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Effects of Lactation Stage and Individual Performance on Milk *cis*-9, *trans*-11 Conjugated Linoleic Acids Content in Dairy Cows

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ABSTRACT: The goal of this study was to evaluate the effects of lactation stage and individual performance on milk *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content in dairy cows. In experiment 1, the milk *cis*-9, *trans*-11 CLA content from dairy cows in early (0.33±0.014%), middle (0.37±0.010%), and late stages (0.44±0.020%) showed significant differences ($p<0.05$); and the individual contents of the major fatty acids, especially *cis*-9, *trans*-11 CLA in cows of the same lactation were also variable. In the second experiment design as a validation test, our results once again showed that the individual contents of *cis*-9, *trans*-11 CLA were various, and a difference of about 2-fold (0.55% vs 0.95%) was observed, although the animals were offered same diet. These data demonstrated that lactation stage and individual performance have considerable effects on milk *cis*-9, *trans*-11 CLA contents. (**Key Words:** *cis*-9, *trans*-11 CLA, Individual Performance, Lactation Stage)

INTRODUCTION

Conjugated linoleic acid (CLA) is a group of isomers that has beneficial health effects in humans and animals (Bauman et al., 2000). Of the various potential isomers, *cis*-9, *trans*-11 CLA accounts for 82 to 97% and 57 to 85% of the total CLA content in milk and beef, respectively (Dhiman et al., 2005). This specific isomer exhibits several health benefits that are not shown by other CLA isomers (Wang and Lee, 2012) thereby leading to heightened interest in the manufacture of *cis*-9, *trans*-11 CLA-fortified products (Dhiman et al., 2005; Rodríguez-Alcalá and Fontecha, 2007). The majority of the *cis*-9, *trans*-11 CLA found in milk is synthesized in mammary glands, in a process catalysed by stearoyl-CoA desaturase 1 (SCD1), while the remaining part is derived from the rumen (Bauman et al., 2000; Griinari et al., 2000; Wang and Lee, 2012). SCD1 is one of the two identified SCD isoforms in the bovine (Paton and Ntambi, 2009). It is an enzyme that predominantly exists in the endoplasmic reticulum (ER) membrane of liver, mammary gland, adipose tissue, etc. (Wang and Lee, 2012). SCD5 is another SCD isoform which is highly expressed in the brain (Lengi and Corl, 2007) and it is also expressed in the bovine mammary tissue (Jacobs, 2011).

Factors that regulate the SCD1 activity or rumen fermentation conditions can alter the milk content of *cis*-9, *trans*-11 CLA. The effects of breed, diet, feeding strategies and seasonal variations have been reported (Dhiman et al., 2005; Slots et al., 2008). However, the effects of lactation stage and individual variation among dairy cows on the *cis*-9, *trans*-11 CLA content are still not well understood. Therefore, in this study, two experiments were conducted in order to evaluate the effects of lactation stage and individual performance on milk *cis*-9, *trans*-11 CLA content in dairy cows.

MATERIALS AND METHODS

Cows and milk samples

Two experiments were performed in this study. All experimental procedures were in accordance with the "Guidelines for the Care and Use of Experimental Animals of Pusan National University". Experiment 1 employed thirty-one multiparous Holstein cows fed the same total mixed ration (TMR) (Tables 1 and 2). Experiment 2 used seventeen multiparous Holstein cows (589±29.6 kg of body

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Table 1. Design of experiment 1

Item	Early stage	Middle stage	Late stage
Number	11	11	9
Weight (kg)	576±20.1	570±20.0	573±19.5
Parity	3.0±0.38	2.8±0.50	1.7±0.22
Milk yield (kg)	32.6±1.66	30.8±1.58	25.8±1.73
Dry matter intake (kg)	23.2±2.80	21.9±2.72	18.7±2.59

weight) in the middle lactation stage (142.5±29.65 d) and fed the same diet. The ratio of roughage to concentrates was about 0.77:1 (dry matter basis); 10 kg of oat hay (dry matter 91.5%, crude protein 1.2%, ether extract 2.3%, neutral detergent fiber 58.3%, acid detergent fiber 36.7%, and ash 8.3%) and 13 kg of concentrate were fed daily. The ingredient (% DM), proximate (% DM), and fatty acid (% total fatty acids) compositions of the concentrate fed to the dairy cows in experiment 2 are shown in Table 3. Water was available at all time for all the animals. Cows were milked twice daily (12-h milking interval). The morning milk was collected from animals individually after machine milking from the four quarters. The bulk tank somatic cell counts (SCC) were monitored and the values were less than 100,000 cells/ml. The milk samples were stored at -20°C till milk fat analysis.

