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Variations in Conjugated Linoleic Acid Concentrations in Cows Milk, Depending on Feeding Systems in Different Seasons

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ABSTRACT : Variations in conjugated linoleic acid (CLA) concentrations in Holstein dairy cows milk, depending on feeding systems in different seasons was investigated. Milk samples were collected from Holstein dairy cows, which either grazed for whole days (WG), only daylight hours (TG), or were offered a total mixed ration (TMR) and experienced no grazing (NG), from April to December of 2005. In April, November and December, the cows in TG and WG treatments received grass silage and some concentrate, while from May to October, the cows grazed on temperate pasture. The cows in NG treatment received the TMR throughout the season. The major fatty acid obtained in the pastures was linolenic acid. There was no significant difference in the pasture's linolenic acid concentrations from May to September, but there was a significant decrease in October. However, the linolenic acid concentrations obtained in the pasture were always much higher than those obtained from the TMR. Linoleic acid was also the major fatty acid in the TMR, but these concentrations were higher in the TMR than in the pasture. There was no significant difference in milk *cis9trans11*CLA (c9t11CLA) concentrations between the three feeding systems while the cows were fed on conserved pasture in April, November and December. Although c9t11CLA concentrations were lower in the TMR, it was found that the cows which grazed in fresh pasture experienced significantly higher concentrations of c9t11CLA in their milk than those which received only TMR. It was also found that cows in the WG treatment experienced higher c9t11CLA concentrations than those in the TG treatment. In the WG and TG treatments, c9t11CLA concentrations were highest in June, after which, they gradually decreased ($p<0.01$) until October. For the NG treatment, there was no significant change in the concentrations of c9t11CLA ($p>0.05$) with season. Overall, *trans11*C18:1 and c9t11CLA were greatly influenced by season, with higher variation in the WG treatment than in the TG treatment and no variation in the NG treatment. (**Key Words** : Feeding System, Seasonal Change, Conjugated Linoleic Acid, Milk Fatty Acids Composition)

INTRODUCTION

In recent years there has been extensive research on the potential benefits of conjugated linoleic acid (CLA) for human health. The CLA is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds, which are unique components of ruminant lipids that have beneficial effects on human health. The major dietary source of CLA from ruminant dairy products and the isomer *cis9trans*11 (c9t11) is major isomer of the CLA in milk fat (Bauman et al., 2000).

Milk production systems in northern Japan utilize pasture for lactating cows in order to reduce production cost and increase profit per cow. However, on some farms there is often an insufficient quantity of vegetation in the pasture for the lactating cows to graze for an entire day. For this reason, owners often carry out time restricted grazing. Also some farmers prefer to feed their cows on a total mixed ration (TMR) in order to increase milk production. Cows that feed only on pasture have higher CLA concentrations in their milk (specifically the c9t11 isomer) than cows that feed only on TMR (Kay et al., 2005; Khanal and Dihman, 2007). The content of CLA in cow's milk has been found to vary with season, because the Δ^9 desaturase activity was found to be highest in summer when cows grazed on fresh pasture, compared to winter when they were offered silage (Lock and Garnsworthy, 2003).

It has also been demonstrated that grazing feeding systems may be influenced by type, quality and herbage

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mass of pasture those vary with changes in seasons (Nakatuji et al., 2006). Seasonal factors such as heat stress, humidity and photo-period (such as light quality and density at sun rise and sun set) influence the dry matter and nutrient intake of pasture (Linnane et al., 2001; West, 2003). Thus, it is possible that the quality and dry matter intake of pasture can affect the concentration of CLA in milk (Chilliard et al., 2000).

All these previous studies compared the concentration of CLA in milk between winter silage and normal grazing feeding systems, but they did not compare the variation of CLA concentration in cow's milk, depending on feeding systems in different seasons. Therefore, the objective of this study was to investigate the effect of seasons on the variation of CLA concentrations in cow's milk using these feeding systems within northern Japan.

