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Effects of forage type, forage to concentrate ratio, and crushed linseed supplementation on milk fatty acid profile in lactating dairy cows

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ABSTRACT

The effects of an increasing proportion of crushed linseed (CL) in combination with varying forage type (grass or corn silage) and forage to concentrate ratio (F:C), and their interactions on milk fatty acid (FA) profile of high-producing dairy cows was studied using a 3-factor Box-Behnken design. Sixteen Holstein and 20 Swedish Red cows were blocked according to breed, parity, and milk yield, and randomly assigned to 4 groups. Groups were fed different treatment diets formulated from combinations of the 3 main factors each containing 3 levels. Forage type (fraction of total forage dry matter, DM) included 20, 50, and 80% grass silage, with the remainder being corn silage. The F:C (DM basis) were 35:65, 50:50, and 65:35, and CL was supplied at 1, 3, and 5% of diet DM. Starch and neutral detergent fiber content (DM basis) of the treatment diets ranged from 117 to 209 g/kg and 311 to 388 g/kg, respectively. Thirteen treatment diets were formulated according to the Box-Behnken design. During 4 experimental periods of 21 d each, all treatment diets were fed, including a repetition of the center point treatment (50% grass silage, 50:50 F:C, 3% CL) during every period. Intake, production performance, and milk FA profile were measured, and response surface equations were derived for these variables. Shifting from 80% grass silage to 80% corn silage in the diet linearly increased dry matter intake (DMI), net energy for lactation (NE_L) intake, *cis*-9,*cis*-12-C18:2 (C18:2n-6) intake, and milk yield, and linearly decreased *cis*-9,*cis*-12,*cis*-15-C18:3 (C18:3n-3) intake and milk fat content. Shifting from a high forage to a high concentrate diet linearly increased DMI, NE_L intake, C18:2n-6 intake, and milk yield, and decreased milk fat content. Supplementation of CL linearly increased C18:3n-3 intake, but had no effect on DMI, NE_L intake, milk yield, or milk fat content. Shifting from 80% grass silage to 80% corn silage linearly increased proportions of *trans*-10-C18:1 and C18:2n-6 in milk fat, whereas the proportions of *trans*-11,*cis*-15-C18:2 and C18:3n-3 linearly decreased. Significant interactions between CL supplementation and F:C were found for proportions of *trans*-10-C18:1, *trans*-15-C18:1, *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2, and C18:3n-3 in milk fat, with the highest levels achieved when the diet contained 5% CL and a 35:65 F:C ratio. The effect of supplementing CL on several milk FA proportions, including C18:2n-6 and C18:3n-3, depends significantly on the F:C ratio and forage type in the basal diet.

Key words: linseed, grass silage, forage to concentrate ratio, milk fatty acid

INTRODUCTION

Because of its relatively large proportion of saturated fatty acids (**FA**), dairy milk fat has been associated with human cardiovascular health problems (Bauman and Lock, 2010; Elwood et al., 2010). On the contrary, monounsaturated FA such as oleic acid (*cis*-9-C18:1), long-chain n-3 FA, and conjugated linoleic acid in milk fat have been associated with potential benefits for human health (Bauman and Lock, 2010). Because of these effects of milk FA profile on human health, the manipulation of milk FA profile has been the subject of extensive research in recent years. The FA profile of milk fat is largely dependent on FA intake and FA metabolism in the rumen (Jenkins et al., 2008), and on lipid mobilization and FA metabolism in the mammary gland (Chilliard et al., 2007). Dietary FA are extensively metabolized and hydrogenated in the rumen, resulting in a wide range of ruminal biohydrogenation intermediates (Chilliard et al., 2007). Ruminal biohydrogenation of *cis*-9,*cis*-12-C18:2 (C18:2n-6) and *cis*-9,*cis*-12,*cis*-15-C18:3 (C18:3n-3) results in the secretion of various *trans*-C18:1, *cis*-C18:1, and nonconjugated

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and conjugated C18:2 and C18:3 isomers in milk fat. Chilliard et al. (2007) reported that the main factor in the variation of biohydrogenation is the forage to concentrate ratio (**F:C**) in the diet. After adding linseed oil to a high concentrate diet, the major biohydrogenation intermediates secreted in milk fat were *trans*-11-C18:1, *trans*-13+14-C18:1, *cis*-9,*trans*-13-C18:2, and *trans*-11,*cis*-15-C18:2 (Loor et al., 2005), whereas *trans*-15-C18:1 and *cis*-15-C18:1 were increased in duodenal flow (Loor et al., 2004). Compared with grass silage, corn silage inclusion in a diet supplemented with fish oil and sunflower oil resulted in higher proportions of *trans*-C18:1 and lower proportions of C18:0 and *trans*-C18:2 in milk fat (Shingfield et al., 2005). Basal diet appears to have a profound effect on ruminal metabolism of FA from supplemental fat sources (Shingfield et al., 2005; Soita et al., 2005), which might be related to shifts in rumen pH and microbial populations. Feeding a high starch diet markedly affects the ratio of cellulolytic to propionogenic, lactogenic, and amylolytic bacteria, which in turn affects ruminal biohydrogenation (Latham et al., 1972; Loor et al., 2004). Thus, interactions between level of lipid supplementation and other dietary changes are likely to occur.

Few direct comparisons exist between the different characteristics of the basal diet, such as type of forage and F:C, and lipid supplements. In addition, a large diversity of diets exists and quantifying interactions is important. To our knowledge, the effects of adding crushed linseed (**CL**) to diets that vary in F:C and in the proportion of grass silage to corn silage and their interactions on milk FA profile within a single experiment have not been reported. Designing an experiment in which multiple factors are considered simultaneously allows quantification of the curvature in relationships as well as interactions among factors (St-Pierre and Weiss, 2009). The Box-Behnken design (Box and Behnken, 1960) is a multifactor experimental model specifically designed for the exploration of response surfaces and it involves a smaller number of experimental points compared with a full-factorial design. The objective of this study was therefore to evaluate the effects of CL supplementation, varying forage type and F:C, and their mutual interactions, on intake, production performance, and milk FA profile. The study was carried out by varying grass silage at the expense of corn silage, F:C ratio, and level of CL supplementation in a 3-factor multivariate Box-Behnken design.

MATERIALS AND METHODS

Experimental Design and Diets

The experimental design was a 3-factor Box-Behnken design with forage type (grass silage or corn silage), F:C, and proportion of CL supplementation as the main factors. Forage type included 20, 50, and 80% grass silage (DM basis), with the remainder being corn silage. Forage to concentrate ratio was 35:65, 50:50, and 65:35 (DM basis) and CL was supplied at 1, 3, and 5% of diet (DM basis). Thirteen treatment diets with varying levels of grass silage, corn silage, F:C, and CL were formulated according to the Box-Behnken design, including the center point treatment (50% grass silage, 50:50 F:C, and 3% CL). The experiment consisted of 4 experimental periods of 21 d each, with 4 treatments evaluated, including the center point treatment, during each period (Table 1). To formulate the treatment diets, 3 commercial concentrate mixtures were used and the treatment diets were balanced for CP content. Contents of starch and NDF were allowed to differ for the different treatment diets because of the varying forage type and F:C ratio. Starch and NDF content (DM basis) in the treatment diets ranged from 117 to 209 g/kg and 311 to 388 g/kg, respectively. The treatment diets met or exceeded the requirements for NE_L (Dutch NE_L system; Van Es, 1975) and intestinal digestible protein (**DVE**; Tamminga et al., 1994). All treatment diets were offered as TMR diets. The CL was obtained from Vegolia (Falkenberg, Sweden). The specified ingredient and chemical composition of the diets are shown in Tables 2 and 3, respectively. Increasing the grass silage percentage mainly decreased starch and C18:2n-6 contents, whereas C18:3n-3 content increased (Table 3). Increasing the forage proportion mainly increased NDF and forage NDF contents, whereas starch, NE_L, DVE, C12:0, C14:0, C16:0, *cis*-9-C18:1, C18:2n-6, and C18:3n-3 contents decreased. Increasing the CL proportion mainly increased C18:3n-3 content in the diets.

