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Fatty Acid Composition of Yak (*Bos grunniens*) Cheese Including Conjugated Linoleic Acid and *trans*-18:1 Fatty Acids

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The esterified fatty acid composition of cheese (YC) from yak (Bos grunniens), reared in the highlands of the Nepalese Himalayas, was studied using capillary gas-liquid chromatography and compared with that of dairy cow Cheddar cheese (DC) purchased in a local market. The YC was collected from Dolakha, Nepal. The YC had a lower (P < 0.001) myristic acid (C14:0; 6.7 vs 10.3%, YC vs DC, respectively) and palmitic acid content (C16:0; 23.3 vs 29.2%, YC vs DC, respectively) compared to DC. The YC had a lower (P < 0.01) total medium-chain saturated fatty acids (C10:0-C16:0) content compared to DC (36.7 vs 47.3%, YC vs DC, respectively). On the other hand, the YC had a 24.8% higher (P < 0.01) level of total long-chain saturated fatty acids (C17:0–C26:0) and a 3.2 times higher (P < 0.001) content of total n-3 PUFA than DC. The ratio of n-3 PUFA to n-6 PUFA in YC was 0.87 compared to 0.20 in DC. YC had a 2.8 times higher (P < 0.001) total trans-18:1 (9.18 vs 3.31%, YC vs DC, respectively) content. The percentage of vaccenic acid (trans-11-C18:1) in YC was 4.6 times higher (6.23 vs 1.35% of total fatty acids, YC vs DC, respectively) than in DC. Vaccenic acid constituted 67.9% of total trans-C18:1 in YC. The Δ 9-desaturase index for YC was lower than that of DC. The total conjugated linoleic acid (CLA) content in YC was 2.3% of total fatty acids compared to 0.57% in DC. The cis-9, trans-11 CLA isomer in YC constituted 88.5% of the total CLA. The results suggest that cheese from yak, grazed on Himalayan alpine pastures, may have a more healthful fatty acid composition compared to cheese manufactured from dairy cattle fed grain-based diets.

KEYWORDS: Cheese; fatty acid composition; conjugated linoleic acid; trans-18:1 fatty acids; yak

INTRODUCTION

The yak is placed in the subfamily Bovine and belongs to the classification of *Bos grunniens* (1). Yak is one of a few domesticated animals that can survive in a cold and low-oxygen environment (2). They can survive at temperatures as low as -40 °C and at atmospheric pressure of 550 hPa (3). The total yak population in the world is estimated at around 14.2 million (4); they are mainly found in the highlands of the Nepalese Himalayas, Indian Kashmir, Tibet, Mongolia, and Bhutan (as cited by ref 3). Yak milk is a component of the diet in those areas and contains 16.9-17.7% dry matter (DM), 4.9-5.3%protein, 5.5-7.2% fat, 4.5-5.0% lactose, and 0.8-0.9% minerals (5). Nepal was the first country in the world to produce cheese from yak milk, and cheese production is viewed as a viable commercial enterprise (6) due to its high demand locally and market value, which can also be exported to other countries (as cited by ref 6). Yak cheese contains 46.8% butterfat on a DM basis (as cited by ref 6). Gross composition and chemical constituents of yak milk and cheese have been reported (7–10).

The nutritive value of dairy products is, in part, related to its fatty acid composition (11); however, little is known about the fatty acid (FA) composition of yak cheese including conjugated linoleic acid (CLA), *trans*-18:1, and odd- and branched-chain FA. Much attention has been directed toward CLA since the discovery of its anticarcinogenic properties almost three decades ago (12, 13). As the biomedical studies with CLA expanded, it became apparent that CLA had a range of positive health effects in experimental animal models, including beneficial effects on reducing body fat accretion, delaying the onset of type II diabetes, retarding the development of atherosclerosis, improving the mineralization of bone, and modulating the immune system (14). CLA are found naturally in ruminant food products due

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to the process of bacterial biohydrogenation of linoleic acid in the rumen (15-17).

Although the FA composition of cow (18), goat (19), and sheep (20, 21) cheese has been reported, there is no literature available for yak cheese. The present study was undertaken to investigate the FA composition of yak cheese (YC) including CLA, *trans*-18:1, and odd- and branched-chain FA and to compare this FA profile with that of dairy cow Cheddar cheese (DC).

MATERIALS AND METHODS

Samples. Yak cheese was collected from Dolakha, Nepal, and was produced by Jiri Yak Cheese Factory located at Cherdung at an altitude of 2600 m. Thapa (22) has described the YC-making procedures used by the Nepalese manufacturers. Dairy cow (Holstein) Cheddar cheese (Loblaws Inc., Toronto, Canada) was sourced commercially from Guelph, ON, Canada. The result of analyses was given as the mean of four samples from each variety. The yak milk was obtained from yak grazed in a community forest area that falls under lower and upper temperate broadleaf and coniferous forest at an altitude of between 1800 and 3700 m. These areas are rich in shrubs and herbs, many of which are known for medicinal, aromatic, and nutritive properties. Some of these plants include Arundinaria spp., Daphne bholua, Edgeworthia gardnerii, Gaultheria fragrantissima, Girardiana diversifolia, Swertia chirayita, Paris polyphylla, Rheum australe, Valeriana jatamansi, Rhododendron anthopogon, Berginia ciliata, Rubia manjith, Cordyceps sinensis, and Buki grass (Kobresia spp.). According to the herders, the major species that yak prefer to eat are Buki grass (Kobresia spp.), Quercus spp., G. diversifolia, Taxus baccata, R. anthopogon, Rhododendron arboretum, C. sinensis, Arundinaria spp., S. chiraiyita, R. australe, and R. manjith. Seasonally, the biomass of sward ranges from <2.9 to 11.4% for crude protein and from 2.4 to 4.9% for crude fat (23). The main forage species grazed by yak in this region have been described by Pradhan (24). In contrast, dairy cows in southwestern Ontario are fed corn silage/alfalfa haylage based diets supplemented with mixed grains or corn, protein sources, minerals, and vitamins.

Chemical Analysis. Cheese samples were analyzed for DM by ovendrying at 60 °C for 48 h [method 930.15 (25)], and chemical composition was determined in triplicate at a commercial laboratory (Agri-Food Laboratories, Guelph, ON, Canada). The samples were analyzed for CP using a LECO FP 428 nitrogen analyzer [LECO Corp., St. Joseph, MI; method 4.2.08 (25)], ether extract [method 920.39 (25)], and total ash [method 942.05 (25)].

