Chapter 5

Fatty Acid Profile and Conjugated Linoleic Acid Content of Milk from Confined Holstein Cows During the Summer and Winter Seasons

Araceli Pinelli-Saavedra, Alfonso Martínez-Borraz,
Silvia Y. Moya-Camarena, Humberto González-Ríos and Jesús Hernández

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http://dx.doi.org/10.5772/50774

1. Introduction

In recent decades, research in dairy cattle has been focused on the evaluation of factors that may cause changes in the lipid composition of milk, due to the fact that unsaturated fatty acids, conjugated linoleic acids (**CLA**) and high monounsaturated fatty acids/saturated fatty acids (MUFA/SFA) and polyunsaturated fatty acids/saturated fatty acids (PUFA/MUFA) ratios in milk have shown beneficial properties, including antiatherogenic and anticarcinogenic effects in humans. The principal factors that determine these effects are the breed (Lawless et al., 1999; Wood et al., 1980) and feeding regime (Cooper et al., 2004) in addition to less-studied factors, such as the parity, days in milk (**DIM)** and extreme temperatures. CLA are a mixture of linoleic acid isomers that contain conjugated double bonds. Studies in experimental animals have demonstrated that CLA has properties that may be beneficial for humans, providing anticarcinogenic, antidiabetic, antiobesity, antiatherogenic and immune stimulatory effects (Huth et al., 2006; Nirvair et al., 2007; Pariza et al., 2001; Parodi., 1999). Milk and dairy products are the primary sources of CLA, and approximately 75 to 90% of the total CLA content in milk fat is represented by *cis*-9, *trans*-11-CLA (Chin et al., 1992; Kay et al., 2004). CLA (9-*cis*, 11-*trans*-CLA) in milk fat is produced in the mammary glands via an endogenous synthesis pathway in which Δ^9-desaturase converts vaccenic acid (*trans*-11 C18:1) to CLA (Bauman et al., 2001; Kay et al., 2005). Under conditions fostered by a certain combination of feed production system, additives, diet, breed, stage of lactation, and season, vaccenic acid is increased in the rumen, which results in a concomitant increase in the 9-*cis*, 11-*trans*-CLA content in milk fat (Griinari et al., 2000; Lock & Bauman, 2004). However, the factors mentioned above have been studied under

controlled conditions. In contrast, our study focused on the evaluation of some of these factors on the fatty acid profile and 9-*cis*, 11-*trans*-CLA content in milk under commercial conditions where extreme temperatures occur. To this end, we evaluated the fatty acid profile and 9-*cis*, 11-*trans*-CLA content in Holstein cow milk during the winter (14 °C) and summer (40 °C) on a commercial dairy farm in Northwestern Mexico (Sonora) by studying the effects of feed composition, parity, stage of lactation (DIM), and milk yield.

2. Material and methods

2.1. Location and sampling

This study was carried out on a commercial dairy farm in Sonora, México, which was located at 29.07 ° longitude and 110.90 ° latitude. A total of 240 Holstein dairy cows were included in the study, and the parity of the cows ranged from 1 to 6. The cows were exposed to all of the environmental elements that can affect the behaviour and milk yield of dairy cattle, including solar radiation, rain, and wind, as shown in Figure 1. The cows were divided into parity groups, including the following: primiparous (**P,** 1 parity, n = 84); earlier multiparous (**EM**, from 2 to 3 parities, n = 96); and late multiparous (**LM**, from 4 to 6 parities; n = 60). The milk was sampled in proportion to the parity of the cows; exclusion criteria included the presence of mastitis and more than 350 DIM.

Figure 1. Physical and environmental conditions of the dairy farm.

The diets were formulated using the Cornell-Penn-Miner Dairy model (Moate et al., 2004), and the ingredients and chemical compositions of summer and winter feed are shown in Table 1. Milking was carried out twice per day (0400 h and 1600 h), and the two milk samples (50 mL each) were combined. Milk samples were collected during the summer (from June to August 2006) and the winter (December 2006 to February 2007). The milk yield was measured using a Waikato MKV milk meter (Waikato MKV, Milking Systems, NZ) and evaluated on the last day of each month, which was standard practice on this dairy farm. The samples were transported on ice to the laboratory and stored at -20 °C for later analysis. Feed samples were collected on each sampling date. The DIM were also recorded.

The meteorological station "El Perico", which was located 5 km from the farm, recorded the daily weather data, and this information was used to calculate the temperature-humidity index (**THI**) for the winter and summer seasons. The THI was calculated as follows: THI = td – (0.55 – 0.55RH) × (td – 58), where td = the dry bulb thermometer in °F and RH = the relative humidity expressed as a decimal (NOAA, 1976). A THI < 72 was taken as an indicator of zero stress for the dairy cows. THI values from 72 to 79 indicated that the dairy cows were under mild stress, whereas THI values from 79 to 88 indicated that cows were under high stress (Armstrong, 1994).

2.2. Feed analysis

2.2.1. Feed chemical composition

The chemical composition of the diets was analysed by the AgroLab México laboratory (Gómez Palacio, Dgo). The components of the feeds that were evaluated in winter and summer were the crude protein, neutral detergent fibre, acid detergent fibre, ether extract, and net absorbable energy.

