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Influence of Supplementing Dairy Cows Grazing on Pasture with Feeds Rich in Linoleic Acid on Milk Fat Conjugated Linoleic Acid (CLA) Content*, **

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ABSTRACT : Three experiments were conducted to investigate the hypothesis that cows grazing on pasture produce the highest proportion of *c*-9 *t*-11 CLA in milk fat and no further increase can be achieved through supplementation of diets rich in linoleic acid, such as full-fat extruded soybeans or soybean oil. In experiment 1, 18 lactating Holstein cows were used in a randomized complete block design with measurements made from wk 4 to 6 of the experiment. In experiment 2, three cannulated lactating Holstein cows were used in a 3×3 Latin square design. Each period was 4 wk with measurements made in the final wk of each period. Cows in both experiments were assigned at random to treatments: a, conventional total mixed ration (TMR); b, pasture (PS); or c, PS supplemented with 2.5 kg/cow per day of full-fat extruded soybeans (PES). In both experiments, feed intake, milk yield, milk composition, and fatty acid profile of milk and blood serum were measured, along with fatty acid composition of bacteria harvested from rumen digesta in experiment 2. In experiment 3, 10 cows which had continuously grazed a pasture for six weeks were assigned to two groups, with one group (n = 5) on pasture diet alone (PS) and the other group (n = 5) supplemented with 452 g of soy oil/cow per day for 7 d (OIL). In experiment 1, cows in PS treatment produced 350% more *c*-9, *t*-11 CLA compared with cows in TMR treatment (1.70 vs. 0.5% of fat), with no further increase for cows in PES treatment (1.50% of fat). Serum *c*-9, *t*-11 CLA increased by 233% in PS treatment compared with TMR treatment (0.21 vs. 0.09% of fat) with no further increase for cows in PES treatment (0.18% of fat). In experiment 2, cows in PS treatment produced 300% more *c*-9 *t*-11 CLA in their milk fat compared with cows in TMR treatment (1.77 vs. 0.59% of fat), but no further increase for cows in PES treatment (1.84% of fat) was observed. Serum *c*-9, *t*-11 CLA increased by 250% for cows in PS treatment compared with cows in TMR treatment (0.27 vs. 0.11% of fat), with no further increase for cows in PES treatment (0.31% of fat). The *c*-9, *t*-11 CLA content of ruminal bacteria for cows in PS treatment was 200% or more of TMR treatment, but no further increase in bacterial *c*-9, *t*-11 CLA for cows in PES treatment was observed. Supplementation of soy oil in experiment 3 also did not increase the *c*-9 *t*-11 CLA content of milk fat compared with cows fed a full pasture diet (1.60 vs. 1.54% of fat). Based on these findings, it was concluded that supplementing with feeds rich in linoleic acid, such as full-fat extruded soybeans or an equivalent amount of soy oil, to cows grazing perennial ryegrass pasture may not increase milk fat *c*-9 *t*-11 CLA contents. (**Key Words :** Conjugated Linoleic Acid, Milk, Serum, Bacteria)

INTRODUCTION

The term conjugated linoleic acid (CLA) refers to a group of compounds formed during the biohydrogenation of linoleic acid in the rumen (Kepler and Tove, 1967) or its subsequent synthesis in milk or meat from a precursor, *t*-11 $C_{18:1}$ (transvaccenic acid, TVA), of rumen origin (Griinari and Bauman, 1999). Although occurring naturally in foods, its principal dietary sources are milk and meat products from ruminants. A growing interest in increasing CLA content of food products is associated with its potential health benefits including, anticarcinogenic, antidiabetic, and antiatherogenic effects observed in several experimental animal models (Parodi, 2001; Banni et al., 2003). The primary isomer of CLA that has been associated with various health benefits is *c*-9, *t*-11 $C_{18:2}$, and accounts for more than 80% of the total isomers in dairy products (Parodi, 2003).

A volume of research is now available on increasing the

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CLA content of milk and meat products from ruminants (Enser et al., 1999; Loor et al., 2002a; Whitlock et al., 2002) and non-ruminants (Fritsche and Steinhart, 1998; Khanal et al., 2004). Manipulation of animal diet has been the focus for increasing the CLA content of foods from ruminants. It involves supplying $C_{18:2}$, $C_{18:3}$ or FA with 20 or more carbons as substrates for rumen biohydrogenation. During the process, *c*-9, *t*-11 CLA or its precursor *t*-11 $C_{18:1}$ escapes further biohydrogenation. The *t*-11 $C_{18:1}$ is then endogenously converted to *c*-9, *t*-11 CLA in the milk and meat by the mammary gland and adipocytes, respectively, using Δ^9-desaturase (Griinari and Bauman, 1999). Depending on the type, plant oils or oil seeds provide either $C_{18:2}$ (e.g., soybean oil or its seed) or $C_{18:3}$ (e.g., linseed oil or its seed), while fish oil provides FA with 20 or more carbons, and grazing cows on pasture provides $C_{18:3}$. So far, the highest *c*-9, *t*-11 CLA contents in milk or meat have been found from animals grazed on pasture (Dhiman et al., 1999a; Kay et al., 2004). A recent New Zealand study (Kay et al., 2003) showed that supplementation with fish oil or a combination of fish oil and sunflower oil to cows grazing on pastures increased milk fat *c*-9, *t*-11 CLA compared with the unsupplemented control. However, studies are limited that compare the milk fat CLA contents of milk from cows grazing fresh pastures with or without supplementing with feeds rich in linoleic acid. Therefore, we hypothesized that milk from cows grazing on pasture will have the highest *c*-9, *t*-11 CLA content and no further increase could be achieved through supplementation of oils or oil seeds rich in linoleic acid. To test this hypothesis, three experiments were conducted with the objective of determining the FA composition of milk, blood serum, and rumen bacteria.

MATERIALS AND METHODS

Experiment 1

Eighteen Holstein cows (12 multiparous and six primiparous) producing 31.0±6.9 kg of milk/day and 204±37 days in milk were blocked into six groups according to milk yield. Within blocks, cows were randomly assigned to one of three treatments, with equal numbers of multi (4) and primiparous (2) cows in each treatment group. Treatments were: a, a total mixed ration containing 50% conserved forage and 50% grain (TMR); b, a pasture diet (PS); and c, PS diet supplemented with 2.5 kg of full fat extruded soybeans (FFES)/cow per day (PES). Cows in TMR treatment were housed in a tie stall barn and fed individually twice daily at 0800 and 1600 h to yield 5-10% orts on an as fed basis and were allowed *ad libitum* access to water. Cows in PS and PES treatments were allowed to graze together with *ad libitum* access to water and minerals. Cows on PES diet were fed FFES individually after each milking in equal proportions. Full-fat soybeans were prepared by extruding whole soybeans at 146-149°C using an Insta-Pro® Extruder Model 2500 (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA). The experiment was carried out at the Caine Dairy Teaching and Research Facility, Utah State University, Logan. Animal care and procedures were approved and conducted under established standards of the Utah State University Institutional Animal Care and Use Committee.

Amounts of feed offered and orts were recorded individually for cows in TMR treatment. Samples of TMR, TMR ingredients, and FFES were collected every week and analyzed separately for each week. Orts were removed daily and samples collected individually from wk 4 through 6 were mixed for the respective wk. Pasture samples were collected by using a 2 ft^2 quadrant during wk 4 through 6 from four different locations per 0.4 ha of paddock. Samples collected from two locations were used for determining botanical composition and samples from the other two locations were stored at -20°C until further chemical analysis. Dry matter content of TMR, feed ingredients, and orts was determined by drying in an oven at 60°C for 48 h, whereas that of pasture samples was determined by drying in a freeze drier (Labconco Freeze Dry System, Labconco, Kansas City, MO). Samples were ground to pass through a 1-mm screen (Wiley Mill; Arthur

Table 1. Chemical composition of TMR, pasture (PS) and full-fat extruded soybeans (FFES)

	DM (%)	CP (%)	NDF (%)	ADF (%)	NE_L[1] (Mcal/kg)	Total fatty acids[2] (%)
TMR[3]	68.0	17.2	35.7	25.1	1.79	5.97
PS						
Experiment 1	20.2	21.2	48.1	25.4	1.64	4.29
Experiment 2	22.8	19.8	50.8	26.7	1.56	4.33
FFES[4]	96.9	40.9	25.9	17.5	3.41	19.8

[1] The NE_L content of forages was estimated from the equation, NE_L (Mcal/kg) = 0.0245×TDN%-0.12. The NRC (2001) values for individual ingredients were used to calculate NE_L content of TMR.

[2] Calculated by addition (sum of $C_{10:0}$ to $C_{22:0}$).

[3] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively. Its chemical composition was calculated from individual ingredient's chemical composition. TMR was same in experiment 1 and 2.

[4] Full-fat extruded soybeans. It does not represent the treatment PES. It was same in both experiments. Full-fat soybean was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA).