Analysis of Fatty Acid. Total cheese fat was extracted according to the method by Bligh and Dyer (26) with minor modifications. Ten milligrams of cheese plus 0.90 mL of water with 2.5 mL of methanol and 1.25 mL of chloroform were mixed into a 15 mL culture tube with a screw-cap Teflon lining. The content of the culture tube was kept for 60 min at room temperature and vortexed frequently during the period. After 1 h, 1.25 mL of chloroform, 1.15 mL of water, and 0.1 mL of 3 M HCl were added, vortex-mixed, and centrifuged. The acid (i.e., 3 M HCl) was added to ensure the pH of the extract was acidic. The chloroform layer (bottom phase) containing fat was removed using two Pasteur pipets, one inserted into another. The methanol/water phase was extracted with an additional 1.25 mL of chloroform, and the chloroform phases were combined, dried over anhydrous Na₂SO₄, filtered, and then transferred into a 4 mL vial. Chloroform was removed from the vial under a stream of N₂, and three drops of benzene were added and mixed. The fat content (esterified FA) in the vial was methylated using NaOCH3 as catalyst (27) as follows (28): 1.5 mL of hexane and 50 μ L of methyl acetate were added into the vial one after another and mixed. Later, 100 µL of NaOCH₃ (0.5 M solution in methanol, Sigma-Aldrich, St. Louis, MO) was added for methylation. The vial was heated at 50 °C for 15 min. After cooling, 100 µL of oxalic acid (10% solution in diethyl ether) was added into the vial and vortexed, and then 0.4 mL of water was added and centrifuged to settle a sodium oxalate precipitate. The upper portion containing esterified fatty acid methyl esters (i.e., hexane layer; FAME) was transferred into another vial, and the volume was reduced using a stream of N2 to

 Table 1. Chemical Composition of Yak and Dairy Cow Cheddar Cheese

 (YC and DC, Respectively; Percentage of As-Is Basis)

parameter ^a	YC	DC	SEM ^b	P value
dry matter	62.7	63.6	0.4	0.16
crude protein	26.1	20.6	0.8	0.008
total ash	3.91	3.72	0.04	0.10
fat	26.2	33.5	0.1	<0.001

^a Means of four samples per treatment. ^b Standard error of the mean.

500–600 μ L. The hexane containing FAME was analyzed by gas–liquid chromatography (GLC) as described by Odongo et al. (28). For the determination of individual isomers of 18:1 FA (6*t*–16*t*), original samples of FAME were further diluted by hexane (28).

Statistical Analysis. The data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (v. 9.1; SAS Institute Inc., Cary, NC) using the model $Yj = \mu + \beta j + \epsilon j$, where μ = overall mean, β = effect of treatment (j = YC or DC), and ϵj = random residual error. Effects were considered to be significant at a probability of P < 0.05.

RESULTS AND DISCUSSION

Cheese Composition. The protein percentage in YC was higher (P < 0.008) than that of DC (**Table 1**). Yak cheese had a lower (P < 0.001) fat content than DC. The total ash percentages in YC and DC were not different (P = 0.10). There were no differences (P = 0.16) in the DM contents.

Fatty Acid Composition. In the present study, NaOCH₃ was used for the trans-methylation of total extracted lipids. Generally, bovine milk contains a negligible amount of free FA [0.28% of total lipids (29)], and NaOCH₃ does not methylate free FA to FAME (27). The percentage of individual FA, in this study, was calculated relative to total esterified FA.

The esterified individual FA compositions of YC and DC cheese varieties are shown in Tables 2-4. The saturated FA were grouped in three classes: total short-chain saturated fatty acids (SC_SFA, C6:0-C8:0), total medium-chain saturated fatty acids (MC_SFA, C10:0-C16:0), and total long-chain saturated fatty acids (LC_SFA, C17:0-C26:0). Total saturated fatty acids (total SFA, Table 2) in YC were 8.6% lower (P < 0.02) than in DC. Caprylic acid (C8:0), among SC_SFA, and capric acid (C10:0), among MC_SFA, were significantly lower (P < 0.004) in YC than in DC (0.56 and 1.68% vs 0.69 and 2.4%, YC vs DC, respectively). Dairy products from goats have high contents of these two FA (2.7% of total FA for 8:0 and 9.9% for 10:0) as reported by Alonso et al. (30). The percentage of lauric acid (C12:0) was 1.8 times lower (P < 0.001) in YC than in DC. The percentages of myristic acid (C14:0) and palmitic acid (C16:0) were 35.2 and 20.2%, respectively, lower (P < 0.001) in YC than in DC. Although stearic acid (C18:0) content in YC was numerically 23.8% higher than that in DC, the difference was not significant. The major saturated FA in yak butter were C16:0, C18:0, and C14:0 as reported by Neupaney et al. (31). A similar pattern was also found in the present study. All of the FA, from C6:0 to C14:0 and predominantly C16:0, are regarded as products of de novo synthesis within the mammary gland of the ruminants, and acetate and β -hydroxybutyrate are believed to be the precursors for de novo FA synthesis in mammary tissue (32). Total LC_SFA was 32.3% higher (P < 0.01) in YC than in DC (20.5 vs 15.5%, YC vs DC, respectively) and total MC_SFA was 22.4% lower (P < 0.01) in YC than DC (36.7 vs 47.3% YC vs DC, respectively). However, percentage of total SC_SFA did not differ (P =(0.12) between the treatments.

 Table 2. Fatty Acid Composition (Percentage of Total FAME on a Weight

 Percent Basis) of Yak and Dairy Cow Cheddar Cheese (YC and DC,

 Respectively)