MATERIALS AND METHODS

Experimental design

This experiment was conducted from April to December in 2005 on dairy farms in Ashoro, Hokkaido, northern Japan. Twelve Holstein dairy cow farms were chosen for this experiment. Four farms had cows grazing the whole day (WG), four farms had cows grazing from 8:00 to 17:00 h (TG) and four farms had their cows fed on total mixed rations (TMR) and so experienced no grazing (NG), respectively. At the beginning of the study in April, cows in the WG and TG treatments were fed with grass silage and corn based concentrate of 3.0 kg fresh matter (FM)/cow/day and 8.0 kg FM/cow/day twice daily after milking, for the WG and TG treatments, respectively. Cows in the NG treatment received TMR in quantities of 40 kg FM/cow/day after their morning milking through to December. The TMR was made up of forage (grass, corn silage and alfalfa hay) and a concentrate (ratio of 6:4). Cows in the WG and TG treatment were allowed to graze from the beginning of May and their concentrate was reduced to 2 kg FM/cow/day and 6 kg FM/cow/day. Cows in the TG treatment were only allowed to graze between the morning milking and evening milking, and were offered grass silage thereafter in the cowshed. After May cows in WG treatment were grazed for the whole day. In November, after the grazing season, cows in WG and TG received grass silage again and the concentrate was increased to 3 kg FM/cow/day and 8 kg FM/cow/day, respectively.

The pasture area included a variety of grass species such as orchard grass (*Dactylis glomerata*), timothy (*Phleum pratense L.*), Kentucky bluegrass (*Poa pratensis L.*), perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens L.*).

Sampling procedures

Sampling of grass silage and concentrate was collected in April, November and December. While from May to October, herbage was collected for the TG and WG treatments in each farm once a month. In the case of NG treatment, sampling of grass silage, maize silage, concentrate, and mixed feeds was performed every month. The feed samples were cut into small pieces and were freeze-dried for 40 h. After freeze-drying, feed samples were ground to pass through a 1mm sieve mesh and were used for the analyses of neutral detergent fiber (NDF), ether extract (EE), water soluble carbohydrate (WSC) and fatty acids.

About 1,000 milliliter (ml) of milk was collected from the bulk once a month for sampling after the morning milking in every farm. The samples were mixed for 5 min at 4.5°C. 200 ml of the milk sample was stored at -30°C for fatty acid analyses.

Chemical and fatty acid analyses

The feed samples were analyzed for dry matter (DM) and EE following the method describe by Aibibula et al. (2007). NDF was measured using the Van Soest et al. (1991) method. The WSC was measured using the Morimoto (1971). The feed samples were analyzed for fatty acid following the method described by Renaguli et al. (2004). The fatty acid methylester was analyzed using a capillary gas chromatography (Shimadzu GS2010, Kyoto, Japan) equipped with a flame ionization detector and capillary column. The column used was a VARIAN CP-Sil 88 (50 m×0.25 mm). The column temperature was adjusted from 80°C to 180°C at a rate of 15°C/min and then increased to 220°C at a rate of 2°C/min, and finally increased to 240°C at a rate of 4°C/min. Inlet and detector temperature were at set at 250°C.

For the estimation of fatty acid composition, milk samples were centrifuged for 15 min at 15,000×rpm at 4°C, in order to separate the fat cake from the milk. The fat cake was then dissolved in a chloroform-methanol solution (2:1, v/v), after which water was added to separate the chloroform layer containing the lipids. The chloroform was then dried with nitrogen gas leaving the lipids. The lipids were then transmethylated with 5% HCl in methanol at 60°C for 30 min. Hexane was then used to extract the fatty acid methylester which was analyzed by gas chromatography. The column temperature was programmed to 70°C and held for 4 min, then increased to 160°C at a rate of 8°C/min and was held for 10 min, then increased to 225°C at a rate of 4°C/min, and finally increased to 240°C at a rate of 4°C/min and held for 5 min. Retention times were determined with the pure methyl ester standard GLC-

Table 1. Changes in ether extract, neutral detergent fiber, water soluble carbohydrate and fatty acid content of pasture with season