Table 2. Ingredients (kg, wet matter) and proximate (% dry matter) composition of the TMR fed to dairy cows (Experiment 1)

Ingredients	
Grass haylage	8.0
Corn silage	10.0
Alfalfa hay	3.8
Organic cows 1a[1]	7.5
Organic cows 1b[1]	3.5
Organic cows 6[1]	5.0
Salt	0.05
Na_2CO_3	0.15
Mineral/vitamin premix[2]	0.55
Total (wet matter)	38.75
Dry matter intake (kg)	24.2
Proximate composition	
Total digestible nutrients (TDN)	71
Crude protein	18.5
Crude fat	3.5
Acid detergent fiber (ADF)	9.8
Neutral detergent fiber (NDF)	18.8
Ashes	3.9
Calcium	0.9
Phosphorus	0.4

[1] The details of these three ingredients are not shown here.
[2] Nutrients provided/kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vitamin A, 2,650,000 IU; Vitamin D_3, 530,000 IU; Vitamin E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

Table 3. Ingredients (% dry matter), proximate (% dry matter) and fatty acid (% total fatty acids) composition of the concentrate[1] fed to dairy cows (Experiment 2)

Ingredients	
Ground corn	5.92
Wheat	30.00
Salt	0.82
Molasses	3.75
Magnesium oxide	0.50
Corn gluten meal	10.82
Sodium bicarbonate	0.70
Extruded soybean	5.00
Palm kernel meal	2.22
Urea	1.00
Rapeseed meal	4.56
Dried distilled grain solubles	2.61
Soyhull	6.54
Corn gluten feed	18.51
Limestone	1.70
Extruded linseed	5.00
Mineral/vitamin premix[2]	0.35
Total	100.00
Proximate composition	
Ashes	7.0±0.02
Crude fat	5.2±0.02
Crude protein	23.6±0.04
Neutral detergent fiber (NDF)	19.3
Acid detergent fiber (ADF)	8.9
Calcium	0.7±0.00
Phosphorus	0.4±0.00
Fatty acid composition	
C16:0	14.1±0.09
C18:0	4.6±0.01
Oleic acid (C18:1n9c)	20.6±0.03
Linoleic acid (C18:2n6c)	39.0±0.15
Linolenic acid (C18:3n3)	16.8±0.09

[1] The ratio of roughage to concentrates is about 0.77:1 (10 kg of oat hay and 13 kg of concentrate were fed daily).
[2] Nutrients provided/kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany): Vitamin A, 2,650,000 IU; Vitamin D_3, 530,000 IU; Vitamin E, 1,050 IU; Niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg.

Lipid analysis

Briefly, total milk lipids were extracted with 20 ml chloroform/methanol (2:1, v/v) from 6 ml of milk. The extracted lipids were then converted into fatty acid methyl esters with 14% (w/v) boron trifluoride-methanol (B1252; Sigma-Aldrich Corp., St. Louis, MO, USA) according to a previously published method (Folch et al., 1957). The fatty acid methyl esters were then injected into a gas chromatograph (GC) (Agilent 7890A GC system, Agilent Technologies, USA) using a system equipped with a 7863 series auto-sampler, 7683B series injector, and flame ionization detector (FID). A SP™-2560 fused silica capillary column (100 m×0.25 mm, inner diameter with 0.2-µm film thickness; Supelco Inc., Bellefonte, PA, USA) was used and the oven temperature was programmed to increase from 70 to 225°C at a rate of 5°C per min to 100°C, held for 2 min, increased 10°C per min to 175°C, held for 40 min, increased 5°C per min to 225°C, and held for 40 min. Front inlet and FID temperatures were maintained at 255°C and 260°C, respectively. The hydrogen flow rate to the detector was 55 ml/min, the airflow rate was 400 ml/min, and the make-up gas flow rate was 20 ml/min. The split ratio was 30:1. Peaks were routinely identified by comparing the retention time with those of authentic standards including Supelco® 37 Component Fatty acid methyl esters (FAME) Mix (47885-U), *trans*-11-octadecenoic methyl ester (46905-U; Sigma-Aldrich Corp.) and *cis*-9, *trans*-11 CLA (1255; Matreya LLC, Pleasant Gap, PA, USA). The percentage of individual fatty acid was calculated as the ratio of individual area to that of total identified fatty acids.