fatty acida	YC	DC	SEM ^b	P value
C8:0	1.67 0.56	1.72 0.69	0.05 0.02	0.52 0.004
C10:0	1.68	2.40	0.02	<0.004
C11:0	0.148	0.273	0.003	< 0.001
C12:0	1.53	2.81	0.04	<0.001
anteiso-C13:0	0.031	0.057	0.002	<0.001
C12:1	0.07	0.08	0.01	0.56
C13:0	0.033	0.075	0.001	<0.001
<i>iso</i> -C14:0	0.21	0.12	0.01	0.008
C14:0 <i>iso</i> -C15:0	6.7 0.37	10.3 0.20	0.3 0.02	<0.001 0.002
anteiso -C15:0	0.37	0.20	0.02	< 0.002
<i>cis</i> -9-C14:1	0.31	0.69	0.02	<0.001
C15:0	1.78	1.15	0.04	< 0.001
iso-C16:0	0.29	0.27	0.01	0.61
C16:0	23.3	29.2	0.5	0.001
<i>iso</i> -C17:0	0.43	0.27	0.01	0.001
trans-9-C16:1	0.35	0.08	0.01	<0.001
anteiso-C17:0	1.11	0.45	0.04	< 0.001
<i>cis</i> -9-C16:1	0.89	1.27	0.04	0.003
C17:0 C18:0	0.81 17.2	0.51 13.9	0.04 0.7	0.006 0.06
C19:0	0.092	0.033	0.003	<0.001
trans-11, cis-15-C18:2	0.93	0.06	0.000	<0.001
C18:2n6	2.1	2.8	0.2	0.11
C20:0	0.35	0.16	0.02	0.002
C18:3n6	0.033	0.022	0.006	0.004
<i>cis</i> -9-C20:1	0.154	0.113	0.004	0.001
<i>cis</i> -11-C20:1	0.072	0.051	0.003	0.007
C18:3n3	1.68	0.49	0.04	< 0.001
C21:0	0.157	0.031	0.002	< 0.001
C20:2n6 C22:0	0.053 0.183	0.029 0.048	0.003 0.007	0.01 <0.001
C20:3n6	0.043	0.102	0.007	<0.001
<i>cis</i> -13-C22:1	0.0093	0.0057	0.0004	0.01
C20:3n3	0.0158	0.0071	0.0007	< 0.001
C20:4n6	0.122	0.153	0.007	0.03
C23:0	0.102	0.019	0.005	<0.001
C20:4n3	0.042	0.031	0.002	0.04
C22:2n6	0.021	0.015	0.001	0.04
C24:0	0.104	0.033	0.002	< 0.001
C20:5n3 <i>cis</i> -15-C24:1	0.068 0.0047	0.041 0.0028	0.004 0.0003	0.003 0.02
C22:4n6	0.0047	0.0028	0.0003	0.02
C26:0	0.022	0.010	0.003	0.38
C22:5n3	0.141	0.048	0.004	< 0.001
C22:6n3	0.023	0.006	0.001	0.002
others ^c	0.60	0.34	0.03	0.002
total CLA ^d	2 07	0.67	0.06	-0.001
total SFA ^e	2.27 59.5	0.57 65.1	0.06 1.1	<0.001 0.02
total SC_SFA (C6:0-C8:0) ^f	2.23	2.41	0.07	0.12
total MC_SFA (C10:0-C16:0) ^g	36.7	47.3	0.7	0.01
total LC_SFA (C17:0-C26:0) ^h	20.5	15.5	0.5	0.01
total MUFA ⁱ	32.4	30.1	0.7	0.07
total n-6 PUFA	2.4	3.2	0.2	0.12
total n-3 PUFA ^k	2.11	0.66	0.04	<0.001
n-3:n-6 PUFA	0.87	0.20	0.03	< 0.001
total trans-18:1	9.2	3.3	0.2	< 0.001
total <i>cis</i> -18:1 total odd and branched FA	21.3 6.1	24.5 3.8	0.9 0.1	0.06 <0.001
	0.1	0.0	0.1	<0.001

^a Means of four samples per treatment. ^b Standard error of the mean. ^c Unidentified fatty acids. ^d Total CLA: *cis*-9, *trans*-11-C18:2; *trans*-9, *cis*-11-C18:2; *trans*-10, *cis*-12-C18:2; *trans*-11, *trans*-13-C18:2; and *trans*-9, *trans*-11-C18:2 + *trans*-10, *trans*-12-C18:2. ^e Total SFA: all saturated fatty acids (without any double bonds, C6:0-C26:0). ^f Total SC_SFA: short-chain SFA (C6:0-C8:0). ^g Total MC_SFA: medium-chain SFA (C10:0-C16:0). ^h Total LC_SFA: long-chain SFA (C17:0-C26:0). ⁱ Total MUFA: all fatty acids with a single double bond (C12:1-C24:1). ^j Total n-6 PUFA: C18:2n6; C18:3n6; C20:2n6; C20:3n6; C20:4n6; C22:2n6, and C22:4n6. ^k Total n-3 PUFA: C18:3n3; C20:3n3; C20:4n3; C20:5n3, C22:5n3, and C22:6n3.

Table 3. 18-Monoene Composition (Percentage of Total FAME on aWeight Percent Basis) of Yak and Dairy Cow Cheddar Cheese (YC andDC, Respectively)

fatty acid ^a	YC	DC	SEM ^b	P value
trans-4-C18:1	0.039	0.021	0.004	0.05
trans-5-C18:1	0.022	0.024	0.002	0.85
trans-6-8-C18:1	0.38	0.29	0.02	0.04
trans-9-C18:1	0.54	0.42	0.02	0.01
trans-10-C18:1	0.58	0.46	0.03	0.04
trans-11-C18:1	6.23	1.35	0.09	< 0.001
trans-12-C18:1	0.91	0.51	0.03	< 0.001
<i>cis</i> -9-C18:1	19.8	22.5	0.9	0.13
<i>cis</i> -11-C18:1	1.32	1.55	0.05	0.03
<i>cis</i> -12-C18:1	0.21	0.44	0.04	0.01
<i>cis</i> -13–18:1	0.038	0.043	0.004	0.35
trans-16-C18:1	0.46	0.23	0.02	0.002

^a Means of four samples per treatment. ^b Standard error of the mean.

 Table 4. Conjugated Linoleic Acid Composition (Percentage of Total FAME on a Weight Percent Basis) of Yak and Dairy Cow Cheddar Cheese (YC and DC, Respectively)

fatty acid ^a	YC	DC	SEM ^b	P value
cis-9, trans-11 CLA	2.01	0.48	0.07	<0.001
trans-9, cis-11 CLA	0.13	0.03	0.01	0.004
trans-10, cis-12 CLA	0.038	0.009	0.001	<0.001
trans-11, trans-13 CLA	0.039	0.018	0.001	< 0.001
trans-9, trans-11 CLA + trans-10, trans-12 CLA	0.057	0.031	0.003	0.003

^a Means of four samples per treatment. ^b Standard error of the mean.

Yak cheese had 59% higher (P < 0.001) total odd- and branched-chain FA content than DC (**Table 2**). Microbes synthesize a significant amount of odd- and branched-chain FA in rumen, which are transferred into milk and body fat (33). The content of these FA in ruminant animal products, such as cheese, therefore, is considered a reflection of microbial activities in the rumen. Bas et al. (34) and Vlaeminck et al. (35) reported that the odd- and branched-chain FA content in mixed rumen bacteria changed relative to diet.