	May	Jun	Jul	Aug	Sep	Oct	SEM	Significance
% DM								
EE[1]	4.6^{a}	3.7^{c}	3.8^{bc}	3.7^{c}	4.3^{ab}	4.2^{ab}	0.15	**
NDF[2]	39.5^{c}	45.2^{b}	46.5^{ab}	47.4^{ab}	49.5^{a}	43.9^{b}	1.18	**
WSC[3]	20.3^{b}	23.0^{ab}	16.5^{c}	15.1^{c}	24.1^{a}	15.7^{c}	0.90	***
Fatty acids (mg/g DM)								
C14:0	0.19^{c}	0.19^{c}	0.27^{b}	0.33^{a}	0.24^{bc}	0.25^{b}	0.02	***
C16:0	3.59^{b}	4.07^{a}	4.11^{a}	4.29^{a}	4.03^{a}	4.03^{a}	0.11	**
c9 C16:1	0.79^{a}	0.69^{b}	0.66^{b}	0.66^{b}	0.62^{b}	0.65^{b}	0.03	**
C18:0	0.43^{c}	0.44^{c}	0.47^{c}	0.57^{bc}	0.69^{ab}	0.74^{a}	0.05	***
c9 C18:1	0.58^{d}	0.78^{cd}	0.77^{cd}	0.96^{bc}	1.14^{b}	1.88^{a}	0.10	***
t11 C18:1	0.08^{d}	0.13^{ab}	0.10^{c}	0.13^{ab}	0.11^{bc}	0.14^{a}	0.01	***
C18:2	4.00^{b}	4.48^{a}	4.35	4.52^{a}	3.95^{b}	3.77^{b}	0.09	***
C18:3	23.23^{a}	23.87^{a}	22.87^{a}	23.17^{a}	22.01^{a}	20.40^{b}	0.44	***
Total	32.90^{abc}	34.65^{a}	33.60^{ab}	34.63^{a}	32.79^{bc}	31.77^{c}	0.57	**

[1] Ether extract. [2] Neutral detergent fiber. [3] Water soluble carbohydrate.
[a,b,c,d,e] Means within a row with different superscripts are significantly different ($p<0.05$).
Significance: ** $p<0.01$; *** $p<0.001$.

Table 2. Concentrations (% DM) of ether extract, neutral detergent fiber, water soluble carbohydrate and fatty acids in TMR and its constituents

	TMR	Grass silage	Maize silage	Concentrate
EE[1]	3.4	3.9	3.6	3.7
NDF[2]	59.2	61.9	39.5	20.1
WSC[3]	17.2	12.7	17.5	20.5
Fatty acid (mg/g DM)				
C14:0	0.11	0.27	0.34	0.46
C16:0	3.87	3.46	3.51	4.10
C9 C16:1	0.09	0.07	0.35	0.04
C18:0	0.74	0.40	0.90	0.90
C9 C18:1	5.19	2.71	6.43	5.15
t11 C18:1	0.07	0.02	0.07	0.02
C18:2	11.60	3.69	14.20	13.15
C18:3	2.58	10.01	1.70	1.10
Total	24.25	20.63	27.50	24.92

[1] Ether extract. [2] Neutral detergent fiber. [3] Water soluble carbohydrate.

85 (GLC-Reference Standard, Fatty Acid Methyl Esters). Retention time of c9t11CLA and t10c12CLA were determined using the Cayman Chemical Company standard (Conjugated linoleic acid methyl). All analyses were repeated three times and average values were reported.

Calculation and statistical analyses

The Δ^9 desaturase index was calculated using the method described by Kay et al. (2005). The effects of seasonal changes on herbage fatty acid levels were analyzed using a one-way ANOVA (SAS Inst. Inc., Cary, NC, USA, 2001), but were not statistically analyzed between the individual treatment. Milk fatty acid level changes in each treatment were analyzed using a one-way ANOVA every month. For all analytical procedures, p values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical and fatty acid composition of pasture