Statistical analysis

Data were presented as mean±SE and analyzed using a one-way analysis of variance (one-way ANOVA) (SPSS Inc., Chicago, USA). In all cases, significant differences were accepted if $p<0.05$. The relationship between *cis*-9, *trans*-11 CLA content and desaturation indexes were analyzed using the correlation procedures (PROC CORR) of SAS (SAS, 2000).

RESULTS AND DISCUSSION

Effects of lactation stage on milk *cis*-9, *trans*-11 CLA content

Previous studies have shown that lactation stage significantly contributes to variation in the milk fat profile (Kay et al., 2005; Stoop et al., 2009). Normally, linoleic acid can be isomerized into *cis*-9, *trans*-11 CLA in the rumen, with the majority being further hydrogenated into *trans*-11 18:1, and then potentially saturated into stearic acid (Wang and Lee, 2012). In experiment 1, both the linoleic acid and stearic acid levels significantly decreased from the early stage of lactation (before 100 d) to the late stage (after 200 d) ($p = 0.032$ and $p = 0.022$, respectively; Table 4). Because the animals were offered the same diet, the decreased linoleic acid and stearic acid levels in milk may suggest increased activity of rumen microorganisms and more rumen output of *trans*-11 18:1 by dairy cows in the late stage of lactation.

The milk *trans*-C18:1 content of dairy cows in middle stage of lactation was significantly lower than that in early stage of lactation ($p = 0.003$) whereas no significant difference was found between the late stage and early stage ($p = 0.087$) (Table 4). Three desaturation indexes were calculated representing the ratio of the Δ9-desaturase product to the sum of the Δ9-desaturase product and substrate (Kelsey et al., 2003) (Table 4). Although not statistically significant, the desaturation indexes for *cis*-9 C14:1 and *cis*-9 C18:1 in the late stage of lactation were all

Table 4. Differences in major milk fatty acids (%) from dairy cows in Experiment 1

Items	Early stage		Middle stage		Late stage	
Fatty acid (%)	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
C12:0 (Lauric acid)	1.88±0.258[a1]	0.71-3.45	2.75±0.092[b]	2.23-3.30	2.67±0.144[ab]	2.07-2.99
C14:0 (Myristic acid)	9.17±0.813[a]	5.26-13.69	12.38±0.194[b]	11.2-13.41	12.04±0.417[b]	10.05-13.92
C14:1 (Myristoleic acid)	0.56±0.073[a]	0.29-1.08	0.89±0.044[b]	0.63-1.06	0.85±0.060[b]	0.64-1.21
C16:0 (Palmitic acid)	27.66±0.536[a]	25.25-30.53	30.20±0.503[b]	27.16-32.63	29.78±1.000[b]	26.61-35.29
C16:1 (Palmitoleic acid)	1.47±0.177[b]	0.92-2.66	1.11±0.071[a]	0.92-1.77	1.13±0.068[ab]	0.79-1.45
C18:0 (Stearic acid)	17.70±0.719[b]	13.92-22.40	15.17±0.444[a]	12.91-17.71	15.00±1.076[a]	10.58-20.31
trans-11 C18:1	1.47±0.050[b]	1.23-1.75	1.26±0.036[a]	1.09-1.49	1.35±0.047[ab]	1.04-1.59
C18:1n9c (Oleic acid)	27.32±1.464[b]	20.47-35.20	22.69±0.571[a]	19.01-25.31	23.75±0.863[a]	19.68-27.83
C18:2n6c (Linoleic acid)	3.20±0.112[b]	2.81-3.98	2.99±0.079[ab]	2.61-3.66	2.88±0.091[a]	2.48-3.25
C18:3n3 (Linolenic acid)	0.35±0.016	0.28-0.48	0.36±0.008	0.32-0.43	0.36±0.014	0.29-0.40
cis-9, *trans*-11 CLA	0.33±0.043[a]	0.27-0.38	0.37±0.010[a]	0.30-0.42	0.44±0.021[b]	0.36-0.57
Other	8.88±0.500	6.19-11.56	9.83±0.172	8.67-10.68	9.75±0.188	8.80-10.40
Desaturation index[2]						
cis 14:1	0.06±0.004	0.03-0.08	0.07±0.003	0.05-0.08	0.07±0.004	0.06-0.09
cis 16:1	0.04±0.003	0.03-0.05	0.04±0.002	0.03-0.05	0.04±0.001	0.03-0.04
cis 18:1	0.60±0.015	0.54-0.70	0.60±0.010	0.54-0.63	0.62±0.015	0.54-0.69