The percentages of odd-chain saturated FA, such as C11:0 and C13:0, were lower (P < 0.001) in YC than in DC, whereas C15:0, C17:0, C19:0, C21:0, and C23:0 were higher (P < 0.01) in YC (**Table 2**). The contents of branched-chain saturated FA, such as *iso*-C14:0, *iso*-C15:0, *iso*-C17:0, *anteiso*-C15:0, and *anteiso*-C17:0, were higher (P < 0.008) in YC than in DC, excluding *iso*-C16:0, which was not different (P = 0.61) and *anteiso*-C13:0, which was lower (P < 0.001) in YC compared to DC. The YC contained 2.5 times higher (P < 0.001) *anteiso*-C17:0 (1.11% of total FA vs 0.45% in DC). This observation suggests that yak grazing natural pasture might have a higher protozoal activity in the rumen. Or-Rashid et al. (*36*) reported that rumen protozoa had 2.1 times higher *anteiso*-C17:0 content than bacteria and proposed *anteiso*-C17:0 as a marker to quantify protozoal biomass.

There were no differences (P > 0.05) in either total MUFA and n-6 PUFA contents (**Table 2**) or *cis*-9-C18:1 (**Table 3**, P > 0.05) and C18:2n6 (**Table 2**, P > 0.05) contents between YC and DC cheeses. Conversely YC had 3.2 times higher percentage of total n-3 PUFA compared to DC (**Table 2**, P <0.001). The α -linolenic acid (C18:3n3) made up the majority of total n-3 FA measured, and its content was higher (P < 0.001) in YC (1.68 vs 0.49%, YC vs DC, respectively). The high α -linolenic acid content in YC indicates that pasture-based yak diet contained higher levels of this n-3 FA than grain-based diets fed to the dairy cows. The YC contained also a significant

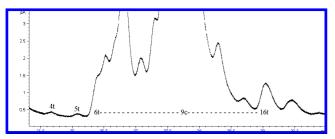


Figure 1. Partial gas chromatographic profile of 18:1 region showing the separation of the C18:1 FAME isomers from dairy cow Cheddar cheese. The GLC temperature program from 45 to 215 $^{\circ}$ C was used.

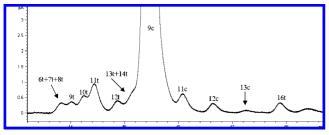


Figure 2. Partial gas chromatographic profile of 18:1 region showing the separation of the C18:1 FAME isomers from dairy cow Cheddar cheese, which was further diluted for the determination of individual isomers (6*t*–16*t*). The GLC temperature program from 45 to 218 °C was used.

percentage of *trans*-11, *cis*-15-C18:2 FA (0.93 vs 0.06%, YC vs DC, respectively), which flows from the rumen to the lower gut in the case of yak, being produced during biohydrogenation of α -linolenic acid (37). Eicosapentaenoic acid (C20:5n3) and docosahexaenoic (C22:6n3) were also present in a higher percentage of total FA in YC than in DC (**Table 2**, *P* < 0.003), and both come from α -linolenic acid metabolism in animal tissues. The ratio of n-3 PUFA to n-6 PUFA in YC was higher (*P* < 0.001) than in DC (0.87 vs 0.20, YC vs DC, respectively). This ratio should be at least 0.25 in fatty food for adults (38). Consequently, YC could be classed as a more healthful food, and its incorporation in human diets could increase the ratio n-3/n-6 PUFA to a more desirable level.

The profiles of 18:1 isomers are illustrated in **Figures 1** and **2**. It was not possible to achieve a complete separation of the cis and trans isomers of 18:1 through a single chromatographic run (**Figure 1**), while other FA were analyzed. Therefore, the FAME sample was further diluted and analyzed under the separate temperature program [see Odongo et al. (28)] as shown in **Figure 2**. The GLC chromatogram (**Figure 2**) shows that the *trans*-6-, *trans*-7-, and *trans*-8-C18:1 isomers and the *trans*-13- and *trans*-14-C18:1 isomers remained unresolved as single peaks, but the major isomers of interest (e.g., *trans*-10 and *trans*-11) were separated.

Among the MUFA, oleic acid (*cis*-9-C18:1) content was highest in both cheeses (**Table 3**). Oleic acid was also the most abundant among the FA of both cheeses. Sheng et al. (*10*) reported the content of oleic acid of yak milk (18.9% of total FA), which was comparable with the present data (**Table 3**). The contents of other *cis*-18:1 fatty acids, such as *cis*-11, *cis*-12, were lower (P < 0.03) in YC than in DC, except *cis*-13, which did not differ significantly between YC and DC. Overall, there was no difference (P = 0.06) in YC and DC percentage of total *cis*-C18:1 (**Table 2**). On the other hand, the level of total *trans*-C18:1 in YC was 2.8 times higher (P < 0.001) than in DC (9.18 vs 3.31%, YC vs DC, respectively; **Table 2**). There is no information in the literature on YC (or any other yak milk products) *trans*-18:1 content. A value of 2.68% for *trans*-C18:1

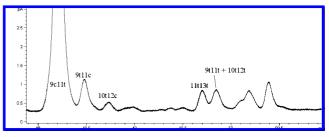


Figure 3. Partial gas chromatographic profile of CLA region showing the separation of the CLA FAME isomers from yak cheese. The GLC temperature program from 45 to 215 °C was used.