The changes in EE, NDF, WSC and fatty acid content of pasture during the grazing period can be seen in Table 1. The amount of EE in pasture was higher in May and lower in June. The NDF content was significantly lower ($p<0.01$) in May but showed an increasing trend from June to September. The WSC content of pasture was significantly higher in June and September and significantly lower in July and August. The major fatty acids found in the pastures were α-linilenic acid (c9c12c15C18:3, C18:3), linoleic (c9c12C18:2, C18:2) and C16:0. There was no significant difference in the C18:3 content of the pasture from May to September, though there was a significant difference found in October (Table 1). The C18:3 concentrations in the pasture varied from 64 to 70 g/100 g FA, which concurs with the study performed by Scharoeder et al. (2004). The content of C18:2 in the pasture had a tendency to be higher in June, July and August compared to May, September and October. The changes in EE, NDF, and the fatty acid content of TMR can be seen in Table 2. Within the treatments, the concentrations of C18:3 were higher in the pasture than in the TMR. The C18:2 content in the pasture was significantly low when compared with the TMR (Table 2).

Milk fatty acid compositions

Fatty acid compositions in milk within the different treatments can be seen in Table 3. Concentrations of short and medium-chain fatty acids (SMFA) in milk over all treatments were significantly higher ($p<0.01$) from late autumn such as September or winter, but were significantly lower ($p<0.001$) in summer (August) compared to the other months. Bargo et al. (2006) reported that the concentrations

Table 3. Monthly changes in milk fatty acid composition in different feeding systems