H. Thomas. Philadelphia. PA). The CP content was determined using a macro Kjeldahl for digestion and Kjeltec System 1026 Distillation Unit for distillation (Tecator, Hoganas. Sweden). The NDF and ADF contents were determined by the filter bag technology of Ankom (Ankom Technology Corp., Fairport. NY). During analysis. samples were further dried at 105°C for 8 h to determine absolute DM. Chemical analysis was expressed on the basis of this final DM. Chemical composition of TMR was calculated from chemical analysis of the individual ingredients (Table 1). Dietary ingredients were analyzed for total FA content and FA profile (Sukhija and Palmquist. 1988).

Cows were weighed 2 d in a row at the beginning (740±50.5 kg) and end (746.4±59.8 kg) of the experiment. The difference between the amount of feed DM offered and refused was used to determine DMI of cows on TMR treatment. For cows in PS and PES treatments. average weights at the beginning and end of the experiment were taken for calculating predicted DM intake (DMI). The amount of DM consumed from pasture was estimated from calculated net energy needs. The NE_L intake from pasture was calculated using milk energy output (Tyrrell and Reid, 1965) plus energy spent for maintenance and BW gain (NRC. 2001) for cows in PS treatment. minus energy intake from FFES for cows in PES treatment. The estimated NE_L value for grass was 1.64 Mcal/kg of grass DM. Milk samples were collected without preservative for fatty acid analysis and with preservatives (Broad Spectrum Microtabs II; D & F Control Systems. Inc., San Ramon. CA) for milk composition. Samples from six consecutive milkings for the respective wk were collected from individual cows and stored in a refrigerator at 4°C. All six samples from the individual cows was analyzed for milk composition by the Rocky Mountain Dairy Herd Improvement Association Laboratory. Logan. Utah with mid-infrared wave bands (2-15 μm) using a Bentley 2000 (Bentley Instruments. Chaska. MN). Final milk composition was expressed on weighted milk yield for each wk during sampling time.

Blood samples were collected from wk 4 through 6 from individual cows through the coccygeal vein or artery approximately 5 h post-feeding. Blood samples were collected in serum separation tubes (Vacutainer brand SST Gel and clot activator. Becton Dickinson and Co., Franklin, NJ) and were allowed to clot overnight before separating serum by centrifugation at 2,000×g for 15 minutes at 4°C. Extracted serum was stored at -20°C until FA was determined later.

Weighted composite milk samples from six consecutive milkings obtained from each cow from wk four to six were analyzed for fatty acid composition. Detailed methods for milk fat extraction, methylation, and analysis on a gas chromatograph have been described previously (Dhiman et al., 2002).

For fat extraction from serum. 20 ml of 2:1 chloroform:methanol mixture was added to 1 ml of serum. The contents were shaken thoroughly and 5 ml of 4% KCl was added. The top layer was removed. sodium sulfate was added. the contents then filtered and the filters and tubes were rinsed with chloroform. The filtrate was dried down with nitrogen and methylation was carried out with 4 ml of 4% MeOH-HCl. The contents were heated in a water bath at 60°C for 20 minutes and the tubes were shaken every five minutes. After cooling to room temperature, 1 ml of distilled water and 2 ml of hexane were added. the mixture was then vortexed and the bottom layer was removed. The contents were washed twice with 1 ml of distilled water, with centrifugation at 2,000 × g for 5 min during the second wash. The bottom layer discarded. the contents dried down with nitrogen and transferred to GC vials.

Experiment 2

Three multiparous cannulated cows with an initial milk yield of 46.0±1.7 kg were used in a 3×3 Latin square design with three periods of 4 wk each. The first 3 wk of each period was for adaptation to diet and measurements were made in the final wk. Rumen digesta samples were collected three days in a row during each period. Sampling on the first day started at 0800 h. the second day at 0900 h and the third day at 1000 h with an interval of 3 h for subsequent sampling. Thus samples were collected at an hourly interval starting from 1 h after feeding through 12 h over 3 consecutive days. Samples were collected from four different sites of the ventral sac of the rumen and pooled to make a composite sample. Approximately 500 g of digesta samples was collected at each time point. Rumen fluid pH was measured immediately. Samples were transported to the laboratory in ice packs and stored at -20°C until centrifugation. Other details of the treatments and feeding, sampling of blood. milk. and dietary ingredients and their processing and laboratory analyses were the same as in experiment 1.

Fluid was squeezed manually from frozen digesta samples after thawing in a water bath at 40°C. Digesta was washed twice with 100 ml of 0.1 M phosphate buffer. Bacteria rich pellets from rumen digesta samples were harvested by centrifuging the samples at 1,000×g for 10 minutes to remove food particles and protozoa. Supernatant fluid was transferred to another set of centrifuge tubes and centrifuged at 12,000×g for 15 minutes. The supernatant was discarded and 250 ml of 0.1 M phosphate buffer was added to it. It was centrifuged again at 12,000 rpm and repeated for a total of three times to wash the bacterial pellets. Harvested bacterial pellets were freeze dried (Labconco Freeze Dry System. Labconco, Kansas City, MO) and stored at -20°C until FA analysis. Bacteria rich

Table 2. Fatty acid composition (% of fat) of TMR, pasture (PS), and full-fat extruded soybeans (FFES)

	$<C_{14:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:1}$	$C_{18:0}$	*t*-9 $C_{18:1}$	*c*-9 $C_{18:1}$	$C_{18:1}$ *c*-11	$C_{18:2}$	$C_{18:3}$	CLA	$>C_{20:2}$
Experiment 1														
TMR[1]	0.28	0.44	0.18	22.1	0.85	0.79	2.57	0.01	15.8	0.09	40.7	16.0	ND	0.19
PS	0.05	0.42	ND[2]	19.7	0.05	1.05	1.05	ND	2.13	0.12	19.8	55.6	ND	ND
FFES[3]	0.03	0.27	0.15	11.0	0.02	0.73	3.50	ND	22.8	0.29	56.0	5.13	ND	0.08
Experiment 2														
TMR	0.28	0.44	0.18	22.1	0.85	0.79	2.57	0.01	15.8	0.09	40.7	16.0	ND	0.19
PS	0.11	0.43	ND	22.6	0.04	0.97	1.58	ND	1.26	0.09	20.2	52.5	ND	0.19
FFES	0.03	0.27	0.15	11.0	0.02	0.73	3.50	ND	22.8	0.29	56.0	5.13	ND	0.08

[1] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively. Its chemical composition was calculated from individual ingredient's chemical composition. TMR was same in experiment 1 and 2.

[2] ND = Not detected.

[3] Full-fat extruded soybeans. It does not represent the treatment PES. It was same in both experiments. Full-fat soybean was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA).

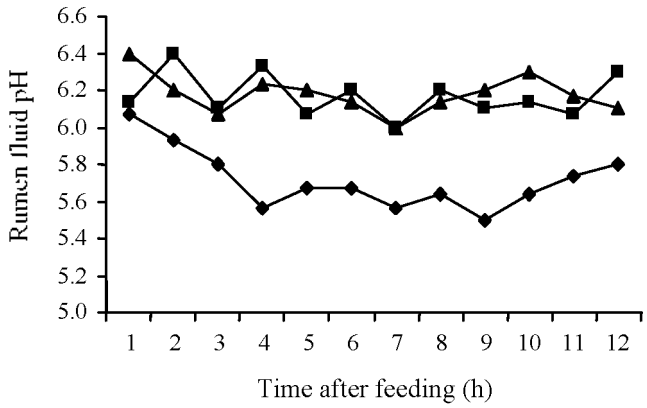

Figure 1. Rumen fluid pH of cows fed TMR (♦), pasture (■), or pasture supplemented with full-fat extruded soybeans (▲) (Experiment 2).

pellets were analyzed for total FA content and FA profile (Sukhija and Palmquist, 1988).

Experiment 3

Ten lactating multiparous Holstein cows with 270±59 days in milk, producing 24.6±4.5 kg/d of milk, and grazing a perennial ryegrass pasture continuously for 6 wk were randomly assigned to one of two dietary treatments. Cows continued to graze on pasture with one treatment group (n = 5) receiving no supplement (PS) and another group (n = 5) drenched with 452 g/cow per day of soybean oil (OIL) for 7 consecutive days. Soy oil was drenched in equal amounts after each milking. The amount of oil drenched was approximately the same as provided through FFES in experiments 1 and 2. Milk FA was determined daily for composite samples collected from a.m. and p.m. milking as described in experiment 1.

Statistical analysis

All statistical analysis was performed in SAS (SAS, 2000). In experiment 1, DMI, milk yield, milk composition, FA profile of milk and blood serum were analyzed using proc MIXED in a repeated measures design. Treatment, block, week, and treatment×week were included in the model as fixed effects with week as the repeated measure on the cows. In experiment 2, DMI, milk yield, milk composition, milk FA, and serum FA were analyzed in a Latin square design with treatment and period included in the model. For bacterial FA, effects of treatment, period, day, and treatment by period were included in the final model. Here, data obtained from digesta samples collected every 3 hrs on a particular day were pooled for daily FA values. Analysis was performed as repeated measures. In experiment 3, treatment, day, and treatment×day effect were included in the model with day being used as the repeated measure on the cows.