Animals and Housing

The experiment was approved and carried out under the Swedish Law on Animal Experimentation. Sixteen Holstein and 20 Swedish Red cows (620 ± 50 kg of BW; 2.1 ± 0.9 parity; 72 ± 17 DIM; 48.1 ± 5.3 kg/d milk; values expressed as means ± SD) were blocked according to breed, parity, and milk yield, and randomly assigned to 4 groups. Groups were fed the different treatment diets during the 4 experimental periods. Cows were housed in freestalls with slatted floors and boxes bedded daily with sawdust on top of rubber mattresses. Individual feed intake was continuously monitored using automated feed bins with weighing equipment (BioControl A/S, Rakkestad, Norway). Each group of 9 cows had ad libitum access to 5 automated feed bins. Cows were fitted with transponders to enable individual feed intake recording from the automated feed bins. Weight changes of the bins (accuracy 0.1 kg) were

Table 1. Experimental design for the different cow groups, periods, and treatment combinations with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis)

Cow group	Period	Forage type (% grass silage)	F:C	Crushed linseed (%)
1	1	80	35:65	3
1	2	50	50:50	3
1	3	80	65:35	3
1	4	50	65:35	5
2	1	20	50:50	5
2	2	20	65:35	3
2	3	50	50:50	3
2	4	50	35:65	1
3	1	50	35:65	5
3	2	20	50:50	1
3	3	80	50:50	5
3	4	50	50:50	3
4	1	50	50:50	3
4	2	20	35:65	3
4	3	50	65:35	1
4	4	80	50:50	1

recorded and the bins were calibrated at the start of the experiment. Cows had free access to water and were milked thrice daily at 0600, 1300, and 2100 h.

Measurements and Sampling

The DMI and milk production were recorded daily during each experimental period. Milk samples were collected over 9 consecutive milkings during the last 3 d of each period, pooled per day (equal volume), and stored at 4°C using sodium azide bronopol as preservative pending analysis for fat, protein, lactose, and MUN. A second set of milk samples was taken at the same 9 consecutive milkings and immediately stored at −20°C pending FA analysis. These samples were pooled (equal volume) per cow per period during the first step in the FA analysis. Samples of all individual feed components were taken on the last 3 d of each period, pooled per period, and stored at −20°C pending analysis.

Analytical Procedures

Contents of fat, protein, lactose, and MUN in milk samples were analyzed by a Milkoscan FT 6000 (A/S N, Foss Electric, Hillerød, Denmark) at Steins Laboratory (Jönköping, Sweden). Milk samples for FA analysis were heated to 40°C and 3 mL of each individual cow milk sample was taken and pooled to form a representative milk sample of 27 mL per cow per period. These samples were then subjected to the same procedure as described by Jacobs et al. (2011). The composite samples of the individual feed components were analyzed for DM, ash, nitrogen, crude fat, starch, sugars, NDF, ADF, and acid detergent lignin as described by Abrahamse et al. (2008a,b). Preparation of feed samples for FA analysis was carried out as described by Khan et al. (2009).

Fatty acid methyl esters from milk and feed samples were quantified using gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, Waltham, MA) with a fused-silica capillary column (100 m × 0.25 mm and 0.2-μm film thickness; Restek, Rt-2560, Bellefonte, PA). The carrier gas was hydrogen at a constant flow of 0.9 mL/min and the flame-ionization detector was set at 280°C. For milk samples, a time-temperature program was used starting with an initial temperature of 70°C and held for 4 min, increasing by 1°C/min to 165°C and then held for 20 min, increasing by 2°C/min to 170°C and then held for 10 min, and increasing by 4°C/min to a final temperature of 215°C and held for 20 min. A second time-temperature program was used to separate the C18:1 isomers; initial temperature of 70°C and held for 1 min, increasing by 5°C/min to 100°C and then held for 2 min, increasing by 10°C/min to 175°C and then held for 40 min, and increasing by 10°C/min to a final temperature of 215°C and held for 20 min. For feed samples, a shorter time-temperature program was used, starting with an initial temperature of 140°C and held for 4 min, and increasing by 4°C/min to a final temperature of 240°C and held for 20 min. Fatty acid methyl esters were identified using external standards (S37, Supelco, Bellefonte, PA; odd- and branched-chain fatty acids, *trans*-11-C18:1, *cis*-9,*trans*-11-C18:2, *trans*-10,*cis*-12-C18:2, Larodan Fine Chemicals AB, Malmö, Sweden). The fatty acids *trans*-4-C18:1, *trans*-5-C18:1, *trans*-6+7+8-C18:1, *trans*-10-C18:1, *trans*-12-C18:1, *trans*-13+14-C18:1, *trans*-15-C18:1, *cis*-12-C18:1, *cis*-13-C18:1, *cis*-14+trans-16-C18:1, *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2 were identified according to the elution sequence reported by Loor et al. (2004) and Shingfield et al. (2006).

Table 2. Ingredient composition (DM basis; g/kg of DM) for diets with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis)

	20% grass silage				50% grass silage					80% grass silage			
	F:C 35	F:C 50		F:C 65	F:C 35		F:C 50	F:C 65		F:C 35	F:C 50		F:C 65
Ingredient	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL
Grass silage[1]	70	100	100	130	175	175	250	325	325	280	400	400	520
Corn silage[2]	280	400	400	520	175	175	250	325	325	70	100	100	130
Crushed linseed[3]	30	10	50	30	10	50	30	10	50	30	10	50	30
Wheat, ground	131	94	87	48	142	135	105	66	57	148	123	114	75
Oats, ground	120	86	79	43	130	123	95	60	52	135	112	104	68
Rapeseed meal, heat treated	92	63	58	29	99	94	66	41	35	101	76	70	46
Soybean meal	41	60	55	75	29	24	41	52	47	13	22	18	24
Soypass[4]	53	48	44	39	53	50	47	38	33	51	48	44	33
Sugar beet pulp	63	39	36	14	67	63	34	19	17	65	36	34	22
Rapeseed meal	22	33	30	41	16	13	22	28	26	7	12	10	13
Rapeseed, crushed	3	4	4	5	2	2	3	4	3	1	2	1	2
Oat bran	25	13	12	2	26	25	8	3	3	24	6	6	4
Wheat bran meal	9	4	4	—	9	8	1	—	—	8	—	—	—
Triticale, ground	7	3	3	—	7	7	1	—	—	6	—	—	—
Palm expeller	3	3	3	3	4	4	5	4	3	5	7	6	4
DDGS[5]	5	5	4	4	5	5	7	5	4	6	9	8	5
Other[6]	44	32	30	17	47	45	33	22	19	48	37	34	23
Premix[7]	1	1	1	1	1	1	1	1	1	1	1	1	1

[1] Grass silage, g/kg of DM: 252 DM (g/kg), 89 crude ash, 184 CP, 36 crude fat, 486 NDF, 15 sugar, 64 DVE (intestinal digestible protein; Tamminga et al., 1994), 5.82 MJ NE_L (calculated with VEM system; Van Es, 1975), 18.0 total fatty acids, 0.1 C12:0, 0.1 C14:0, 3.8 C16:0, 0.3 C18:0, 0.4 *cis*-9-C18:1, 3.3 *cis*-9,*cis*-12-C18:2, 9.6 *cis*-9,*cis*-12,*cis*-15-C18:3.

