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Grazing Period Variations in Cow Milk Vaccenic Acid (VA) and Conjugated Linoleic Acid (CLA)

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Abstract

The primary objective of this study was to examine the effect of breed, lactation and grazing period on the *cis 9*, *trans* 11-Conjugated Linoleic Acid (*c*9,*t*11-CLA) and *trans* 11-Vaccenic Acid (VA) levels in cow's milk. The secondary objective was to compare the properties of cheese products made from the study milk to commercially available cheeses. Milk samples from a commercial dairy farm representing 3 breeds (Fleckvieh Cross, n=19; Holstein, n=18; Jersey, n=12) were analyzed at three points in the grazing period. Daily yield, CLA yield, VA, yield and the percentage of VA in milk were greatest in June, intermediate in July and lowest in September. The percentage of *c*9,*t*11-CLA in milk was lower in September compared to June and July. The VA and *c*9,*t*11-CLA content of cheese made from the milk samples were ~2-fold higher than commercially available samples of Cheddar and ~1.5-fold higher than commercially available samples of Mozzarella. In conclusion, the c9,t11-CLA and VA contents of milk decrease over the June through September grazing period, likely due to the grazing material.

Abbreviations: c: cis; CLA: Conjugated Linoleic Acid; LA: Linoleic Acid; SFA: Saturated Fatty Acids; t: trans; VA: Vaccenic Acid

Introduction

Conjugated linoleic acid (CLA) is a term used to describe positional (carbon 6,8 to 12,14) and geometric [*cis(c)-cis, cis-trans(t), trans-cis and trans-trans*] isomers of linoleic acid (LA or C18:2n6) [1,2]. CLAs are produced from dietary LA by rumen bacteria such as *Butyrivibrio fibrisolvens* that isomerize *c*12 to *t*11 bonds, and *Megasphaera elsdenii* that isomerize *c*9 to *t*10 [1,3-6]. CLAs are also synthesized in ruminant and human tissues from *t*-11-18:1 (Vaccenic acid, VA) by Δ^9desaturase [7-10]. The major CLA in the milk of cows is *c*9,*t*11-CLA or rumenic acid [11]. Both VA and *c*9,*t*11-CLA may provide health benefits, although the degree of benefit to human health is still being investigated. For example, *c*9,*t*11-CLA and VA may provide anticarcinogenic, antiatherosclerotic, antidiabetic and anti-inflammatory effects [12,13]. These fatty acids in milk may be beneficial to consumer health.

The fatty acid composition of milk is influenced greatly by diet, while data on the effects of cow breed, stage of lactation and grazing period are less well defined. Ruminants that are exclusively pasture fed produce greater levels of VA and *c*9,*t*11-CLA compared to those fed indoors, although this effect is dependent on the specific diet composition [10,14-16]. Up to a three-fold variation in fatty acid composition, including VA and *c*9,*t*11 CLA, may occur between individual cows [17]. It has been reported that Holstein cows produced more *c*9,*t*11-CLA compared to Jersey cows, while in a separate study, Jersey cows produced more than Friesian cows [15,18]. Stage of lactation appears to have little effect on the final VA and *c*9,*t*11-CLA content of milk [17] but the data are limited. The CLA content of milk is up to 4 times higher in summer compared to winter [19], however, less information is available on the levels of VA and *c*9,*t*11-CLA throughout the summer grazing period.

Thus, the primary objective of the following study was to examine the effect of breed, lactation and grazing period on the VA and *c*9,*t*11-CLA levels in cow's milk. The secondary objective was to compare the properties of cheese products made from the experimental milk to commercial cheese products (Mozzarella and Cheddar).

Materials and Methods

Milk samples and analysis

Individual cow milk samples from three different breeds (Fleckvieh Holstein Crosses, n=19; Holstein, n=18; Jersey, n=12) were taken twice daily (5 AM, 5 PM) using diverter bottles attached to the milk hose. Milk samples were taken twice per day to represent when the cows were normally milked and samples were pooled for analysis. These animals were located on a 400 cow commercial dairy facility, 15 km south east of Winnipeg, Canada. All animals sampled were classified into three representative stages of lactation based on days in milk [DIM; defined as early (0-79 DIM), middle (80-129 DIM) and late (129-300 DIM)]. Milk was sampled at three time points within the grazing period (June 28, July 20, and September 5, 2007). Milk yield was taken using daily measures from De laval MM15 milk meters with Alpro software. According to a lead feeding schedule (Table 1), daily concentrate feeding was controlled using Alpro software. Cows were also pastured on mixed perennial rye grass and alfalfa swards between milkings. All animals were cared for in accordance with NFACC codes of practice for care and handling for dairy cattle [20].