[1, a-c] within a row, values with different superscripts is significantly different ($p<0.05$).

[2] Desaturation index are calculated as following: ratio of the Δ9-desaturase product divided by the sum of the Δ9-desaturase product and substrate. For example, the desaturase index for *cis*-9 14:1 would be (*cis*-9 14:1)/(*cis*-9 14:1+14:0).

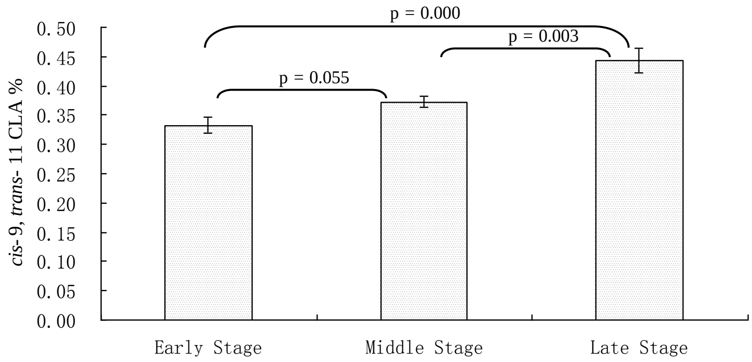

Figure 1. *cis*-9, *trans*-11 CLA content in milk from dairy cows at different lactation stages (Experiment 1).

a little higher than that in the early stage of lactation (p = 0.116 and p = 0.609, respectively; Table 4) and these results may indirectly imply a higher activity of Δ9-desaturase of the mammary glands of animals in the late stage of lactation. However, no difference was found for the desaturation index for *cis*-9 C16:1. As we have speculated, the *cis*-9, *trans*-11 CLA continually increased from the early stage of lactation to the late stage of lactation (0.33% vs 0.44%; Figure 1). A similar result was also found in the milk of Canadian Jersey cows, with the *cis*-9, *trans*-11 CLA content in the late stage (after 200 d) being significantly higher than during early lactation (before 100 d) (Kgwatalala et al., 2009). In a dairy ewe research, greatly increased *cis*-9, *trans*-11 CLA content was observed in Transylvania Merino sheep (2.03 g vs 2.70 g/100 g FAME), Tsigay sheep (2.05 g vs 2.60 g/100 g FAME) and Turcana sheep (2.36 g vs 3.64 g/100 g FAME) from 2 wks to 14 wks of lactation (Mierlita et al., 2011). However, in another report it was said that the *cis*-9, *trans*-11 CLA content of bovine milk and Δ9-desaturase activity in the mammary gland are independent of stage of lactation under normal conditions (Lock et al., 2005).

