Indian Journal of Animal Sciences **85** (8): 889–896, August 2015/Article

Fatty acids profile of milk and milk products in cow and buffalo fed roughage based diet

AMRISH KUMAR TYAGI[1], UMESH K SHANDILYA[2], ANIMA SRIVASTAVA[3], VISHNU R KALE[4] and NEELAM J KEWALRAMANI[5]

National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 9 February 2015; Accepted: 18 March 2015

ABSTRACT

Diet composition is an important determinant of milk production and composition, including milk fatty acid profile. Present experiment evaluated the influence of diet on milk fatty acid composition of cow and buffaloes, which have varying genetic potential of milk secretion and mammary lipogenesis. Sixteen of each multiparous crossbred cows and Murrah buffaloes were divided in 2 equal groups of each species with 8 animals in each group making 4 groups altogether (groups 1 and 2 for cows and groups 3 and 4 for buffaloes). Cows and buffaloes of groups 1 and 3 were fed *ad lib.* berseem (*Trifolium alexandrinum*) fodder along with wheat straw, whereas, animals of groups 2 and 4 were offered concentrate mixture and wheat straw. Fatty acid profiles were determined of milk samples and ghee (clarified butter oil) prepared at fortnight intervals by indigenous and creamery (commercial) methods from the total milk collected from each group. Total milk fat, protein, total solids and solid not fat contents of milk were similar between cow and buffaloes irrespective of dietary changes. Total CLA content in milk was higher in berseem fed groups of both the species. In conclusion, total PUFA and SFA levels of cow and buffalo milk with same feeding regimen were nonsignificant. However, the total MUFA content significantly increased in cows when fed with green fodder as compared to buffaloes. Furthermore, total CLA content in ghee prepared using indigenous method was higher as compared to that of creamery method.

Key words: Conjugated linoleic acid, Dietary manipulation, Fatty acids, Green fodder

Fatty acid composition of milk and milk products is an important economic trait of dairy animals. Fat not only provides energy in the diet, but also has an important role in promoting health status of humans (Parodi 1994). Although studies (Tremblay and Gilbert 2009, Elwood *et al.* 2010) have suggested that foods rich in fat with a high percentage of saturated fatty acids in the human diet might increase the risk factor for cardiovascular disease and insulin resistance. Altering milk fat so as to lower overall intakes of saturated fatty acids, particularly 12:0, 14:0, 16:0, and enhancement of unsaturated fatty acids (UFAs) constituents such as CLA, in these foods will make them more suitable and acceptable for human consumption.

Diet-related factors are most important in terms of influencing milk PUFA/MUFA concentration (Khanal and Olson 2004). Animals offered high forage diets have higher concentrations of PUFA (Dewhurst *et al.* 2006, Elgersma *et al*. 2006). The content of both mono and polyunsaturated fatty acids was found to be higher in cow milk (Tyagi *et al.* 2007). Additionally, there are very few studies examining the variation in milk fatty acid (FA) concentration and more so CLA concentration among different species, viz. cows and buffaloes.

Besides improving CLA content in milk through dietary manipulation, there are reports indicating that processing of milk can also alter fatty acid as well as CLA content. Ghee was prepared by many commercial methods involving heating of butter or cream at different temperatures. However, traditional method involves conversion of whole milk to yoghurt by fermentation, and butter is removed by churning and is heated at high temperature for a prolonged period to prepare ghee. Such ghee was reported to have considerably higher levels of CLA (Aneja and Murthi 1990).

The present study encompasses these 2 aspects and examines the effect of feeding fresh cut green fodder and concentrate on the milk fat, solid not fat, total solids and milk protein along with CLA content in milk from both the ruminant species.

MATERIALS AND METHODS

Animals: Crossbred (Holstein Friesian × Tharparkar) cows (16) and Murrah buffaloes (16) in early lactation,

Present address: [1](amrishtyagi1963@yahoo.com), [2]Research Associate (ukshandilya@gmail.com), [3]Senior Research Fellow (animasrivastava1987@gmail.com), [4]Ph.D. Scholar (drvishnukale @gmail.com), [5]Principal Scientist (neelamjk@gmail.com), Dairy Cattle Nutrition Division.

having similar body weight (cows 405–425 kg and buffaloes 537–560 kg) and milk yield (cow 11–12 kg and buffalo 7.2–7.8 kg) were selected from a herd maintained at National Dairy Research Institute, Karnal, India. Cows and buffaloes were housed in well ventilated stalls having arrangements for individual feeding and watering during the 90 days experimental period.