2.2.2. Feed fatty acid analysis

Total lipids were extracted with a chloroform and methanol mixture (2:1, vol:vol), as described by Folch et al., (1957). Fatty acid methyl esters (**FAMEs**) were prepared by the standard method (AOAC, 1995). FAMEs were identified and quantified by gas chromatography using a Varian Star 3400 CX Gas Chromatograph (VARIAN Inc, Walnut Creek, CA) with a DB-23 (J & W Scientific, Folsom CA) capillary column (30 m x 0.25 mm). Helium was used as the carrier gas with a flow rate of 1 mL/min; the airflow was 300 mL/min, and the hydrogen flow was 30 mL/min. The column temperature was initially set at 50 °C and held for 1 min. It was then increased at 10 °C/min to 166 °C, and then at 1 °C/min to 174 °C and held for 30 s; next, it was increased at 2 °C/min to 194 °C and held for 30s. As a final step, the column temperature was increased at a rate of 3.5 °C/min to 215 °C and held for 5 min. The total running time was 42.6 min. Identification of the fatty acid profile was performed with an external standard FAME mix (C4-C24, Sigma, USA).

2.3. Milk quality

The milk composition (fat, protein, lactose, and total solids) was determined by near infrared spectrometry (Milkoscan Minor FT120).

2.3.1. Fatty acid analysis of the milk

2.3.1.1. Extraction

Milk fat extraction was carried out according Luna et al., (2005). In brief, 30 mL of milk was centrifuged at 17,800 × g for 30 min at 8 °C (Beckman Coulter centrifuge, Mod. Allegra 64R).

Approximately 350 mg of fat was subsequently removed for lipid extraction with 18 mL of a hexane:isopropanol mixture (3:2 v:v) per g of fat; next, 12 mL of sodium sulphate per g of fat was added as described by Hara & Radin (1978).

2.3.1.2. Methylation

The milk fatty acids were transesterified with methoxide sodium according the method of Christie (1982) as modified by Chouinard et al., (1999). Briefly, the fatty acids were mixed with 2 mL of hexane per 40 mg of fatty acid, and 40 μL of methyl acetate was added. We then vortexed the mixture and added 40 μL of methylation reagent (1.75 mL of methanol mixed with 0.4 mL of 5.4 M sodium methoxide). The mixture was again vortexed and allowed to react for 10 min. We then added 60 μL of termination reagent (1 g of oxalic acid in 30 mL of diethyl ether). Next, the sample was centrifuged for 5 min at 2400 × g at 5 °C, and the hexane layer was removed and transferred to a new tube for evaporation with nitrogen. When the resulting methylated fatty acids had dried, 50 mg was transferred to an amber vial, and 200 μL of hexane was added. At that point, the sample was ready for quantification of fatty acids and 9-*cis*, 11-*trans*-CLA.

2.3.1.3. Identification and quantification of FAMEs by gas chromatography

The FAMEs were identified and quantified by gas chromatography using a Varian Star 3400 CX Gas Chromatograph (VARIAN Inc., Walnut Creek, CA), with a DB-23 (J & W Scientific, Folsom, CA) capillary column (30 m x 0.25 mm). The procedure was identical to that described above for the *Feed Analysis of Fatty Acids* with the same total running time of 42.6 min. Identification of the fatty acid profile was performed with an external standard fatty acid methyl ester mix (C4-C24, Sigma, USA). Conjugated linoleic acid isomers were identified using 98% pure standards (Matreya Inc., PA).

2.3.2. Desaturase index

The desaturase index was calculated as reported by Kelsey et al. (2003), using four pairs of fatty acids where each pair represented one product and one substrate of Δ^9-desaturase. The fatty acid pairs (product and substrate) were as follows: *cis*-9 14:1 and 14:0; *cis*-9 16:1 and 16:0; *cis*-9 18:1 and 18:0; and *cis*-9 *trans*-11 CLA and *trans*-11 18:1. Kelsey et al. (2003) have defined the desaturase index as follows: [product of Δ^9-desaturase]/[product of Δ^9-desaturase + substrate of Δ^9-desaturase]. For example, the desaturase index for *cis*-9 16:1 would be (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0).

2.4. Statistical analysis

The PROC MIXED procedure provided in the SAS software (SAS Inst. Inc., Cary, NC, 2001) was used to adjust a model for analysing the fatty acid data. The model included the fixed effects of season and month within the season and parity; the random effects included milk yield and DIM (with linear and quadratic effects) and the residual term of animal per parity. The data were considered significant when $P < 0.05$. The response variable was reported as the least square mean ± SEM. The adjusted model was evaluated as follows:

$$Y_{ijklmn} = \mu + A_i + B_j(A_i) + C_k + D_l + E_m + F_n + \varepsilon_{ijklmn},$$

where Y_{ijklm} is the response variable (fatty acid content), A_i is the season, B_j is the month in the season, C_k is the parity, D_l is the days in milk, E_m is the quadratic of days in milk, F_n is the milk yield, and ε_{ijklmn} is the residual error term.

When significant effects of the factors studied were found, mean comparisons of the response variables were performed by the LSMEANS procedure of SAS.

3. Results and discussion

3.1. Temperature-humidity index

In desert climates, the ambient temperatures are extreme and vary widely between winter and summer. To our knowledge, the previous studies that have evaluated variations in the milk 9-*cis*, 11-*trans*-CLA content in different seasons had been performed in countries with moderate ambient temperatures. Therefore, our objective was to evaluate the 9-*cis*, 11-*trans*-CLA content in Holstein cow milk in the winter and summer in a hot environment in the Northwestern region of México. We used the THI as an indicator of the heat stress of the dairy cattle in both seasons (Figure 2). As anticipated, during the winter, with an average maximum temperature of 24 °C and 20% RH, the dairy cows were not under heat stress according to the maximum THI measured (average 57 ± 8.7, range from 33 to 74). In contrast, with an average maximum temperature of 43 °C and 50% RH, the dairy cows were under heat stress during the summer (average THI maximum = 80 ± 5.1, range from 63 to 88).

