.0	0.03	0.1	0.04	0.31	0.70
*t*11,*c*15	0.4	1.9	1.7	3.1	0.1	0.01	0.01
Conjugated 18:2							
*c*9,*t*11	0.9	1.1	1.2	1.9	0.2	0.01	0.01
*t*10,*c*12	0.01	0.0	0.0	0.0	0.0	...	...
18:3n3	53	54	46	80	8.1	0.36	0.01
20:3n6	20	9.5	9	17	2.1	0.08	0.01
20:4n6	22	14	13	25	2.0	0.04	0.01
20:5n3	24	18	16	27	1.7	0.05	0.01
Total	943	551	554	1013	98	0.05	0.01

[1]Values are the average of means obtained at wk 4 during grazing.

[2]TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

were similar to those reported for grazing cows supplemented with grain mixtures or oilseeds (Precht and Molkentin, 1997; Lawless et al., 1998) and confirmed that *trans*11-18:1 is the major isomer resulting from hydrogenation of 18:2n6 or 18:3n3 in pastures or grain supplements.

The major conjugated intermediate arising from isomerization of dietary 18:2n6 during the first step of the biohydrogenation process is *cis*9,*trans*11-18:2 (Kepler and Tove, 1967). However, *cis*9,*trans*12-18:2 also is a likely product of isomerization of 18:2n6 during fermentation (Kemp et al., 1975). Yields of *cis*9,*trans*11-18:2 and *cis*9,*trans*12-18:2 in milk fat (Table 6) were greater due to MES or MESM compared with SES. Higher yields of *cis*9,*trans*11-18:2 also were found when grazing cows were supplemented with various levels of grain or oilseed supplements (Kelly et al., 1998; Lawless et al., 1998; Dhiman et al., 1999a). Furthermore, *cis*9,-*trans*11-18:2 concentration and yield in milk fat was up to 1.5-fold greater as cows derived increasing portions of their daily DMI from pasture (Dhiman et al., 1999a).

Rumen-derived *cis*9,*trans*11-18:2 undoubtedly contributes to the pool of *cis*9,*trans*11-18:2 secreted in milk fat. In a recent study, however, it was estimated that up to 64% of the *cis*9,*trans*11-18:2 in milk fat may be synthesized endogenously from *trans*11-18:1 via Δ^9 desaturase (EC 1.14.99.5; Griinari et al., 2000). A portion of the *trans*11-18:1 may be desaturated in the enterocyte during absorption, but the remainder may be desaturated in adipose tissue or the mammary gland. The activity of Δ^9 desaturase in adipose tissue of grazing beef cattle was 73% higher compared with feedlot cattle (Yang et al., 1999).

Table 6. Milk fatty acid yields by grazing cows supplemented with solvent extracted soybean meal (SES), mechanically extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM).[1]

