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Status of Milk Fat Conjugated Linoleic Acid (CLA) in Selected Commercial Dairies* **

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ABSTRACT: Because of the increasing evidence of potential benefits of conjugated linoleic acid (CLA) on human health, there is a need to investigate its status in commercial dairies and develop feeding strategies to enhance the content and supply of CLA in milk and milk products. A two-year experiment was conducted to study the status of milk fat c-9, t-11 CLA on four selected commercial dairy farms in Utah (two) and Idaho (two), USA. Farms A and C grazed cows on pasture and supplemented with 7.0 kg/cow per day of their respective grain mixes during summer, while conserved forage and grain mix was fed during winter. Farm B fed a total mixed diet all year, with 10% of diet dry matter as fresh cut pasture during summer. Farm D had 1/3 of its cows grazed on pasture and supplemented with a total mixed diet during summer, while the rest were fed a total mixed diet. All cows in Farm D were fed a total mixed diet during winter. Farms A, B, C, and D had on average 80, 400, 150, and 500 milking cows, respectively, with Holstein or its crosses as the major breed. On a year-round basis, Farms A and C produced milk with 60% or more milk fat c-9, t-11 CLA and transvaccenic acid (TVA) contents than Farm B. Similarly, Farm D produced 30% or more c-9, t-11 CLA and TVA in milk than Farm B. Milk fat content of CLA and TVA was 150-200% more during summer compared with winter. Individual cows varied from 0.16 to 2.22% in milk fat c-9, t-11 CLA contents and 89% of the cows had c-9, t-11 CLA contents between 0.3 and 1.0% of milk fat. Individual cow variation was larger on Farms A and C compared with Farm D, with least variation on Farm B. Variation was larger in summer than in winter. The bulk tank milk c-9, t-11 CLA content varied from 0.27 to 1.35% of milk fat. Cows on Farms A and C produced similar or higher amounts of milk fat c-9, t-11 CLA on a daily basis even though their milk yield was lowest among the dairies. Concentration and supplies of c-9, t-11 CLA and TVA were highest from June through September and lowest from February through April, which should be the months for targeting improvement in the content and supply of milk fat c-9, t-11 CLA and TVA. (**Key Words**: Milk Fat, CLA (Conjugated Linoleic Acid), TVA (Transvaccenic Acid), Commercial Dairies)

INTRODUCTION

In the United States a vast majority of dairy farms are operated in confinement in an intensive feeding and management system with the objective to produce maximum milk yield. Dairy cows in such operations are fed a total mixed diet comprised of conserved forage and grain in about equal proportions. Dairies that graze cows during summer supplement some form of grain or a mixture of

grains to minimize the loss in milk yield while on pasture.

There may be an increasing interest among dairy producers to enhance conjugated linoleic acid (CLA) due to its potential health benefits (see Parodi, 2001; Khanal, 2004 for review). Two isomers of CLA have been identified as having potential health benefits: c-9. t-11 CLA against cancer (Banni et al., 2003), diabetes (Belury, 2003), atherosclerosis (Kritchevsky, 2003), and in immune regulation (Cook et al., 2003), and t-10, c-12 CLA on expenditure, lipid metabolism, and body composition (Terpstra, 2002; Keim, 2003). Steroylcoenzyme A desaturase present in the mammary gland of cattle as well as in the small intestine and adipose tissue of humans is able to convert transvaccenic acid (t-11 C_{181}): TVA) into c-9. t-11 CLA (Adolf et al., 2000; Griinari et al., 2000). Thus, an increased supply of TVA may also lead to the positive health benefits associated with c-9, t-11 CLA.

Many foods contain CLA naturally, but those of ruminant origin are the richest sources. Dairy products are

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^{**} Trade names and the names of commercial companies are used in this report to provide more specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the Utah State University or endorsement over products not mentioned.

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Table 1. Description of commercial dairies involved in the study

Description	Farm A	Farm B	Farm C	Farm D
Location	Preston, Idaho	Smithfield, Utah	Logan, Utah	Kuna, Idaho
Total area (ha)	55	600	200	950
Area under forage (ha)	48	500 (~45 for fresh cut)	160	375
Area grazed (ha)				
 Season start 	30	none	80	180
b. Season end	45	none	115	180
Herd size	200	650	350	900-1,200
Milking cows	75-90	380-410	140-160	450-500
Major breeds	Holstein, with crosses of Ayrshire, Jersey,	Holstein with a few crosses of Jersey and	Holstein, with crosses of Jersey and Brown	2/3 Holstein×Jersey, 1/3 Jersey
	Brown Swiss, and Normandy	Brown Swiss	Swiss	1/3 Jetsey
Major forages grown	Fescue, brome grass, alfalfa, perennial ryegrass, white clover	Alfalfa, fescue, brome grass, perennial ryegrass	Fescue, brome grass, alfalfa, perennial ryegrass, white clover	Alfalfa, perennial ryegrass, fescue, brome
Production system				
Summer	Pasture+grain	Chopped fresh forage (10%) mixed in TMR	Pasture+grain	1/3 on pasture+TMR, 2/3 on TMR
Winter	Conserved forage (ad lib)+grain	TMR (no com silage)	Conserved forage (ad lib)+grain	TMR
Grain (DM kg/cow/d)	6.25		6.25	
TMR (DM kg/cow/d)		18.5		17.2

thus among the major sources of CLA, of which c-9, t-11 $C_{18:2}$ is the major isomer. Research has shown that c-9, t-11 CLA and TVA contents in milk and meat can be increased by feeding animal diets that include pasture, plant and fish oils, and oil seeds (Zheng et al., 2005; Allred et al., 2006; Choi et al., 2006; Zhang et al., 2006; Khanal, et al., 2007a,b). Similarly, increased CLA and TVA contents have been observed in vitro as well as in vivo in the ruminal bacteria and blood plasma of dairy cows, sheep, and steers fed pasture, oils and oil seeds (Khanal et al., 2007a: Song and Choi, 2005; Song et al., 2005; Tanaka et al., 2003; Song et al., 2002). Highest concentrations were achieved in milk from cows grazing lush green pastures (Dhiman et al., 1999; Khanal et al., 2007a) and further increase from supplementation with feeds rich in linoleic acid appears unlikely (Kay et al., 2004; Khanal et al., 2005, 2007a).