3.2. Feed composition

According to the components and chemical analysis of the feed (Table 1), the summer and winter diets were similar in caloric, nitrogen, and fibre content. Nevertheless, changes in the ingredients were made according to the standard practice on the dairy farm. These changes included the use of whole cottonseeds, maize silage and less alfalfa content in the feed during the winter. The final forage-to-concentrate ratios of the diets were 58:42 and 59:41 for winter and summer, respectively. Accordingly, the fatty acid composition of the diets showed variations between the two seasons (Table 1). Because these compounds are precursors of CLA in both ruminal and mammary gland biosynthesis, the discussion is focused on the concentrations of *cis*-9 18:1, C18:2 and C18:3 in the feed. Despite a lower 18:2 concentration in the summer diet (37.21% of FAMEs) compared to the winter diet (45.45% of FAMEs), the 9-*cis*, 11-*trans*-CLA content in the milk during the summer was higher (9.36 mg/g fatty acids) compared to that observed during the winter (7.03 mg/g fatty acids). However, the concentration of the other CLA precursors *cis*-9 18:1 and C18:3 were higher ($P < 0.05$) during the summer compared to the winter diets. This finding may explain, in part, the increased content of CLAs in the milk during the summer. Although the *cis*-9 18:1 isomer is not a well-recognised CLA precursor, Mosley et al. (2002) have demonstrated the ability of mixed ruminal microbes to convert *cis*-9 18:1 to several *trans*

positional isomers, including *trans*-11 18:1, which is a precursor of CLA (Griinari et al., 2000; Lock & Bauman, 2004). These data suggest that biosynthesis in the mammary gland may explain the increased milk 9-*cis*, 11-*trans*-CLA content observed during the summer (Soyeurt et al., 2008).

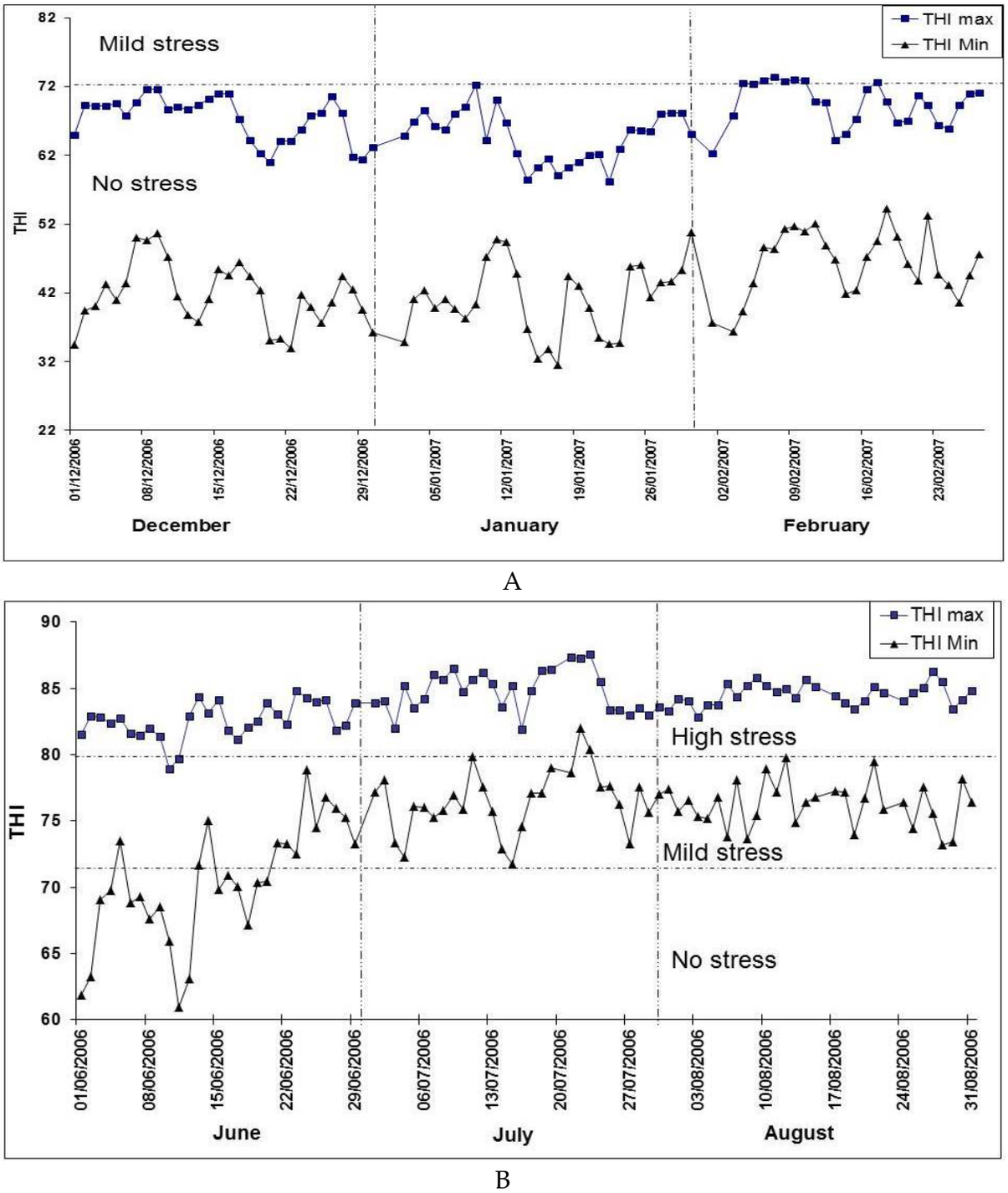


Figure 2. Maximal and minimal THI values during the winter (A) and summer (B) and the relation of THI to heat stress in dairy cows.