Fatty acid	Treatment	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	SEM	Sign.
		g/100 g FAME										
SMFA[1]	NG[2]	21.65^{bcz}	21.28^{bcy}	21.60^{bc}	21.03^{bc}	20.35^{cx}	22.00^{ab}	22.03^{ab}	22.95^{ay}	21.80^{aby}	1.22	N
	TG[3]	23.05^{ay}	22.20^{abcxy}	22.18^{abc}	21.20^{bcd}	20.43^{dx}	20.98^{cd}	21.15^{bcd}	23.75^{axy}	22.80^{abxy}	1.29	***
	WG[4]	24.20^{abx}	22.95^{bcx}	23.15^{abc}	20.73^{d}	17.90^{ey}	20.53^{d}	21.63^{cd}	24.85^{ay}	23.53^{abx}	1.45	***
C18:0	NG	11.43^{cdex}	12.28^{abc}	12.31^{abc}	12.43^{ab}	12.76^{ax}	11.30^{cde}	10.56^{e}	10.86^{ex}	11.77^{bcdx}	0.34	*
	TG	9.11^{dy}	11.59^{ab}	12.10^{a}	12.20^{a}	12.56^{ax}	11.32^{ab}	10.44^{bc}	8.97^{dy}	9.71^{cdy}	0.41	***
	WG	7.89^{ez}	11.75^{bc}	12.28^{ab}	13.20^{ab}	14.27^{ay}	12.03^{bc}	10.52^{cd}	7.95^{ey}	9.27^{dey}	0.55	***
t11C18:1	NG	1.54^{dy}	1.91^{az}	1.80^{abcz}	1.75^{abcz}	1.57^{cdz}	1.52^{dz}	1.54^{dz}	1.62^{bcdy}	1.83^{abx}	0.08	**
	TG	1.58^{dex}	3.40^{aby}	4.22^{ay}	3.53^{aby}	3.36^{aby}	2.77^{bcy}	2.33^{cdy}	1.30^{ez}	1.47^{dey}	0.31	***
	WG	1.66^{dx}	5.93^{ax}	6.53^{ax}	5.51^{abx}	5.48^{abx}	4.26^{bcx}	3.37^{cx}	1.88^{dx}	1.80^{dx}	0.42	***
*c*9C18:1	NG	20.05^{abx}	24.33^{abx}	24.37^{ab}	24.39^{ab}	25.11^{a}	23.77^{ab}	22.27^{bz}	22.39^{bx}	22.90^{abx}	0.76	N
	TG	21.16^{bcy}	23.47^{abxy}	22.62^{ab}	23.60^{a}	24.58^{a}	24.72^{a}	23.63^{ay}	20.07^{cy}	19.87^{cy}	0.77	**
	WG	20.84^{dey}	22.27^{cdy}	22.79^{cd}	24.41^{bc}	26.92^{a}	25.60^{ab}	23.20^{cdx}	17.98^{fz}	19.89^{efy}	0.77	***
C18:2	NG	3.14^{ax}	3.12^{ax}	3.23^{ax}	3.11^{ax}	3.26^{ax}	3.02^{ax}	2.80^{ax}	2.74^{ax}	2.76^{ax}	0.30	N
	TG	1.54^{ay}	1.59^{ay}	1.54^{ay}	1.41^{aby}	1.41^{aby}	1.36^{aby}	1.38^{aby}	1.23^{by}	1.20^{by}	0.09	*
	WG	1.26^{az}	1.34^{ay}	1.22^{ay}	1.15^{ay}	1.43^{ay}	1.06^{ay}	1.04^{ay}	0.89^{ay}	1.39^{ay}	0.18	N
c9t11 CLA	NG	0.43^{bcx}	0.50^{bz}	0.60^{az}	0.51^{ab}	0.44^{bcz}	0.44^{bcz}	0.47^{bcz}	0.38^{cy}	0.46^{bc}	0.03	N
	TG	0.47^{dx}	0.96^{bcy}	1.25^{ay}	1.12^{ab}	0.94^{bcy}	0.88^{cy}	0.78^{cy}	0.45^{dy}	0.51^{d}	0.07	***
	WG	0.48^{dx}	1.47^{bx}	1.96^{ax}	1.55^{b}	1.41^{bcx}	1.43^{bcx}	1.10^{ex}	0.56^{dx}	0.57^{d}	0.12	***
C18:3	NG	0.49^{x}	0.48^{y}	0.48^{y}	0.44^{z}	0.48^{y}	0.49^{y}	0.46^{y}	0.44^{y}	0.45	0.03	N
	TG	0.43^{cy}	0.74^{ax}	0.55^{bcy}	0.59^{by}	0.52^{bcy}	0.51^{bcxy}	0.52^{bcxy}	0.41^{cy}	0.43^{c}	0.04	**
	WG	0.54^{dx}	0.84^{ax}	0.76^{abcx}	0.81^{abx}	0.67^{bcdx}	0.60^{cdx}	0.64^{bcdy}	0.57^{dx}	0.51^{d}	0.06	**
LFA[5]	NG	78.35^{abx}	78.65^{abx}	78.38^{ab}	78.98^{ab}	79.65^{ay}	78.00^{bc}	77.95^{bc}	76.98^{cy}	78.18^{bcx}	1.12	N
	TG	76.90^{dy}	77.73^{bcdxy}	77.83^{bcd}	78.80^{abc}	79.55^{ay}	78.98^{ab}	78.83^{abc}	76.23^{dxy}	77.13^{cdxy}	1.29	**
	WG	75.80^{dez}	77.05^{cdy}	76.85^{cde}	79.28^{b}	82.10^{ax}	79.48^{b}	78.38^{bc}	75.15^{ey}	76.48^{dey}	1.45	***

[1] SMFA: Total short and medium-chain fatty acids (C6:0, C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C15:0).
[2] NG = No grazing; [3] TG = Daytime grazing; [4] WG = Whole day grazing.
[5] LFA = Total long-chain fatty acids (C16:0, c9 C16:1, C18:0, c9 C18:1, t11 C18:1, C18:2, C18:3, c9 t11CLA, t10,c12 CLA, C20:0).
[a, b, c, d, e] Treatment within a row with different superscripts are significantly different.
[x, y, z] Treatment within a column with different superscripts are significantly different ($p<0.05$).
Significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N = No significance.

of short and medium chain fatty acids in milk increased with an increase in DM and energy intake. From this experiment, the results suggest that the reduction of SMFA concentrations in milk over all the treatments in August was probably due to heat stress during the summer season, resulting with a reduced DM intake of cows (West, 2003), leading to a reduced concentrations of SMFA in milk.