While it is important to increase the concentrations of CLA in milk fat through various feeding and management strategies, it may not be wise to assume similar conditions prevailing in commercial dairies; the set up and principle of which are different from those of research. Although a volume of research is now available for increasing milk fat CLA contents, the same is not true with regards to the status of CLA in commercial dairies. Since current dietary recommendations include eating less animal fat, dietary CLA concentration and potential health benefits thereof are believed to have declined during the last two decades (Stanton et al., 1997), which needs to be rectified if we are to derive the health benefits associated with CLA. Therefore, it is important to have an estimate on the year-round status and supply of CLA in milk fat from

commercial dairies of different sizes with varied feeding practices during different times of the year. This is essential for maintaining a constant level and steady supply of CLA in dairy products for maximum human benefits. Therefore, the objective of the current study was to investigate the year-round concentration and supply status of milk fat CLA in selected commercial dairies of Utah and Idaho.

MATERIALS AND METHODS

Selection of commercial dairies

Four commercial dairy farms in Utah and Idaho, USA were selected for a two-year study. Farms were selected to represent cows grazing pastures during summer with supplementation of grain/grain mix, mixed group of cows with cows grazed on pastures and on total mixed diets, and cows with the provision of some fresh cut pasture fed along with total mixed diets. Farms A and C were of a semi-intensive type, while farms B and D were intensive in operation. Description of the farms is given in Table 1.

Feeding and management

The experiment started in July, 2001 and ended in June. 2003. During summer (mid to late May through September) cows in Farms A and C were grazed day and night except during milking. Access to drinking water and mineral mixture was *ad libitum* while grazing on pasture. Major forages grown on pasture in Farm A were alfalfa (Medicago sativa), brome grass (Bromus commutatus). fescue (Festuca arundinacea). perennial ryegrass (Lolium perenne). and white clover (Trifolium repens), whereas Farm C grew

Table 2. Composition of feed used in four commercial dairies (During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3^{cd} of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.)

Feed	DM	CP	NDF	ADF
Farm A				
Pasture clippings	24.5±4.4	14.5±3.4	46.2±5.6	28.4±3.9
Grain mix	88.3±2.3	10.8±1.2	7.3 ± 0.4	6.3±0.4
Alfalfa hay	87.9 ± 2.3	17.8±2.6	43.6±4.2	33.4±3.9
Oat hay	88.1±7.3	9.3±2.3	43.3±5.0	31.8±3.7
Oat haylage	36.5±6.2	8.6±1.7	45.4±4.8	33.5±3.2
Grass hay	82.5±4.9	8.6±1.5	58.6±5.5	40.8±4.6
Farm B				
Fresh cut pasture	24.6±3.8	11.6±2.0	52.5±4.7	31.7±3.5
Flaked com	91.0±0.9	8.5±1.1	8.6 ± 0.8	5.4±0.7
Sugar beet pulp	91.7±1.7	10.6±1.0	46.0±4.3	26.2±1.8
Wheat bran	91.7 ± 1.4	15.4±1.7	40.6±4.5	32.3±3.3
Oat grain	94.0±2.3	12.3±1.5	32.6±3.4	20.7±2.3
Whole cotton seed	92.9±1.8	22.7±2.8	52.2±3.7	43.9±4.3
Alfalfa hay	92.3±2.5	17.6±2.4	48.4±4.6	34.3±2.8
Grass hay	92.4±3.2	6.8±1.1	60.6±5.9	43.3±5.2
Oat haylage	30.0±3.6	7.3±1.5	55.5±5.2	32.6±2.7
Farm C				
Pasture clippings	30.6 ± 4.5	13.4 ± 2.2	49.3±5.7	32.6±3.4
Grain mix	89.5±1.8	9.8±1.3	18.2±1.9	13.3±1.6
Alfalfa hay	89.9±2.7	16.4±2.9	46.0±3.1	32.4±3.8
Mixed hay	89.3±2.3	13.6±3.4	49.9±4.3	41.3±3.9
Farm D				
Pasture clippings	22.6±2.1	19.5±3.8	42.6±5.0	29.6±2.3
Flaked com	91.3±2.6	8.3±0.6	7.9 ± 0.8	6.5±0.6
Sugar beet pulp	92.8±2.4	11.1±0.9	43.3±3.0	26.1±2.1
Whole cotton seed	92.6±1.6	23.4±2.7	54.2±4.6	$42.4\pm\pm3.4$
Com silage	35.4±4.3	7.3±1.4	46.6±5.2	25.9±2.2
Distiller's grain				
Alfalfa hay	91.6 ± 2.8	18.3±2.9	44.5±4.8	35.2±3.1

fescue (Festuca arundinacea), brome grass (Bromus commutatus), rye grass (Lolium perenne), alfalfa (Medicago sativa), and some white clover (Trifolium repens) as the major forages. Cows in Farm A were supplemented with 6.25 DM kg/cow per day of flaked corn and barley (60:40). Cows in Farm C were also supplemented with 6.25 DM kg/cow per day of a grain mix containing 10% molasses, 60% flaked corn, and 30% sugar beet pulp pellets. Grain mix in both of these farms was provided in approximately equal proportions during each milking. During winter months, Farm A fed cows with oat hay, alfalfa hay, and some alfalfa haylage on an ad libitum access basis, whereas Farm C let the cows have ad libitum access to alfalfa and meadow hay in year I and alfalfa hay alone in year 2. The amount of grain supplemented to cows remained the same throughout the year in both the farms. In Farms A and C, mineral and vitamin mixture was provided on an ad libitum access basis in the form of lick blocks. Farm B fed a diet in the form of total mixed ration (TMR) with no access to grazing during any time of the year. During summer months, however, Farm B mixed fresh cut pasture grass, mostly meadow brome (*Bromus commutatus*) and fescue (*Festuca arundinacea*), with TMR at approximately 10% of the DM. Oat haylage was fed occasionally in Farm B, but corn silage was not fed throughout the study period. Farm D had a mixed production system with 1/3 of the cows grazing during summer months along with the supplementation of a mixed diet, while the rest were stall-fed on a TMR diet. Detailed composition (DM, CP, NDF, and ADF) for each of the feed component in each of the four farms is given in Table 2 and their fatty acid (FA) composition presented in Table 3.

Sampling and laboratory analysis

Feed samples were collected every other month. Pasture samples were obtained by using a two-ft² quadrant from four different locations/0.4 ha of paddock during grazing season. Pasture samples were harvested at a height of 1.5

Table 3. Fatty acid composition (% of reported fat) of feeds offered to cows in four commercial dairies (During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.)