content in goat cheese was reported by Wolf (39), which was 3.4 times lower than that of YC in our study. Jensen (29) reported the minimal and maximal range (2.65-5.53%) for the trans-C18:1 FA content in cow milk fat from different countries. Vaccenic acid (VA; trans-11-C18:1) was found to be the most abundant component, being 67.9% of total trans-C18:1 in YC, and was 4.6 times higher (P < 0.001) in YC than DC (6.23 vs 1.35% of total FA, YC vs DC, respectively). Mammals, including humans (40), are capable of converting trans-11-C18:1 into cis-9, trans-11 CLA by the Δ 9-desaturase enzyme. An increase in milk VA as well as an increase in milk CLA is desirable (41). The percentage of *trans*-10-C18:1 was higher (P < 0.04) in YC than in DC (0.58 vs 0.46%, respectively). Griinari et al. (42) showed that milk fat depression was associated with an increase in the milk fat content of the trans-10-C18:1 isomer (2.9% of total FA), but milk fat percent was not depressed when the range of trans-10-C18:1 was much lower (0.33-0.70% of total FA). If the cows' diets are high in oils, the level of trans-10-C18:1 in milk fat may increase in comparison to trans-11-C18:1 because the oils induce a shift in the ruminal biohydrogenation process, resulting in production of higher amounts of *trans*-10-C18:1 in the rumen (43). In the present study, the trans-10-C18:1 content was 10.7 times lower than trans-11-C18:1 in YC and 3.0 times lower in DC. In addition, yak are generally grazed on natural swards within the Himalayas (3). The biomass of these swards may contain crude fat from 2.4 to 4.9% [on dry matter basis (23)], of which the major lipid class is glycolipids (44). On the other hand, dairy cows in southwestern Ontario are fed corn silage/alfalfa haylage based diets supplemented with a concentrate mixture and may contain 3.0-6.4% crude fat [on a dry matter basis (28, 45)], but the major lipid class of concentrates is triglycerides (44).

The partial gas chromatographic profile of the CLA region is shown in Figure 3. The biggest peak in this region was cis-9, trans-11-C18:2, and the trans-9, trans-11-C18:2 and trans-10, trans-12-C18:2 isomers remained unresolved as a single peak (Figure 3). It is surprising that the total CLA content in YC was 2.3% of total FA (Table 2); the cis-9, trans-11 CLA was the major isomer within the total CLA (88.5% of total CLA). Dairy cow milk products generally contained $\sim 0.6\%$ total CLA of total FA, with the *cis*-9, *trans*-11 isomer representing \sim 80% of the total CLA (46). This level can be increased severalfold with the supply of CLAenhancing feeds, but milk fat percentage drops significantly in most cases (47). In the case of yaks, Himalayan mountain pasture is a natural feed source to enhance the level of CLA in cheese. Collomb et al. (48) reported that milk from cows grazed on mountain and highland ranges contained a higher CLA content (1.86–2.87% of total FA), compared with milk obtained from cows grazing lowland areas (0.87% of total FA). These authors reported also that these elevated CLA contents are correlated with the nonleguminous herbal dicotyledonous species, particularly Compositae, Rosaceae, and Plantaginaceae, often found in mountain and highland swards.

 Table 5. Desaturase Index^a

fatty acid ratio ^b	YC	DC	SEM ^c	P value
<i>cis</i> -9-C14:1/C14:0	0.046	0.067	0.002	0.002
<i>cis</i> -9-C16:1/C16:0 <i>cis</i> -9-C18:1/C18:0	0.038 1.15	0.044 1.63	0.001 0.08	0.01 0.04
cis-9, trans-11-C18:2/trans-11-C18:1	0.32	0.35	0.02	0.36

^{*a*} Values represent the ratio of product/substrate for Δ 9-desaturase. The Δ 9-desaturase index serves as a proxy for Δ 9-desaturase activity and/or expression. ^{*b*} Means of four samples per treatment. ^{*c*} Standard error of the mean.

In the present study, YC contained 4.2 times higher (P < 0.001) cis-9, trans-11-CLA compared to DC (2.01 and 0.5% of total FA, respectively). Neupaney et al. (3) found cis-9, trans-11 CLA constituted 2.25% of total FA in yak butter. In ruminants, cis-9, trans-11 CLA is produced by microbial isomerization of linoleic acid (C18:2n6) in rumen, absorbed from the digestive tract, and transferred to milk fat later (49). The majority of milk fat cis-9, trans-11 CLA (78.0-93.0% of total CLA) is derived by endogenous synthesis from Δ 9-desaturation of *trans*-11-C18:1, an intermediate in the rumen biohydrogenation of linoleic and linolenic acids, in tissues of the host animals (50, 51). The trans, -10, cis12-CLA isomer was present in small amounts in both cheeses with a higher (P < 0.001) proportion in YC. Only two of the isomers, cis-9, trans-11 CLA and trans-10, cis-12 CLA, are shown to have biological activity (52). The most important natural source of CLA for human is dairy products, which contain predominantly *cis*-9, trans-11 CLA (53). This isomer is the most biologically active, and some of the biological effects include lowering the plasma low-density lipoprotein/high-density lipoprotein (LDL/HDL) ratio and the total cholesterol/HDL ratio (54), modulating the immune system (55), or anticarcinogenic activity (56). Some other minor isomers were also detected, such as trans-9, cis-11; trans-11, trans-13; and trans-9, trans- 11 + trans-10, trans-12 in both YC and DC cheeses.

The Δ 9-desaturase enzyme activity is important in the maintenance of the fluidity of cellular membranes and milk fat (57, 58). There are four fatty acid pairs in milk fat, and ratios for these pairs of FA represent a proxy for $\Delta 9$ -desaturase activity (59). Peterson et al. (60) suggested that the ratio of myristoleic acid to myristic acid (cis-9-C14:1/C14:0) might represent the best proxy for Δ 9desaturase activity in the mammary gland, because myristic acid originates almost exclusively via de novo synthesis within the mammary gland, and, as a consequence, all of the myristoleic acid present in milk fat would also be synthesized in the mammary gland by $\Delta 9$ -desaturase. The ratios of *cis*-9-C14:1 to C14:0, of cis-9-C16:1 to C16:0, and of cis-9-C18:1 to C18:0 were lower (P < 0.002) in YC than in DC, suggesting that yak might have lower levels of $\Delta 9$ -desaturase activity in the tissues. Explanation of this observation can be found in the different contents of n-3 PUFA in the two studied cheeses (YC and DC) from yak and from cow, respectively, and consequently in the milk produced by the two animal species. The n-3 PUFA were higher in YC than in DC, and high levels of these polyunsaturated FA are able to inhibit the Δ 9-desaturase activity in the mammary gland (61). Cabiddu et al. (62) found that Δ 9-desaturase activity (*cis*-9-C14:1/C14:0) was lower in the milk of Sarda sheep as compared with results from previous studies on dairy cattle fed on pasture. This supports the hypothesis of a species-specific regulation of Δ 9-desaturase activity, as suggested by Jahreis et al. (63). The Δ 9-desaturase index was not different (P = 0.36) for the pair of *cis*-9, *trans*-11 CLA/*trans*-11-C18:1 in YC and DC cheeses (Table 5). It is generally accepted that trans-11-C18:1 is the main source in the mammary gland for the synthesis of cis-9, trans-11 CLA, and higher cis-9, trans-11 CLA is positively correlated with higher level of trans-11-C18:1

(64). Therefore, an increased index of Δ 9-desaturase activity could be found in milk fat (64). In the present comparative study, the Δ 9-desaturase index in YC was lower than that of DC (**Table 5**), although the content of *cis*-9, *trans*-11 CLA was 4.2 times higher (**Table 4**), which appeared to suggest that a high percentage of *cis*-9, *trans*-11 CLA might originate directly from the rumen of yak.