[2] Corn silage, g/kg DM: 271 DM (g/kg), 38 crude ash, 86 CP, 19 crude fat, 483 NDF, 210 starch, 45 DVE, 6.05 MJ NE_L, 13.2 total fatty acids, 0.0 C12:0, 0.1 C14:0, 3.4 C16:0, 0.3 C18:0, 2.3 *cis*-9-C18:1, 6.3 *cis*-9,*cis*-12-C18:2, 0.6 *cis*-9,*cis*-12,*cis*-15-C18:3.

[3] Crushed linseed, g/kg of DM: 932 DM (g/kg), 42 crude ash, 198 CP, 433 crude fat, 293 NDF, 9 starch, 22 sugar, 70 DVE, 12.11 MJ NE_L, 335.6 total fatty acids, 0.0 C12:0, 0.2 C14:0, 19.9 C16:0, 7.5 C18:0, 53.9 *cis*-9-C18:1, 50.1 *cis*-9,*cis*-12-C18:2, 203.6 *cis*-9,*cis*-12,*cis*-15-C18:3 (Vegolia, Falkenberg, Sweden).

[4] Soypass: heat-, xylose-, and lignosulfate-treated soybean meal (Cargill, Amsterdam, the Netherlands).

[5] Dried distillers grains and solubles from wheat.

[6] Containing blendmeal ruminants (Lantmännen, Lidköping, Sweden), magnesium oxide, monocalcium phosphate, salt, limestone, Akofeed 45 (AarhusKarlshamn, Karlshamn, Sweden), Lipitec Bovi 85 (Lipitec, Vantinge, Denmark), Lignobond DD (Borregaard Lignotechn, Sarpsborg, Norway).

[7] Contained per kilogram of mix: 58 g of Ca, 416 g of Mg, 1 g of S, 5 g of Cu, 10 g of Mn, 350 mg of I, 90 mg of Co, 200 mg of Se, 2,000,000 IU of vitamin A, 1,000,000 IU of vitamin D, 20,000 mg of vitamin E (all-*rac* tocopherol acetate; Premix KO, Lantmännen, Sweden).

Table 3. Chemical composition of diets with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis)

	20% grass silage				50% grass silage					80% grass silage			
	F:C 35	F:C 50		F:C 65	F:C 35		F:C 50	F:C 65		F:C 35	F:C 50		F:C 65
Nutrients/component, g/kg of DM	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL
DM, g/kg	504	428	425	373	474	510	416	352	344	518	389	412	356
CP	177	176	178	172	183	182	185	180	177	187	189	189	187
Crude fat	60	45	60	45	57	69	56	42	59	64	54	65	53
NDF	311	344	346	382	314	317	340	369	388	317	345	326	365
Forage NDF	172	245	244	319	171	171	245	318	318	171	244	244	317
ADF	176	202	200	229	193	185	211	232	242	193	223	210	240
ADL[1]	29	29	29	29	35	31	32	31	32	33	34	33	34
Starch	209	187	184	155	204	197	170	151	134	189	154	151	117
Sugar	53	50	49	44	43	51	46	41	36	50	37	44	38
Crude ash	57	56	57	54	62	64	63	64	59	70	66	71	74
NE_{L},[2] MJ/kg of DM	7.55	7.12	7.28	6.85	7.45	7.60	7.15	6.73	6.89	7.50	7.04	7.20	6.78
DVE[3]	98	96	93	89	98	96	94	89	86	96	94	91	85
Fatty acids													
C12:0	1.0	0.6	0.6	0.2	1.1	1.0	0.4	0.2	0.2	1.0	0.2	0.3	0.2
C14:0	0.6	0.4	0.4	0.2	0.7	0.6	0.4	0.2	0.2	0.6	0.4	0.3	0.2
C16:0	12.8	10.4	10.2	8.1	14.8	12.9	11.1	8.5	9.6	13.1	13.2	11.2	8.8
C18:0	3.0	2.3	2.3	1.6	2.1	3.1	2.5	2.2	1.6	3.1	2.2	3.5	2.4
cis-9-C18:1	10.7	8.0	9.6	7.0	11.9	11.5	9.0	5.8	8.5	10.6	9.2	9.0	6.3
cis-9,*cis*-12-C18:2	11.4	9.3	10.6	8.8	10.3	11.6	9.5	7.7	9.5	10.5	8.7	9.7	7.8
cis-9,*cis*-12,*cis*-15-C18:3	7.9	3.9	11.3	8.3	4.7	12.0	9.3	6.0	14.2	9.1	6.7	14.8	11.9
Total fatty acids[4]	47.7	35.1	45.2	34.6	45.8	52.9	42.4	30.9	44.3	48.4	40.9	49.1	38.1

[1] ADL = acid detergent lignan.

[2] Calculated with VEM system (Van Es, 1975).

[3] DVE = intestinal digestible protein (Tamminga et al., 1994).

[4] Total fatty acids: Σ (C12:0, C14:0, C16:0, C18:0, *cis*-9-C18:1, *cis*-9,*cis*-12-C18:2, *cis*-9,*cis*-12,*cis*-15-C18:3, C20:0).

Statistics

Intake, milk yield, and milk composition were averaged within cow and period for the 3-d collection periods. Results were analyzed using the MIXED procedure (SAS version 9.2; SAS Institute Inc., Cary, NC) according to the model described by St-Pierre and Weiss (2009). Using this model, response surface equations were derived for intake, milk yield, milk composition, and selected milk FA [main milk FA: C4:0 to C14:0 saturated FA (**C4-C14**), C14:0, C16:0, C18:0, *cis*-9-C18:1, C18:2n-6, C18:3n-3, and main biohydrogenation intermediates: *trans*-10-C18:1, *trans*-11-C18:1, *trans*-13+14-C18:1, *trans*-15-C18:1, *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2, and *cis*-9,*trans*-11-C18:2]. The model included linear and quadratic main effects (forage type, F:C, and CL) and all 2-way interactions as fixed effects. Random effects included cow group, period within cow group, and cow nested within cow group. Nonsignificant fixed effects ($P > 0.10$) were removed from the model. Nonsignificant ($P > 0.10$) linear effects remained in the model when they were included in a quadratic effect or an interaction effect. Linear changes in parameters for a main factor were described at the medium levels of the other main factors.

RESULTS

Intake and Performance

Individual treatment means for DMI, NE_L intake, FA intake, milk yield, and milk composition are shown in Table 4. Equations for response surfaces were derived for DMI, NE_L intake, C18:2n-6 intake, C18:3n-3 intake, milk yield, and milk composition (Table 5). Dry matter intake, NE_L intake, C18:2n-6 intake, and C18:3n-3 intake averaged 23.0 ± 3.6 kg/d, 166 ± 24 MJ/d, 179 ± 199 g/d, and 172 ± 107 g/d, respectively. Shifting from 80% grass silage to 80% corn silage in the diet linearly increased DMI ($P = 0.038$), NE_L intake ($P = 0.030$), and C18:2n-6 intake ($P = 0.007$) by 2.7 kg/d, 20 MJ/d, and 43 g/d, respectively, and decreased C18:3n-3 intake ($P = 0.003$) by 42 g/d. Shifting from a high forage (65:35 F:C) to a high concentrate (35:65 F:C) diet linearly increased DMI ($P < 0.001$), NE_L intake ($P < 0.001$), and C18:2n-6 intake ($P < 0.001$) by 5.3 kg/d, 54 MJ/d, and 109 g/d, respectively. Increasing the CL proportion in the diet linearly increased ($P < 0.001$) C18:3n-3 intake by 180 g/d.