The component testing of the milk, including fat, and protein were completed with Fossomatic FC by the optical somatic cell counting method with Milkoscan 6000 FT by mid infrared spectoscopy.

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Cheese preparation and analysis

Cheddar and Mozzarella cheeses were prepared from bulk tank samples of study milk received at The University of Manitoba dairy plant in August 2007. Cheese varieties were prepared according to the University of Manitoba recipe of manufacture procedures. Samples of commercially prepared Cheddar and Mozzarella cheeses were obtained from a local cheese plant within the same time frame to represent bulk milk pooled from several farms using standard production practices. The manufactured cheese was analyzed for compositional factors according to Standard Methods for the Examination of Dairy Products [21]. The cheeses were analyzed for mechanical properties including stress at fracture, strain at fracture and elastic modulus using a Zwick Roell materials testing analyzer. Mozzarella cheese melting properties were determined using the industry standard Schreiber melt test [22]. The test involves placing a 0.5 cm high plug of cheese in a glass petri dish, heating in an oven at 232°C for 5 minutes, cooling for 30 minutes, and measuring sample spread. The sensory properties of the cheese were analyzed in February 2008 by a panel of professionals using the American Dairy Science Association (ADSA) Cheddar cheese scorecard as a guideline [23].

Fatty acid analysis

Lipids were extracted from a 1.0 ml sample of milk or 0.5 g sample of cheese using a modified [24] extraction procedure. The samples were added to 10 mL of chloroform:methanol (2:1, vol/vol) with 0.01% BHT (Sigma-Aldrich, Oakville, Ontario, Canada) and extracted as previously described [25]. All solvents were from Fisher Scientific (Nepean, Ontario, Canada). The VA standards (C18:1n7t, C18:1n7c) were purchased from Sigma (Oakville, Ontario, Canada). The CLA standards (*c*9,*t*11 and *t*10,*c*12) were purchased from Larodan Fine Chemicals (Burlington, Ontario, Canada). Samples were split and methylated with sodium hydroxide (NaOCH3; 15 minutes at 50°C) or methanolic HCl (mHCL; 2 hours at 80°C) and results were combined as previously described [26]. The samples were separated on a Chrompack CP-select CB column (100 m × 0.25 mm diameter and 0.25 µm film thickness; Varian Canada Inc., Mississauga, Ontario) using a Varian CP-3800 GC with FID. The temperature program was 50°C x 3 minutes, 20°C/minute to 180°C x 10 minutes, 1°C/ minute to 205°C x 3 minutes, 20°C/minute to 240°C x 5 minutes. Total run time was 54.25 minutes. Samples were run with a 20:1 split ratio. Individual fatty acid results are expressed as the percentage of total fatty acids (g/100 g fatty acids). In order to get good separation in the very long chain fatty acid region of the chromatogram, data for C4:0 to C8:0 was not obtained, and therefore total Saturated Fatty Acids (SFA) were estimated based on standard milk composition of C4:0, C6:0 and C8:0 of 3.0%, 2.0% and 1.5%, respectively [27]. These three fatty acids represent less than 10% of the saturated fatty acids in milk fat, however, not including them would artificially lower the % total SFA.

The percentage of CLA, VA and LA in whole milk and daily yield were calculated as shown in equations 1 and 6.

- Eq. 1 CLA (% of whole milk) = % butterfat in whole milk × percentage CLA in butterfat/100
- Eq. 2 Daily CLA yield (ml) = CLA % of whole milk × daily yield (ml)
- Eq. 3 VA (% of whole milk) = % butterfat in whole milk × percentage VA in butterfat/100
- Eq. 4 Daily VA yield (ml) = CLA % of whole milk × daily yield (ml)
- Eq. 5 LA (% of whole milk) = % butterfat in whole milk × percentage LA in butterfat/100
- Eq. 6 Daily LA yield (ml) = CLA % of whole milk × daily yield (ml)

The CLA-delta-9 desaturase index was calculated as shown in equation 7 [28].