Farm/feed	C_{140}	$C_{16.0}$	C_{161}	C_{180}	C _{18 1}	C _{18.2}	C _{18.3}	Others
Farm A								
Pasture clippings	2.3	22.6	0.6	3.0	3.5	17.2	49.4	0.4
Grain mix	0.0	21.5	0.6	1.9	25.8	47.9	2.1	0.2
Alfalfa hay	15.1	23.5	0.3	5.0	4.7	19.5	25.2	6.7
Oat hay	2.8	18.9	0.2	3.5	2.9	18.9	42.4	10.4
Oat haylage	6.0	22.2	0.5	2.2	7.7	22.9	26.2	12.3
Grass hay	1.8	21.6	0.6	1.8	2.1	20.5	50.4	1.2
Farm B								
Pasture clippings	3.4	16.2	0.9	5.4	3.4	23.9	45.4	1.4
Flaked corn	0.1	17.3	0.0	2.3	29.7	46.9	3.1	0.4
Barley	0.0	27.4	0.7	1.4	20.6	44.2	3.4	2.3
Sugar beet pulp	6.8	25.5	1.2	4.9	14.0	29.5	14.6	3.5
Wheat bran	7.4	20.9	1.6	6.6	12.2	35.4	12.7	3.2
Oat grain	0.1	20.1	0.9	1.5	37.2	37.7	2.2	0.3
Cotton seed	0.2	23.2	0.5	2.2	17.1	56.4	0.2	0.2
Alfalfa hay	17.2	24.6	0.2	4.1	3.8	18.2	20.3	11.6
Grass hay	6.3	20.8	0.4	3.9	3.9	19.2	40.2	5.3
Oat haylage	5.5	22.7	0.6	3.4	7.5	19.8	26.6	16.9
Farm C								
Pasture clippings	3.6	22.2	1.3	4.5	3.4	16.6	48.2	0.2
Grain mix	0.0	21.4	0.7	1.7	25.9	47.3	2.7	0.3
Alfalfa hay	14.3	22.8	0.3	3.1	2.8	22.4	28.9	5.4
Mixed hay	10.2	18.4	1.7	3.5	4.2	22.8	35.9	3.3
Farm D								
Pasture clippings	1.4	21.2	0.8	2.4	5.2	18.6	50.4	0.0
Flaked corn	0.0	17.6	0.1	1.3	33.9	43.7	3.4	0.0
Cotton seed	0.0	23.8	0.4	2.0	17.9	56.4	0.2	0.1
Com silage	1.0	14.9	0.7	3.6	18.8	41.6	5.7	13.7
Alfalfa hat	12.8	22.5	0.4	6.0	4.9	22.6	24.4	6.4
Distiller's grain	0.0	16.1	0.1	2.9	23.5	55.2	1.9	1.3

cm. Duplicate milk samples from the bulk tank were collected every month throughout the experiment. Milk samples from individual cows were collected twice a year from two consecutive milkings, once during grazing season in July/August and once during winter in January/February. A minimum of 20 or 15% of the total milking cows, whichever was greater at the time of sampling, were selected for milk sampling. These cows were not selected to be the same every time, if any, it was by chance during sampling. Bulk tank and individual cow milk samples from all four farms were collected within seven days of each other in plastic vials with preservative (Broad Spectrum Microtabs II; D & F Control Systems, Inc., San Ramon, CA). Milk samples were stored in a refrigerator at 4°C until further processing within 72 h, and feed samples were stored in a freezer at -20°C until laboratory analyses.

Milk samples were obtained from two consecutive milkings from individual cows as well as from the bulk tank.

Samples were analyzed for milk composition (fat. protein. lactose, SNF, milk urea nitrogen (MUN), and somatic cell count (SCC)) with mid infrared wave bands (2-15 $\mu m)$ using a Bentley 2000 (Bentley Instruments, Chaska, MN) by the Rocky Mountain Dairy Herd Improvement Association Laboratory, Logan, Utah, Fat was extracted from duplicate milk samples from the bulk tank and weighted composite milk samples (two) from individual cows. Details about fat extraction, methylation, and analysis on a gas chromatograph have been described (Dhiman et al., 2002).

Dry matter content of feed was determined by drying in a forced air 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). Feed samples were ground to pass through a 1-mm screen (Wiley Mill; Arthur H. Thomas, Philadelphia, PA). Crude protein content was determined using a macro

Table 4. Composition of milk collected from four commercial dairies (During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.)

Parameters -			F	amı		Season					
A A	А	В	С	D	SEM	<u>р</u>	Summer	Winter	Overall	SEM	p
Bulk tank											
Fat (%)	3.84 ^a	3.00°	3.59 ^b	3.87a	0.06	< 0.01	3.69^{b}	3.46°	3.58	0.04	< 0.01
Protein (%)	3.29a	2.91°	3.11 ^b	3.18 ^b	0.03	< 0.01	3.05^{b}	3.19^{a}	3.12	0.02	< 0.01
Lactose (%)	4.80^{a}	4.69^{b}	4.78	4.82	0.02	< 0.01	4.79^{a}	$4.75^{\rm b}$	4.77	0.01	0.04
SNF ¹ (%)	8.99 ^a	8.51°	8.79 ^b	8.91	0.02	< 0.01	$8.75^{\rm b}$	8.85°	8.80	0.03	< 0.01
MUN ² (mg/dl)	9.9°	12.3 ^b	15.1a	14.0^{a}	0.54	< 0.01	11.8 ^b	14.0^{a}	1 2 .9	0.38	< 0.01
SCC^{3} , 000	407.0°	154.6°	259.8 ^b	164.5 ^a	24.1	< 0.01	230.5	262.4	246.2	16.9	0.19
Individual cow											
Fat (%)	3.98^{a}	3.46^{10}	3.65 ^b	3.34°	0.06	< 0.01	3.71^{a}	$3.50^{\rm b}$	3.61	0.04	< 0.01
Protein (%)	3.33a	3.13^{b}	3.14^{b}	3.18^{b}	0.02	< 0.01	3.11 ^b	3.29a	3.21	0.02	< 0.01
Lactose (%)	4.79^{b}	4.89^{a}	4.78 ^b	4.87^{a}	0.02	< 0.01	4.85	4.82	4.84	0.01	0.09
SNF ¹ (%)	9.09^{a}	8.98°	8.82 ^b	8.99	0.03	< 0.01	8.91^{b}	9.03 ^a	8.97	0.03	< 0.01
MUN ² (mg/dl)	10.9^{c}	12.7^{b}	12.7 ^b	13.8 ^a	0.18	< 0.01	11.1 ^b	14.0°	12.6	0.15	< 0.01
SCC3, :000	520.8°	303.7^{b}	317.1 ^b	125.9°	50.5	< 0.01	300.4	333.3	316.8	43.1	0.59

^{a, b, c} Means with different superscripts within farm or within season in the same row differ significantly,

¹ Solids-not-fat. ² Milk urea nitrogent. ³ Somatic cell count.