		Supplements				Contrasts	
Fatty acids	TMR[2]	SES	MES	MESM	SEM	MES+MESM vs. SES	MESM vs. MES
	g/d						
4:0	89.0	55.7	65.5	58.6	3.2	0.53	0.14
6:0	44.9	26.9	28.7	27.4	1.2	0.73	0.44
8:0	23.5	17.1	15.0	14.5	1.9	0.33	0.86
10:0	49.6	28.8	30.6	29.0	1.6	0.95	0.46
12:0	48.1	28.4	29.4	27.9	1.5	0.82	0.50
14:0	151.3	91.6	92.7	91.5	3.8	0.98	0.83
*cis*9-14:1	9.1	5.8	6.4	6.6	0.5	0.66	0.89
16:0	402.2	222.2	223.3	219.2	9.2	0.88	0.76
6:0-16:0	719.6	414.8	418.0	410.7	16.8	0.75	0.55
*cis*9-16:1	18.1	10.7	11.3	11.5	0.7	0.70	0.84
*trans*9-16:1	3.8	3.6	3.9	3.9	0.2	0.33	0.84
18:0	166.9	97.0	113.8	111.1	4.8	0.05	0.75
*Cis*18:1							
9	275.9	170.5	199.6	204.3	10.4	0.04	0.75
11	8.4	4.2	4.9	4.5	0.3	0.14	0.30
12	11.1	2.5	2.2	2.1	0.2	0.25	0.88
13	2.3	0.9	1.1	1.2	0.1	0.15	0.41
15	3.2	1.8	1.9	2.2	0.1	0.09	0.13
*Trans*18:1							
6,7,8	4.5	2.3	2.7	2.7	0.01	0.01	0.95
9	4.2	2.4	2.5	2.6	0.01	0.14	0.41
10	7.7	2.8	2.5	2.8	0.3	0.78	0.61
11	17.8	35.1	42.6	43.9	1.7	0.01	0.59
12	9.4	3.9	3.7	3.7	0.2	0.40	0.84
13,14	18.3	7.5	6.5	6.4	0.3	0.03	0.76
16	7.3	3.2	3.0	3.0	0.2	0.45	0.92
Isolated 18:2							
*c*9,*c*12	38.8	22.5	28.2	28.2	0.7	0.01	0.97
*c*9,*t*12	0.2	0.7	1.2	1.3	0.01	0.01	0.12
*t*9,*c*12	0.9	1.0	1.3	0.4	0.2	0.47	0.24
*t*9,*t*12	4.9	2.9	2.6	3.0	0.1	0.15	0.06
*t*11,*c*15	0.7	4.1	3.6	4.1	0.3	0.35	0.22
Conjugated 18:2							
*c*9,*t*11	5.1	8.9	12.1	13.6	0.6	0.01	0.13
*t*10,*c*12	0.4	0.7	0.7	0.9	0.1	0.55	0.28
18:3n3	5.7	4.4	5.5	5.0	0.2	0.05	0.17
20:3n6	1.5	0.8	0.9	0.9	0.05	0.13	0.54
20:4n6	2.0	1.1	1.2	1.2	0.06	0.21	0.94
20:5n3	1.2	0.7	0.6	0.7	0.04	0.73	0.49
Total	1429.8	812.5	887.2	881.4	34.1	0.09	0.91

[1]Values are the average of means obtained at wk 4, 8, and 12 during grazing.

[2]TMR = The means of observations at the end of a 2-wk preliminary period in which cows were fed a TMR are shown for comparison only.

On the basis of their profiles in rumen fluid and milk fat, it is thought that conjugated 18:2 isomers other than *cis*9,*trans*11-18:2 arise via several microbial *cis,trans* isomerases (Griinari and Bauman, 1999). Thus, *cis*9,*trans*10 isomerase may alter 18:2n6 to *trans*10,*cis*12-18:2. Low rumen pH and availability of 18:2n6 appear to be the major factors responsible for the synthesis of this isomer (Griinari and Bauman, 1999). Although not affected by the grain supplements fed in this study, the yield of *trans*10,*cis*12-18:2 in milk fat increased gradually from wk 0 through 12, when it was fivefold greater (data not shown). Thus, it appears that rumen conditions for the production of this isomer became more favorable over time. A major factor contributing to the greater yield of *trans*10,*cis*12-18:2 may have been the constant supply of 18:2n6 and starch from the grain supplement accompanied by a gradual decline in pasture intake as the study progressed. Certain propionogenic bacteria can isomerize 18:2n6 to *trans*10,*cis*12-18:2 (Verhulst et al., 1987), and their proportions in the rumen of grazing cows supplemented with grain mixtures increased in proportion with the level of supplementation (Elias et al., 1996).