- Eq. 7 (*c*9,*t*11 CLA)/(*c*9,*t*11CLA +VA)

Statistical analysis

Milk data were analyzed with the Statistical Analysis System (SAS V9.1 for Windows, SAS Institute Inc., Cary, NC) using PROC MIXED and main effects for breed, lactation and time. Post-hoc analysis was completed using preplanned contrast statements. The level of

Holstein, Fleckvieh							
Milk (L)			15.0	20.0	25.0	30.0	35.0	40.0
Concentrate (kg)[y]			1.6	2.5	3.3	4.2	5.2	6.4
Jersey								
Milk (L)	7.0	10.0	15.0	20.0	25.0	30.0		
Concentrate (kg)[y]	1.4	1.8	2.4	3.2	4.1	5.0		

[z]Litres of milk produced determined the amount of concentrate fed
[y]Concentrate composed of: protein (12.2%), fat (4.2%), crude fibre (3.6%), calcium (1.1%), phosphorus (0.7%), salt (1.0%), sodium (0.4%), potassium (0.5%), magnesium (0.4%), sulfur (0.2%), vitamin A (17.0 IU/kg), vitamin D (2.7 IU/kg), vitamin E (100.0 IU/kg)

Table 1: Lead feeding schedule[z]

	June	July	September	Main Effects Pr>F		
				Breed[Y]	Lactation[Y]	Time
Fat (%)	4.3 ± 0.3[a]	3.5 ± 0.1[b]	3.7 ± 0.1[b]	0.0008	NS	0.0021
Protein (%)	3.4 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	NS	NS	NS
Milk Yield (kg/d)	28.0 ± 0.8[a]	23.3 ± 0.6[b]	21.0 ± 0.5[c]	0.0006	NS	<0.0001
VA (% of whole milk)	0.119 ± 0.014[b]	0.136 ± 0.005[a]	0.086 ± 0.005[c]	0.0100	0.0275	<0.0001
VA Yield (ml/d)	1.209 ± 0.064[a]	0.897 ± 0.036[b]	0.508 ± 0.024[c]	NS	NS	<0.0001
LA (% of whole milk)	0.101 ± 0.008[a]	0.076 ± 0.004[c]	0.080 ± 0.004[bc]	0.0036	NS	0.0132
LA Yield (ml/d)	0.648 ± 0.027[a]	0.501 ± 0.022[b]	0.468 ± 0.012[b]	0.0040	NS	0.0006
CLA (% of whole milk)	0.051 ± 0.004[a]	0.040 ± 0.002[b]	0.030 ± 0.002[c]	NS	0.0003	<0.0001
CLA Yield (ml/d)	0.012 ± 0.002[a]	0.009 ± 0.001[b]	0.007 ± 0.000[c]	NS	NS	<0.0001

[z]Means ± SEM, n=49. Different superscript letters indicate differences over time, by contrasts (p<0.05). Main effects were for Breed (Fleckvieh, Holstein, Jersey), Lactation (early, mid, late) and Time (June, July, September). [y] means for significant main effects due to breed or lactation are provided in the text. NS=not significant.

Table 2: Milk macronutrient composition, milk yield and CLA yield during the grazing period[z]

significance was $p<0.05$. All values were reported as means ± Standard Error of the Mean (SEM), except Table 4 which has means ± Standard Deviation (SD).

Results

Milk macronutrient composition, yield, CLA, VA and LA yield

Total fat, daily yield, VA yield, LA yield and CLA yield, but not protein, of the milk, differed over time (Table 2). Fat content was higher in June compared to July and September. Daily yield, CLA yield and VA yield were greatest in June, intermediate in July and lowest in September. LA yield was also lower in July compared to June, but did not drop further in September.

The Jersey cows, compared to the Fleckvieh and Holstein cows, had the greatest fat content in milk (4.7 ± 0.3 vs. 3.5 ± 0.2 and 3.6 ± 0.1%, respectively) and lowest daily yield (20.4 ± 0.8 vs. 25.1 ± 0.6 and 25.7 ± 0.8 kg/d, respectively). Total protein and CLA yield did not differ by breed (data not shown), however Jersey cows had a lower LA yield compared to Fleckvieh and Holstein cows (0.481 ± 0.034 vs. 0.580 ± 0.024 and 0.568 ± 0.025 ml/d, respectively).