RESULTS AND DISCUSSION

Botanical composition of pasture, chemical and FA composition of the diet, and rumen fluid pH

Botanical composition of pasture on a DM basis was 2.49, 0.15, 0.31, and 0.36 t/ha of grass (predominantly perennial ryegrass; *Lolium perenne*), legumes (predominantly white clover; *Trifolium repens*), weeds, and dead materials, respectively, in experiment 1 and 2.09, 0.17, 0.61, and 0.45 t/ha of grass (predominantly perennial ryegrass; *Lolium perenne*), legumes (predominantly white clover; *Trifolium repens*), weeds, and dead materials, respectively, in experiment 2. Protein (Table 1) and total FA contents (Table 2) were normal for the respective diets, but NDF and ADF contents were higher than recommended by NRC (2001). The NE_L content of TMR, PS, and FFES were found to be at the upper end of normal values reported in NRC (2001). As expected, $C_{18:2}$ was the predominant FA in FFES as was $C_{18:3}$ in pasture samples. However, $C_{18:3}$ content was relatively low and $C_{18:2}$ and $C_{16:0}$ contents were

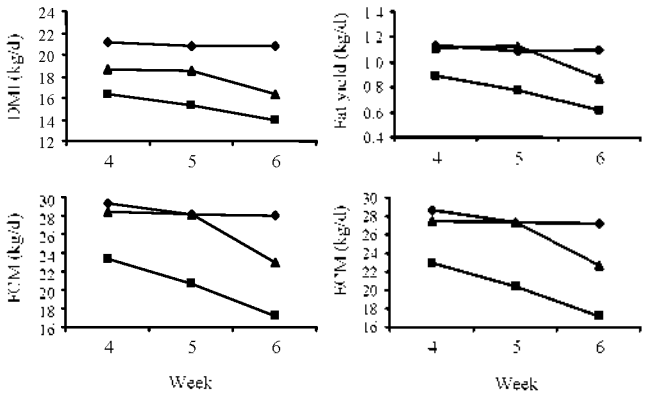

Figure 2. Dry matter intake (kg/d), fat corrected milk yield (kg/d), energy corrected milk yield (kg/d), and fat yield (kg/d) of cows fed TMR (♦), pasture (■), or pasture supplemented with full-fat extruded soybeans (▲) (Experiment 1).

high in pasture samples from experiment 2 compared with experiment 1. This was the result of a more mature pasture with increased proportions of weeds and dead materials in experiment 2. Increased $C_{18:2}$ and $C_{16:0}$ concomitant with decreased $C_{18:3}$ result from increased pasture maturity (Loor et al., 2002a). Neither *c*-9, *t*-11 CLA nor TVA were detected in any of the feeds.

As expected, rumen fluid pH was above 6.0 for cows in PS and PES treatments, but it was below 6.0 for cows in TMR treatment. The pH was consistently higher for cows in PS and PES treatments than for cows in TMR treatment (Figure 1). Relatively higher pH values in the rumen fluid of grazing cows can be attributed to higher contents of non-protein nitrogen compounds in pasture. Ammonia content in the rumen and blood urea nitrogen are also generally high in grazing animals. Their possible effects on milk fat CLA and TVA are discussed later.

Dry matter intake, milk yield, and milk composition

Cows in TMR treatment in both experiments were fed to meet nutrient demand according to NRC (2001). In experiment 1, there was a significant treatment×week effect on DMI, FCM, ECM, and fat yield (Figure 2) with a tendency for the same on milk yield ($p = 0.09$) and fat content ($p = 0.06$) (Table 3). Cows in PS treatment ate less than cows in PES treatment which in turn ate less than cows in TMR treatment during all weeks ($p<0.05$). Reduced DMI is a normal phenomenon when cows derive most or all of their DM from the pasture (Dhiman et al., 1999a; Loor et al., 2002b), and supplementation of FFES was not sufficient to fully offset the reduction in DMI. The DMI for cows in PS

Table 3. Dry matter intake, milk yield, and milk composition of cows fed TMR (TMR), pasture (PS) and pasture supplemented with full fat extruded soybeans (PES)

Parameters	Treatment			SEM[1]	P	
	TMR[2]	PS	PES[3]		Trt	Trt×week[4]
Experiment 1						
Milk yield (kg/d)	24.3[a]	18.6[b]	22.2[a]	1.07	0.05	0.09
Milk fat (%)	4.40	4.08	4.31	0.27	0.36	0.06
Milk protein (%)	3.11	3.16	3.18	0.06	0.71	0.96
Milk protein yield (kg/d)	0.75	0.58	0.70	0.06	0.20	0.16
Milk lactose (%)	4.39	4.41	4.59	0.18	0.70	0.50
Milk SNF (%)	8.32	8.41	8.63	0.21	0.57	0.76
Experiment 2						
DMI[5] (kg/d)	28.4[a]	24.9[b]	26.8[ab]	0.92	0.04	0.32
Milk yield (kg/d)	36.3[a]	23.5[b]	25.8[b]	2.03	0.04	0.23
3.5% FCM[6] (kg/d)	36.5[a]	24.8[b]	27.2[b]	3.01	0.05	0.22
ECM[7] (kg/d)	36.3[a]	24.6[b]	27.1[b]	2.95	0.05	0.20
Milk fat yield (kg/d)	1.28[a]	0.91[b]	0.97[b]	0.10	0.05	0.09
Milk protein yield (kg/d)	1.08[a]	0.72[b]	0.80[ab]	0.08	0.05	0.15
Milk fat (%)	3.51	3.87	3.84	0.44	0.62	0.20
Milk protein (%)	2.98	2.91	3.03	0.11	0.62	0.57
Milk lactose (%)	4.67	4.45	4.39	0.13	0.13	0.24
Milk SNF (%)	8.52	8.20	8.18	0.09	0.10	0.18

[a,b,c] Means with different superscripts in the same row differ significantly.

[1] Standard error of mean.

[2] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively.

[3] Full-fat extruded soybean used in PES treatment was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA.

[4] Period replaces the trt×week for experiment 2.

[5] The amount of DMI for cows on pasture was estimated from calculated net energy needs. The NE_L intake from grass was calculated using milk energy output (Tyrrell and Reid, 1965) plus energy spent for maintenance and BW gain (NRC, 2001) minus energy intake from the supplement as appropriate.

[6] 3.5% FCM (Fat corrected milk (kg)) = 0.432 (kilograms of milk)−16.2 (kilograms of fat).

[7] ECM (Energy corrected milk (kg)) = (0.327×milk yield (kg))−(12.95×fat yield (kg))− (7.2×protein yield (kg)). (Moe and Tyrrell, 1965).

Table 4. Milk yield and composition (means±standard deviation) of cows fed pasture (PS) or PS supplemented with soy oil (OIL) (Experiment 3)

Parameters	Treatment		P
	PS	OIL[1]	
Milk yield (kg/d)	24.8±5.45	25.3±4.46	0.87
3.5% FCM[2]	25.9±5.79	25.3±4.83	0.86
ECM[3] (kg/d)	25.4±5.91	25.2±4.71	0.94
Fat (%)	3.78±0.16[a]	3.49±0.13[b]	0.01
Protein (%)	2.86±0.17	2.96±0.17	0.40
Lactose (%)	4.46±0.19	4.55±0.24	0.53
SNF[4] (%)	8.17±0.38	8.38±0.45	0.46
MUN[5] (mg/dl)	22.0±2.53	22.1±2.91	0.96

[a, b, c] Means with different superscripts in the same row differ significantly.
[1] Cows in OIL treatment were drenched with 452 g of soy oil/cow per day in equal amounts after each milking.
[2] 3.5% FCM (Fat corrected milk (kg)) = 0.432 (kilograms of milk)−16.2 (kilograms of fat).
[3] ECM (Energy corrected milk (kg)) = (0.327×milk yield (kg))−(12.95×fat yield (kg))−(7.2×protein yield, kg). (Moe and Tyrrell, 1965).
[4] Solids-not-fat. [5] Milk urea nitrogen.

treatment reduced linearly from wk 4 to 6 and resulted in a linear reduction in milk yield, FCM, ECM, and fat yield compared with cows on TMR or PES treatments. There was no difference ($p>0.10$) between the cows in TMR or PES treatments for milk yield, FCM, ECM, and fat yield for wk 4 and 5, but cows in PES treatment produced less milk in wk 6 ($p<0.05$). When pooled for weeks, there was no significant difference ($p>0.05$) between the two dietary treatments for any of these parameters. This has practical implications for dairy producers to enhance *c*-9, *t*-11 CLA content and yield by supplementing FFES to cows grazing on pasture without compromising the milk yield (Table 4), which is desirable to increase the overall CLA supply in the market. Moreover, such a practice has the potential to reduce cost of production while at the same time obtaining a premium price for high CLA milk and milk products.