The dietary allowance of CLA needed to inhibit carcinogenesis in humans is still unknown. However, Baer et al. (32) extrapolated from animal studies the amount of CLA that might contribute to significantly reduce the incidence of certain forms of cancer in humans to be 1.33 g/day from dairy products with 90% *cis-9*, *trans-11* CLA of total CLA. In the present study, the percentages of *cis-9*, *trans-11* CLA and *trans-11-C18:1* of total FA in YC were 2.0 and 6.23%, respectively (**Tables 3** and **4**). Assuming a 50% conversion rate of *trans-11-C18:1* to *cis-9*, *trans-11* CLA in the human body (32, 65), 100 g of YC within the diet would supply an efficacious quantity of *cis-9*, *trans-11* CLA (ca. 1.33 g, including the amount obtained from the conversion of *trans-11-C18:1*).

In summary, the composition of FA in YC was very different from that in DC. When compared with DC, the content of n-3 PUFA in YC was extremely high (3.2 times). The ratio of n-3 PUFA and n-6 PUFA in YC was 0.87, and the value for DC was 0.20. According to the set value of this ratio [>0.25; (38)], YC could be classified as a healthy food in human diets. The most notable fatty acid increases, in YC compared to DC, concerned *cis*-9, *trans*-11 CLA, a proven anticarcinogen, and trans-11-C18:1. The amounts of cis-9, trans-11 CLA and trans-11-C18:1 in YC were 4.2 and 4.6 times higher, respectively, than those in DC. However, the decreased $\Delta 9$ -desaturase index in YC compared to DC appeared to suggest that more *cis*-9, trans-11 CLA might have originated directly from the rumen. On the basis of animal trial data extrapolation, 100 g of YC in the human diet might be enough to supply the necessary amounts of cis-9, trans-11 CLA and trans-11 C18:1 to promote health. Our results suggest that cheese from yak, grazed on Himalayan alpine pastures, might have a more healthful fatty acid composition compared to cheese manufactured from dairy cattle fed grain-based diets. Further work needs to consider the effect of cheese from yak on human health.

LITERATURE CITED

- Silk, T. M.; Guo, M.; Haenlein, G. F. W.; Park, Y. W. Yak milk. In <u>Handbook of Milk of Non-Bovine Mammals.</u>; Park, Y. W., Haenlein, G. F. W., Eds.; Blackwell Publishing: Ames, IA, 2006; pp 345–353.
- (2) Palmieri, P. Yak. In *The Cambridge World History of Food*; Kiple, K. F., Ornelas, K. C., Eds.; Cambridge University Press: Cambridge, U.K., 2000; pp 607–615.
- (3) Neupaney, D.; Kim, J.-B.; Ishioroshi, M.; Samejima, K. Study on some functional and compositional properties of yak butter lipid. <u>Anim. Sci. J.</u> 2003, 74, 391–397.
- (4) Origins, Domestication and Distribution of Yak. *The Yak*, 2nd ed.; Wiener, G. H., Jianlin, H., Ruijun, L., Eds.; RAP Publication; FAO (Food and Agricultural Organization of the United Nations) Regional Office for Asia and the Pacific: Bangkok, Thailand, 2003; pp 1–16.
- (5) Production characteristics of yak. *The Yak*, 2nd ed.; Wiener, G. H., Jianlin, H., Ruijun, L., Eds.; RAP Publication; FAO (Food and Agricultural Organization of the United Nations) Regional Office for Asia and the Pacific: Bangkok, Thailand, 2003; pp 119–171.
- (6) Products from yak and their utilization. *The Yak*, 2nd ed.; Wiener, G. H., Jianlin, H., Ruijun, L., Eds.; RAP Publication; FAO (Food and Agricultural Organization of the United Nations) Regional

Office for Asia and the Pacific: Bangkok, Thailand, 2003; pp 237–256.