	Winter	Summer
Ingredients[1]		
Concentrate	42.21	41.13
Maize ground	21.09	25.49
Whole Cottonseed	6.48	0
Soya 47% Protein	3.53	5.59
Soybean 70	4.27	2.91
Vitamins and minerals	2.28	2.08
Fat mixture[2]	1.42	1.85
Cane Molasses	3.15	3.07
Alfalfa	30.19	58.87
Maize silage	27.60	0
F:C[3]	58:42	59:41
Chemical analysis[1]		
CP	15.7	15.4
NDF	24.2	25.2
ADF	33.6	34.4
EE	5.56	4.86
ENl	1.55	1.54
Fatty acids of the diet[4]		
C12:0	0.00^{b}	0.78^{a}
C14:0	0.00^{b}	0.77^{a}
C16:0	20.54^{a}	20.04^{a}
C18:0	6.71^{a}	5.70^{a}
C18:1 c9	21.56^{b}	26.15^{a}
C18:2	45.45^{a}	37.21^{b}
C18:3	5.74^{b}	9.35^{a}

[1]Values were determined using dry matter (% of DM); [2]Tallow and soybean oil; [3]Forage:concentrate ratio; [4]Fatty acid values were calculated as the percentage of methylated fatty acids; Means within a row with different superscripts were significantly different.

Table 1. Ingredients and chemical composition of the feed in the winter and summer.

3.3. Milk yield and composition

The different climate conditions also had effects on the milk yield and composition (fat, protein, lactose, and total solids) as shown in Table 2. The milk yield was significantly lower ($P < 0.05$) in the summer (15.8 ± 0.5 kg/d) compared to the winter (18.9 ± 0.4 kg/d). This reduced yield may have been due, at least in part, to the reduced energy intake and the heat stress observed during the summer season, similar to the results reported by others (Fuquay, 1981; Granzin, 2006, Rhone et al., 2008; West, 2003). In addition, the reduction in

milk yield agreed with the findings of West (2003), who has reported a reduction in the milk yield of 0.2 kg per unit increase in THI when the THI was above 72. Accordingly, in our study, the milk yield was 3.1 kg/d lower in the summer than in the winter; this reduction corresponded to the 16 THI units above 72 that were recorded during the summer (average 80 ± 5.1, range from 63 to 88). The percentages of fat, protein, lactose, and total solids in the milk were also lower during the summer season. This result may also have been attributable to the reduced feed intake induced by heat stress (Collier et al., 2006).

	Winter	Summer
Milk yield, kg/d	$18.9^{a} \pm 0.40$	$15.8^{b} \pm 0.50$
Milk fat, %	$2.69^{a} \pm 0.10$	$1.91^{b} \pm 0.06$
Milk protein, %	$3.45^{a} \pm 0.03$	$3.34^{b} \pm 0.03$
Lactose, %	$4.41^{a} \pm 0.02$	$4.30^{b} \pm 0.02$
Total solids, %	$11.5^{a} \pm 0.11$	$10.4^{b} \pm 0.09$

[a,b] Means within a row with different superscripts were significantly different ($P < 0.05$)

Table 2. Cow milk yield and composition during the summer and winter.

3.4. Fatty acid profile in milk

Table 3 shows the significance, determined by the analysis of variance, of the effects of the season, month in the season, parity, DIM, and milk yield on the content of CLA, individual fatty acids, SFA, MUFAs, PUFAs, and the desaturase index. Most of the individual fatty acids in milk and the sum of the saturated fatty acids, the sum of the monounsaturated fatty acids and the sum of the polyunsaturated fatty acids, the MUFA/SFA and PUFA/SFA ratios and desaturase index were affected ($P<0.05$) by the season. In contrast, only the C14:0, C16:0, and unsaturated fatty acids, such as C16:1, C18:1 t11, C18:2 and 9-*cis*, 11-*trans*-CLA, and the sum of PUFA and both ratios MUFA/SFA and PUFA/SFA were affected by parity. Only the season, month in the season, parity, and DIM had significant effects ($P < 0.001$) on the milk CLA content. In addition, the season, month in the season, and milk yield had significant effects ($P<0.05$) on the desaturase activity, as evaluated indirectly by the desaturase index for *cis*-9 14:1.

Table 4 shows the fatty acid profiles of the milk during both seasons. A low content of saturated fatty acids (C14:0, C16:0, and C18:0) and a low sum of saturated fatty acids were observed during the summer. In contrast, the sum of monounsaturated fatty acids, the sum of polyunsaturated fatty acids, 9-*cis*, 11-*trans*-CLA, and the MUFA/SFA and PUFA/SFA ratios were higher in the summer than in the winter. The milk fat samples obtained during the summer had higher contents of 9-*cis*, 11-*trans*-CLA ($P < 0.001$) than those obtained during the winter (9.36 ± 0.28 mg/g fatty acids vs. 7.03 ± 0.29 mg/g fatty acids, respectively). As mentioned above, this difference in the 9-*cis*, 11-*trans*-CLA content was attributable to the summer feed content of 18:2 and mammary gland precursors of CLA (*cis*-9 18:1 and 18:3).