No significant difference in milk composition was observed between the three treatments. This is important because the objective is to increase both the concentration and yield of *c*-9, *t*-11 CLA in milk without altering the milk composition. Fat content of milk, >4%, in all treatments was higher than would be considered normal for Holstein cows. Most studies have used either early- or mid-lactation cows that had a milk fat content of 3.5% or less (White et al., 2001; Giesy et al., 2002; Whitlock et al., 2002) as opposed to the use of late-lactation cows in experiment 1. Protein, lactose, and SNF contents observed were normal for Holstein cows and did not differ significantly between treatments ($p>0.1$). Similar results were obtained previously when lactating cows were fed an all pasture diet or diets supplemented with extruded oil seeds (Dhiman et al., 1999a,b).

In experiment 2, there was a significant effect of treatment ($p = 0.04$), but not of period ($p = 0.32$) on DMI (Table 3) and milk yield. Cows in TMR treatment had higher DMI compared with cows in PS but not with PES treatment, and produced higher milk yield than either of the other treatments. This was a notable difference from experiment 1 in which cows in TMR and PES treatments produced similar milk yields. It is quite possible that cows in experiment 2 were more affected by a pasture diet, since they were quite early in lactation compared with those in experiment 1 where cows were a little late in lactation. No difference in DMI or milk yield was observed between cows in PS and PES treatments. Overall, DMI was higher for cows in experiment 2 than in experiment 1. Pasture DMI for cows in PS or PES treatment was similar within experiments. Overall, cows in all treatments produced higher milk yields than in experiment 1 by about 6 kg/d, owing to their higher initial milk yield and earlier stage of lactation. Supplementation of FFES to cows grazing on pasture raised the milk yield by 2.3 kg/d compared to cows on PS treatment, which will have economic implications. Though it was not sufficient to bring back the milk yield to the level of cows in TMR treatment, it would help increase the overall CLA output from the farm. Consistent with experiment 1, no significant effect ($p>0.1$) of treatment was observed on milk composition that resulted in higher 3.5% FCM, ECM, and fat yield for cows in TMR treatment. No significant effect ($p>0.1$) of period was found on milk yield or milk composition.

In experiment 3, milk yield remained constant (24.6±4.5 kg/d) during the relatively short duration of the experiment with no difference ($p>0.1$) in DMI and milk composition between PS and OIL treatments, except for milk fat content (Table 4). Increased proportion of free oil such as fish or plant oils in the diet is associated with reduced milk fat content (Baer et al., 2001). This will reduce the overall CLA yield from a cow, thus decreasing the importance of free oil in enhancing the concentration of milk fat *c*-9, *t*-11 CLA.

Fatty acid composition of milk

In experiment 1, treatment×week effect was not significant for FA content of milk, except for $C_{18:2}$ and $C_{18:3}$.

Table 5. Fatty acid composition of milk from cows fed TMR (TMR), pasture (PS) and PS pasture supplemented with full fat extruded soybeans (PES) (Experiment 1)

Fatty acids (% of reported fat)	Treatment			SEM[1]	P	
	TMR[2]	PS	PES[3]		Trt	Trt×week
$C_{8:0}$	0.78	0.84	0.76	0.05	0.58	0.70
$C_{10:0}$	0.65	0.67	0.60	0.03	0.30	0.86
$C_{12:0}$	1.68	1.71	1.47	0.08	0.12	0.50
$C_{14:0}$	8.45^{ab}	8.96^{a}	7.73^{b}	0.25	0.02	0.19
$C_{14:1}$	2.10	2.10	1.81	0.09	0.07	0.30
$C_{15:0}$	1.22^{b}	1.84^{a}	1.48^{b}	0.11	<0.01	0.39
$C_{16:0}$	32.3^{a}	26.9^{b}	24.6^{c}	0.38	<0.01	0.84
$C_{16:1}$	1.12^{b}	1.82^{a}	1.61^{ab}	0.17	0.04	0.25
$C_{17:1}$	0.22^{c}	0.46^{a}	0.37^{b}	0.07	<0.01	0.07
$C_{18:0}$	16.1	14.0	15.6	0.62	0.06	0.20
TVA	2.98^{b}	5.74^{a}	5.93^{a}	0.30	<0.01	0.25
Other *trans*-$C_{18:1}$	2.00^{a}	1.16^{b}	2.00^{a}	0.14	<0.01	0.90
c-9, $C_{18:1}$	25.0^{b}	28.0^{a}	29.4^{a}	0.82	0.01	0.58
Other *cis*-$C_{18:1}$	0.66	0.81	0.76	0.04	0.07	0.07
CLA	0.50^{b}	1.70^{a}	1.50^{a}	0.08	<0.01	0.26
$C_{20:2}$	0.04	0.04	0.05	0.005	0.74	0.42
$C_{20:3}$	0.02	0.02	0.01	0.004	0.32	0.61
$C_{20:4}$	0.18^{a}	0.09^{c}	0.12^{b}	0.001	<0.01	0.13
$C_{22:4}$	0.15	0.13	0.13	0.005	0.06	0.13
Saturated (%)	61.1^{a}	53.2^{b}	52.2^{b}	2.3	<0.01	0.76
Unsaturated (%)	38.9^{b}	46.7^{a}	47.8^{a}	2.3	<0.01	0.76

[a, b, c] Means with different superscripts in the same row differ significantly. [1] SEM = Standard error of mean.

[2] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively.

[3] Full-fat extruded soybean used in PES treatment was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA.

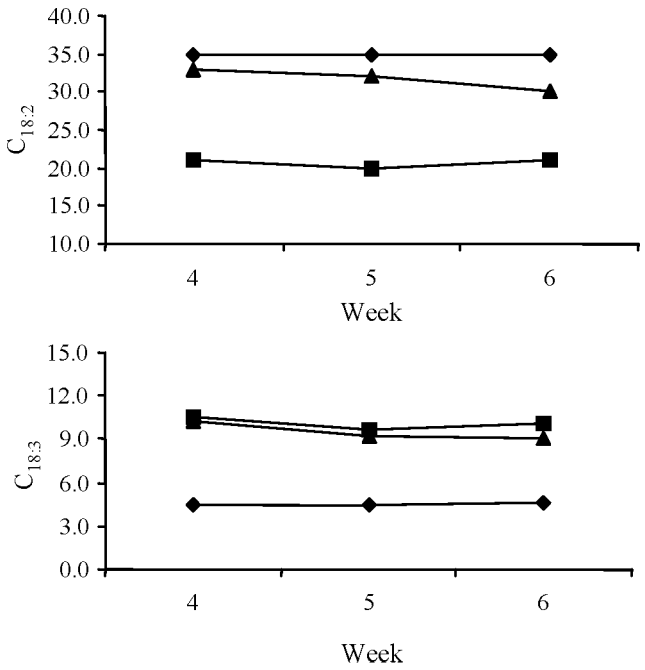

Figure 3. $C_{18:2}$ and $C_{18:3}$ content (%of fat) in milk from cows fed TMR (♦), pasture (■), or pasture supplemented with full-fat extruded soybeans (▲) (Experiment 1).

Therefore, their least square means are given in Table 5. For $C_{18:2}$ and $C_{18:3}$, weekly means from wk 4 through 6 are presented in Figure 3. The effect of treatment was variable on individual short and medium chain FA. An overall reduction in the sum total of short and medium chain FA occurred from TMR to PS to PES treatment which was contributed mostly by a reduction in milk fat $C_{16:0}$ content. Such a reduction in short and medium chain FA was expected because supplementation of long chain FA such as $C_{18:2}$ in the diet have an inhibitory effect on *de novo* FA synthesis (Grummer, 1991; Lock and Garnsworthy, 2002). There was a tendency ($p = 0.06$) for reduced milk fat $C_{18:0}$ content in PS treatment. Effect of a full pasture diet on milk fat $C_{18:0}$ is conflicting. While decreased $C_{18:0}$ content in milk fat has been reported previously (Dhiman et al., 1999a; Loor et al., 2002b), a more recent study showed increased milk fat $C_{18:0}$ content in cows fed similar pastures (Khanal et al., 2005). The reasons for such conflicting results could not be ascertained properly. Decreased $C_{18:0}$ in milk fat has been reported previously for cows receiving a pasture diet. A linear increase in milk fat *c*-9 $C_{18:1}$ content was observed for cows from TMR to PS to PES treatments, which was the result of feeding diets rich in 18-carbon FA which are extensively bihydrogenated in the rumen (Grummer, 1991).