Experimental design: Cows and buffaloes were randomly divided into 4 equal groups of 8 animals each (groups 1 and 2 for cows and groups 3 and 4 for buffaloes) on the basis of body weight, milk yield, stage of lactation and parity. All the animals were fed an isocaloric and isonitrogenous diet as per NRC (2001) feeding standard for cows and Kearl (1982) feeding standard for buffaloes. Cows and buffaloes of groups 1 and 3 were fed *ad lib* berseem (*Trifolium alexandrinum*) fodder along with wheat straw, whereas, animals of groups 2 and 4 were offered concentrate mixture and wheat straw. The ingredient composition of concentrate mixture is given in Table 1. Berseem fodder was offered 4 times in a day. The ratio of concentrate and wheat straw was 60:40 in groups 2 and 4. After the completion of 4 weeks adaptation period, feed intake, body weight and milk yield were recorded daily for 8 weeks of experimental period. The animals were milked twice at 06:00 and 18:00 h according to the routine practice of farm.

Analysis of feed sample and their fatty acid profile: The roughages and concentrates were sampled at the beginning, middle and end of the experimental period. All the samples were grounded individually, labelled and analysed for proximate principles as per AOAC (2005) and cell wall constituents as per Van Soest et al. (1991). Fatty acids were analysed as per direct trans-esterification method of O'Fallon *et al.* (2007). Methyl esters were separated by using a GC 5700 and equipped with a SGE Forte GC capillary column (120m × 0.25mm × 70 µm- BPX70). Helium was used as carrier gas at constant inlet pressure (205 kPa) with a constant flow rate of 2ml/min. The injector and detector temperature were 260°C and 270°C respectively and the split ratio was 1:10. The initial oven temperature was 120°C and increased by 2°C/min to 240°C for 55 min. The identification of individual fatty acid was based on a commercial standard mixture and published isomeric profiles.

Table 1. Chemical composition of experimental feeds (% DM basis)

Attribute	Berseem fodder	Concentrate mixture	Wheat straw
DM	14.50	94.03	95.57
OM	89.20	92.99	89.72
CP	14.84	21.29	3.49
CF	25.50	4.78	39.31
EE	2.52	4.70	0.84
ASH	10.80	7.01	10.28
NFE	46.34	56.24	41.65
NDF	45.12	44.16	82.20
ADF	29.63	9.15	59.52
HC	15.49	35.01	22.68

*Concentrate mixture contains maize 33%; groundnut cake (expeller) 22%; mustard oil cake (expeller) 11%; wheat bran 31%; mineral mixture 2%; and common salt 1%.*Mineral mixture (2 kg) contain dicalcium phosphate 1.65 kg; chalk powder 0.3312 kg; magnesium carbonate 0.0900 kg; sodium chloride 0.0900 kg; ferrous sulphate 0.01500 kg; copper sulphate 0.0021 kg; manganese oxide 0.0021 kg; cobalt chloride 0.0015 kg; potassium iodide 0.0003 kg; sodium fluoride 0.0003 kg; zinc sulphate 0.0075 kg.

Collection and analysis of milk samples: Milk samples were collected at fortnight intervals for the analysis of milk fat (BIS 1989), total solids and milk protein (AOAC 1995). Milk samples were transported in a cooled box to the laboratory and preserved with bronopol-B2 at 4°C until further analysis. Solid not fat content in milk was calculated by subtracting fat percentage from total solids. Another milk sample (100 ml) from each cow and buffalo was collected from the each milking at fortnightly intervals from 2 consecutive milking. Weighted composite milk samples from AM and PM milking were analyzed for fatty acid composition, including CLA using gas chromatograph. Samples containing methyl esters of fatty acids in hexane (1 to 3 µl) were directly injected through the splitless injection port onto a supelco wax 10, fused silica capillary column (100m × 0.25m i.d.0.25 µm film thickness. Injector temperature of the gas chromatograph was maintained at 250°C. Initial oven temperature was 50°C which was programmed to increase at 20°C per min up to 200°C and was held for 50 min. Thereafter, it was raised to 225°C at 10°C per min and held for 20 min. Detector temperature of GC was 250°C. Different fatty acids including CLA (cis-9, trans-11 18:2) were identified by comparing the retention time with the methylated fatty acid standards. The percentage of each fatty acid was calculated by dividing the peak area under particular fatty acid by the total peak area under reported fatty acids.

Ghee preparation: Ghee (clarified butter oil) was prepared at fortnight intervals by 2 different methods i.e. indigenous and creamery (commercial) from the total milk of each group on that particular day.

Indigenous method: The indigenous method of ghee making involves heating of milk at 80°C (until boiling) followed with overnight fermentation with NCDC 167 culture containing *Lactococcus lactis* sp. Lactis. The *dahi* (yoghurt) thus formed is churned in a mixer for separating the butter. The butter thus obtained is heated to 110–115°C in a metal pan on an electric heater until all the moisture was removed and prepared ghee was separated. The contents were left undisturbed to settle down the curd particles at the bottom of the pan and clear fat (ghee) was carefully decanted off into the storage vessel (Rangappa *et al.* 1974).