Effects of individual performance on milk *cis*-9, *trans*-11 CLA content

The individual contents of the major fatty acids, especially *cis*-9, *trans*-11 CLA in cows of the same lactation were found variable in experiment 1. In milk, *cis*-9, *trans*-11 CLA contents were 0.27 to 0.38%, 0.30 to 0.42%, and 0.36 to 0.57% in the early stage, middle stage and later stage, respectively (Table 4). These data suggested large individual differences in the *cis*-9, *trans*-11 CLA synthesis ability of cows offered the same diet and this may be related to both rumen biohydrogenation and Δ9-desaturase activity in the mammary gland (Lock and Garnsworthy, 2002; Peterson et al., 2002).

In order to confirm if individual performance really had effect on milk *cis*-9, *trans*-11 CLA content, experiment 2 was designed as a validation test. More animals in middle lactation were used in this experiment (17 multiparous Holstein cows) compared with that in experiment 1 (11 multiparous Holstein cows). Our results once again showed that the individual contents of *cis*-9, *trans*-11 CLA were various, and a difference of about 2-fold (0.55% vs 0.95%) was observed although the animals were offered same diet (Figure 2). Corresponding differences were also evident in the desaturation indexes for *cis*-9 C14:1 (0.069 vs 0.137) and *cis*-9 C16:1 (0.036 vs 0.064) (Table 5). These substantial variations in the desaturation indexes may demonstrate a huge diversity of the Δ9-desaturase activity in mammary glands of dairy cows. A positive correlation (R^2 = 0.558) existed between *cis*-9, *trans*-11 CLA content

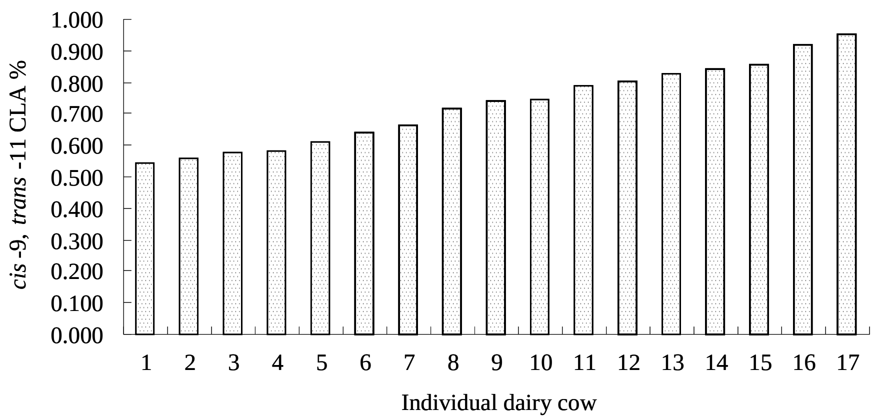

Figure 2. *cis*-9, *trans*-11 CLA content of milk from individual multiparous dairy cows in the middle lactation stage (Experiment 2).

Table 5. Important parameters and major milk fatty acids composition (%) from dairy cows in Experiment 2

Items	Mean±SE	Range
Milk yield (L)	19.89±2.32	17-24
Milk protein (%)	3.28±0.32	2.93-4.22
Milk lactose (%)	5.01±0.11	4.83-5.15
Fat content (%)	3.55±0.85	2.37-5.21
Fatty acid composition (%)		
C12:0 (Lauric acid)	2.87±0.197	1.57-4.46
C14:0 (Myristic acid)	10.34±0.283	7.9-11.60
C14:1 (Myristoleic acid)	1.08±0.073	0.64-1.60
C16:0 (Palmitic acid)	28.01±0.488	24.81-31.16
C16:1(Palmitoleic acid)	1.40±0.051	1.06-1.76
C18:0 (Stearic acid)	11.97±0.471	9.05-15.66
trans-11 C18:1	2.09±0.082	1.76-2.92
C18:1n9c (Oleic acid)	26.39±0.617	22.37-31.10
C18:2n6c (Linoleic acid)	3.79±0.172	2.47-5.32
C18:3n3 (Linolenic acid)	0.46±0.027	0.34-0.68
cis-9, *trans*-11 CLA	0.73±0.031	0.55-0.95
Other	10.88±0.461	8.22-14.25
Desaturation index[1]		
cis 14:1	0.09±0.005	0.069-0.137
cis 16:1	0.05±0.002	0.036-0.064
cis 18:1	0.69±0.009	0.601-0.735