As expected, the proportion of $C_{18:2}$ was higher in milk from cows in TMR or PES treatments compared with PS treatment during all weeks, while $C_{18:3}$ was higher in milk

Table 6. Fatty acid composition of milk fat from cows fed TMR (TMR), pasture (PS), and PS supplemented with full fat extruded soybeans (PES) (Experiment 2)

Fatty acids (% of reported fat)	Treatment			SEM[1]	P	
	TMR[2]	PS	PES[3]		Trt	Period
$C_{8:0}$	1.08	1.03	0.69	0.30	0.67	0.59
$C_{10:0}$	0.67	0.60	0.57	0.10	0.71	0.10
$C_{12:0}$	1.82	1.58	1.41	0.21	0.42	0.35
$C_{14:0}$	9.58	8.82	7.95	0.79	0.44	0.82
$C_{14:1}$	2.27	1.95	1.76	0.25	0.42	0.46
$C_{15:0}$	1.61	1.92	1.71	0.15	0.21	0.39
$C_{16:0}$	33.2^{a}	28.2^{b}	25.2^{c}	0.96	<0.01	0.25
$C_{16:1}$	1.33	2.11	1.72	0.35	0.34	0.94
$C_{17:1}$	0.24^{b}	0.45^{a}	0.38^{ab}	0.05	0.05	0.97
$C_{18:0}$	12.7	12.8	14.5	1.03	0.49	0.42
TVA	3.21^{b}	5.15^{a}	5.35^{a}	0.21	0.02	0.41
Other *trans*-$C_{18:1}$	2.09^{a}	1.07^{b}	2.03^{a}	0.14	0.02	0.80
c-9, $C_{18:1}$	24.2	27.9	28.9	2.01	0.16	0.53
Other *cis*-$C_{18:1}$	0.80	0.94	0.87	0.16	0.82	0.54
$C_{18:2}$	3.72^{a}	2.40^{b}	3.50^{a}	0.30	0.05	0.54
CLA	0.61^{b}	1.77^{a}	1.84^{a}	0.08	0.01	0.27
$C_{18:3}$	0.54^{b}	1.04^{a}	1.08^{a}	0.04	0.02	0.58
$C_{20:2}$	0.05^{b}	0.05^{b}	0.08^{a}	0.005	0.08	0.07
$C_{20:4}$	0.14	0.09	0.09	0.02	0.08	0.34
$C_{22:4}$	0.13	0.13	0.10	0.01	0.08	0.18
Saturated (%)	60.7^{a}	54.9^{b}	52.0^{b}	2.1	0.04	0.19
Unsaturated (%)	39.3^{b}	45.1^{a}	48.0^{a}	2.1	0.04	0.19

[a, b, c] Means different superscripts in the same row differ significantly.

[1] SEM = Standard error of mean.

[2] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively.

[3] Full-fat extruded soybean used in PES treatment was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA.

from cows in PS treatment than either TMR or PES treatments (Figure 3). Higher levels of $C_{18:3}$ are obtained in milk fat when cows are grazed on pasture, which has 50% or more of its FA as $C_{18:3}$, while reduced supply of $C_{18:2}$ in the diet for cows in PS treatment resulted in decreased $C_{18:2}$ content. Supplementation of FFES, which is high in $C_{18:2}$, increased $C_{18:2}$ content in the milk from cows fed PES diet compared with cows fed PS diet. A linear increase in milk fat $C_{18:3}$ and a linear decrease in $C_{18:2}$ content had been found in milk fat when DMI from pasture increased from 33 to 100% (Dhiman et al., 1999a). Moreover, such a trend in $C_{18:2}$ and $C_{18:3}$ contents reversed completely when cows were withdrawn from pasture and put back on a 50:50 conserved forage:concentrate diet (Khanal et al., 2003).

The *c*-9, *t*-11 CLA content in milk fat from cows in PS treatment was 350% of milk fat *c*-9, *t*-11 CLA from cows in TMR treatment. Supplementation of FFES to cows grazing fresh pastures did not increase *c*-9, *t*-11 CLA content in milk ($p>0.10$) indicating that supplementation of fat sources rich in $C_{18:2}$, such as FFES, under the conditions described for this experiment, is not likely to further enhance the *c*-9, *t*-11 CLA content in the milk of cows grazing similar pastures. A 200% or more increase in milk fat TVA contributed to the increased concentration of milk fat *c*-9, *t*-11 CLA in PS and PES treatments since TVA could be converted into *c*-9, *t*-11 CLA endogenously in the mammary gland (Griinari et al., 2000). It was evident in a high correlation ($r^2 = 0.87$) of milk fat *c*-9, *t*-11 CLA with TVA. Although the milk fat *c*-9, *t*-11 CLA content in the current study was lower than observed previously (Dhiman et al., 1999a; Chouinard et al., 2001), it was similar to the values observed for cows at a similar stage of lactation grazing a similar pasture during a similar time of the year (Khanal et al., 2005). Loor et al. (2002b) and Kay et al. (2004), on the other hand, reported even lower concentrations of milk fat *c*-9, *t*-11 CLA for cows grazing lush, green pastures supplemented with or without soybean meal or soy oil. By feeding higher levels of full fat soy or rapeseed, Lawless et al. (1998) were able to increase the milk fat *c*-9, *t*-11 CLA content of cows grazing on pasture over those supplemented with unmolassed beet pulp. However, they did not have cows receiving a pasture only diet. Such differences in milk fat *c*-9, *t*-11 CLA between experiments were the result of variation among cows, stage of lactation, diet, and production system (Lal and Narayanan, 1984; Jahries et al., 1997; Lock and Garnsworthy, 2002) or some other yet unknown factors (Kay et al., 2005). The relationship of milk fat *c*-9, *t*-11 CLA with serum *c*-9, *t*-11 CLA ($r^2 = 0.67$) or

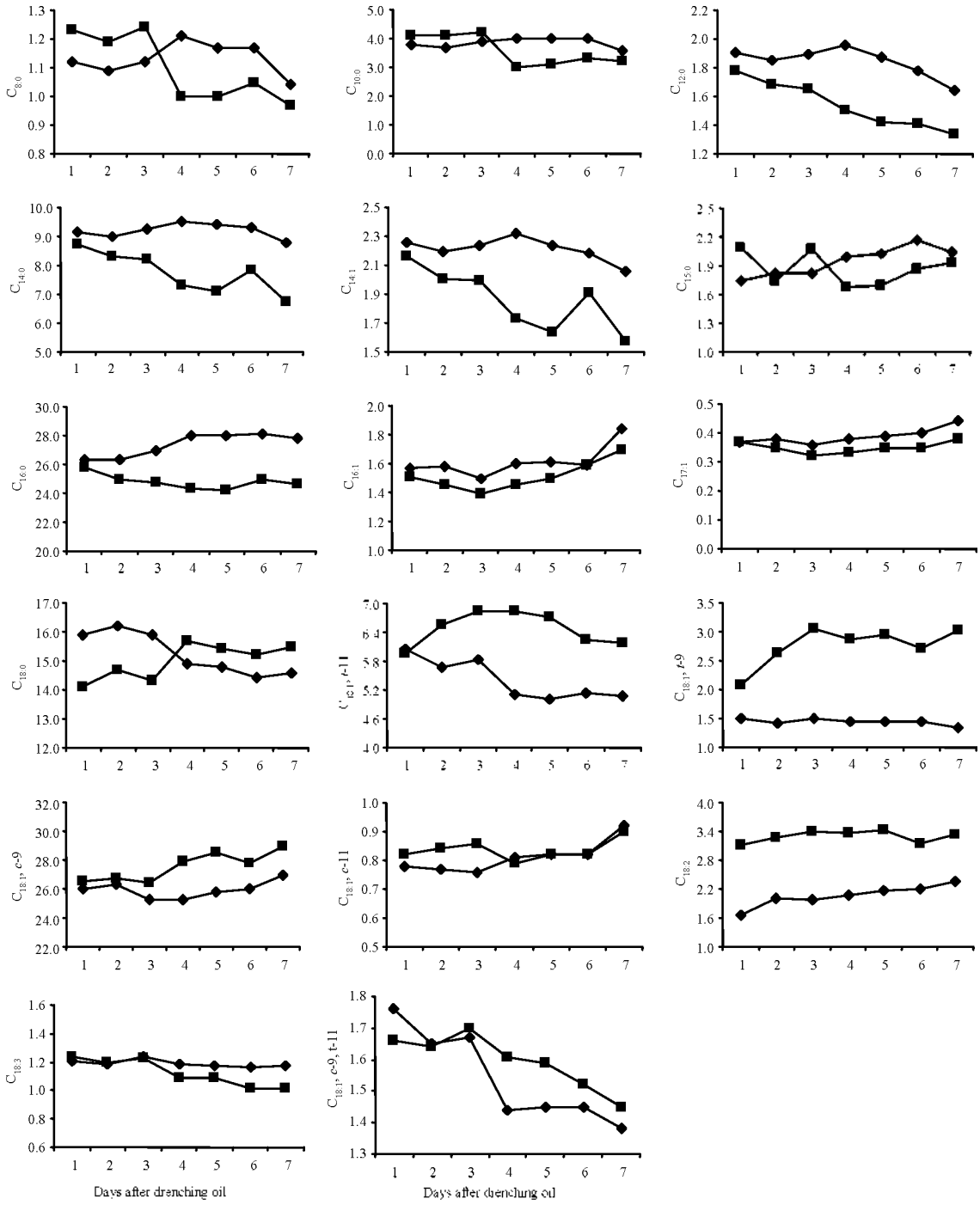

Figure 4. Fatty acid composition (% of fat) of milk from cows grazed on pasture, PS (♦) or PS drenched with soybean oil, OIL (■) (Experiment 3). Although, some fatty acids, viz. $C_{20:2}$, $C_{20:4}$, and $C_{22:4}$ were detected, they are not reported here for they were present in very small amounts (≤0.01%) and neither a diet nor diet by day effect was observed ($p>0.1$).

serum TVA (r^2 = 0.80) was positive suggesting that an increase in milk fat *c*-9, *t*-11 CLA is likely through an increased concentration of *c*-9, *t*-11 CLA or TVA in the serum which has its origin in the rumen, indicating the incomplete biohydrogenation of available substrates.