- (7) Jain, Y. C.; Yadava, R. S. Yield and composition of milk of Himachali yak, yak hybrid and hill cow. *Indian J. Anim. Sci.* 1985, 55, 223–224.
- (8) Mondal, D. B.; Pal, R. N. Yak milk—chemical constituents. *Indian J. Dairy Sci.* 1996, 46, 413–414.
- (9) Neupaney, D.; Kim, J.; Ishioroshi, M.; Samejima, K. Study on composition of Nepalese cheeses, yak milk, and yak cheese whey. <u>*Milk Sci.*</u> 1997, 46, 95–102.
- (10) Sheng, Q.; Li, J.; Alam, M. S.; Fang, X.; Guo, M. Gross composition and nutrient profiles of Chinese yak (Maiwa) milk. *Int. J. Food Sci. Technol.* **2007**,doi: 10.1111/j.1365–2621. 2006.01463.x.
- (11) Clapham, W. M.; Foster, J. G.; Neel, J. P.; Fedders, J. M. Fatty acid composition of traditional and novel forages. <u>J. Agric. Food</u> <u>Chem.</u> 2005, 53, 10068–10073.
- (12) Pariza, M. W.; Ashoor, S. H.; Shu, F. S.; Lund, D. B. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Cancer Lett.* **1979**, *7*, 63–70.
- (13) Gayda, D. P.; Pariza, M. W. Activation of 2-amino-3-methylimidazo-[4,5-f]quinone and 2-amino fluorine for bacterial mutagenesis by primary monolayer cultures of adult rat hepatocytes. <u>*Mutat.*</u> <u>*Res.*</u> 1983, 118, 7–14.
- (14) Belury, M. A. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. <u>Annu. Rev. Nutr.</u> 2002, 22, 505–531.
- (15) Chin, S. F.; Liu, W.; Storkson, J. M.; Ha, Y. L.; Pariza, M. W. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. <u>J. Food Compos. Anal.</u> 1992, 5, 185–197.
- (16) Lin, H.; Boylston, T. D.; Chang, M. J.; Luedecke, L. O.; Shultz, T. D. Survey of the conjugated linoleic acid contents of dairy products. *J. Dairv Sci.* **1995**, 78, 2358–2365.
- (17) Parodi, P. W. Cows' milk fat components as potential anticarcinogenic agents. <u>J. Nutr.</u> 1997, 127, 1055–1060.
- (18) Dhiman, T. R.; Helmink, E. D.; McMahon, D. J.; Fife, R. L.; Pariza, M. W. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. <u>J. Dairv Sci</u>. **1999**, 82, 412– 419.
- (19) Álvarez, S.; Fresno, M.; Méndez, P.; Castro, N.; Fernández, J. R.; Sanz, M. R. Alternatives for improving physical, chemical, and sensory characteristics of goat cheeses: the use of arid-land forages in the diet. *J. Dairy Sci.* 2007, *90*, 2181–2188.
- (20) Addis, M.; Cabiddu, A.; Pinna, G.; Decandia, M.; Piredda, G.; Pirisi, A.; Molle, G. Milk and cheese fatty acid composition in sheep fed mediterranean forages with reference to conjugated linoleic acid *cis*-9,*trans*-11. *J. Dairy Sci.* **2005**, 88, 3443–3454.
- (21) Nudda, A.; McGuire, M. A.; Battacone, G.; Pulina, G. Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. <u>J. Dairv Sci.</u> 2005, 88, 1311–1319.
- (22) Thapa, T. B. Diversification in processing and marketing of yak milk based products. In *Yak Production in Central Asian Highlands*; Proceedings of the Third International Congress on Yak, Lhasa, China, Sept 4–9, 2000; Jianlin, H., Richard, C., Hanotte, O., McVeigh, C., Rege, J. E. O., Eds.; ILRI (International Livestock Research Institute): Nairobi, Kenya, 2002; pp 1–572.
- (23) Linghao, H. Shujie, L.; Shatuo, C. Advances in yak nutrition research. In Yak Production in Central Asian Highlands; Proceedings of the Third International Congress on Yak, Lhasa, China, Sept 4–9, 2000; Jianlin, H., Richard, C., Hanotte, O., McVeigh, C., Rege, J. E. O., Eds.; ILRI (International Livestock Research Institute): Nairobi, Kenya, 2002; pp 1–572.
- (24) Pradhan, S. M.; Pariyar, D.; Adhikari, J. R.; Ghimire, R. C. Range Systems Study of Ramechap District (Thodung Region) of Nepal; available Jan 22, 2007; http://www.fao.org/ag/agp/agpc/doc/ pasture/peshawarproceedings/rangesystem.pdf.
- (25) AOAC. Official Methods of Analysis, 15th ed.; AOAC: Arlington, VA, 1990.

- (26) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. <u>Can. J. Biochem. Physiol.</u> 1959, 37, 911–917.
- (27) Cruz-Hernandez, C.; Deng, Z.; Zhou, J.; Hill, A. R.; Yurawecz, M. P.; Delmonte, P.; Mossoba, M. M.; Dugan, M. E. R.; Kramer, J. K. G. Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. <u>J. AOAC Int.</u> 2004, 87, 545–562.
- (28) Odongo, N. E.; Or-Rashid, M. M.; Kebreab, E.; France, J.; McBride, B. W. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. *J. Dairy Sci.* 2007, *90*, 1851–1858.
- (29) Jensen, R. G. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 2002, 85, 295–350.
- (30) Alonso, L.; Fontecha, J.; Lozada, L.; Fraga, M. J.; Juarez, M. Fatty acid composition of caprine milk: major, branched-chain, and trans fatty acids. *J. Dairy Sci.* 1999, 82, 878–884.
- (31) Neupaney, D.; Sasaki, S.; Kim, J.; Ishioroshi, M.; Samejima, K. Yak butter lipid composition and vitamins in comparison with cow butter lipids. <u>*Milk Sci.*</u> 2003, *52*, 33–39.
- (32) Baer, R. J.; Ryali, J.; Schingoethe, D. J.; Kasperson, K. M.; Donovan, D. C.; Hippen, A. R.; Franklin, S. T. Composition and properties of milk and butter from cows fed fish oil. *J. Dairy Sci.* 2001, 84, 345–353.
- (33) Diedrich, M.; Henschel, K. P. The natural occurrence of unusual fatty acids. 1. Odd numbered fatty acids. <u>*Nahrung*</u> 1990, 34, 935– 943.
- (34) Bas, P.; Archimède, H.; Rouzeau, A.; Sauvant, D. Fatty acid composition of mixed-rumen bacteria: effect of concentration and type of forage. *J. Dairv Sci.* 2003, 86, 2940–2948.
- (35) Vlaeminck, B.; Fievez, V.; Demeyer, D.; Dewhurst, R. J. Effect of forage:concentrate ratio on fatty acid composition of rumen bacteria isolated from ruminal and duodenal digesta. *J. Dairy Sci.* 2006, 89, 2668–2678.
- (36) Or-Rashid, M. M.; Odongo, N. E.; McBride, B. W. Fatty acid composition of ruminal bacteria and protozoa with emphasis on conjugated linoleic acid, vaccenic acid, and odd-chain and branched-chain fatty acids. *J. Anim Sci.* 2007, 85, 1228–1234.
- (37) Kemp, P. R.; White, W.; Lander, D. J. The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. <u>J. Gen. Microbiol</u>. **1975**, 90, 100– 114.
- (38) Simopoulos, A. P. The importance of the ratio of omega-6/ omega-3 essential fatty acids. <u>Biomed. Pharmacother</u>. 2002, 56, 365–79.
- (39) Wolf, R. L. Content and distribution of *trans*-18:1 acids in ruminant milk and meat fats. Their importance in European diets and their effect on human milk. *JAOCS* **1995**, 72, 259–272.
- (40) Adlof, R. O.; Duval, S.; Emken, E. A. Biosynthesis of conjugated linoleic acid in humans. *Lipids* 2000, *35*, 131–135.
- (41) Whitlock, L. A.; Schingoethe, D. J.; AbuGhazaleh, A. A.; Hippen, A. R.; Kalscheur, K. F. Milk production and composition from cows fed small amounts of fish oil with extruded soybeans. <u>J.</u> <u>Dairy Sci.</u> 2006, 89, 3972–3980.
- (42) Griinari, J. M.; Dwyer, D. A.; McGuire, M. A.; Bauman, D. E.; Palmquist, D. L.; Nurmela, K. V. V. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. *J. Dairy Sci.* 1998, *81*, 1251–1261.
- (43) Pottier, J. M.; Focant, C.; Debier, G.; De Buysser, C.; Goffe, E.; Mignolet, E.; Froidmont, E.; Larondelle, Y. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. <u>J. Dairv Sci.</u> 2006, 89, 685–692.
- (44) Bauman, D. E.; Perfield, J. W., II; de Veth, M. J.; Lock, A. L. New perspectives on lipid digestion and metabolism in ruminants. *Proc. Cornell Nutr. Conf.* 2003, 175–189.
- (45) Calberry, J. M.; Plaizier, J. C.; Einarson, M. S.; McBride, B. W. Effects of replacing chopped alfalfa hay with alfalfa silage in a total mixed ration on production and rumen conditions of lactating dairy cows. <u>J. Dairy Sci</u>. 2003, 86, 3611–3619.