[1] Desaturation index are calculated as following: ratio of the Δ9-desaturase product divided by the sum of the Δ9-desaturase product and substrate. For example, the desaturase index for *cis*-9 14:1 would be (*cis*-9 14:1)/(*cis*-9 14:1+14:0).

and the desaturation index for *cis*-9 C14:1 (Figure 3). However, no obvious correlation was found between *cis*-9, *trans*-11 CLA content and the desaturation index for *cis*-9 C16:1 (R^2 = 0.087) or *cis*-9 C18:1 (R^2 = 0.218) (Figure 3). Moreover, large variations were found in the milk content of linoleic acid (2.47% to 5.32%) and linolenic acid (0.34% to 0.68%) further suggesting considerable differences in the rumen environments of these animals. In addition, various individual *cis*-9, *trans*-11 CLA contents were also found in two crossbreed dairy ewes including Karakachan× Blackhead Pleven (13.5 to 28.8 mg/g fat) and Tsigay× Blackhead Pleven (13.7 to 43.6 mg/g fat) (Mihailova and Odjakova, 2011). Lastly, two additional aspects were again confirmed from the results of these 2 experiments (Table 4; Figure 3). First, higher desaturation indexes correspond to higher *cis*-9, *trans*-11 CLA content and second, the desaturation index of *cis*-9 C14:1 appears to be a much more reliable indicator than that of *cis*-9 C16:1 or *cis*-9 C18:1.

In conclusion, our results demonstrated that lactation stage and individual performance have considerable effects on the milk fat profile in dairy cows, especially in the content of *cis*-9, *trans*-11 CLA. Furthermore, the basis for the variation of *cis*-9, *trans*-11 CLA content at the

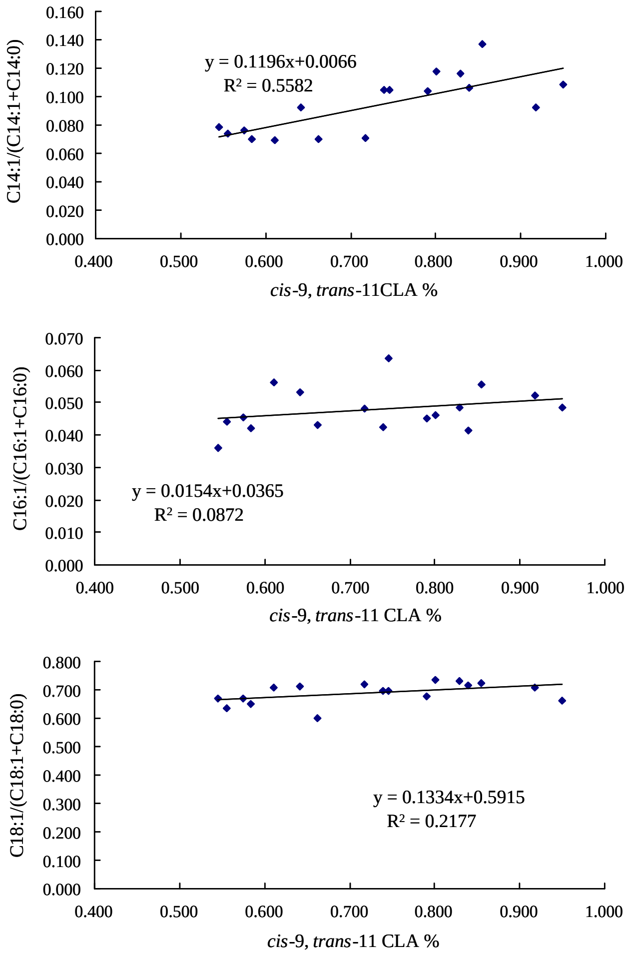

Figure 3. Correlation between *cis*-9, *trans*-11 CLA content and desaturation indexes (Experiment 2).

individual level and during lactation may be related to the rumen output of *trans*-11 18:1 and to the activity of Δ9-desaturase in the mammary glands.

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