Total CLA yield was lowest in early lactation (0.038 ± 0.003 vs. 0.043 ± 0.004 and 0.042 ± 0.003% in mid and late lactation, respectively) but stage of lactation did not affect fat, protein or daily yield (data not shown). There were no interactions of time with breed or stage of lactation for milk fat, protein, daily yield and CLA yield (Table 2).

Milk fatty acid composition

The percentage of VA in milk was highest in June, intermediate in July and lowest in September (Figure 1). The percentage of *c*9,*t*11-CLA was lower in September compared to June and July. The CLA-delta-9 desaturase index and LA were not affected throughout the study period. VA and the CLA-delta-9 desaturase index were not different among breeds or by stage of lactation (data not shown). The *c*9,*t*11-CLA concentration was affected by breed, as the Jersey cows had less *c*9,*t*11-CLA in milk compared to the Fleckvieh and Holstein cows (0.92 ± 0.05 vs.1.15 ± 0.06 and 1.12 ± 0.06%, respectively).

There were several variations in the fatty acid composition of the milk throughout the grazing period (Table 3). Estimated total SFA was higher in September compared to June and July, attributable to higher C16:0. However, the C18:0 and C20:0 were lowest in September, compared to June and July. C10:0 and C12:0 were lowest in June compared to July and September. Total MUFA was not different during the study period although C16:1n9 was lowest in June and C20:1n9 was lowest in September compared to the other months. Total PUFA also did not differ but the C18:3n3 content was highest in September.

Breed did not affect total SFA, MUFA, PUFA or individual fatty acids in milk, except the C15:0 content was lower in the Jersey compared to Fleckvieh and Holstein cows (1.16 ± 0.04 vs. 1.31 ± 0.04 and 1.35 ± 0.03 g/100 g fatty acids, respectively). There was a significant main effect of breed on C16:0 but the post-hoc analysis failed to confirm any differences (26.62 ± 0.50, 28.21 ± 0.50 and 25.90 ± 0.55% for Fleckvieh, Holstein and Jersey cows, respectively).

Stage of lactation did not affect the total SFA, MUFA or PUFA composition of milk; however, a few individual fatty acids differed slightly depending on the stage of lactation including C10:0 (2.34 ± 0.10 in mid- and 1.91 ± 0.09 in late-lactation versus 1.71 ± 0.11 g/100 g fatty acids in early lactation), C14:1 (1.16 ± 0.06 in mid and 1.17 ± 0.06 in late lactation versus 0.90 ± 0.05 g/100 g fatty acids in early lactation), C18:1n9 (20.11 ± 0.76 in early lactation versus 17.66 ± 0.38 in mid- and 18.45 ± 0.48 g/100 g fatty acids in late-lactation) and C18:2n6 (2.42 ± 0.07 in late lactation versus 2.24 ± 0.10 in early- and 2.06 ± 0.08 g/100 g fatty acids in mid-lactation).