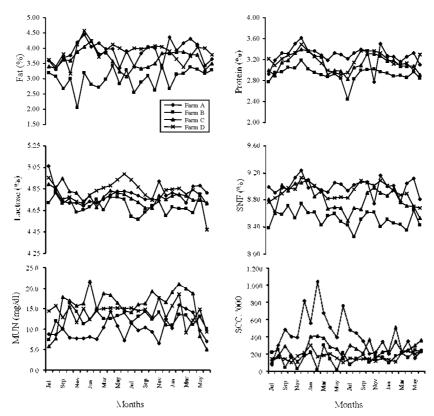


Figure 1. Temporal pattern of milk composition collected from four commercial dairies. During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.

Kjeldahl for digestion and Kjeltec System 1026 Distillation Unit for distillation (Tecator, Hoganas, Sweden). The NDF and ADF contents were determined by filter bag technology of Ankom (Ankom Technology Corp., Fairport, NY). Samples were further dried at 105°C for 8 h to determine absolute DM. Chemical composition of the feed (Table 2) was expressed on the basis of absolute DM. Fatty acid composition of feed was determined as per Sukhija and Palmquist (1988).

Table 5. Fatty acid composition of bulk tank milk samples collected from individual cows from four commercial dairy farms (During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.)

Fatty acids			Fa	ım					Season		
(% reported fat)	A	В	C	D	SEM	р	Summer	Winter	Overall	SEM	p
$C_{6:0}$	1.24ª	0.98°	1.18 ^{ab}	1.06^{lx}	0.05	< 0.01	1.09	1.14	1.12	0.03	0.28
C _{8 0}	0.84^{a}	0.67^{b}	0.81^{ab}	0.72^{ab}	0.04	0.02	0.65 ^b	0.87^{a}	0.76	0.03	< 0.01
$C_{10:0}$	3.28 ^a	2.54 ^b	3.04^{a}	2.51 ^b	0.06	< 0.01	2.67 ^b	3.01^{a}	2.84	0.04	< 0.01
$C_{12:0}$	4.18^{a}	3.12 ^b	3.96°	2.98^{b}	0.08	< 0.01	$3.30^{\rm b}$	3.81°	3.56	0.06	< 0.01
C_{140}	12.8a	11.1 ^b	13.0^{a}	10.9^{b}	0.18	< 0.01	11.6 ^b	12.4^{a}	12.0	0.13	< 0.01
C_{141}	0.86^{a}	$0.47^{\rm b}$	0.81^{a}	0.55^{b}	0.03	< 0.01	0.66	0.69	0.68	0.02	0.26
$C_{15:0}$	1.88°	1.60 ^b	1.99^{a}	1.55^{b}	0.06	< 0.01	1.71 ^b	1.90^{a}	1.81	0.04	< 0.01
$C_{16:0}$	32.4^{b}	30.9°	34.1^{a}	32.1 ^{bc}	0.44	0.04	30.9^{b}	34.8 ^a	32.9	0.31	< 0.01
C_{161}	1.68 ^a	1.29^{b}	1.56 ^a	1.36 ^b	0.04	< 0.01	1.36 ^b	1.59^{a}	1.48	0.03	< 0.01
C_{171}	0.36^{a}	0.25 ^b	0.33^{a}	0.24^{b}	0.01	< 0.01	0.30	0.29	0.30	0.01	0.18
$C_{18:0}$	10.7^{6}	15.0^{a}	10.5 ^b	14.6°	0.32	< 0.01	13.6°	11.8^{b}	12.7	0.23	< 0.01
c-9 C _{18 1}	20.7^{b}	22.5 ^a	19.5 ^b	22.2°	0.34	< 0.01	22.7^{a}	$19.7^{\rm b}$	21.2	0.24	< 0.01
other cis-C _{18 1}	0.69^{b}	0.99^{a}	0.66^{b}	0.75^{b}	0.05	< 0.01	0.82^{a}	0.72^{b}	0.77	0.03	0.04
TVA	3.30^{b}	2.45°	3.72°	3.09^{b}	0.10	< 0.01	3.73^{a}	2.55 ^b	3.15	0.07	< 0.01
other trans-C _{18:1}	0.86^{b}	1.70°	$0.83^{\rm b}$	1.90^{a}	0.07	< 0.01	1.42^{a}	$1.23^{\rm b}$	1.35	0.05	< 0.01
C_{182}	2.36^{bc}	3.23°	2.19^{c}	2.40^{b}	0.05	< 0.01	2.51	2.58	2.55	0.04	0.24
c-9, t-11 CLA	0.66 ^b	0.40^{d}	0.75^{a}	0.58°	0.03	< 0.01	0.79^{a}	0.42 ^b	0.61	0.02	< 0.01
t-10, c-12 CLA	0.05^{a}	ND^{l}	0.05^{a}	$0.04^{\rm b}$	0.005	0.02	0.04	0.04	0.04	0.005	0.25
C ₁₈₃	0.90^{a}	0.57^{b}	0.84^{a}	0.57^{b}	0.03	< 0.01	0.72	0.73	0.73	0.02	0.34
C _{20.2}	0.03	0.03	0.03	0.03	0.004	0.78	0.035	0.027	0.031	0.003	0.07
$C_{20:3}$	0.07^{b}	0.10^{a}	0.06^{b}	$0.07^{\rm b}$	0.007	< 0.01	$0.07^{\rm b}$	0.09^{a}	0.08	0.005	< 0.01
$C_{20:4}$	0.10^{b}	0.12a	0.10^{b}	0.09°	0.004	< 0.01	0.10^{b}	0.11^{a}	0.11	0.002	< 0.01

a.b.e Means with different superscripts within farm or within season in the same row differ significantly.

Statistical analysis

Statistical analysis was performed in SAS (SAS, 2000) using proc MIXED in a factorial structure. Farm, year, season, farm×year, farm×season, year×season, and farm× year×season were included in the model. Relationship of CLA with TVA was determined by regression analysis using proc REG in SAS. Arithmetic means and standard deviations are given for feed composition data. Only the averages are given for FA composition data.