Milk yield averaged 41.2 ± 7.3 kg/d with 3.81 ± 0.55% fat, 3.14 ± 0.24% protein, and 4.82 ± 0.29% lactose. Shifting from 80% grass silage to 80% corn silage in the diet linearly increased ($P = 0.085$) milk yield by 3.4 kg/d, whereas fat content linearly decreased ($P = 0.099$) by 0.3%. Shifting from a high forage to a high concentrate diet linearly increased ($P < 0.001$) milk yield by 8.2 kg/d and linearly decreased ($P < 0.001$) fat content by 0.8%. Increasing the proportion of CL in the diet did not affect milk yield and fat content.

The F:C ratio in the diet had a quadratic relationship ($P = 0.012$) with fat- and protein-corrected milk (**FPCM**) yield, with the highest FPCM yield achieved at a 50:50 F:C. In addition, an interaction ($P = 0.032$) was found between forage type and F:C for FPCM yield. When F:C was 35:65, FPCM yield reached a plateau for diets containing 80% grass silage in the diet. However, when F:C was 65:35, FPCM yield was higher when 20% grass silage was included in the diet.

Interactions were found between forage type and CL proportion ($P = 0.031$) and between F:C and CL proportion ($P = 0.052$) for milk protein content. In the 80% grass silage diet, milk protein content showed the highest level when 1% CL was included, whereas in the 80% corn silage diet, milk protein content showed the highest level in combination with 5% CL. An F:C ratio of 35:65 resulted in the highest milk protein content in combination with 1% CL.

None of the main factors affected milk lactose content.

Milk FA Profile

Individual treatment means for milk FA profile are shown in Table 6. Equations for response surfaces are derived for selected milk FA; namely, C4-C14, C14:0, C16:0, C18:0, *cis*-9-C18:1, C18:2n-6, and C18:3n-3, and selected biohydrogenation intermediates; namely, *trans*-10-C18:1, *trans*-11-C18:1, *trans*-13+14-C18:1, *trans*-15-C18:1, *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2, and *cis*-9,*trans*-11-C18:2 (Table 7).

When shifting from 80% grass silage to 80% corn silage in the diet, the proportions of *trans*-10-C18:1 ($P = 0.035$) and C18:2n-6 ($P = 0.002$) in milk fat linearly increased by 0.34 and 0.21 g/100 g of FA, respectively, whereas the proportions of *trans*-11,*cis*-15-C18:2 ($P = 0.084$) and C18:3n-3 ($P < 0.001$) linearly decreased by 0.08 and 0.14 g/100 g of FA, respectively. Increasing the forage proportion in the diet linearly increased ($P < 0.001$) the proportion of C18:0 by 1.67 g/100 g of FA and decreased ($P = 0.004$) the proportion of *trans*-13+14-C18:1 by 0.34 g/100 g of FA. The F:C ratio in the diet showed a quadratic relationship with C4-C14 ($P = 0.011$), C14:0 ($P = 0.090$), C16:0 ($P = 0.050$), *trans*-10-C18:1 ($P = 0.007$), *trans*-11-C18:1 ($P = 0.032$), *trans*-15-C18:1 ($P = 0.075$), *cis*-15-C18:1 ($P = 0.006$), *trans*-11,*cis*-15-C18:2 ($P = 0.006$), C18:2n-6 ($P = 0.014$), and C18:3n-3 ($P = 0.027$) proportions in milk fat. At the medium level of grass silage (50%

Table 4. Treatment means for DMI, NE_L intake, fatty acid intake, milk yield, and composition for cows fed diets with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis)

	20% grass silage				50% grass silage					80% grass silage			
	F:C 35	F:C 50		F:C 65	F:C 35		F:C 50	F:C 65		F:C 35	F:C 50		F:C 65
Parameter	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL
DMI, kg/d	26.5	24.2	24.8	23.0	24.8	25.7	22.7	20.1	21.2	24.9	22.5	22.3	17.8
NE_L intake,[1] MJ/d	200	173	180	158	185	195	163	135	146	187	159	160	121
Fatty acid intake, g/d													
C16:0	339	252	252	187	368	331	250	171	202	326	297	248	157
C18:0	80	55	56	38	51	79	59	43	36	78	49	79	44
cis-9-C18:1	284	194	239	161	294	294	203	117	176	265	207	200	113
cis-9,*cis*-12-C18:2	302	225	263	203	255	298	215	154	198	262	196	216	139
cis-9,*cis*-12,*cis*-15-C18:3	209	94	279	191	117	308	210	120	296	228	152	331	212
Milk yield, kg/d	46.9	42.4	43.9	39.7	41.6	44.5	41.6	37.1	37.0	48.0	38.9	38.6	35.1
FPCM yield,[2] kg/d	42.1	40.7	41.4	38.9	40.1	39.5	40.7	36.7	36.5	42.7	38.4	38.6	35.1
Milk lactose													
%	4.86	4.81	4.86	4.74	4.74	4.82	4.79	4.99	4.78	4.80	4.80	4.71	5.08
kg/d	2.29	2.04	2.13	1.89	1.97	2.15	1.99	1.85	1.77	2.31	1.87	1.82	17.9
Milk fat													
%	3.35	3.81	3.54	3.90	3.70	3.18	3.95	4.12	4.02	3.18	3.96	4.18	4.18
kg/d	1.49	1.59	1.55	1.54	1.52	1.39	1.63	1.53	1.49	1.52	1.54	1.61	1.47
Protein													
%	3.21	3.16	3.29	3.26	3.38	3.13	3.13	2.92	3.06	3.10	3.22	2.99	2.96
kg/d	1.49	1.33	1.43	1.29	1.40	1.38	1.30	1.08	1.13	1.49	1.25	1.15	1.04
MUN, mg/dL	14.1	14.5	14.9	15.7	15.4	12.0	15.5	14.7	17.2	14.6	16.5	14.3	14.4

[1] Calculated with VEM system (Van Es, 1975).

[2] FPCM = fat- and protein-corrected milk (0.337 + 0.116 × fat % + 0.06 × protein %) × milk yield (kg/d).