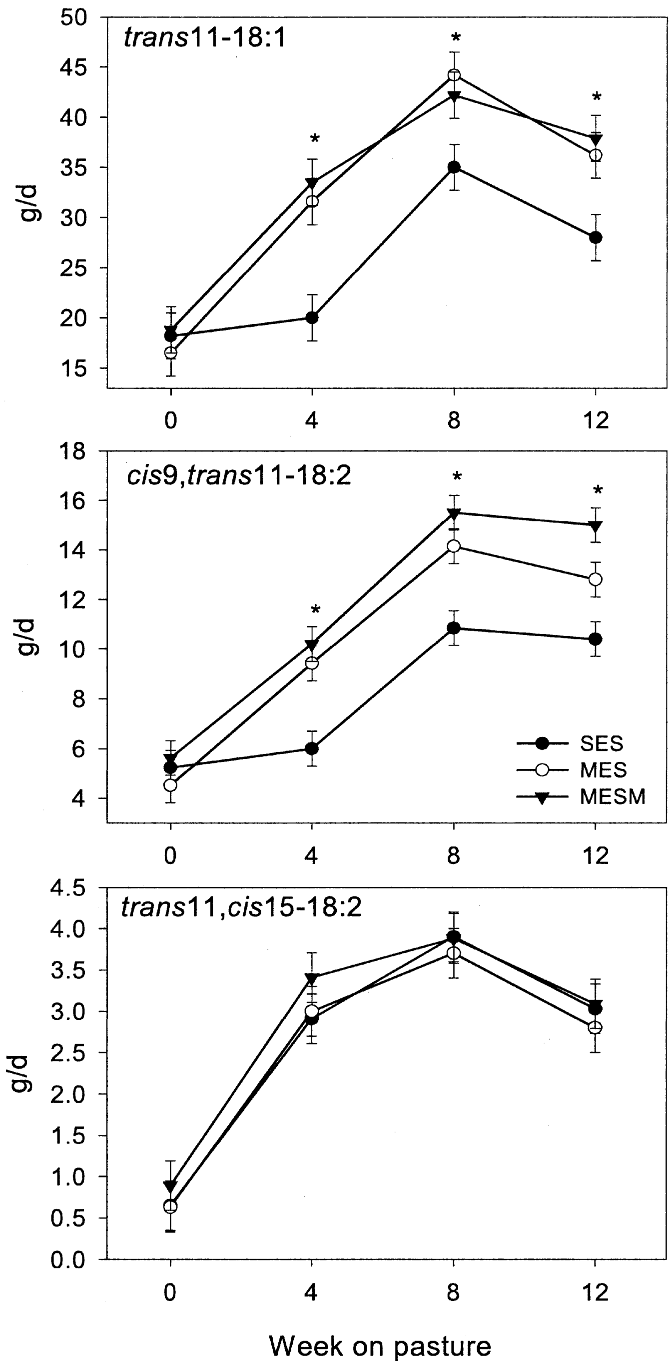

Figure 2. Yields of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in milk fat from grazing cows supplemented with solvent-extracted soybean meal (SES), mechanically-extracted soybean meal (MES), or MES plus methionine hydroxy analog (MESM). Asterisks denote differences ($P < 0.05$) due to feeding MES or MESM versus SES.

Compared with feeding a TMR during the preliminary period, grazing caused a fivefold increase in *trans*11,*cis*15-18:2 yield regardless of supplement, even with a lower total fatty acid yield. A similar increase in concentration was observed in milk fat from grazing cows compared with cows fed preserved forages (Precht and Molkentin, 2000). Using rumen fermenters fed orchardgrass or red clover, our laboratory has confirmed that incomplete hydrogenation of 18:3n3 resulted in production of *trans*11,*cis*15-18:2, which accounted for up to 15% of total daily fatty acid outflow from the fermenters.

CONCLUSIONS

Cows grazing mixed pastures, especially in the spring, have a greater intake of 18:3n3 compared with cows fed a TMR. Incomplete ruminal hydrogenation of 18:2n6 and 18:3n3 during grazing leads to greater concentrations of *trans*11-18:1, *cis*9,*trans*11-18:2, and *trans*11,*cis*15-18:2 in blood plasma and greater yields in milk fat. Yields of *trans*11-18:1, 18:2n6, *cis*9,-*trans*11-18:2, and 18:3n3 in milk fat of grazing cows can likely be increased by feeding MES, rather than SES as part of a grain supplement.

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