RESULTS AND DISCUSSION

Milk composition

A significant difference occurred among farms in milk composition (p<0.01) for both bulk tank and individual cow milk samples (Table 4). Fat content was lowest for Farm B in bulk tank milk: it was lowest in individual cows in Farm D. While Farm B was based on TMR diets. Farm D had 2/3 of cows on TMR, and Farms A and C were based on forage. Higher milk fat contents could be expected when the diet contains more forage. Milk protein content was lowest for Farm B, both for bulk tank and individual cow milk samples. Milk fat and protein contents in Farm B could be regarded as less than normal (NRC, 2001). A lack of consistency was observed between bulk tank and individual

cow milk samples for some of the milk components within farms. For example, SCC was higher for individual cows compared with bulk tank, except in Farm D, and fat content was higher for individual cows in Farm B, but lower in Farm D compared with bulk tank samples. Since only 15% of the cows were selected for individual cow sampling, such differences should not be considered unlikely. Somatic cell count was significantly higher for Farms A and C compared with Farms B and D, where cows were mostly fed in confinement and under more controlled conditions. Temporal pattern showed monthly fluctuation of milk components within farms (Figure 1). While protein, lactose, and SNF contents fluctuated less from month to month in all farms, fat content and MUN values varied highly from one month to the other, which was probably due to variation in feed. Milk fat content was higher during summer (p< 0.01) when cows received fresh cut forage or were grazed on pasture, which is common with increased proportion of forage in the diet. It could be an added advantage for producers interested in increasing milk fat CLA and TVA. Milk protein content was lower (p<0.01) during summer. which was also reflected in lower MUN values in milk, an indicator of protein nutrition in dairy cows. Farms B and D produced milk with less than 200,000 SCC in their bulk tanks and were more consistent over the months than Farms

¹ Not detected.

Table 6. Fatty acid composition of milk collected from individual cows from four commercial dairy farms (During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.)

Fatty acids (% reported fat)		Farm							Season					
	A	В	С	D	SEM	р	Summer	Winter	Overall	SEM	р			
$\overline{C_{o:0}}$	1.21 ^{ab}	0.92°	1.28	1.08^{b}	0.02	< 0.01	1.08^{b}	1.21ª	1.15	0.01	< 0.01			
C _{8.0}	1.00^{a}	0.60^{d}	0.77^{b}	0.52^{e}	0.01	< 0.01	0.65^{b}	0.80^{a}	0.73	0.01	< 0.01			
C _{10:0}	3.25	2.38°	2.90^{b}	2.47°	0.04	< 0.01	$2.42^{\rm b}$	3.08^{a}	2.75	0.03	< 0.01			
$C_{12:0}$	4.11 ^a	3.05°	3.78^{b}	3.04°	0.06	< 0.01	$3.09^{\rm b}$	3.91^{a}	3.50	0.04	< 0.01			
C_{140}	12.8°	11.1 ^b	12.9°	11.0 ^b	0.12	< 0.01	11.6 ^b	12.7^{a}	12.2	0.08	< 0.01			
$C_{14 1}$	0.92^{a}	0.55 ^b	0.88^{a}	0.57^{b}	0.01	< 0.01	0.70^{b}	0.76^{a}	0.73	0.01	< 0.01			
$C_{15:0}$	1.984	1.71 ^b	$1.84^{\rm ab}$	1.43°	0.03	< 0.01	$1.54^{\rm b}$	1.94^{a}	1.74	0.02	< 0.01			
$C_{16:0}$	32.4^{b}	31.1°	34.0°	31.9^{b}	0.22	< 0.01	30.5^{b}	35.2°	33.4	0.16	< 0.01			
C _{16 1}	1.68°	1.32 ^b	1.61^{a}	1.27 ^b	0.03	< 0.01	1.38^{b}	1.56^{a}	1.47	0.02	< 0.01			
$C_{17.1}$	0.35^{a}	0. 24 ^b	0.34^{a}	0.21^{b}	0.005	< 0.01	0.30^{a}	0.28^{b}	0.29	0.004	< 0.01			
C _{18:0}	10.5 ^b	14.9^{a}	$10.7^{\rm b}$	15.3	0.19	< 0.01	14.3	11.4^{b}	12.9	0.13	< 0.01			
c-9 C _{18 1}	20.5°	22.5°	19.8°	21.7 ⁶	0.21	< 0.01	23.0^{a}	$19.3^{\rm b}$	21.2	0.18	< 0.01			
other cis-C _{18.1}	0.60°	1.01^{a}	0.73^{b}	0.79^{b}	0.02	< 0.01	0.86^{a}	0.71^{b}	0.79	0.01	< 0.01			
TVA	3.53	2.48°	3.72	2.98^{b}	0.07	< 0.01	3.75°	2.01 ^b	2.98	0.05	< 0.01			
other trans-C _{18:1}	0.82°	1.77^{b}	0.72^{c}	2.10^{a}	0.03	< 0.01	1.43^{a}	$1.27^{\rm b}$	1.35	0.02	< 0.01			
C ₁₈₋₂	2.26 ^{bc}	3.08°	2.14^{c}	2.34^{b}	0.03	< 0.01	2.44	2.47	2.46	0.02	0.26			
c-9, t-11 CLA	0.73^{a}	0.43°	0.75^{a}	0.55^{b}	0.02	< 0.01	0.78^{a}	0.40^{b}	0.59	0.01	< 0.01			
<i>t</i> -10, <i>c</i> -12 CLA	0.05°	0.03^{b}	0.05^{a}	ND^1	0.002	< 0.01	0.04°	0.02^{b}	0.03	0.001	0.01			
C ₁₈₋₃	0.92°	0.53°	0.87^{a}	0.47^{b}	0.01	< 0.01	0.73^{a}	0.66^{b}	0.70	0.01	< 0.01			
C _{20 2}	0.06^{a}	0.03°	0.04^{b}	0.05^{b}	0.002	< 0.01	0.05	0.05	0.05	0.001	0.88			
$C_{20:3}$	$0.07^{\rm b}$	ND^{l}	0.07^{b}	0.09^{a}	0.002	< 0.01	$0.02^{\rm b}$	$0.03^{\rm b}$	0.03	0.001	< 0.01			
C _{20:4}	0.10^{b}	0.12	0.11^{b}	0.09^{b}	0.002	< 0.01	0.10^{a}	0.12^{b}	0.11	0.002	< 0.01			

a.b.c Means with different superscripts within farm or within season in the same row differ significantly.