Table 5. Effects of varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis) on DMI (kg/d), NE_L intake (MJ/d), *cis*-9,*cis*-12-C18:2 intake, *cis*-9,*cis*-12,*cis*-15-C18:3 intake (g/d), and milk yield (kg/d) and composition[1]

Dependent variable	Intercept	Grass silage %	Forage %	CL %	Forage % × Forage %	CL % × CL %	Grass silage % × CL %	Forage % × CL %	Grass silage % × Forage %	RMSE[2]
DMI	34.0 (2.21)	−0.0444 (0.0196)	−0.1770 (0.0383)	NS	NS	NS	NS	NS	NS	2.1007
NE_L intake	272.5 (16.05)	−0.3394 (0.1421)	−1.8150 (0.2777)	NS	NS	NS	NS	NS	NS	20.5851
cis-9,*cis*-12-C18:2 intake	438.8 (27.01)	−0.7194 (0.2357)	−3.6089 (0.4704)	NS	NS	NS	NS	NS	NS	41.2586
cis-9,*cis*-12,*cis*-15-C18:3 intake	40.6 (14.24)	0.6984 (0.1964)	NS	44.9203 (2.9052)	NS	NS	NS	NS	NS	66.0955
Milk yield	57.94 (3.601)	−0.0567 (0.0308)	−0.2746 (0.0589)	NS	NS	NS	NS	NS	NS	3.1547
FPCM yield[3]	7.46 (10.138)	0.2117 (0.0954)	1.2693 (0.0385)	NS	−0.0116 (0.0037)	NS	NS	NS	−0.0047 (0.0019)	2.1722
Fat %	2.30 (0.314)	0.0049 (0.0028)	0.0251 (0.0053)	NS	NS	NS	NS	NS	NS	0.2861
Protein %	3.73 (0.246)	0.0037 (0.0024)	−0.0141 (0.0044)	−0.0788 (0.0749)	NS	NS	−0.0019 (0.0008)	0.0030 (0.0014)	NS	0.1062

[1] The full model included linear and quadratic effects of forage type, forage to concentrate ratio, crushed linseed, and all 2-way interactions. The final models include significant effects ($P < 0.10$). The SE of the coefficient is given within parentheses.

[2] RMSE = root mean square error.

[3] FPCM = fat- and protein-corrected milk.

grass silage), the lowest proportions of *trans*-10-C18:1 and *trans*-15-C18:1 were achieved when the diet contained 55 to 65% forage. The lowest proportions of *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2, and C18:3n-3 were achieved when the diet contained 50:50 F:C, whereas the lowest proportion of C18:2n-6 was achieved when the diet contained 65:35 F:C. Proportions of C4-C14, C14:0, C16:0, and *trans*-11-C18:1 were maximal when the diet contained 50:50 F:C. An increasing proportion of CL in the diet linearly increased proportions of C18:0 ($P < 0.001$), *trans*-11-C18:1 ($P < 0.001$), *trans*-13+14-C18:1 ($P < 0.001$), and *cis*-9,*trans*-11-C18:2 ($P = 0.046$) in milk fat by 2.03, 0.21, 0.52, and 0.04 g/100 g of FA, respectively. In contrast, the proportions of C14:0 ($P = 0.084$) and C16:0 ($P < 0.001$) decreased linearly with an increasing proportion of CL in the diet. The proportion of CL showed a quadratic relationship with C4-C14 ($P = 0.094$), C18:2n-6 ($P = 0.017$), and C18:3n-3 ($P = 0.073$) proportions in milk fat; the proportion of C4-C14 reached a minimum at 3% CL, whereas the proportion of C18:2n-6 reached a maximum at 3% CL, and the proportion of C18:3n-3 reached a plateau at 5% CL.

Interactions between F:C and CL proportion were found for *trans*-10-C18:1 ($P = 0.023$), *trans*-15-C18:1 ($P = 0.039$), *cis*-15-C18:1 ($P = 0.014$), *trans*-11,*cis*-15-C18:2 ($P = 0.066$), and C18:3n-3 ($P = 0.034$) proportions in milk fat. From 80 to 20% of grass silage, the proportion of *trans*-10-C18:1 (1.64 to 1.98 g/100 g of FA), *trans*-15-C18:1 (0.75 g/100 g of FA), *cis*-15-C18:1 (0.63 g/100 g of FA) *trans*-11,*cis*-15-C18:2 (0.68 to 0.59 g/100 g of FA), and C18:3n-3 (1.08 to 0.93 g/100 g of FA) showed the highest levels when the diet contained 5% CL in combination with a 35:65 F:C.

Interactions between forage type and F:C were found for the proportions of C4-C14 ($P = 0.032$), C14:0 ($P = 0.033$), and *cis*-9-C18:1 ($P = 0.045$) in milk fat. The combination of a high forage proportion with 80% grass silage or a low forage proportion with 20% grass silage in the diet gave the highest *cis*-9-C18:1 proportions (19.81 and 21.94 g/100 g of FA, respectively), whereas these combinations resulted in the lowest C4-C14 (20.12 and 21.51 g/100 g of FA, respectively) and C14:0 proportions (9.59 and 10.17 g/100 g of FA, respectively).

DISCUSSION

The aim of the current study was to simultaneously evaluate different levels of CL supplementation in combination with variation in the characteristics of the basal diet (forage type and F:C) on intake, production performance, and milk FA profile. Multiple mechanisms regulate DMI of ruminants, but DMI generally declines with increasing NDF, especially forage NDF, content of the diet (Allen, 2000). Increasing the concentrate

Table 6. Treatment means for milk fatty acid (FA) profile (g/100 g of FA) for cows fed diets with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (1, 3, and 5% CL; DM basis)