Cheese properties

The VA and *c*9,*t*11-CLA contents of cheese made from the study

				Main Effects				
Fatty Acids (%)	**June**	**July**	**September**	**Breed**	**Lactation**	**Time**	**Breed x Time**	**Lacation x Time**
ΣSFA^{y}	**61.42 ± 0.55^{b}**	**61.19 ± 0.58^{b}**	**64.04 ± 0.47^{a}**	**NS**	**NS**	**0.0055**	**NS**	**NS**
C10:0	1.72 ± 0.08^{b}	2.20 ± 0.11^{a}	2.18 ± 0.11^{a}	NS	0.0012	0.0129	NS	0.0417
C12:0	2.68 ± 0.10^{b}	3.20 ± 3.24^{a}	3.24 ± 0.08^{a}	NS	NS	0.0013	NS	NS
C14:0	10.35 ± 0.22	10.72 ± 0.46	11.34 ± 0.13	NS	NS	NS	NS	NS
C15:0	1.25 ± 0.04	1.35 ± 0.04	1.29 ± 0.03	0.0457	NS	0.0317	NS	NS
C16:0	26.90 ± 0.42^{b}	25.44 ± 0.52^{b}	29.61 ± 0.39^{a}	0.0215	NS	0.0001	NS	NS
C18:0	10.72 ± 0.46^{a}	10.53 ± 0.4^{a}	8.90 ± 0.29^{b}	NS	NS	0.0455	NS	NS
C20:0	0.69 ± 0.05^{a}	0.69 ± 0.01^{a}	0.24 ± 0.06^{b}	NS	NS	<0.0001	NS	NS
ΣMUFA	**26.39 ± 0.63**	**24.99 ± 0.54**	**24.06 ± 0.42**	**NS**	**NS**	**NS**	**NS**	**NS**
C14:1	0.98 ± 0.05	1.07 ± 0.07	1.12 ± 0.07	NS	0.0130	NS	NS	NS
C16:1n9	0.94 ± 0.05^{b}	1.11 ± 0.05^{a}	1.21 ± 0.07^{a}	NS	NS	0.0023	NS	NS
C18:1n9	19.20 ± 0.62	17.97 ± 0.53	18.54 ± 0.36	NS	0.0455	NS	NS	0.0433
C20:1n9	0.40 ± 0.04^{a}	0.50 ± 0.03^{a}	0.19 ± 0.01^{b}	NS	NS	<0.0001	NS	NS
ΣPUFA^{x}	**4.24 ± 0.11**	**4.18 ± 0.11**	**4.44 ± 0.13**	**NS**	**NS**	**NS**	**NS**	**NS**
C18:3n3	0.25 ± 0.04^{b}	0.15 ± 0.01^{b}	0.80 ± 0.06^{a}	NS	NS	<0.0001	NS	NS
Σn-9	**20.14 ± 0.63**	**19.08 ± 0.52**	**19.75 ± 0.35**	**NS**	**0.0323**	**NS**	**NS**	**NS**
Σn-6	**2.39 ± 0.09**	**2.30 ± 0.09**	**2.34 ± 0.09**	**NS**	**0.0061**	**NS**	**NS**	**NS**
Σn-3	**0.25 ± 0.04^{b}**	**0.15 ± 0.01^{b}**	**0.80 ± 0.06^{a}**	**NS**	**NS**	**<0.0001**	**NS**	**NS**

^{z}Means ± SEM, n=39. Different superscript letters indicate differences over time by contrasts ($p<0.05$); NS=not significant; %=g/100 g fatty acids. Only fatty acids >0.5% reported. Means for significant main effects of breed and lactation are provided in the text.
^{y}Total SFA includes estimates for C4:0, C6:0 and C8:0 (3.0%, 2.0% and 1.5%, respectively) as explained in the methods.
^{x}Data for vaccenic acid, conjugated linoleic acid and linoleic acid are shown in Figure 1

Table 3: Milk fatty acid composition during the grazing period^{z}.

Cheese	Stress at Fracture (kN/m²)	Strain at Fracture (mm/mm)	Elastic Modulus (kN/m²)
Commercial Cheddar – Mild	46.2 ± 9.2	0.50 ± 0.07	113.1 ± 9.7
Commercial Cheddar – Medium	55.3 ± 1.6	0.43 ± 0.03	230.1 ± 37.6
Study Cheddar	59.4 ± 4.3	0.52 ± 0.04	156.3 ± 12.4
Commercial Mozzarella	59.4 ± 5.9	0.33 ± 0.02	223.8 ± 37.8
Study Mozzarella	62.2 ± 9.4	0.59 ± 0.05	77.2 ± 15.4

[z]Means ± SD, n=4, crosshead speed = 3.75 mm/min

Table 4: Mechanical properties of cheese[z].