The concentrations of C18:0 and c9 C18:1 in milk over all the treatments, was significantly higher ($p<0.01$) in summer (August) and lower ($p<0.01$) in winter (November) (Table 3). These fatty acids were highly concentrated in the milk when the cows had a negative energy balance (Chilliard et al., 2000). A high variation in C18:0 and c9 C18:1 concentrations were observed in the WG treatment. The cows experienced a greater negative energy balance from the pasture based feeding system than that from the TMR (Kolver and Muller, 1998). This study suggested that in August, reduced DM and energy intake of cows from heat stress resulted in an increase in the concentration of C18:0 and c9 C18:1 in milk. Furthermore, concentrations of C18:0 in blood are formed in rumen from polyunsaturated fatty acids such as C18:2 and/or C18:3. These polyunsaturated fatty acids are completely biohydrogenated by rumen bacteria (Moate et al., 2004). In this study, the concentrations of these fatty acids decreased in summer in over all the treatment. Therefore, it is suggested that the negative energy balance has a greater influence on the concentration of these fatty acids than the biohydrogenation of polyunsaturated fatty acid in the rumen. In the WG treatment and NG treatments, the concentration C18:2 in milk did not significantly change with the seasons. This result concurs with the previous studies performed by Lock and Garnsworthy (2003), who found that the concentration of C18:2 in milk did not vary with season. For the NG treatment, the concentrations of C18:3 in milk did not change with season, but in the two grazing treatments, the concentrations of C18:3 in milk were highest in spring, most notably in early spring. After spring, the concentration gradually decreased ($p<0.01$) until October, which supports the results from the experiments performed by Lock and Garnsworthy (2003), who found that for cows fed solely on pasture, the concentration of C18:3 in milk was two to three times greater than that from cows fed only on TMR.

This study found that there was no significant difference in the concentrations of t11C18:1 in milk between the three feeding systems when the cows were offered conserved

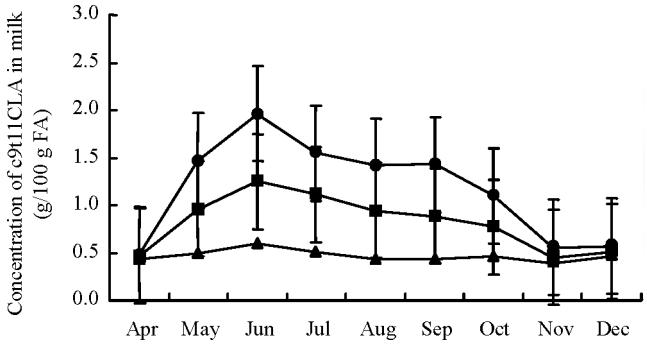

Figure 1. Monthly change the concentration of CLA in milk fatty acids in each treatment. (●) WG, whole day grazing; (■) daylight hours grazing; (▲) NG, experienced no grazing.

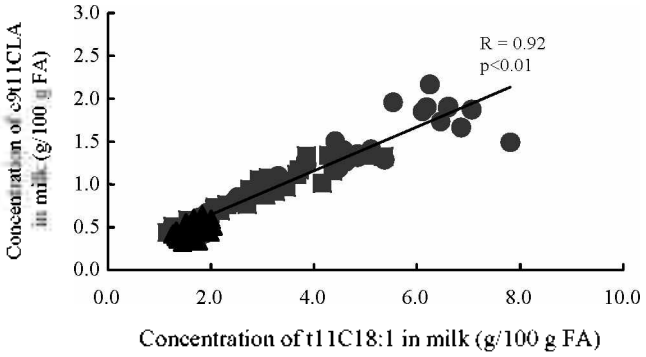

Figure 2. Relationship between CLA and trans-11 C18:1 in milk. (●) WG, whole day grazing; (■) daylight hours grazing; (▲) NG, experienced no grazing.