	20% grass silage				50% grass silage					80% grass silage			
	F:C 35	F:C 50		F:C 65	F:C 35		F:C 50	F:C 65		F:C 35	F:C 50		F:C 65
Milk FA profile, g/100 g of FA	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL
C4:0	2.70	3.21	3.07	2.94	2.83	2.88	3.18	3.03	3.66	3.13	3.28	3.37	3.43
C6:0	1.73	2.18	2.17	1.99	1.88	1.87	2.08	2.00	2.19	2.08	2.13	2.11	2.06
C8:0	1.10	1.39	1.47	1.28	1.22	1.18	1.30	1.24	1.27	1.31	1.34	1.23	1.19
C10:0	2.52	3.19	3.44	2.90	2.81	2.66	2.86	2.74	2.58	2.97	3.01	2.53	2.42
C11:0	0.06	0.07	0.07	0.05	0.07	0.06	0.05	0.04	0.03	0.06	0.06	0.05	0.03
C12:0	3.12	3.65	4.05	3.34	3.64	3.21	3.28	3.12	2.83	3.61	3.51	2.77	2.64
C13:0	0.07	0.08	0.10	0.08	0.09	0.08	0.07	0.07	0.06	0.09	0.07	0.06	0.06
iso-C13:0	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02
anteiso-C13:0	0.07	0.07	0.09	0.07	0.10	0.06	0.07	0.07	0.06	0.08	0.08	0.06	0.05
C14:0	9.82	11.23	11.60	10.98	11.10	10.00	10.56	10.62	10.08	10.74	10.91	9.78	9.99
iso-C14:0	0.05	0.06	0.06	0.07	0.06	0.05	0.07	0.10	0.09	0.05	0.10	0.09	0.10
cis-9-C14:1	0.93	0.92	1.12	0.97	1.28	0.82	0.89	0.89	0.80	0.97	0.93	0.80	0.81
C15:0	0.73	0.82	0.83	0.80	0.90	0.81	0.78	0.86	0.79	0.84	0.87	0.73	0.88
iso-C15:0	0.14	0.17	0.16	0.18	0.15	0.14	0.18	0.21	0.19	0.15	0.19	0.18	0.19
anteiso-C15:0	0.37	0.39	0.36	0.37	0.34	0.36	0.37	0.40	0.39	0.36	0.40	0.36	0.37
C16:0	28.39	31.41	27.75	28.68	31.95	27.53	29.31	29.58	26.30	29.32	30.95	27.75	29.07
cis-9-C16:1	1.90	1.72	1.44	1.62	1.84	1.56	1.56	1.91	1.34	1.58	1.67	1.57	1.68
C17:0	0.46	0.49	0.47	0.54	0.49	0.44	0.47	0.58	0.47	0.45	0.50	0.44	0.53
cis-9-C17:1	0.16	0.14	0.11	0.15	0.12	0.12	0.13	0.20	0.12	0.12	0.13	0.14	0.19
C18:0	10.50	9.98	10.91	10.96	8.64	10.99	11.04	11.04	13.01	9.68	10.33	12.35	11.40
Total *trans*-C18:1[1]	6.46	4.46	5.84	4.91	4.59	8.06	5.36	3.93	6.20	7.10	4.40	5.29	4.71
trans-4-C18:1	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.02	0.04	0.03	0.03	0.03	0.03
trans-5-C18:1	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02
trans-6+7+8-C18:1	0.53	0.37	0.41	0.39	0.39	0.57	0.41	0.31	0.45	0.53	0.37	0.37	0.33
trans-9-C18:1	0.39	0.29	0.31	0.30	0.31	0.42	0.32	0.25	0.34	0.40	0.28	0.29	0.26
trans-10-C18:1	1.67	0.62	0.57	0.46	0.63	1.88	0.58	0.35	0.45	1.35	0.42	0.37	0.34
trans-11-C18:1	1.01	1.10	1.43	1.19	0.95	1.20	1.30	1.12	1.58	1.50	1.18	1.30	1.37
trans-12-C18:1	0.56	0.42	0.55	0.50	0.45	0.66	0.52	0.37	0.61	0.60	0.40	0.53	0.45
trans-13+14-C18:1	1.19	0.86	1.35	1.02	1.03	1.76	1.16	0.74	1.42	1.48	0.93	1.16	0.91
trans-15-C18:1	0.51	0.34	0.55	0.47	0.35	0.79	0.48	0.34	0.60	0.58	0.34	0.57	0.46
trans-16+*cis*-14-C18:1	0.58	0.41	0.63	0.54	0.44	0.74	0.55	0.43	0.68	0.60	0.43	0.66	0.55
Total *cis*-C18:1[2]	22.58	19.55	19.32	21.61	20.50	20.61	20.81	22.23	21.76	18.98	20.08	22.63	22.74
cis-9-C18:1	21.11	18.43	18.12	20.34	19.28	18.90	18.55	21.08	20.37	17.50	19.00	21.38	21.53
cis-11-C18:1	0.64	0.53	0.46	0.50	0.59	0.58	0.51	0.56	0.44	0.54	0.50	0.43	0.50
cis-12-C18:1	0.32	0.29	0.36	0.38	0.30	0.33	0.37	0.26	0.45	0.40	0.27	0.36	0.30
cis-13-C18:1	0.10	0.07	0.07	0.09	0.08	0.10	0.08	0.09	0.09	0.10	0.08	0.08	0.10
cis-15-C18:1	0.41	0.21	0.31	0.30	0.24	0.70	0.31	0.23	0.41	0.44	0.23	0.38	0.32
Total nonconjugated C18:2[3]	3.67	2.70	2.86	2.91	3.01	3.79	2.94	2.63	2.93	3.50	2.64	2.86	2.72
trans-9,*trans*-12-C18:2	0.04	0.01	0.01	0.01	0.03	0.05	0.02	0.01	0.02	0.03	0.02	0.01	0.01
trans-11,*cis*-15-C18:2	0.41	0.18	0.30	0.28	0.22	0.70	0.32	0.26	0.48	0.49	0.24	0.39	0.42
cis-9,*cis*-12-C18:2	2.28	1.87	1.69	1.78	1.93	1.95	1.76	1.71	1.44	1.99	1.69	1.60	1.55
Total conjugated C18:2[4]	0.57	0.53	0.69	0.60	0.60	0.60	0.62	0.54	0.69	0.74	0.57	0.59	0.63
cis-9,*cis*-12,*cis*-15-C18:3	0.82	0.51	0.80	0.74	0.61	1.00	0.79	0.72	0.86	0.89	0.68	0.99	0.89
FA ≥C:20[5]	0.47	0.45	0.47	0.51	0.41	0.46	0.47	0.52	0.43	0.45	0.43	0.50	0.46
C20:0	0.11	0.10	0.10	0.11	0.10	0.11	0.12	0.12	0.14	0.10	0.12	0.12	0.12
C20:1	0.06	0.05	0.04	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.04	0.05	0.05
C20:2	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.04	0.01
C21:0	0.03	0.02	0.03	0.02	0.03	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02

Continued

Table 6 (Continued). Treatment means for milk fatty acid (FA) profile (g/100 g of FA) for cows fed diets with varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (F:C; 35, 50, and 65% forage; DM basis), and proportion of crushed linseed (1, 3, and 5% CL; DM basis)

Milk FA profile, g/100 g of FA	20% grass silage				50% grass silage					80% grass silage			
	F:C 35	F:C 50		F:C 65	F:C 35		F:C 50	F:C 65		F:C 35	F:C 50		F:C 65
	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL	1% CL	5% CL	3% CL
C22:0	0.04	0.05	0.09	0.08	0.00	0.04	0.05	0.06	0.01	0.04	0.00	0.06	0.06
Total unknown	0.59	0.59	0.72	0.68	0.69	0.64	0.68	0.64	0.79	0.68	0.68	0.72	0.66
SFA[6]	60.03	66.39	64.64	63.26	64.18	60.48	63.80	63.56	62.09	62.99	65.58	62.07	62.38
C4-C14[7]	20.99	24.84	25.79	23.43	23.49	21.79	23.26	22.75	22.62	23.84	24.18	21.78	21.72
OBCFA[8]	2.11	2.31	2.27	2.32	0.35	2.15	2.21	2.55	2.23	2.24	2.42	2.11	2.43
MUFA[9]	31.92	26.70	27.77	29.17	28.25	31.11	28.67	29.02	30.14	28.70	27.12	30.36	30.00
PUFA[10]	5.30	3.97	4.57	4.51	4.48	5.59	4.59	4.17	4.70	5.35	4.15	4.68	4.46
UFA[11]	37.22	30.67	32.34	33.68	32.73	36.70	33.26	33.19	34.84	34.05	31.27	35.04	34.46

[1] Total *trans*-C18:1: Σ (*trans*-4-C18:1, *trans*-5-C18:1, *trans*-6+7+8-C18:1, *trans*-9-C18:1, *trans*-10-C18:1, *trans*-11-C18:1, *trans*-12-C18:1, *trans*-13+14-C18:1, *trans*-15-C18:1, *trans*-16+*cis*-14-C18:1).

[2] Total *cis*-C18:1: Σ (*cis*-9-C18:1, *cis*-11-C18:1, *cis*-12-C18:1, *cis*-13-C18:1, *cis*-15-C18:1).

[3] Total nonconjugated C18:2: Σ (*trans*-9,*trans*-12-C18:2, *cis*-9,*trans*-13-C18:2, *trans*-8,*cis*-13-C18:2, *cis*-9,*trans*-12-C18:2, *trans*-9,*cis*-12-C18:2, *trans*-11,*cis*-15-C18:2, *cis*-9,*cis*-12-C18:2, *cis*-9,*cis*-15-C18:2).

[4] Total conjugated C18:2: Σ (*cis*-9,*trans*-11-C18:2, *trans*-10,*cis*-12-C18:2).

[5] Total $\geq$C20:0: Σ (C20:0, C20:1, C20:2, C20:3n-3, C20:4n-6, C21:0, C22:0, *cis*-13-C22:1, C22:5, C22:6, C24:0).