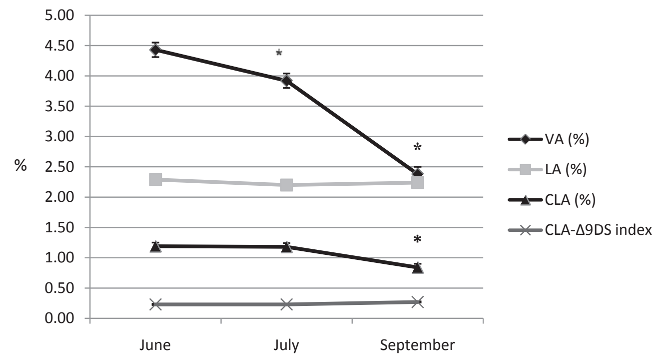

Figure 1: Vaccenic acid (VA), linoleic acid (LA), *c*-9,*t*-11-conjugated linoleic acid (CLA) and the Δ⁹desaturase index in milk during the grazing period. *September different from June and July for VA and different from June for CLA by contrasts ($p<0.05$). %=g/100 g fatty acids.

samples were ~2-fold higher than commercially available samples of Cheddar and ~1.5-fold higher than commercially available samples of Mozzarella (Figure 2). Based on the ADSA scorecard, the sensory panel scored the 6 month study Mozzarella cheese higher than the commercial Mozzarella cheese for taste and texture. The study Cheddar cheese was noted to be young with little flavor development but no obvious flavor defects, while the texture was rubbery (data not shown).

The analytical properties of the cheeses were within regulatory standards (data not shown). The mechanical properties including stress at fracture, strain at fracture and elastic modulus were similar between commercial and study cheeses, except the study Mozzarella cheese had less elasticity compared to the commercially available Mozzarella (Table 4). Although elasticity can be assessed by instrumental analysis, the melting properties of cheese are more important. The melting properties of the study Mozzarella cheese were better than the commercial varieties (Figure 3); when heated, the study Mozzarella cheese spread out over a greater distance without oiling off.

Discussion

In the current study, milk VA and *c*9,*t*11-CLA declined as the grazing period progressed (Figure 1). The CLA content mirrored the VA rather than the LA content, with a drop in VA beginning in July and occurring before the drop in *c*9,*t*11-CLA in September. This result is partially explained by the fact that CLA in milk is mainly from the delta-9 desaturation of VA in the mammary gland [29,30]. VA in milk is primarily from rumen biohydrogenation in the pathway LA → *c*9,*t*11-CLA → VA → C18:0 → C16:0 [31,32]. Therefore, when there is a decline in one fatty acid in these pathways, the other would follow. We cannot explain currently, however, why the CLA and VA contents dropped when the LA content of milk was consistent over time, although when expressed as daily yield, the LA content of milk also dropped in July compared to June (Table 2). C16:0 may have also been expected to decrease to be consistent with this pathway of rumen biohydrogenation, however, it increased, and we cannot explain this based on the data available.

As Chilliard and Ferlay [32] discussed in their review article, the CLA and VA composition of milk is affected by (i) lipid precursors such as sources of C18:2 or C18:3 available in the diet and (ii) dietary factors such as fiber that alter microbial activity in the rumen, as well as the interaction of these two factors. With regards to lipid precursors, although forages typically only contain 2-3% fat, early pasture feeding increases 18-carbon fatty acid intake and production, making the substrates for VA and CLA metabolism more readily available [32]. With regards to dietary fiber, the cows received more dietary fiber at the beginning of the grazing period when the alfalfa content of the forage was greater and voluntary food intake would expected to be higher as all cows would be earlier in lactation. This may significantly affect the type of rumen microflora present and the biohydrogenation or isomerization processes [33-36]. It has recently been reported that higher fiber diets enhance the level of longer chain SFA, LA and *c*9,*t*11-CLA, but not VA, compared to lower fiber diets when the level of PUFA is low and controlled [37]. Additionally, the type of dietary fiber may be important for VA and CLA production. *In vitro*, lignified fibre (wheat straw), compared to highly digestible fibre (soybean hulls) and purified cellulose, significantly increased the production of *c*9,*t*11-CLA and total CLA by ruminal microorganisms [36]. Generally longer chain fatty acids, specifically C18 levels decrease as the alfalfa plant matures [38,39]. This is not as pronounced in other forage legumes such as clover [40]. Other studies looking at milk CLA levels on mixed clover pastures have shown little variation over the grazing period [16,41]. The variation in our results over the season may be specific to alfalfa grass mixes and is in agreement with other studies [34,42]. However, as this data was collected from a commercial not experimental situation the reality is was not as straightforward for a number of reasons: (i) Cows grazed different fields every 2-3 days which may have been at different stages of maturity; (ii) The proportion of alfalfa in the pasture would change over time depending on grazing pressure, soil moisture content and climatic conditions, specifically precipitation. The late grazing season would predictably have less alfalfa and it would be at a more mature stage; (iii) In the hotter months of the grazing period cows will lower their intakes of pasture if they are heat stressed which directly affects the amounts of CLA precursors entering the rumen and consequently the amount expressed in milk [41]. The data does show a general trend of higher CLAs with immature pasture in spring with a decline over the subsequent months which can be attributed to some or all of the factors stated above.