pasture in April, November and December. Also, the concentration of t11C18:1 in milk did not significantly change throughout the seasons in the NG treatment. However, in the grazing season the concentrations of t11C18:1 in milk were significantly higher ($p<0.001$) for the WG and TG treatments than for the NG treatment, supporting the study performed by Lock and Garnsworthy, (2003), with higher t11C18:1 concentrations in grazing cows milk. A high variation in C18:0 and c9C18:1 concentrations were observed in the WG treatment than TG treatment. The experimental results were consistent with the results of Bargo et al. (2006), this experiment found that the concentration of t11C18:1 was reduced by increasing the amount of supplementation, and increasing the amount of pasture DM intake. The C18:3 biohydrogenation pathways in the rumen are hydrogenated to t11c15-octadecadienoic acid (C18:2) this can further be hydrogenated to t11C18:1, after which can then be completely hydrogenated to C18:0 (Moat et al., 2004). Furthermore, in the grazing season, the concentrations of t11C18:1 in milk increased from May to August when the pasture contained a higher C18:3 and a lower NDF content. Martin and Jenkins (2002) reported that a culture pH greatly influenced the production of t11C18:1 by ruminal bacteria in the *in vitro* culture. This study suggested that the aviation of NDF and WSC content within the pasture may cause alterations in the rumen environment such as pH level changes, leading to the production of t11C18:1 in the rumen. It is suggested that the increasing concentration of t11 C18:1 in milk was due to an increased intake of polyunsaturated fatty acid, such as C18:3, from the pasture and influenced by biohydrogenation activity from bacteria (C18:1 to C18:0) in the rumen.

The major objective of this study was to investigate the effect of feeding systems on concentrations of CLA in milk in the different seasons. It was found that the concentration of c9t11CLA in milk did not significantly change ($p>0.05$) with season in the NG treatment. Though there was a higher variation of c9t11CLA concentrations in milk within two grazing treatments (TG and WG) throughout the year. In April, November and December, the c9t11CLA concentration in milk did not differ between the three treatments. Concentrations increased from May till they reached the highest levels in June. After June, the c9t11CLA concentrations in the milk gradually decreased ($p<0.01$) until October in the WG and TG treatments (Figure 1). This result was consistent with previous studies which found that the concentrations of CLA in milk were higher in May and June when cows were grazing on fresh pasture, than in winter when cows were fed on silage (Lock and Garnsworthy, 2003). Thus, it is concluded that diet is a major factor influencing the concentrations of CLA in milk (Chilliard et al., 2000). The concentration of c9t11CLA in milk increased with increasing pasture DM intake (Kay et al., 2005; Bargo et al., 2006). Bargo et al. (2006) found there was a positive regression ($p<0.05$; $r^2 = 0.35$) between content of c9t11CLA in milk and pasture DM intake. Also, Moorby et al. (2006) found there was a positive relationship between the DM intake, digestibility of pasture and WSC content of pasture. From the results, it was concluded that grazing pasture contains a high WSC content with a low NDF content, which resulted in an increased DM intake for cows in June and a decreased DM intake from the pasture due to heat stress in July and August. It is believed that this could affect production of c9t11CLA in milk. Bargo et al. (2006) reported that 41% of the total variation in c9t11CLA was directly related to C18:3 intake. Also, Brown et al., (2008) reported that the concentration of c9t11CLA in milk was increased when supplementing the diet of grazing cows with fish oil and linseed oil which is rich in C18:3 polyunsaturated fatty acid. In this study the content of C18:3 in pasture significantly decreased ($p<0.001$) in autumn compared to the other seasons. It is suggested that the decrease in c9t11CLA of milk in autumn, may have been affected by some seasonal variation of C18:3 in the

Table 4. Monthly changes in ratio of desaturase products to their precursor fatty acids in milk in different feeding systems

		Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	SEM	Sign.
c9C18:1/C18:0	NG[1]	2.08^{ay}	1.98^{a}	1.96^{a}	2.01^{a}	2.01^{a}	2.10^{a}	2.08^{a}	2.09^{a}	1.97^{a}	0.05	N
	TG[2]	2.32^{ay}	2.03^{cd}	1.87^{d}	1.94^{d}	1.96^{d}	2.19^{abc}	2.26^{ab}	2.25^{ab}	2.06^{bcd}	0.07	***
	WG[3]	2.56^{ax}	1.90^{cd}	1.86^{d}	1.85^{d}	1.89^{cd}	2.14^{bc}	2.17^{b}	2.31^{ab}	2.18^{b}	0.09	***
t11C18:1/CLA	NG	0.30^{ab}	0.26^{bcd}	0.33^{a}	0.29^{abcy}	0.28^{bcd}	0.29^{abcy}	0.30^{ab}	0.24^{dz}	0.25^{cdy}	0.01	**
	TG	0.30^{ab}	0.29^{b}	0.30^{ab}	0.33^{abx}	0.29^{b}	0.32^{abxy}	0.33^{ab}	0.35^{ax}	0.35^{ax}	0.02	***
	WG	0.29^{abc}	0.25^{c}	0.30^{abc}	0.28^{bcy}	0.26^{c}	0.34^{ax}	0.33^{ab}	0.30^{abcy}	0.32^{abxy}	0.02	***
Over all	NG	0.32^{abx}	0.32^{abc}	0.32^{a}	0.31^{abc}	0.31^{abc}	0.30^{abc}	0.29^{c}	0.29^{bxc}	0.29^{abcx}	0.01	*
	TG	0.29^{by}	0.32^{a}	0.31^{ab}	0.31^{ab}	0.32^{a}	0.32^{a}	0.31^{ab}	0.26^{cy}	0.26^{cy}	0.01	*
	WG	0.29^{cy}	0.31^{bc}	0.31^{abc}	0.32^{abc}	0.34^{a}	0.33^{ab}	0.31^{bc}	0.25^{dy}	0.27^{dy}	0.01	**

[1] NG = No grazing. [2] TG = Daytime grazing. [3] WG = Whole day grazing.

[a, b, c, d] Treatment within a row with different superscripts are significantly different.

[x, y, z] Treatment within a column with different superscripts are significantly different ($p<0.05$).

Significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; N = No significance.

pasture.

The c9t11CLA in milk is formed in the mammary gland from t11C18:1 due to Δ^{-9} desaturase enzyme activity (Bauman et al., 2000). Kay et al. (2004) reported that 91% of c9t11CLA in milk was produced by endogenous synthesis via the Δ^{-9}-desaturase enzyme when cows feed on fresh pasture. The variation of c9t11CLA concentration occurred in the same sampling time with t11C18:1, and a positive regression between the concentrations of c9t11CLA and t11C18:1 in milk was found ($r = 0.920$, $p<0.001$) (Figure 2). It is suggested that the decrease in t11C18:1 production in rumen is due to the fact that grazing pasture contains a high NDF content and a low WSC content, which leads to increased biohydrogenation of t11C18:1 to C18:0, resulting in reduced endogenous synthesis via the Δ^{-9} desaturase enzyme.

The ratio of Δ^{-9}-desaturase products to their substrate fatty acids in milk, in the different treatments was shown in Table 4. The ratio of CLA/VA was significantly lower ($p<0.01$) in the summer grazing season compared to spring and autumn in WG and TW treatments, especially in the WG treatment. Lock and Garnsworthy. (2003) reported that fresh grass enhance the synthesis of CLA in the dairy cows through an increase in dasaturase activity in the mammary gland. However, the results from this experiment do not support the concept that the desaturase activity is related to the grazing pasture. In September and October the index of desaturase activity was higher than in August, this is possibly due to a higher content of WSC in pasture in autumn. The desaturase activity may be dependant on the concentration and intake of sugar from the pasture (Rearte, 2005). Pollard et al. (1980) reported that desaturase activity could use the CoA ester of *trans* fatty acids including t11C18:1. The increase of c9t11CLA in milk in spring can primarily affect the flow of t11C18:1 to mammary gland and reduce the desaturase activity (Figure 2).

CONCLUSION

This experiment results that the concentration of t11C18:1 and c9t11CLA in milk were greatly influenced by season, with higher variation in the WG treatment than in the TG treatment and no variation in the NG treatment. It is concluded from this experiment, that the variation of t11C18:1 and c9t11CLA concentrations in cows milk can be influenced by the DM intake from pasture, and desaturase activity in mammary gland.

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