[6] Saturated fatty acids: Σ (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C24:0).

[7] C4-C14 saturated fatty acids: Σ (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0).

[8] Odd- and branched-chain fatty acids: Σ (*iso*-C13:0, *anteiso*-C13:0, *iso*-C14:0, *anteiso*-C14:0, *iso*-C15:0, *anteiso*-C15:0, C15:0, *iso*-C16:0, *anteiso*-C16:0, *iso*-C17:0, *anteiso*-C17:0, C17:0, *cis*-9-C17:1).

[9] Monounsaturated fatty acids: Σ (*cis*-9-C14:1, *cis*-9-C16:1, total *cis*-C18:1, total *trans*-C18:1, C20:1, *cis*-13-C22:1).

[10] Polyunsaturated fatty acids: Σ (total nonconjugated C18:2, total conjugated C18:2, C18:3n-6, C18:3n-3, C20:2, C20:3n-3, C20:4n-6, C22:5, C22:6).

[11] Unsaturated fatty acids: Σ (MUFA, PUFA).

Table 7. Effects of varying forage type (20, 50, and 80% grass silage; DM basis), forage to concentrate ratio (35, 50, and 65% forage; DM basis), and proportion of crushed linseed (CL, 1, 3, and 5%; DM basis) on selected milk fatty acids (g/100 g of fatty acids)[1]

Dependent variable	Intercept	Grass silage %	Forage %	CL %	Forage % × Forage %	CL % × CL %	Grass silage % × CL %	Forage % × CL %	Grass silage % × Forage %	RMSE[2]
C4 to C14[3]	4.71 (5.354)	0.1100 (0.0500)	0.7673 (0.2020)	−1.4526 (0.6767)	−0.0065 (0.0020)	0.2067 (0.1095)	NS	NS	−0.0026 (0.0010)	1.1133
C14:0	4.79 (2.249)	0.0460 (0.0225)	0.2200 (0.0914)	−0.1365 (0.0712)	−0.0017 (0.0009)	NS	NS	NS	−0.0011 (0.0004)	0.4048
C16:0	28.61 (2.216)	NS	0.1768 (0.0910)	−1.0133 (0.0750)	−0.0021 (0.0009)	NS	NS	NS	NS	1.5082
C18:0	6.56 (0.562)	NS	0.0555 (0.0092)	0.5051 (0.0731)	NS	NS	NS	NS	NS	0.9423
trans-10-C18:1	4.51 (1.110)	−0.0057 (0.0023)	−0.1494 (0.0418)	0.5236 (0.1600)	0.0014 (0.0004)	NS	NS	−0.0088 (0.0032)	NS	0.4523
trans-11-C18:1	0.02 (0.422)	NS	0.0418 (0.0171)	0.0523 (0.0141)	−0.0004 (0.0002)	NS	NS	NS	NS	0.0958
trans-13+14-C18:1	1.32 (0.179)	NS	−0.0111 (0.0032)	0.1290 (0.0240)	NS	NS	NS	NS	NS	0.2183
trans-15-C18:1	0.66 (0.278)	NS	−0.0180 (0.0106)	0.1555 (0.0390)	0.0002 (0.0001)	NS	NS	−0.0017 (0.0008)	NS	0.1085
cis-9-C18:1	22.89 (3.171)	−0.1194 (0.0586)	−0.0705 (0.0619)	NS	NS	NS	NS	NS	0.0025 (0.0012)	0.8286
cis-15-C18:1	0.97 (0.303)	NS	−0.0361 (0.0115)	0.1730 (0.0429)	0.0004 (0.0001)	NS	NS	−0.0024 (0.0008)	NS	0.1064
trans-11,*cis*-15-C18:2	1.08 (0.361)	0.0014 (0.0007)	−0.0442 (0.0137)	0.1631 (0.0511)	0.0005 (0.0001)	NS	NS	−0.0021 (0.0010)	NS	0.1144
cis-9,*cis*-12-C18:2	3.68 (0.373)	−0.0036 (0.0009)	−0.0608 (0.0155)	0.1209 (0.0536)	0.0005 (0.0002)	−0.0247 (0.0087)	NS	NS	NS	0.1820
cis-9,*trans*-11-C18:2	0.57 (0.033)	NS	NS	0.0122 (0.0060)	NS	NS	NS	NS	NS	0.0176
cis-9,*cis*-12,*cis*-15-C18:3	0.71 (0.245)	0.0025 (0.0050)	−0.0191 (0.0094)	0.2196 (0.0427)	0.0002 (0.0001)	−0.0102 (0.0050)	NS	−0.0017 (0.0007)	NS	0.1235

[1] The full model included linear and quadratic effects of forage type, forage to concentrate ratio, crushed linseed, and all 2-way interactions. The final models include significant effects ($P < 0.10$). The SE of the coefficient is given within parentheses.

[2] RMSE = root mean square error.

[3] C4-C14 saturated fatty acids: Σ (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0).

proportion linearly increased DMI in the current study, which is in agreement with a lower NDF content for the high concentrate diets. In addition, DMI was strongly correlated with NE_L intake ($r = 0.98$, $P < 0.001$). Abrahamse et al. (2008b) observed a significantly higher DMI when corn silage proportion in the diet increased at the expense of grass silage, and Kliem et al. (2008) found a linear increase in DMI when replacing grass silage with corn silage. These results were all in agreement with the results of the current study. The absence of an effect of CL supplementation on DMI in the current experiment is in agreement with a recent review (Petit, 2010) that reported no effect of feeding up to 15% whole linseed on DMI of dairy cows in early lactation. Chilliard et al. (2009) indeed showed no effect on DMI when 12.4% whole linseed was included in the diet, whereas an equal amount of linseed FA fed as extruded linseed or linseed oil did result in decreased DMI, with a greater decrease for cows fed linseed oil. It was therefore concluded that processing of oilseeds might affect DMI, which might be related to the increased availability of oil in the rumen (Petit, 2010). In the current study, the amount of CL was probably not high enough to cause rumen disturbances resulting in decreased DMI. Intake of C18:2n-6 and C18:3n-3 were affected by forage type and reflected the higher proportion of C18:3n-3 in grass silage versus the higher proportion of C18:2n-6 in corn silage. In addition, intake of C18:2n-6 greatly increased when the diet shifted toward a higher concentrate proportion, whereas intake of C18:3n-3 strongly increased when the diet contained a higher proportion of CL.

Milk yield was influenced by both forage type and F:C, but not by CL supplementation, which was consistent with the effect on DMI. For milk fat content, however, the opposite relationship was found for forage type and F:C. Fat content decreased when the diet contained 80% corn silage compared with 80% grass silage and a higher proportion of concentrate. Chilliard et al. (2007) reported a larger decrease in milk fat content when vegetable oils were added to a corn silage-based diet compared with a grass silage-based diet, which was mainly related to an increased proportion of *trans*-10-C18:1 in milk fat. Indeed, an increased proportion of *trans*-10-C18:1 in milk fat related to increased dietary starch and decreased NDF contents are associated with a reduction in milk fat content (Nielsen et al., 2006), which was confirmed in the current study. It should be noted that *trans*-10-C18:1 has often been associated with milk fat depression although this FA is thought to play no regulatory role in milk FA synthesis (Lock et al., 2007). Proportion of *trans*-10-C18:1 has rather been empirically related to milk fat depression probably in relation to its association with CLA, notably *trans*-10, *cis*-12-C18:2 that does play a regulatory role (Shingfield and Griinari, 2007). However, in addition to *trans*-10, *cis*-12-C18:2, other biohydrogenation intermediates might play a regulatory role in milk FA synthesis (Loor et al., 2005). Milk protein content showed interactions between F:C and proportion of CL and between forage type and proportion of CL. This was probably related to the relationship between milk protein content and the supply of glucogenic nutrients (relation between starch content and milk protein content was $R^2 = 0.40$; Jenkins and McGuire, 2006).