Fatty acid	Model adjusted term					
	Season	Month (season)	Parity	DIM	DIM[2, 1]	Milk yield
C4:0	*	**	NS	NS	NS	NS
C6:0	**	**	NS	NS	NS	NS
C8:0	**	**	*	*	*	NS
C10:0	**	**	**	*	*	NS
C12:0	*	**	*	NS	NS	NS
C14:0	*	**	*	NS	NS	NS
C14:1	**	NS	NS	NS	NS	*
C15:0	NS	**	*	NS	NS	NS
C16:0	**	*	*	NS	NS	NS
C16:1	NS	0.064	*	*	*	NS
C17:0	NS	**	NS	NS	NS	NS
C18:0	*	*	NS	NS	NS	NS
C18:1, t11	*	**	*	NS	NS	NS
C18:1, c9	**	**	NS	NS	NS	NS
C18:2	**	**	*	NS	NS	*
C18:3	**	**	NS	NS	NS	NS
CLA[2]	**	**	*	**0.051**	**0.070**	NS
C20:0	*	**	NS	NS	NS	NS
Unknown	NS	NS	*	NS	NS	NS
∑ SFA[3]	**	**	NS	NS	NS	NS
∑ MUFA[4]	**	**	NS	NS	NS	NS
∑ PUFA[5]	**	**	*	NS	NS	*
MUFA/SFA ratio	**	**	**	NS	NS	NS
PUFA/SFA ratio	**	**	*	NS	NS	NS
Desaturase index						
cis-9 14:1	**	*	NS	NS	NS	*
cis-9 16:1	*	*	NS	*	*	NS
cis-9 18:1	**	**	NS	*	*	NS
cis-9, *trans*-11 CLA	**	**	NS	*	*	NS

[1]Quadratic term of days in milk; [2]9-*cis*, 11-*trans*-CLA; [3]Sum of saturated fatty acids; [4]Sum of monounsaturated fatty acids; [5]Sum of polyunsaturated fatty acids
*P < 0.05; **P < 0.001; NS = Not significant

Table 3. Significance of factors of the adjusted model on individual milk fatty acids.

Fatty acid	Winter		Summer		P
	Mean ± SE	CI (95%)	Mean ± SE	CI (95%)	
C4:0	1.80 ± 0.18	1.44 – 2.14	0.90 ± 0.12	0.67 – 1.13	*
C6:0	9.69 ± 0.25	9.19 – 10.18	6.45 ± 0.30	5.86 – 7.03	**
C8:0	10.36 ± 0.14	10.07 – 10.64	8.28 ± 0.18	7.90 – 8.64	**
C10:0	26.10 ± 0.40	25.30 – 26.90	21.21 ± 0.41	20.40 – 22.02	**
C12:0	31.70 ± 0.48	30.74 – 32.65	27.74 ± 0.45	26.85 – 28.63	*
C14:0	109.05 ± 0.97	107.13 – 110.96	100.53 ±1.01	98.51 – 102.54	*
C14:1	8.71 ± 0.21	8.29 – 9.13	11.83 ± 0.28	11.27 – 12.39	**
C15:0	11.53 ± 0.13	11.28 – 11.78	11.69 ± 0.19	11.30 – 12.08	NS
C16:0	332.31 ± 2.37	327.62 – 337.00	299.90 ± 2.73	294.54 – 305.37	**
C16:1	14.04 ± 0.30	13.45 – 14.63	15.97 ± 0.38	15.22 – 16.72	NS
C17:0	6.09 ± 0.10	5.88 – 6.30	5.54 ± 0.11	5.32 – 5.75	NS
C18:0	125.18 ± 1.63	121.90 – 128.42	105.86 ± 1.68	102.50 – 109.21	*
C18:1, t11	9.64 ± 0.23	9.17 – 10.11	8.18 ± 0.28	7.62 – 8.73	*
C18:1, *cis*-9	237.75 ± 2.50	232.80 – 242.70	288.23 ± 2.77	282.70 – 293.70	**
C18:2[1]	22.79 ± 0.29	22.21 – 23.37	39.59 ± 0.84	37.92 – 41.26	**
C18:3[2]	2.82 ± 0.08	2.65 – 2.98	4.37 ± 0.09	4.18 – 4.56	**
CLA[3]	**7.03 ± 0.29**	**6.46 – 7.59**	**9.36 ± 0.28**	**8.81 – 9.91**	**
C20:0	1.25 ± 0.07	1.10 – 1.39	0.68 ± 0.08	0.51 – 0.84	*
Others	32.62 ± 0.30	32.01 – 33.22	33.12 ± 0.63	31.86 – 34.38	NS
Σ SFA[4]	665 ± 28.8	659.8 – 670.3	588.8 ± 41.4	581.3 – 596.3	**
Σ MUFA[5]	270.1± 25.3	265.6 – 274.7	324.2 ± 31.2	318.5 – 329.9	**
Σ PUFA[6]	32.3 ± 4.0	31.5 – 33.0	53.8 ± 11.2	51.7 – 55.8	**
MUFA/SFA ratio	0.408±0.007	0.398- 0.419	0.557±0.007	0.539-0.574	**
PUFA/SFA ratio	0.048±0.001	0.047-0.05	0.092±0.001	0.088-0.097	**

[1]Linoleic acid; [2]Linolenic acid; [3]9-*cis*, 11-*trans*-CLA; [4]Sum of saturated fatty acids; [5]Sum of monounsaturated fatty acids; [6]Sum of polyunsaturated fatty acids; *P < 0.05; **P < 0.001; NS = Not significant; N = 240 cows (120 in winter and 120 in summer); ± Standard error

Table 4. Cow milk fat composition (mg/g fatty acids) by season.