A and C, which had average SCC of 407,000 and 260,000, respectively in their bulk tank milk samples. Although not significant, somatic cell count was higher during winter probably because of the congested environment, particularly in Farms A and C.

Fatty acid composition of milk

Fatty acid composition of milk samples from bulk tank (Table 5) and individual cows (Table 6) within farms were similar to each other for the most part. Farms A and C were higher in milk fat contents of short and medium chain FA $(C_{6,0}$ through $C_{1?,1}$) compared with that of Farms B or D. This was in contrast to previous findings where increased dietary supply of longer chain polyunsaturated FA reduced the short and medium chain FA in milk (Chouinard et al., 2001; Loor et al., 2002; AbuGhazaleh et al., 2003). Farms A and C had permanent pastures, which were infested with weeds that lowered the quality of pasture. With the growth from the previous season, permanent pastures tend to mature faster than annual pastures. This increases the concentration of saturated FA ($C_{14.08}$, $C_{16.08}$ and $C_{18.0}$) and decreases that of C_{18:3} as the pasture matures (Loor et al., 2002), which was reflected in the FA profile (Table 3). As a result, dietary supply of longer chain polyunsaturated FA was reduced and the inhibition such polyunsaturated FA would cause on the de novo synthesis of short and medium chain FA in the mammary gland was probably not as effective. Dhiman et al. (1999) did not find any significant difference in short and medium chain FA when 1/3, 2/3 or all pasture diet was given to lactating cows. Milk fat $C_{18:0}$ and c-9 C₁₈₁ contents were lower for Farms A and C compared with Farms B or D. This might have been the result of the unavailability of enough substrates for desaturation in the rumen, for the reasons explained above. otherwise have produced concentration of C_{18:0} and c-9 C_{18:1} in milk fat. Higher concentration of milk fat C₁₈₃ for Farms A and C was probably the result of its increased supply through predominantly a pasture diet during grazing and a higher proportion of forage in the diet during winter compared with Farms B or D. Similarly, higher proportion of C_{18:2} in the milk fat for Farm B might be due to TMR diet compared with the rest of the farms, which had at least some cows grazing on pastures. Moreover, feed FA composition in Farm B had a higher proportion of $C_{18,2}$ (Table 3).

Milk from Farms A and C had significantly higher c-9. t-11 CLA and TVA than milk from Farm B, with Farm D in between. Farm D produced milk with significantly higher c-9. t-11 CLA than Farm B. Overall, TVA and c-9. t-11 CLA contents were similar between bulk tank and individual cow

¹ Not detected.

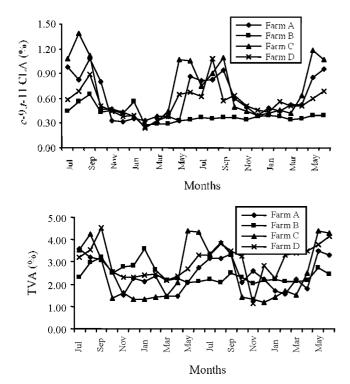


Figure 2. Temporal pattern of *c-9*, *t-11* CLA and TVA for bulk tank milk samples collected from four commercial dairy farms. During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3 of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.

milk samples. The c-9, t-11 CLA and TVA contents in milk fat were a reflection of the production system employed by each farm and the FA composition of the diet. Cows grazed on pasture produce higher milk fat CLA than cows fed mixed diets (White et al., 2001) or those kept in confinement (Jahries et al., 1997; White et al., 2001). The milk fat c-9, t-11 CLA and TVA values for Farms A and C were not as high as reported previously for cows grazing hish green pastures with or without supplementation (White et al., 2001; Loor et al., 2002; Lock and Garnsworthy, 2002; Khanal et al., 2007a,b); one of the reasons being the inclusion of data for milk collected during winter when cows had no access to pasture. However, it could be safe to say that on a year-round basis cows in Farms A and C produced 50% or more c-9, t-11 CLA in milk fat when grazed during summer compared with cows fed diets of 50% conserved forage and 50% grains, which produced milk with $\sim 0.45\%$ of c-9, t-11 CLA in milk fat. Similarly, when 1/3 of the cows are grazed during summer 20% more c-9. t-11 CLA could be expected on a year-round basis. Across the farms, milk fat CLA contents were twice as much in summer than in winter.

Temporal pattern of c-9, t-11 CLA and TVA contents in the bulk tank milk samples (Figure 2) showed that highest

milk fat c-9, t-11 CLA contents could be observed between July and September. While TVA concentration fluctuated slightly from month to month, its overall pattern was similar to c-9, t-11 CLA. Jahries et al. (1997) have observed the lowest milk fat c-9, t-11 CLA and TVA contents for cows kept indoor year round and fed diets of corn silage and cereal-rich concentrates. The content of t-10, c-12 isomer was about 5-7% of total CLA present in milk fat. which was within the normal range. However, it was not detected in bulk tank milk samples from Farm B and individual cow samples in Farm D. It was not clear why individual cow milk samples from Farm B showed the presence of t-10, c-12 CLA, but not the bulk tank milk samples and vice versa for Farm D. Moreover, transfer efficiency of this isomer from dietary supplement into milk fat in sheep has been found only 3.8% (Lock et al., 2006). suggesting that it is not as likely to be affected by feeding regimen as is the c-9. t-11 isomer.

Concentration of milk fat CLA and TVA was 1.5 to 2fold higher during summer compared with winter for both bulk tank and individual cow milk samples. An increased concentration of conjugated FA in milk fat during summer were noticed very early when the FA in milk fat showed greatly increased absorption in the unltraviolet region at 230 nm (Booth et al., 1935). A 3-fold increase in total conjugated dienes in milk fat was observed during summer when cows were switched to pasture from winter-feeding (Kuzdal-Savioe and Kuzdal, 1961). Similarly, conjugated dienes in Canadian milk was 2 to 3 times greater during summer when cows were grazed on pasture compared with winter-feeding (Riel, 1963). It has been established that concentration of milk fat CLA is increased with the inclusion of pasture in the diet (Khanal et al., 2005, 2007a). The increment in the concentration of milk fat CLA in the present study was slightly lower than observed in earlier studies probably because the commercial dairies were heterogeneous in production system and breed, and cows did not derive all of their DM from pasture. In the present study, concentration of CLA in milk fat was lowest during February-March and highest during August-September. which was in agreement with the previous finding (Riel, 1963). With the increase in the proportion of CLA, TVA, and major longer chain FA in milk, the proportion of short and medium chain FA up to $C_{16:1}$, which are synthesized mostly de novo, decreased during summer. Similar results have been observed previously with the increased supply of long chain FA in the diet (Dhiman et al., 1999; Loor et al., 2002).