Milk CLA and VA contents can vary between <0.5 and 5% [32]. Levels beyond those in the current study of up to 1.2%, occur during the grazing period with lipid supplementation of oils, rather than seeds, that are rich in C18:2 and/or C18:3 (e.g. soybean, sunflower, linseed oils) or C20:5 and C22:6 (fish oils). In fact, fish oils are more effective than vegetable oils and the combination of the two are most effective at increasing milk CLA and VA, possibly by inhibiting VA hydrogenation to C18:0 [43-49].

The other changes in milk fatty acids occurring through the grazing period were general patterns of increases in the medium chain SFA and decreases in longer chain PUFA (Table 3). The increase in the medium chain SFAs may be explained by increased mammary gland de novo synthesis of these fatty acids. As the number of long chain fatty acids decreased, acetyl CoA carboxylase for de novo fatty acid synthesis may have been less inhibited [50]. A decrease in the percentage of C18:0

in milk has been related to the amount of VA available for rumen biohydrogenation [32]. Changes in MUFA (increases in C16:1n9 and decreases in C20:1n9) although statistically significant, were minor (~ 0.3%; Table 3). The higher proportional grass intake near the end of the period may explain the ~0.5% increase in milk alpha-linolenic acid (ALA; C18:3n3) to 0.80% since the content of milk is almost exclusively dependent on dietary ALA intake and grass is a dietary source [32]. Although, 0.8% ALA is still a relatively low concentration compared to milk of exclusively grass-fed cows where 2.5% ALA has been reported [34].

The lower fat content and yield of milk at the end of the study (Table 2) was likely due to a combination of stage of lactation and decreasing diet quality. Typically the yield will drop by about 0.30% or 80-100 ml/d after peaking at about 60-80 days in milk and in fact, yield persistency between June and September was 0.25, within the expected ranges for Canadian dairy cattle [51]. Thus, the yield was expected to drop over the grazing period. High quality pasture as present in the early grazing periods, defined by highly digestible forages, may have also yielded higher milk production and butterfat percentages.

The Jersey cows had a higher milk fat content and lower percentage of C15:0 and *c*9,*t*11-CLA along with lower daily yields compared to the Fleckvieh and Holsteins demonstrating slight breed variations in milk production. The CLA yield did not appear to be affected by breed in the current study and the differences in the percentages of *c*9,*t*11-CLA and C15:0 were only about 0.2 g/100 g fatty acids, therefore the biological significance of these variations are questionable. On the other hand, the difference in daily yield among the breeds was about 5 kg/d, which may be quite significant to the producer.

Finally, the VA and *c*9,*t*11-CLA content of commercially available cheese varies considerably depending on the period in which it was made, the nutritional intake of the cows and the ripening period [52,53]. In the current project, the VA and *c*9,*t*11-CLA in cheese made from the milk of grazing cows was up to two-fold higher compared to commercially available cheeses (Figure 2) despite the fact that they were made from milk obtained in August, when VA and *c*9,*t*11-CLA were declined compared to June. Based on a confidential producer survey conducted by Dairy Farmers of Manitoba in 2005 (personal communication), over 95% of dairy producers in MB do not graze their

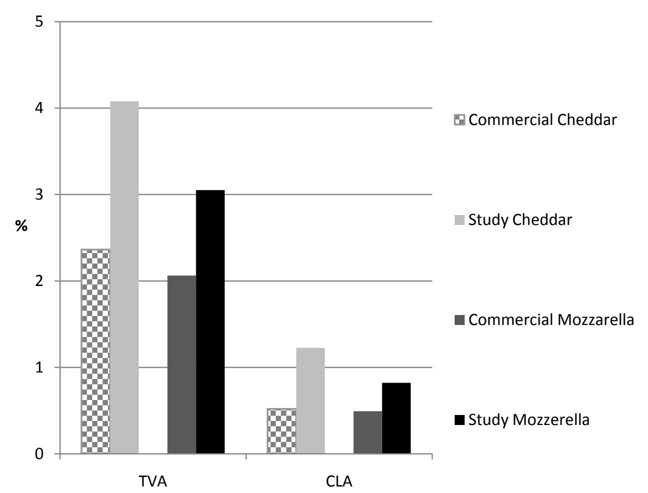

Figure 2: VA and *c*-9,*t*-11-CLA in commercially available cheese or cheese prepared from milk samples obtained during study period. Observational data.