These results show that the attributes of the milk were modified by the season, but this modification of the fatty profile and 9-*cis*, 11-*trans*-CLA content was mainly attributable to the diet because the summer diet (28% more of alfalfa) was richer in PUFA and CLA

precursors than the winter diet, promoting an increase in the *Butirivibrio fibrisolvens* isomerase activity and resulting in an increase in 9-*cis*, 11-*trans*-CLA production and MUFAs and PUFAs during the summer. The importance of manipulating the fatty acid composition in the milk, thus favouring a high content of unsaturated fatty that includes 9-*cis*, 11-*trans*-CLA, is due to the fact that their consumption can help to prevent chronic diseases in humans. A decrease in the SFA levels and/or a concomitant increase in the MUFA and PUFA contents of ruminant milk may confer benefits for human health and may provide a basis for marketing claims. The PUFA/SFA ratio in humans is an important risk factor for cardiovascular diseases (Sacks & Katana, 2002; Simopoulos, 1999), and thus the proportions of total unsaturated and PUFAs in regard to the total SFAs are relevant from a human health perspective. Indeed, the PUFA/SFA ratio has been used to calculate the risk factor of foods. According to Wood et al., (2008), the recommended value is at least 0.4, although, others have suggested that the minimum PUFA/SFA ratio is 0.12 (Hoffman, Muller, & Cloete, 2003). However, in our study, the PUFA/SFA ratios for the milk in both seasons (0.048 for winter and 0.092 for summer season) was lower than 0.12. It is important to note that there was no manipulation on the original formulation of the diets to modify the PUFA/SFA ratios obtained.

In contrast, it is well known that most CLA are produced in the mammary gland via Δ^9 desaturase. As shown in Table 5, the activity of this enzyme was higher in summer than in winter for the four substrates (C14:0, C16:0, C18:1, and *trans*-C18:1) of the desaturated products, C14:1, C16:1, *cis 9*-C18:1, and CLA, respectively. Because the C14:0 content in milk fat is produced via de novo synthesis in the mammary gland, desaturation is the only source of C14:1; therefore, the ratio of C14:1 to C14:0 is the best indicator of Δ^9desaturase activity (Lock & Garnsworthy, 2003). Our results showed that the desaturase index was lower for C14:1 and C16:1 compared to 18:1 and CLA. Kelsey et al. (2003) have reported a similar pattern in the desaturase index (0.06, 0.04, and 0.67 for C14:1, C16:1, and 18:1, respectively).

Desaturase index[1]	Winter		Summer		P
	Mean ± SE	CI (95%)	Mean ± SE[2]	CI (95%)	
cis-9 14:1	0.074 ± 0.001	0.071 – 0.077	0.104 ± 0.002	0.100 – 0.109	**
cis-9 16:1	0.040 ± 0.001	0.038 – 0.042	0.05 ± 0.001	0.048 – 0.052	**
cis-9 18:1	0.65 ± 0.002	0.65 – 0.66	0.73 ± 0.003	0.72 – 0.73	**
c-9, t-11 CLA	0.41 ± 0.007	0.39 – 0.42	0.55 ± 0.008	0.53 – 0.57	**

[1]Calculated as the Δ^9-desaturase product divided by the sum of the Δ^9-desaturase product and substrate. For example, the desaturase index for *cis* 9 16:1 would be (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0) . **P < 0.001; [2]Standard error

Table 5. Estimated Δ9-desaturase activity in milk fatty acids during the winter and summer.

Figure 3 shows the frequency distributions of the 9-*cis*, 11-*trans*-CLA content in milk obtained during the winter and summer. In the winter, the CLA content ranged from 3.49 to

10.45 mg/g fatty acids (with a mean value of 6.65 ± 1.4 mg/g fatty acids), with a peak frequency (59/120) within the range of 5.0 to 6.99 mg/g fatty acids. In the summer, the CLA content ranged from 4.03 to 17.86 mg/g fatty acids (with a mean value of 9.83 ± 2.58 mg/g fatty acids), with a peak frequency (40/120) within the range of 9.0 to 10.99 mg/g fatty acids. Additionally, in the summer, 34 out of 120 cows had CLA values above 10.99 mg/g fatty acids. There was a broad variation in the content of 9-*cis*, 11-*trans* CLA in milk within a season and among animals despite the same diet and environmental conditions. Similar results have been reported by other authors (Bell et al., 2006; Lawless et al., 1999; Stanton et al., 1997; Staszak, 2005;).

In the present study, parity affected the CLA content in milk fat as shown in Figure 4. For this analysis, dairy cows were grouped by parity status by season. In the summer, there were no differences in the 9-*cis*, 11-*trans*-CLA content between parity levels, and the range of CLA was from 9 to 10 mg/g fatty acids. In contrast, we found differences between the parity groups and the 9-*cis*, 11-*trans*-CLA content in milk during the winter where primiparous cows had a higher CLA content (6.96 ± 0.2 mg/g fatty acids, $P < 0.05$) compared to the milk from LM cows (6.17 ± 0.2 mg/g fatty acids), but the difference was not significantly greater than with the milk from EM cows (6.69 ± 0.25 mg/g fatty acids, $P > 0.05$). To date, studies have been scarce on the effect of parity on the CLA content in milk fat. Peng et al. (2008) have reported that the milk from primiparous yak cows had a lower CLA content than multiparous yak cows. These authors have also suggested that the differences found may be related to the stage of growth of the mammary glands, which are one of the sites of the synthesis of fatty acids in milk, or that the differences may be related to forage, as there were differences on forage intake in the Yaks of different parities. In contrast, Kelsey et al., (2003) have studied the effect of parity and did not find differences in the milk CLA content between primiparous and multiparous dairy cows. This discrepancy with respect to our results for the winter season led us to hypothesise that the differences in CLA may be due, at least in part, to the hierarchy of the cows within the paddock area. Multiparous cows are generally older, bigger, and heavier than primiparous cows, therefore, they are the first to attain feed (Phillips & Rind, 2002). This hierarchy may have resulted in the consumption by the multiparous cows of high amounts of concentrate, which provoked a decrease in the ruminal pH, which, in turn, induced an adverse environment for the *Butirivibrio fibrisolvens* bacteria in the rumen that are responsible for CLA production.