An increased pH (Figure 1) may have contributed to some of the increase in *c*-9, *t*-11 CLA and TVA in the milk, because rumen fluid pH above 6.0 has a positive effect on milk fat *c*-9, *t*-11 CLA and TVA contents in rumen cultures (Martin and Jenkins, 2002; Troegeler-Meynadir et al., 2003). Jiang et al. (1996) have observed higher milk fat *c*-9, *t*-11 CLA due to increased pH in cows fed a higher proportion of forage in the diet.

FA of 20 carbons or more were detected in small

amounts in the milk fat, with a significant difference ($p<0.01$) between treatments on $C_{20:4}$ content, the lowest being for cows in PS treatment. This reflected the supply of its precursor $C_{18:2}$ in the diet and its increased concentration in blood serum.

In experiment 2, period had no significant effect ($p>0.1$) on FA profile of milk, except for a tendency ($p = 0.07$) observed on $C_{20:2}$ (Table 6). Since most of the results were similar to those observed in Experiment 1, only the differences observed between the two experiments will be discussed. In experiment 2, no treatment effect ($p>0.1$) was observed on FA up to $C_{15:0}$ and $C_{16:1}$, contrary to the significant difference observed in experiment 1 for FA $C_{14:0}$, $C_{15:0}$, and $C_{16:1}$. Although numerical values for all three FA were similar to those observed in experiment 1, fewer experimental units ($n = 3$) probably did not lend enough power to detect such differences, as could be seen in larger standard error of means. The same was true for *c*-9 $C_{18:1}$, though there was a linear increase from TMR to PS to PES treatment as was observed in experiment 1. The TVA content in the milk fat of cows on PS and PES treatments was lower than in experiment 1 by about 0.6 percentage units, yet the *c*-9, *t*-11 CLA content was slightly higher in experiment 2. This may indicate that higher TVA content does not necessarily result in higher *c*-9, *t*-11 CLA and caution should be observed while making comparisons between experiments. It is probably the result of saturation of the Δ^{-9} desaturase enzyme system (Ntambi, 1999). An important point to be noted, however, is that there was no increase ($p>0.1$) in the *c*-9, *t*-11 CLA content of milk from cows in PES treatment compared with cows in PS treatment. It corroborated the finding in experiment 1 that supplementation of diets rich in linoleic acid is not likely to further enhance the *c*-9, *t*-11 CLA content of milk from cows grazing fresh pastures.

In experiment 3, there was a treatment×day effect on $C_{8:0}$, $C_{10:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, TVA, other *trans*-$C_{18:1}$, and *c*-9 $C_{18:1}$ content of milk fat. Therefore, all FA present in the milk from cows in PS or OIL treatment is presented in Figure 4. This suggests that there was a daily variation in the effects of the two diets on FA profile of milk. The daily fluctuation was higher for cows on OIL treatment than for cows on PS treatment, and the FA derived from the diet fluctuated more than their *de novo* synthesized counterparts. It was probably the result of the short period of drenching oil within which cows, and more importantly, the rumen bacteria were probably not able to adjust to the change in diet. It has been observed that cows receiving a full pasture diet require a minimum of 3 wk for establishment of the FA profile of milk (Khanal et al., 2003). Similarly, AbuGhazaleh et al. (2004) have shown highest milk fat CLA contents in cows fed TMR based diets supplemented with fish meal and extruded soybean for an extended period of time. Adaptation time to OIL may not take as long but sampling from d 1 probably led to such results.

Although TVA content was significantly higher ($p<0.05$) for cows in OIL treatment from d 2 through 7, it neither produced the higher concentration of *c*-9, *t*-11 CLA in milk on corresponding days nor did it increase the overall *c*-9, *t*-11 CLA content in milk fat compared with PS treatment. This leads to the question raised previously regarding whether an increased TVA necessarily means an increased *c*-9, *t*-11 CLA in milk fat. Since Δ^{-9} desaturase enzyme system shows saturation kinetics (Ntambi, 1999), it is probably reasonable to assume that only certain amounts of TVA reaching the mammary gland get converted to *c*-9, *t*-11 CLA. Overall, cows on the PS and OIL treatments produced 1.55 and 1.60% *c*-9, *t*-11 CLA in the milk fat, respectively, which was similar to levels observed in the experiment comparing PS and PES treatments. When data for all 7 d were pooled, no significant increase in overall *c*-9, *t*-11 CLA content of milk for cows in OIL treatment occurred compared with cows in PS treatment. This suggested once again that no significant improvement in the *c*-9, *t*-11 CLA in milk from cows on pasture is likely through supplementation of FFES or soy oil. In a New Zealand study, cows grazing on pasture continuously for several weeks failed to increase *c*-9, *t*-11 CLA concentration in milk fat when sunflower oil, which is high in $C_{18:2}$ was infused for 4 d (Kay et al., 2004). However, another study by the same authors (Kay et al., 2003), showed a significant increase in milk fat *c*-9, *t*-11 CLA from cows grazing pastures supplemented with fish oil or fish oil+sunflower oil, but not on sunflower oil alone. Fish oil is high in FA with 20 or more carbons, whereas sunflower oil is high in $C_{18:2}$, leading to differences in the biohydrogenation of dietary FA in the rumen and thus the results on milk fat *c*-9, *t*-11 CLA contents. Moreover, the differences in type and amount of oil and the type and quality of pastures between experiments may also have led to such differences in milk fat *c*-9, *t*-11 CLA contents.

Although there was no increase in the concentration of CLA or TVA in any of the experiments for PES or OIL treatments over PS treatment, cows in PES treatment produced numerically higher CLA yield (Figure 5). The yield of TVA was also numerically higher for cows supplemented with linoleic acids than for unsupplemented cows with significant difference between the two in experiment 1. A higher milk yield for cows supplemented with linoleic acid resulted in higher milk fat CLA and TVA yields, which has practical significance for dairy producers. Since TVA can be converted to CLA in humans (Adolf et al., 2000), increased yields of TVA would help maintain its constant supply in the market and help derive the potential health benefits associated with CLA.

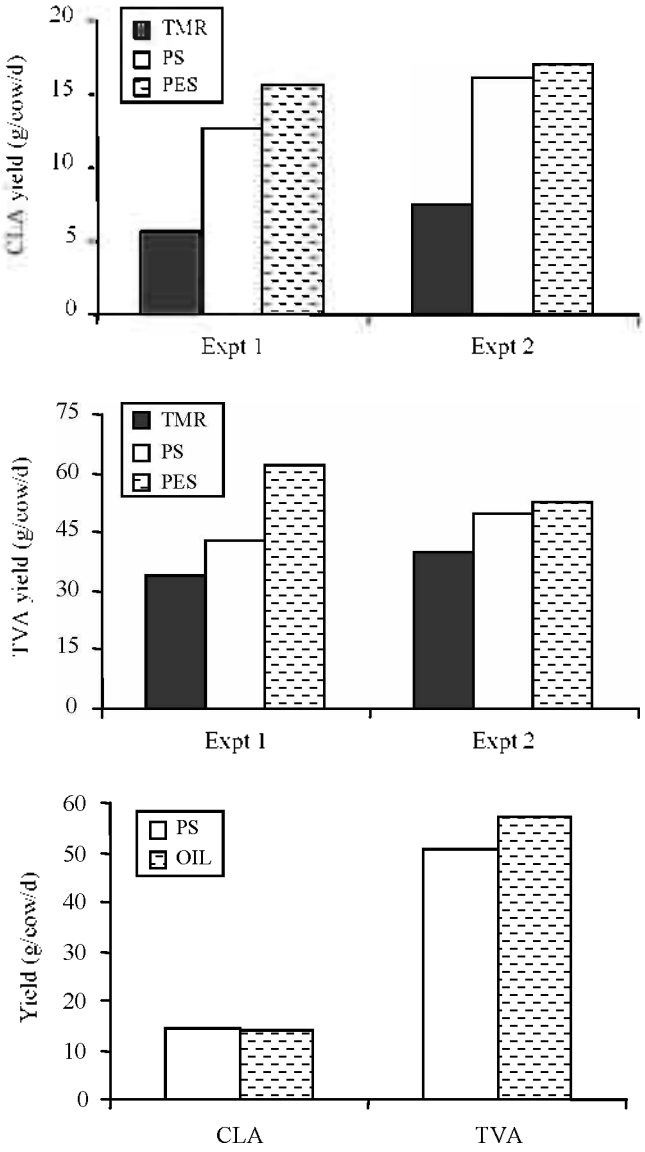

Figure 5. The CLA and TVA yield for cows fed TMR (TMR), pasture (PS), or PS supplemented with full-fat extruded soybeans (PES) (experiments 1 and 2, top two panels) and pasture (PS) or PS supplemented with soy oil (OIL) (Experiment 3, bottom panel).