Relationship of milk fat c-9. t-11 CLA with milk fat TVA was determined by regressing c-9. t-11 CLA with TVA in a number of ways and the results are presented in a series of graphs (Figure 3). Overall, coefficient of variation for individual cow milk samples were consistent between year

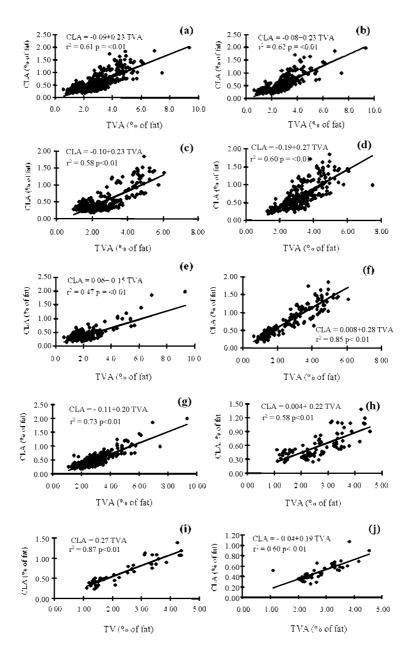


Figure 3. Relationship of milk fat *c*-9, *t*-11 CLA with TVA for samples collected from four commercial dairy farms, (a) all cows all farms, (b) all cows year 1 only, (c) all cows year 2 only, (d) all cows summer only, (e) all cows winter only, (f) Farms A and C all cows, (g) Farm B and D all cows, (h) bulk tank all farms, (i) bulk tank Farms A and C, and (j) bulk tank Farms B and D.

1 ($r^2 = 0.62$) and year 2 ($r^2 = 0.58$) and when pooled for both the years ($r^2 = 0.61$), indicating that 60% of the variation in milk fat c-9, t-11 CLA was contributed by milk fat TVA. When pooled bulk tank milk c-9, t-11 CLA was regressed with TVA, the coefficient of variation ($r^2 = 0.58$) was consistent with individual cow milk samples. Coefficient of variation was higher in summer ($r^2 = 0.60$) than in winter ($r^2 = 0.47$) for individual cow milk samples. When c-9, t-11 CLA was regressed with TVA for bulk tank and individual cow milk samples separately for Farms A and C, r^2 increased to 0.87 and 0.85. However, when the same was done for Farms B and D, r^2 were 0.60 and 0.73

for bulk tank and individual cows, respectively. These results indicated that although some variation among individual cows for milk fat *c*-9. *t*-11 CLA existed in the population, it was more due to the result of diet as could be seen through higher r² values during summer than in winter and for Farms A and C than Farms B or D. Jahries et al. (1997) have observed a slightly higher correlation coefficient of 0.73 for bulk tank milk samples obtained from the cows fed a corn silage-based, cereal-rich concentrate diet, grazed on traditional pasture or on ecological pasture. Correlation of milk fat *c*-9, *t*-11 CLA with TVA for individual milk samples that were obtained

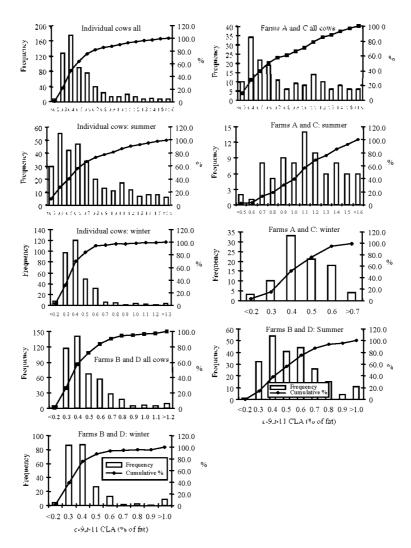


Figure 4. Histograms of individual cow variations on milk fat *c-9*, *t-11* CLA content (%) in four commercial dairies. Vertical bars indicate frequency and line graphs indicate cumulative frequency (%). During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and 1/3rd of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.

from pasture fed cows had previously been observed in the range of 0.78 to 0.90 (Jiang et al., 1996; Offer et al., 1999).

Since c-9. t-11 CLA has been found to have positive effects on immune regulation (Cook et al., 2003), and a negative correlation of c-9, t-11 CLA with milk fat content was observed previously (Giesy et al., 2002). its relationship with somatic cell counts and fat content in milk was determined. The relationship was very weak with an r^2 of 0.05 with somatic cell counts and 0.06 with milk fat content. The results suggested that c-9, t-11 CLA was not related to milk fat content and the health condition of the animal in commercial dairies in the present study. Previously, Lawless et al. (1999) have observed no significant correlation between milk fat c-9, t-11 CLA and milk fat contents.

Variation in individual cows for milk fat c-9, t-11 CLA

content

Variation of milk fat c-9. t-11 CLA content among individual cows is presented in a series of histograms in Figure 4. The c-9, t-11 CLA content varied from as low as 0.16% to 2.2% of the milk fat among the individual cows. A vast majority of the cows (88.6%), however, had milk fat c-9, t-11 CLA contents between 0.3 and 1.0% of fat, with only 1.3% of the cows having CLA contents below 0.2% and the same proportion of cows having more than 1.6% of c-9. t-11 CLA in milk fat. When analyzed separately for summer and winter. 93% of the cows had c-9. t-11 CLA contents between 0.3 and 0.7% of fat during winter whereas only 62% of the cows fell within the same range during summer with 10% of the cows having c-9. t-11 CLA contents of 1.3% or more. Proportion of cows with 0.3% or less c-9, t-11 CLA contents in milk fat was 32.5% during winter. whereas it was less than 10% during summer. Similarly, a

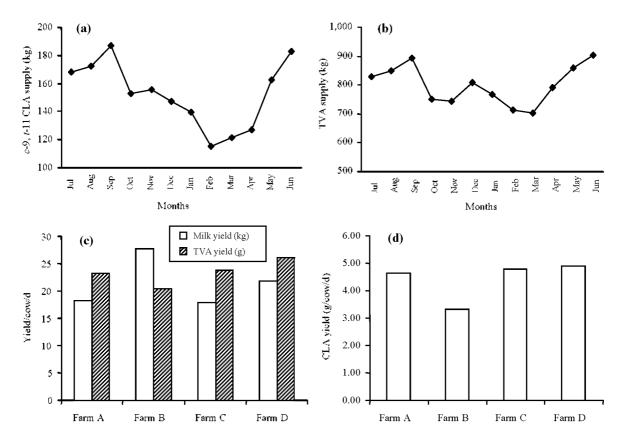


Figure 5. Monthly supply (kg) of c-9, t-11 CLA (a) and TVA (b), and daily yield of milk (c), TVA (c) and c-9, t-11 CLA (d) from cows in four commercial dairies. During summer, cows in Farms A and C were supplemented with a grain mix while grazing on pasture, cows in Farm B received 10% of DM from fresh cut pasture, and $1/3^{rd}$ of cows in Farm D were supplemented with grain while grazing and the rest fed a total mixed diet. During winter cows were fed conserved forage and grain diets in all farms.