The relationships between the milk CLA content and DIM, milk fat, and milk yield are shown in Figure 5 (Panels A, B, and C, respectively). The DIM had an effect on the milk CLAs with an $R^2 = 0.338$, $P < 0.05$. In our study, the DIM explained a third (33.8%) of the variation in the CLA content. This result contrasted with previous reports that have shown that this factor explained less than 10% of the variation (Lock et al., 2005; Kelsey et al., 2003). The milk fat and milk yield did not have effects on the milk CLA content with $R^2 = 0.063$ and $R^2 = 0.017$, respectively; this result is in agreement with Kelsey et al., (2003).

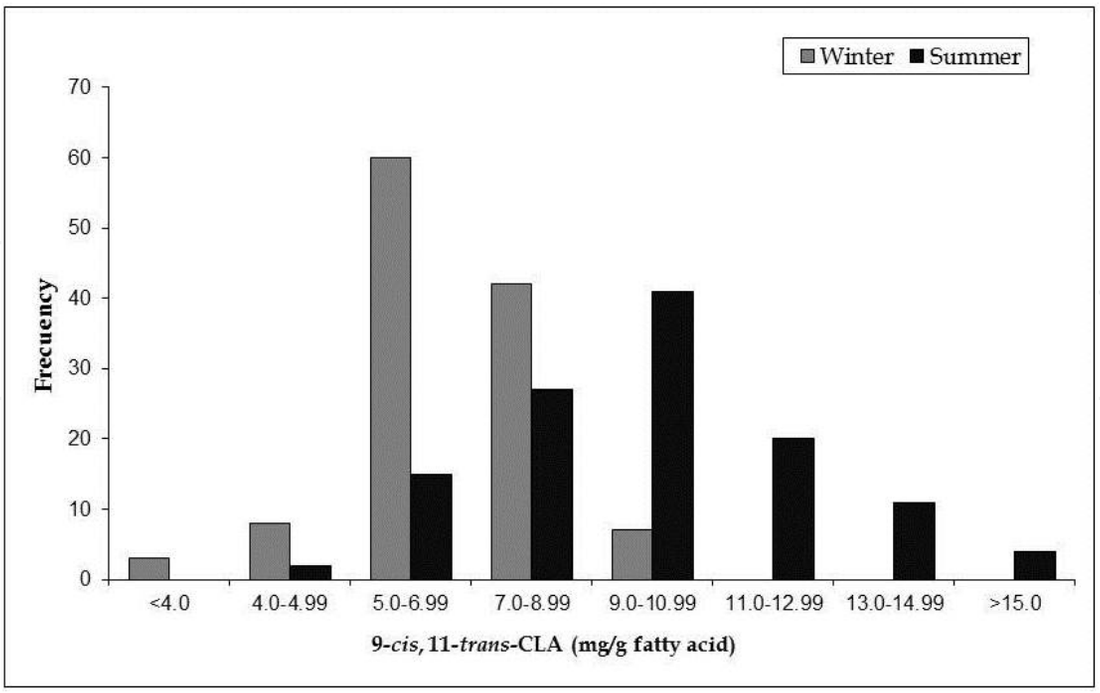


Figure 3. Frequency distribution for the 9-*cis*, 11-*trans*-CLA content in cow's milk collected during the winter and summer seasons.