Fatty acid composition of blood serum

In experiment 1, a significant treatment×week effect on $C_{14:1}$, $C_{15:0}$, $C_{16:0}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:4}$ was observed (Figure 6). The FAs with no treatment×week effect are presented in Table 7. In experiment 2, period had no significant effect ($p>0.05$) on FA profile, except for $C_{20:4}$ and *c*-9 $C_{18:1}$ (Table 8). Overall, FA profile was comparable in both the experiments. As expected, no FA with less than 12 carbon atoms were detected in serum, while FA $C_{14:0}$ and $C_{15:0}$ were detected in very small amounts in both the experiments, owing their presence in milk entirely or mostly to *de novo* synthesis. The proportion of *c*-9 $C_{18:1}$ was much smaller in serum than in milk in both the experiments, suggesting its

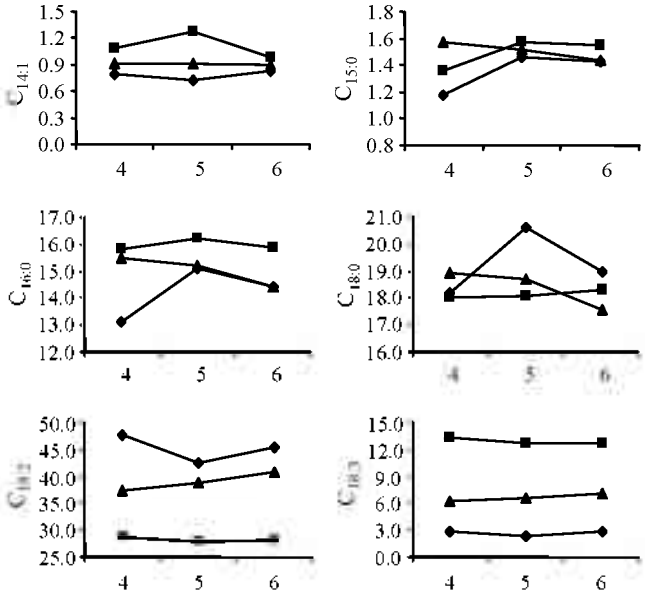

Figure 6. $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:2}$ and $C_{18:3}$ content (% of fat) of serum from cows fed TMR (♦), pasture (■), or PS supplemented with full-fat extruded soybeans (▲) (Experiment 1).

larger proportion in the milk was synthesized through desaturation and isomerization in the mammary gland. Moreover, the same enzyme Δ^{-9} desaturase that converts TVA to *c*-9, *t*-11 CLA also converts $C_{18:0}$ to *c*-9 $C_{18:1}$ in the mammary gland.

A linear increase in experiment 2 and a 200% increase in experiment 1 of TVA together with a similar trend for *c*-9, *t*-11 CLA compared with TMR treatment provided evidence of increased supply of intermediates from incomplete biohydrogenation of unsaturated FA in the rumen for cows in PS and PES treatments. The increases in serum TVA and *c*-9, *t*-11 CLA for cows in PES treatment compared with cows in PS treatment in experiment 2, however, failed to increase milk fat *c*-9, *t*-11 CLA. It once again suggested that supplementation of diets rich in $C_{18:2}$ to cows grazing lush, green pastures does not increase milk fat *c*-9, *t*-11 CLA contents irrespective of their status in the serum. Loor et al. (2002b) obtained similar results in cows grazing on pasture supplemented with solvent-extracted or mechanically extracted soybean meal. Significant treatment×week effect in experiment 1 for a higher number of FA in serum compared with milk suggested a greater variation in serum FA composition over time than in milk FA. This is likely in view of the serum FA being derived from the diet, which is more variable than milk FA that are regulated more tightly. Both $C_{18:2}$ and $C_{18:3}$, the two important substrates for endogenous synthesis of *c*-9, *t*-11 CLA, reflected the dietary regimen of cows with much greater proportions in serum than in milk. The FA $C_{14:1}$ was detected in experiment 1 but not in 2 and $C_{16:1}$ was detected in experiment 2 but not in 1, reasons for which were not clear. Similarly, considerable

Table 7. Fatty acid composition of blood serum from cows fed TMR (TMR), pasture (PS) and PS supplemented with full fat extruded soybeans (PES) (Experiment 1)

Fatty acids[1] (% of reported fat)	Treatment			SEM[2]	P	
	TMR[3]	PS	PES[4]		Trt	Trt×week
$C_{16:1}$	0.56	1.59	1.14	0.12	<0.01	0.69
$C_{17:1}$	0.18[c]	0.42[a]	0.27[b]	0.03	<0.01	0.88
TVA	0.89[b]	2.10[a]	1.76[a]	0.13	<0.01	0.81
Other *trans*-$C_{18:1}$	0.77[a]	0.56[b]	0.76[a]	0.04	<0.01	0.90
c-9, $C_{18:1}$	7.54[b]	11.5[a]	8.32[b]	0.57	<0.01	0.84
Other *cis*-$C_{18:1}$	0.42[c]	0.56[b]	0.73[a]	0.03	<0.01	0.71
CLA	0.09[b]	0.21[a]	0.18[a]	0.02	<0.01	0.61
$C_{22:4}$	2.53[b]	3.12[a]	3.07[a]	0.09	<0.01	0.15
Saturated (%)	39.9	41.8	41.1	3.7	0.89	0.64
Unsaturated (%)	60.1	58.2	58.9	3.7	0.89	0.64

[a, b, c] Means with different superscripts in the same row differ significantly.

[1] Fatty acids of <C14 were not detected. Similarly, $C_{20:2}$ and $C_{20:5}$ were not detected.

[2] SEM = Standard error of mean.

[3] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively.

[4] Full-fat extruded soybean used in PES treatment was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA.

Table 8. Fatty acid composition of blood serum from cows fed TMR (TMR), pasture (PS) and PS supplemented with full fat extruded soybeans (PES) (Experiment 2)

Fatty acids[1] (%of reported fat)	Treatment			SEM[2]	P	
	TMR[3]	PS	PES[4]		Trt	Period
$C_{14:0}$	1.01	1.27	1.08	0.25	0.74	0.26
$C_{14:1}$	0.15	0.22	0.20	0.06	0.68	0.22
$C_{15:0}$	1.44	1.37	1.61	0.06	0.17	0.21
$C_{16:0}$	14.4	15.2	14.2	1.15	0.31	0.09
$C_{17:1}$	0.20[c]	0.33[a]	0.25[b]	0.04	0.05	0.07
$C_{18:0}$	18.5	19.0	20.7	1.32	0.46	0.99
TVA	1.00[b]	1.55[a]	2.28[a]	0.20	0.04	0.73
Other *trans*-$C_{18:1}$	0.87[a]	0.64[b]	0.93[a]	0.07	0.02	0.09
c-9, $C_{18:1}$	8.1[b]	13.7[a]	9.4[b]	0.85	0.01	0.05
Other *cis*-$C_{18:1}$	0.42[b]	0.66[a]	0.56[ab]	0.07	0.03	0.01
$C_{18:2}$	45.9[a]	32.9[b]	36.8[b]	2.05	0.04	0.28
CLA	0.11[b]	0.27[a]	0.31[a]	0.15	0.02	0.10
$C_{18:3}$	2.92[b]	8.18[a]	7.12[a]	1.01	0.04	0.62
$C_{20:4}$	3.12[a]	2.24[b]	2.11[b]	0.22	<0.01	0.02
$C_{22:4}$	1.79	2.51	2.45	0.26	0.13	0.18
Saturated (%)	35.3	36.8	37.5	2.58	0.74	0.33
Unsaturated (%)	64.7	63.2	62.5	2.58	0.74	0.33

[a, b, c] Means with different superscripts in the same row differ significantly.

[1] Fatty acids of <C14 were not detected. Similarly $C_{20:2}$ and $C_{20:5}$ were also not detected.

[2] Standard error of mean.

[3] TMR contained alfalfa hay, alfalfa haylage, brome grass hay, corn silage, flaked corn, USU commodity mix, whole cotton seed, soy plus, custom yeast, beet pulp, molasses, and EnrGII at 18.55, 17.63, 2.33, 17.37, 9.95, 17.91, 7.53, 1.73, 0.15, 4.63, 1.11, and 1.11% on DM basis, respectively.