wider variation in milk fat c-9, t-11 CLA contents was observed for Farms A and C that grazed cows during summer compared with Farms D or B that either didn't graze cows on pasture or only 1/3 of cows grazing during summer. When the data for Farms B and D were analyzed separately, overall variation was greater for Farm D than B (data not shown). For Farms A and C during summer, proportion of cows with less than 0.6% c-9. t-11 CLA in milk fat was only 3.6% with about 32% of the cows having 1.3% or more c-9, t-11 CLA in milk fat. During winter, 92% of the cows in farms A and C had milk fat c-9, t-11 CLA contents between 0.3 and 0.6%, while cows with less than 0.2% and more than 0.7% c-9, t-11 CLA were 4% each. For Farm B, a vast majority of cows (96%) had milk fat c-9. t-11 CLA contents between 0.3 and 0.8% and the ratio did not change much between summer and winter. There was a greater variation among cows on milk fat CLA content in Farm D than B, particularly during summer (data not shown). Since only a small proportion of cows were at the two ends of the curve, variations among individual cows for milk fat c-9, t-11 CLA contents may not be as wide as had been believed previously (Kelly et al., 1998; White et al., 2001; Lock and Garnsworthy, 2002). Moreover, bulk tank milk samples were more homogenous in c-9, t-11 CLA contents and ranged between 0.27 and 1.35% of the milk fat (Table 4), further suggesting that only a small population of animals lies at the far ends of the curve. Smaller variations during winter when diet was more homogenous among farms than during summer indicated that the variations were probably caused more by the diet than by the animal per se. Although the reasons for individual variations are not very clear in the literature, it may be due to one or more factors, such as rumen microflora (Harfoot and Hazlewood, 1988) and pH (Troegeler-Meynadier et al., 2003), feed intake and feeding behavior of the cow, energy content of the diet (Timmen and Patton, 1988). efficiency of incorporation of CLA into milk (Chilliard et al., 2000), lactation number (Lal and Narayanan, 1984) or in the conversion of TVA to milk fat c-9, t-11 CLA by Δ^9 -desaturase in the mammary gland (Grinari et al., 2000).

Supply of milk fat c-9, t-11 CLA and TVA

Highest supplies of *c*-9. *t*-11 CLA and TVA were observed during summer for all farms when cows received some form of pasture either through cut grass or by grazing (Figure 5). On an average, monthly supply of *c*-9. *t*-11 CLA was between 115 and 187 kg and that of TVA being 700-900 kg, the highest being from June through September and

lowest being from February through April. Highest c-9. t-11 CLA and TVA supply resulted from their highest contents in milk fat during the same months (Figure 2), even though the milk supply was one of the lowest during those months at 703,000 to 760,000 kg. Since both the concentration and supply of c-9, t-11 CLA and TVA was lowest from February through April, feeding strategies need to be geared up to enhance the concentration and supply of these two FA in milk and other dairy products so we can derive the potential health benefits. Supplementing milking cows with feed sources high in C_{18:2}, C_{18:3} or 20-carbon FA such as extruded soybeans, sunflower seeds, linseed, or marine algae will help in this regard. Similarly, milk supply needs to be increased from July through September without compromising the supply of c-9, t-11 CLA and TVA to the market if the status of these four commercial dairies were any indication of overall market scenario, at least locally.

The c-9, t-11 CLA and TVA yield (g/cow per day) was lowest at 3.32 and 20.4 g for Farm B even though the milk yield/cow per day was highest at 27.7 kg among these commercial dairies (Figure 5). The c-9, t-11 CLA yield (g/cow per day) in the other three farms was similar at 4.64. 4.79, and 4.89 for Farms A, C, and D respectively. The TVA yield (g/cow per day) was highest for Farm D at 26.1 g/cow/d while the Farms A and C produced similar yields of TVA at 23.2 and 23.8 g/cow per day, respectively. Cows in Farms A and C also produced similar amounts of milk yields on a daily basis. It should be noted that cows in Farms A and C produced the least amount of milk on a daily basis, yet yielded higher or similar amounts of c-9, t-11 CLA compared to that of Farms B or D, which produced higher milk yield/cow on a daily basis. Moreover, cows in Farm D had a good number of Jersey cows or its crosses. which had higher milk fat content (Table 4) that helped to raise overall CLA and TVA yields on a daily basis.

CONCLUSION

Based on the findings of this research, commercial dairies that grazed cows during summer and supplemented with grain mix produced milk with 60% or more milk fat c-9. t-11 CLA and TVA contents on a year-round basis compared with the dairy that fed 10% of dry matter as fresh cut pasture. Similarly, the dairy that grazed 1/3 of its cows during summer and supplemented with mixed diets produced 30% or more c-9. t-11 CLA and TVA in milk compared with the dairy that fed 10% of dry matter as fresh cut forage. Milk fat content of c-9. t-11 CLA and TVA was 150-200% more during summer compared with winter. Although individual cow milk samples varied from 0.16 to 2.22% in milk fat c-9, t-11 CLA contents, 89% of the cows had c-9, t-11 CLA contents between 0.3 and 1.0%.

indicating only a small proportion of cows in the population being at extreme ends of the curve. Individual cow variation was larger in dairies that had cows grazing in summer compared with dairies that either did not graze or grazed only 1/3 of the cows. Variation was larger in summer than in winter. The range in bulk tank milk c-9, t-11 CLA content was smaller than in individual cow milk samples ranging from 0.27 to 1.35% of milk fat. Cows grazed on pasture during summer produced similar or higher amounts of milk fat c-9, t-11 CLA on a daily basis even though their milk yield was lowest among the dairies. Concentration and supply of c-9, t-11 CLA and TVA were highest from June through September and lowest from February through April. which should be the months for targeting improvements in the supply of c-9, t-11 CLA and TVA.

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