- (46) Luna, P.; Fontecha, J.; Juárez, M.; de la Fuente, A. Conjugated linoleic acid in ewe milk fat. J. Dairv Res. 2005, 72, 415–424.
- (47) Bauman, D. E.; Barbano, D. M.; Dwyer, D. A.; Griinari, J. M. Technical note: Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. <u>J.</u> <u>Dairy Sci.</u> 2000, 83, 2422–2425.
- (48) Collomb, M.; Bütikofer, U.; Sieber, R.; Bosset, J. O.; Jeangros, B. Conjugated linoleic acid and trans fatty acid composition of cows' milk fat produced in lowlands and highlands. *J. Dairy Res.* 2001, 68, 519–523.
- (49) Griinari, J. M.; Bauman, D. E. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Press: Champaign, IL, 1999; Vol. 1, pp 180–200.
- (50) Corl, B. A.; Baumgard, L. H.; Dwyer, D. A.; Griinari, J. M.; Phillips, B. S.; Bauman, D. E. The role of Δ9-desaturase in the production of *cis*-9, *trans*-11 CLA. <u>J. Nutr. Biochem</u>. 2001, 12, 622–630.
- (51) Piperova, L. S.; Sampugna, J.; Teter, B. B.; Kalscheur, K. F.; Yurawecz, M. P.; Ku, Y.; Morehouse, K. M.; Erdman, R. A. Duodenal and milk *trans* octadecenoic acid and conjugated linoleic acid (CLA) isomers indicate that post absorptive synthesis is the predominant source of *cis*-9-containing CLA in lactating dairy cows. *J. Nutr.* **2002**, *32*, 1235–1241.
- (52) Pariza, M. W.; Park, Y.; Cook, M. E. The biologically active isomers of conjugated linoleic acid. <u>*Prog. Lipid Res.*</u> 2001, 40, 283–298.
- (53) Rainio, A.; Vahvaselka, M.; Suomalainen, T.; Laakso, S. Reduction of linoleic acid inhibition in production of conjugated linoleic acid by *Propionibacterium freudenreichii* ssp. <u>shermanii. Can.</u> J. Microbiol. 2001, 47, 735–740.
- (54) Tricon, S.; Burdge, G. C.; Russell, J. J.; Jones, E. L.; Grimble, R. F.; Williams, C. M.; Yaqoob, P.; Calder, P. C. Opposing effects of cis-9, trans-11 and trans-10,cis-12 CLA on blood lipids in healthy humans. <u>Am. J. Clin. Nutr.</u> 2004, 80, 614–620.
- (55) Tricon, S.; Burdge, G. C.; Kew, S.; Banerjee, T.; Jones, E. L.; Grimble, R. F.; Williams, C. M.; Calder, P. C.; Yaqoob, P. Effects of *cis-9*, *trans-11* and *trans-10*, *cis-12* conjugated linoleic acid on immune function in healthy humans. <u>Am. J. Clin. Nutr.</u> 2004, 80, 1626–1633.

- (56) Watkins, B. A.; Li, Y. CLA in functional food: enrichment of animal products. In *Advances in Conjugated Linoleic Acid Research*; Sebedio, J. L., Christie, W. W., Adolf, R., Eds.; AOCS Press: Champaign, IL, 2003; Vol. II, pp 174–188.
- (57) Parodi, P. W. Positional distribution of fatty acids in the triglyceride classes of milk fat. <u>J. Dairy Res</u>. 1982, 49, 73–80.
- (58) Chilliard, Y.; Ferlay, A.; Mansbridge, R. M.; Doreau, R. M. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. <u>Ann. Zootech.</u> 2000, 49, 181–205.
- (59) Bauman, D. E.; Corl, B. A.; Baumgard, L. H.; Griinari, J. M. Conjugated linoleic acid (CLA) and the dairy cow. In *Recent Advances in Animal Nutrition*; Garnsworthy, P. C., Wiseman, J., Eds.; Nottingham University Press: Nottingham, U.K., 2001; pp 221–250.
- (60) Peterson, D. G.; Kelsey, J. A.; Bauman, D. E. Analysis of variation in *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in milk fat of dairy cows. <u>J. Dairy Sci.</u> 2002, 85, 2164–2172.
- (61) Sessler, A. M.; Ntambi, J. M. Polyunsaturated fatty acid regulation of gene expression. <u>J. Nutr</u>. 1998, 128, 923–926.
- (62) Cabiddu, A.; Addis, M.; Pinna, G.; Spada, S.; Fiori, M.; Sitzia, M.; Pirisi, A.; Piredda, G.; Molle, G. The incusion of a daisy plant (*Chrysanthemum coronarium*) in dairy sheep diet. 1: effect on milk and cheese fatty acid composition with particular reference to C18:2 cis-9, trans-11. *Livest. Sci.* 2006, *101*, 57–67.
- (63) Jahreis, G.; Fritsche, J.; Möckel, P.; Schöne, F.; Möller, U. The potential anticarcinogenic conjugated linoleic acid, cis-9, trans-11 C18:2, in milk of different species: cow, goat, ewe, sow, mare, woman. <u>Nutr. Res. (N. Y.</u>) **1999**, *19*, 1541–1549.
- (64) Allred, S. L.; Dhiman, T. R.; Brennand, C. P.; Khanal, R. C.; McMahon, D. J.; Luchini, N. D. Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. *J. Dairv Sci.* 2006, *89*, 234–248.
- (65) Banni, S.; Angioni, E.; Murru, E.; Carta, G.; Melis, M. P.; Bauman, D.; Dong, Y.; Ip, C. Vaccenic acid feeding increases tissue levels of conjugated linoleic acid and suppresses development of premalignant lesions in rat mammary gland. *Nutr. Cancer* 2001, *41*, 91–97.

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