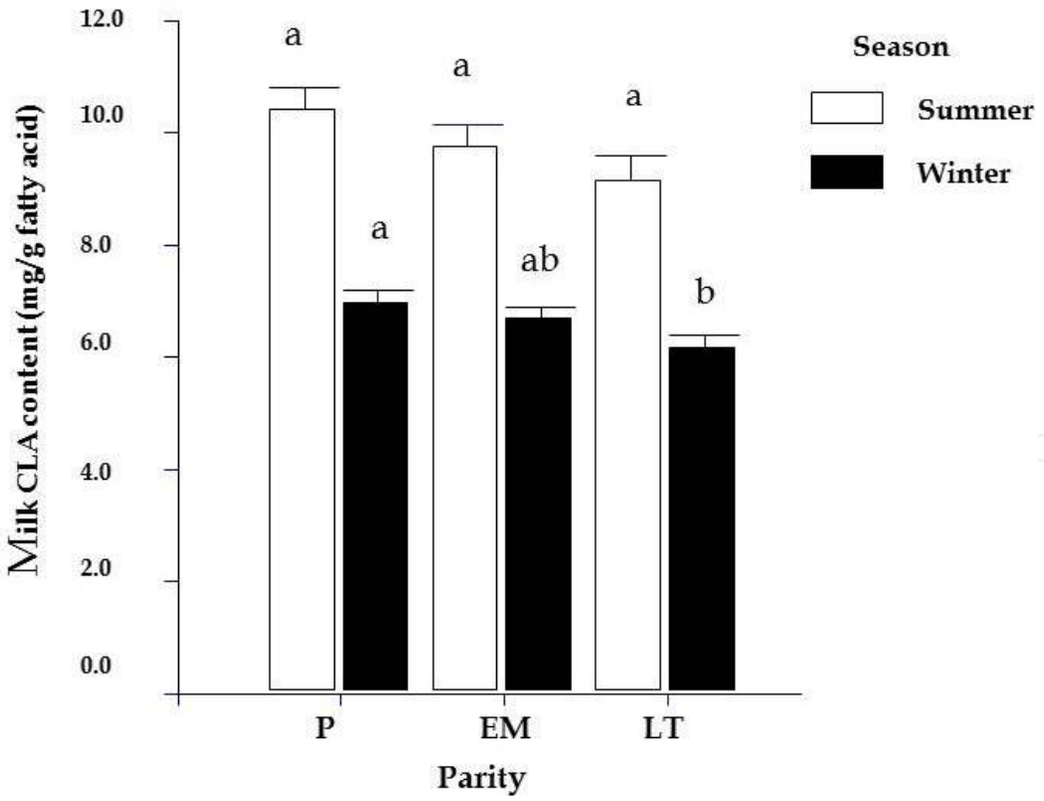
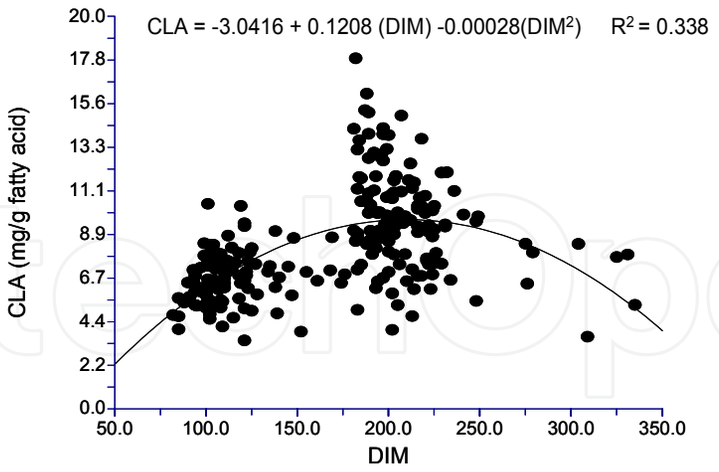
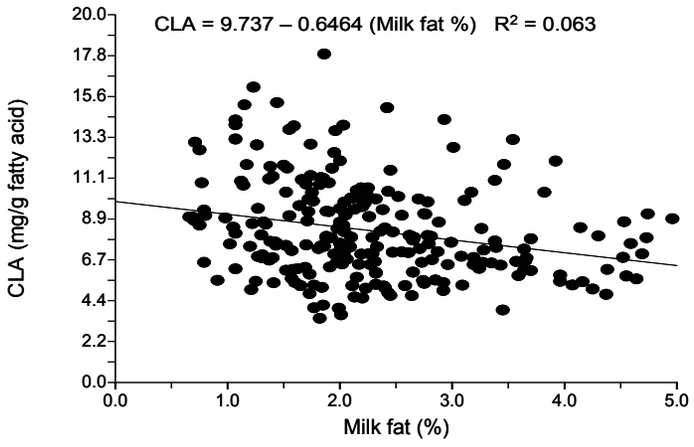


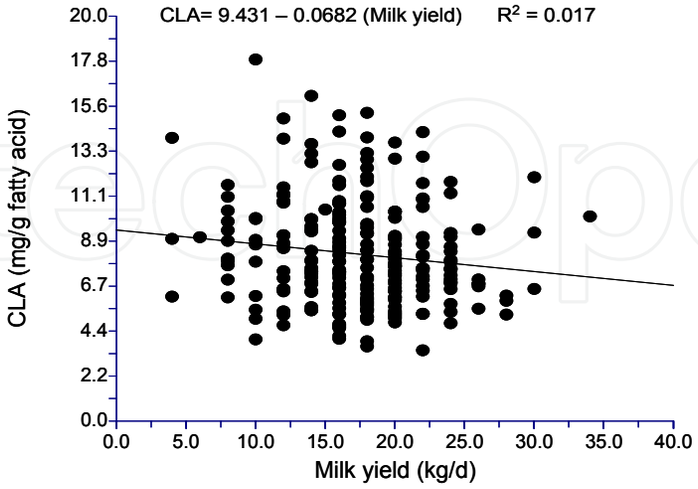
Figure 4. Milk CLA (9-*cis*, 11-*trans*) relative to parity by season. Bars with different superscripts (a, b, and ab) within the season indicate significant differences ($P < 0.05$). Primiparous (P): one parity; early multiparous (EM): dairy cows with two and three parities; late multiparous (LM): dairy cows with four to six parities.



A



B



C

Figure 5. Relationships between the milk CLA content and the **(A)** DIM, **(B)** milk fat, and **(C)** milk yield. Data points represent milk samples collected during both the winter and summer seasons from 240 dairy cows

4. Conclusions

We found that the practical management of the adjustment of the ingredients in the diet to maintain energy intake and avoid decreased milk yield during the summer (when temperatures may exceed 40 °C) was beneficial because this adjustment of the diet allowed an improvement in the unsaturated fatty acid profile and CLA content in the milk, despite the animals' experiencing heat stress. The alterations in the fatty acid profile and CLA content in milk suggest that this milk could be healthier for the consumer in the summer, due to the increased MUFA/SFA and PUFA/SFA ratios.

Author details

Alfonso Martínez-Borraz, Silvia Y. Moya-Camarena,
Humberto González-Rios, Jesús Hernández and Araceli Pinelli-Saavedra*
Centro de Investigación en Alimentación y Desarrollo, A.C., Hermosillo, Sonora, México

Acknowledgement

The authors thank Dr. Arturo Madrid Lopez, Nutritionist, for his advice and assistance. We also thank Ing. Roy Swanson, the manager of the dairy farm where the present study was conducted. We also thank Amparo Nieblas Almada, Ingrid Rebeca Esquerra Brauer and Francisco A. Vazquez Ortiz for their excellent technical assistance.

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* Corresponding Author

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