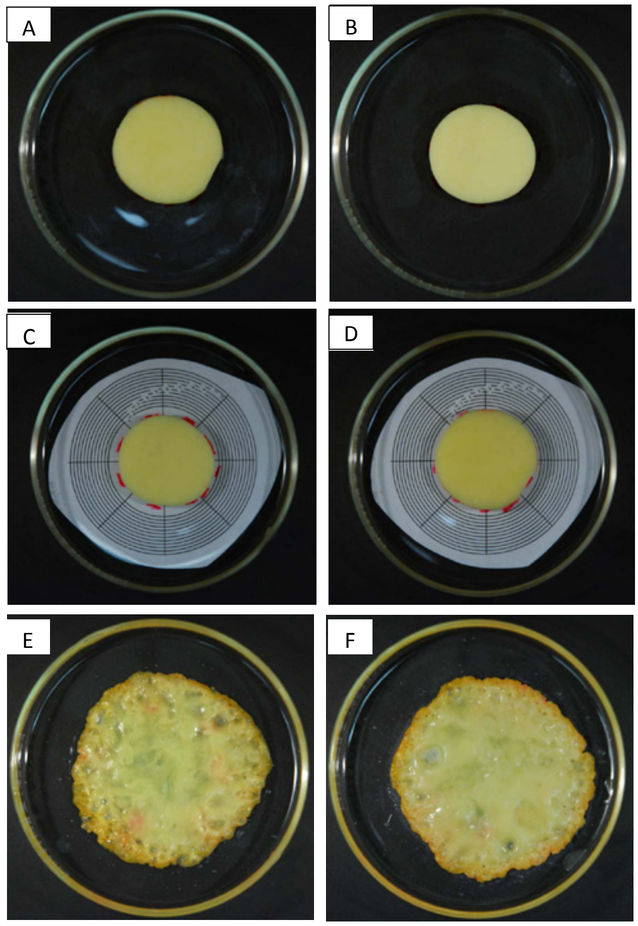

Figure 3: Study Mozzarella cheese (A), commercial Mozzarella cheese (B), Study Mozzarella cheese scale (C) commercial Mozzarella cheese scale (D), study Mozzarella cheese melted (E) and commercial Mozzarella cheese melted (F). Observational data.

lactating cattle. Therefore the milk used to produce the commercial cheese likely came from cattle receiving no fresh pasture but fed preserved forages in the form of silage or hay. As discussed above, fresh pasture produces more CLA than preserved forage rations [16,41,54].

Higher VA and CLA content in cheese has not affected quality or consumer acceptance [52]. In this report, the analytical and mechanical properties of the cheeses were comparable to industry standards, although the study Mozzarella cheese showed less elasticity (Table 4), better melting properties (Figure 3) and improved sensory scores than the commercial Mozzarella cheese. The texture of the study Cheddar cheese was rubbery and may have required a longer aging process at a slightly higher temperature (4-10°C compared to 4°C). Given the possible health benefits of VA and CLA, there may be marketable benefit to enhancing higher fat dairy products such as cheese with these fatty acids, since the total intake of VA and CLA from a serving of cheese would be greater than from a serving of low-fat milk.

In conclusion, the percentages of VA and *c*9,*t*11-CLA, were better early in the grazing period, presumably as the proportion of legume to grass in pasture was higher. The *c*9,*t*11-CLA in milk was influenced more by VA than LA. Cow breed and stage of lactation had minor effects on milk VA and *c*9,*t*11-CLA. Increased levels of VA and CLA in milk and cheese were achieved through grazing practices on a commercial dairy farm. Enhancements in VA and CLA in milk and cheese may have potential health benefits for consumers and marketability for producers.

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