[4] Full-fat extruded soybean used in PES treatment was extruded using Insta-Pro® extrusion technology (Insta-Pro® Int., Division of Triple "F", Inc., Des Moines, IA.

amounts of $C_{20:4}$ and $C_{22:4}$ were detected in experiment 2 while no $C_{20:4}$ was detected in experiment 1. The reasons for such differences between experiments were not very clear. Higher proportions of unsaturated FA in serum than in milk in both the experiments could be explained by the continuous dietary supply of unsaturated FA and less tight mechanisms to control the same in serum than in milk.

Fatty acid composition of ruminal bacteria

Since a significant treatment×period effect occurred for FA $C_{10:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, TVA, $C_{18:3}$, and $C_{20:4}$ content in the bacteria rich pellets harvested from rumen digesta, means by period for all FA are presented in Figure 7. These data indicated that FA composition of bacteria from cows on the same treatment varied over the 3 periods. No $C_{8:0}$, $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, $C_{20:2}$, $C_{20:5}$ and $C_{22:4}$ were observed, though

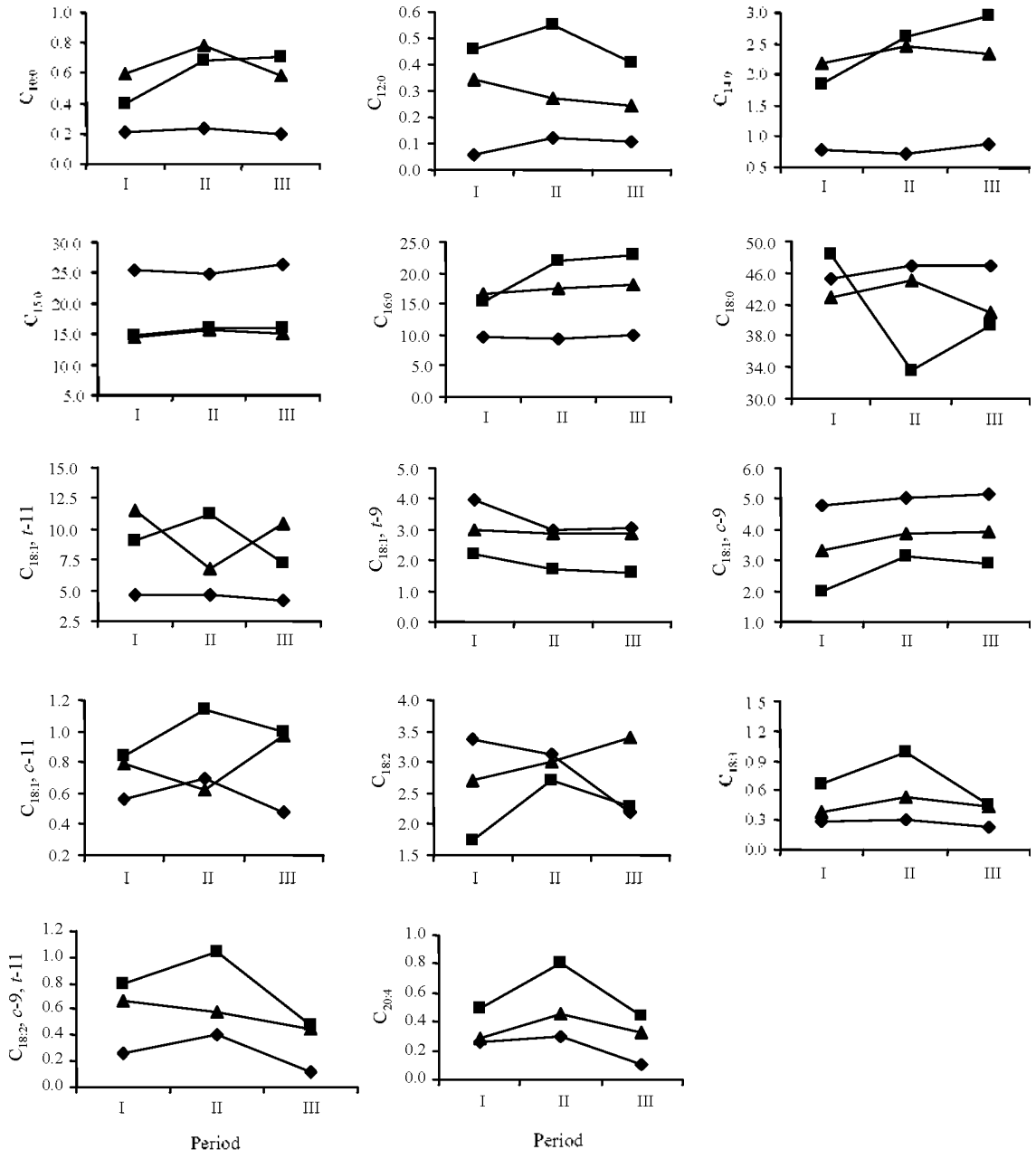

Figure 7. Fatty acid composition (% of fat) of bacteria rich pellets harvested from the rumen of cows fed TMR (♦), pasture (■), or pasture supplemented with full-fat extruded soybeans (▲) (Experiment 2).

some of these were present in the diet. The disappearance of some FA and the appearance of others suggested that some synthesis, elongation, and desaturation of dietary FA took place before finally being incorporated into bacterial cells. Absence of $C_{14:1}$ and $C_{16:1}$ in bacteria and their presence in milk fat suggested that Δ^9-desaturase was active irrespective of the dietary treatments, because both these FA are products of the same enzyme that converts TVA to *c*-9, *t*-11 CLA in the mammary gland. Disappearance of some FA and appearance of others is the result of intense microbial lipid metabolism in the rumen. Lipolysis of dietary lipids leads to more saturated FA. As a result, a considerable amount of $C_{15:0}$ was observed in bacteria harvested from the rumen of cows on all diets. This was in contrast to earlier findings, where only a small proportion of $C_{15:0}$ was reported (Wu and Palmquist, 1991; Pantoja et al., 1996; Loor et al., 2002a). Since the diet had only a small fraction of total FA as $C_{15:0}$, its net synthesis must have taken place in the bacterial cells. Wu and Palmquist (1991) showed that odd numbered FAs were the ones synthesized by bacteria in greatest amounts. Although not measured in the current study, an increase in effective fiber in the diet may have caused this unusually high $C_{15:0}$ content (Pantoja et al., 1996). Both NDF and ADF contents of the diet in the

current study were higher by 6 to 18 percentage units than recommended by NRC (2001).

Fatty acid profile of bacteria from cows in TMR treatment was fairly constant, while that from cows in PS and PES treatments varied considerably. This could probably be attributed to variation in the quality of pasture over the three periods. Though the diet had only a small proportion of $C_{18:0}$, it increased considerably in bacteria from cows on all treatments. This was the result of biohydrogenation of dietary FA $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$. The increased concentrations of TVA and *c*-9, *t*-11 CLA from cows in PS and PES treatments compared with cows in TMR treatment was an indication of incomplete biohydrogenation of $C_{18:2}$ and $C_{18:3}$ supplied through diets. No increase in bacterial TVA and *c*-9, *t*-11 CLA for cows in PES treatment was observed compared with cows in PS treatment, suggesting that supplementation of $C_{18:2}$ to cows grazing on pasture does not enhance the milk fat *c*-9, *t*-11 CLA through its ruminal synthesis. Increased proportions of TVA and *c*-9, *t*-11 CLA in the ruminal digesta have been reported when cows on a total mixed diet were supplemented with fish oil, extruded soybeans or both (AbuGhazaleh et al., 2002).

CONCLUSION

Cows grazing perennial ryegrass pasture produced 300-350% more *c*-9, *t*-11 CLA compared with cows on a TMR diet, with no further increase resulting from supplementation of linoleic acid through full-fat extruded soybeans or soy oil. Concentrations of bacterial and serum *c*-9, *t*-11 CLA were increased by 200 to 300% for cows grazed on pasture compared with cows on a TMR diet, but no further increase was achieved with supplementation of full-fat extruded soybeans. Ruminal synthesis of *c*-9, *t*-11 CLA was also not increased by the supplementation of linoleic acid to cows grazing on pastures. Milk fat *c*-9, *t*-11 CLA was positively correlated with milk fat TVA ($r^2 = 0.87$), serum *c*-9, *t*-11 CLA ($r^2 = 0.67$), and serum TVA ($r^2 = 0.80$). Based on the findings of these experiments and the conditions in which they were carried out, it was concluded that *c*-9, *t*-11 CLA in milk from cows grazing perennial ryegrass pasture is not likely to be further enhanced by supplementing linoleic acid through full-fat extruded soybeans or soy oil. It is, however, possible to increase total *c*-9, *t*-11 CLA and TVA through supplementation of full fat extruded soybeans without compromising the milk yield, particularly from cows in late lactation. This can have practical implications for dairy producers on one hand and consumers of dairy products on the other. Why an increase in the dietary supply of substrates in the form of linoleic acid failed to enhance the milk fat *c*-9, *t*-11 CLA content of cows grazing lush, green pastures needs further investigation.

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