Responses in milk FA profile from lipid supplementation largely depend on characteristics of the lipid (source, physical form, and inclusion rate) and on characteristics of the basal diet (forage type and F:C; Chilliard et al., 2007). To our knowledge, the current experiment is the first to simultaneously vary crushed linseed supplementation, forage type, and F:C to be able to identify and quantify interactions between these factors on milk FA profile in high-producing dairy cows. Various biohydrogenation intermediates (*trans*-C18:1, *cis*-C18:1, and nonconjugated and conjugated C18:2 and C18:3 isomers) are formed from dietary C18:2n-6 and C18:3n-3 (Chilliard et al., 2007). In the current study, supplementation of different levels of CL to a basal diet varying in forage type and F:C affected the proportions of biohydrogenation intermediates in milk fat. Interactions were found between CL supplementation and F:C for proportions of C18:3n-3, *trans*-11,*cis*-15-C18:2, *trans*-10-C18:1, *trans*-15-C18:1, and *cis*-15-C18:1 in milk fat, with the highest levels achieved when the diet contained 5% CL and a 35:65 F:C. These increased levels of C18:3n-3 and biohydrogenation intermediates are in agreement with results of Loor et al. (2005), who found increased proportions of *trans*-10-C18:1, *trans*-11-C18:1, *trans*-11,*cis*-15-C18:2, and total C18:3 isomers for the high concentrate diet with supplemental linseed oil (3% of DM). Previously, Sterk et al. (2010) showed that rumen biohydrogenation kinetics of crushed linseed did not differ from biohydrogenation kinetics of linseed oil. Loor et al. (2004) suggested that the increased dietary starch content in high concentrate diets affects ruminal FA metabolism, resulting in increased biohydrogenation intermediates produced in the rumen and consequently secreted in milk fat.

Diets with high starch and low fiber contents that are supplemented with polyunsaturated FA can inhibit mammary gland short-chain FA synthesis (Kliem et al., 2008). In the current study, interactions between forage type and F:C were found for the proportions of C4:0 to C14:0 in milk fat, with lower levels achieved when the diet contained a high forage proportion in combination with 80% grass silage or a low forage proportion in combination with 80% corn silage. In addition, the proportion of C4:0 to C14:0 in the current study reached a minimum when 3% CL was included in the diet, whereas the proportions of C14:0 and C16:0 in milk fat linearly decreased with increasing CL proportion. During diet-induced milk fat depression, the secretion of all fatty acids in milk is decreased, but the decrease is disproportionally higher for the fatty acids synthesized de novo (Shingfield and Griinari, 2007). Shingfield and Griinari (2007) summarized the major theories explaining diet-induced milk fat depression and concluded that the direct inhibition of milk fat synthesis in the mammary gland by elevated biohydrogenation intermediates was able to explain most cases. In the current study, the decreased proportions of C4:0 to C14:0 saturated FA, C14:0, and C16:0 in milk fat were in accordance with the increased proportions of the biohydrogenation intermediates.

No interactions were found between CL supplementation and forage type for the selected milk FA. Chilliard et al. (2007) suggested rumen biohydrogenation to be less complete when adding vegetable oils to a corn silage-based diet compared with addition to a grass silage-based diet. In the current study, this was not confirmed, which might be related to the relatively low starch content of the corn silage, resulting in a relatively low maximum starch content of 209 g/kg of DM in the treatment diets. However, several linear effects of forage type on milk FA were found. Shifting from 80% grass silage to 80% corn silage linearly increased *trans*-10-C18:1 and C18:2n-6, whereas proportions of *trans*-11,*cis*-15-C18:2 and C18:3n-3 linearly decreased. Kliem et al. (2008) found increased proportions of *trans*-C18:1 isomers, total conjugated C18:2, and C18:2n-6, and a decreased proportion of C18:3n-3 in milk fat when replacing grass silage with corn silage in a diet without supplemental oil.

Glasser et al. (2008) suggested that changes in ruminal biohydrogenation are caused by changes in starch content of the diet affecting ruminal pH and microbial populations. However, Loor et al. (2004) suggested that changes in ruminal biohydrogenation could follow changes in dietary starch content without an effect on ruminal pH. This might be related to the content of dietary NDF (physically effective NDF) in addition to the content of dietary starch playing an important role in the estimation of ruminal pH (Zebeli et al., 2008). In addition, changes in dietary starch content might induce small alterations in the microbial population that are able to affect ruminal biohydrogenation (Loor et al., 2004). Starch and NDF availability and their effects on buffering capacity and alterations in the microbial population in the rumen are linked to a shift in the production of isomers with a *trans*-11 to a *trans*-10 double bond (Loor et al., 2004). An increase in milk *trans*-10-C18:1 was commonly found with either high concentrate diets or corn silage based-diets that were supplemented with polyunsaturated FA rich oils (Chilliard et al., 2007). In the current study, increasing starch content in the diet indeed increased the *trans*-10-C18:1 proportion in milk fat ($R^2 = 0.50$) and this increased proportion was strongly related to the decreased milk fat content ($R^2 = 0.81$). The increased *trans*-10-C18:1 proportion in relation to high starch diets might be related to changes in the bacterial population. Nielsen et al. (2006) reported that high grain diets promote the growth of the bacterial strain *Megasphaera elsdenii* YJ-4 (Kim et al., 2002) in combination with a decrease in the main cellulose digesting bacterial strain *Butyrivibrio fibrisolvens* (Klieve et al., 2003). These different bacterial strains convert C18:2n-6 and C18:3n-3 through different biohydrogenation routes. *Megasphaera elsdenii* YJ-4 can convert C18:2n-6 to *trans*-10,*cis*-12-C18:2 and *trans*-10-C18:1 (Bauman and Griinari, 2001), whereas the bacterial strain *Butyrivibrio fibrisolvens* converts C18:2n-6 to *cis*-9,*trans*-11-C18:2 and *trans*-11-C18:1 (Harfoot and Hazlewood, 1997). Shingfield et al. (2005) suggested that starch content and the ratio of starch to NDF in the diet are important determinants of the *trans*-C18:1 isomer profile in milk due to the effects on the relative abundance and activity of specific populations of bacteria in the rumen.

CONCLUSIONS

Increasing the proportion of CL in combination with varying forage type and F:C in the diet of high-producing dairy cows affects intake, production performance, and milk FA profile. Interactions were found between CL supplementation and F:C for proportions of C18:3n-3 and several biohydrogenation intermediates in milk fat, with the highest levels achieved when the diet contained 5% CL and a 35:65 F:C ratio. No interactions were found between CL supplementation and forage type for the selected milk FA. However, several linear effects of shifting from 80% grass silage to 80% corn silage on milk FA were found. This study showed that the effect of adding CL on the proportions of several FA in milk fat, including C18:2n-6 and C18:3n-3, depends significantly on the F:C and forage type (grass silage versus corn silage) in the basal diet. In addition, this study showed that in FA research other feed characteristics such as forage type and F:C could influence the final effect of a supplemental fat source on milk FA profile.

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