Creamery method: Cream from milk was separated by centrifugation and ghee was prepared directly from cream. Cream was heated to 115°C in a stainless steel jacketed ghee kettle fitted with an agitator, steam control valve,

pressure and temperature gauges and movable, hollow, stainless steel tube centrally bored for emptying out the contents. Heating was discontinued as soon the color of the ghee residue turned to golden yellow (Rajorhia 1993).

Fatty acid analysis of ghee: Fatty acids of ghee were analysed as per direct trans-esterification method of O'Fallon *et al.* (2007). For the methyl ester formation 40µl ghee sample was taken and 1 µl sample was injected in GC. The conditions used for GC was described earlier.

Statistical analysis: Statistical analysis of the data for milk yield and its composition, fatty acid composition in different dietary treatment groups among different species was determined by two way analysis of variance (ANOVA) through SPSS 21.0.0 software package according to Snedecor and Cochran (1994) using general linear model. The data of fatty acid composition of ghee were analyzed by 3 way ANOVA as there were different methods of preparation, species and dietary treatment effects and also their interaction effect. Duncan's method was used for multiple comparisons among means.

RESULTS AND DISCUSSION

Chemical composition of experimental feeds: Chemical composition of different feeds and fodders (compound concentrate mixture, green berseem fodder and wheat straw) offered to the animals during the experimental period of 90 days is presented in Table 1. On % DM basis the fat content was 2.52, 4.70, 0.84 in berseem fodder, concentrate mixture and wheat straw, respectively, whereas crude protein was 14.84, 21.29 and 3.49, while NDF was 45.12, 44.16 and 82.20 in berseem fodder, concentrate mixture and wheat straw, respectively. Fatty acid composition of these feeds and fodder (Table 2) indicated, that berseem forage contained higher C18:3 (14.98 mg/g fat) whereas, concentrate mixture was high in C18:2 (14.22 mg/g fat). Fresh grass contained a high proportion (50–75%) of its total fatty acid content in the form of a-linolenic acid (C18:3). Levels of a-linolenic acid vary with plant and environmental factors such as stage of maturity, genetic differences (Elgersma *et al.* 2003). Concentrate mixture contained higher mono-unsaturated fatty acid whereas, berseem forage provided higher amount of poly-unsaturated fatty acids (PUFA) due to the difference in type of fat in respective feeds and fodder (Bu *et al*. 2007).

Dry matter intake, milk yield and its composition: Body weight of cows and buffaloes in the 2 treatment groups was similar (Table 3). DM intake (Table 3) was higher (P <0.001) in berseem forage fed animals (groups 1 and 3) than concentrate fed animals (groups 2 and 4). Ether extract was low in berseem forage (2.52%) than concentrate mixture (4.70%) but, the total fat intake in all the groups of animals was similar due to higher dry matter intake through green fodder. This results in consistence with the results of Tyagi *et al.* (2006) where higher dry matter intake in berseem forage fed animals (groups 1 and 3) as compared to concentrate fed animals (groups 2 and 4) (Table 3) was recorded (12.6 vs10.3 kg) in buffaloes, resulting in higher

Table 2. Fatty acid composition of forage and concentrate mixture

Fatty Acid1	Total fatty acids (mg/g dry sample)		
	Berseem fodder	Concentrate mixture	Wheat straw
C12:0	0.053	0.000	0.027
C14:0	0.139	0.059	0.064
C14:1 cis	0.339	0.000	0.000
C16:0	4.129	5.835	0.803
C16:1 cis	0.083	0.175	0.027
C18:0	0.639	1.206	0.211
C18:1 c-9	0.912	16.079	0.690
C18:2	3.545	14.224	0.715
C20:0	0.105	0.426	0.048
C18:3	0.101	0.045	0.000
C18:3	14.884	1.320	0.193
Total C18:3	14.985	1.365	0.193
C22:0	0.177	0.642	0.094
C22:1	0.000	4.080	0.048
C24:0	0.190	0.448	0.062
ΣOmega-3	14.884	10.320	0.193
ΣOmega-6	3.735	14.325	0.715
ΣFat (mg)	25.296	44.539	2.982
ΣSFA (%)	5.432	8.616	1.309
ΣUFA (%)	94.568	91.384	98.691
ΣMUFA (mg)	1.334	20.334	0.765
ΣPUFA (mg)	18.53	15.589	0.908

[1]Expressed as number of carbons: number of double bonds; c, cis. SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

total solids as well as solid-not fat content.

The average milk yield (Table 3) in 4 respective groups was 12.0, 11.9, 9.9 and 7.8 kg/ day and recorded higher (P<0.01) in buffaloes fed berseem forage diet as compared to concentrate diet. Total milk fat content was higher (P<0.01) in buffalo milk than cow milk (740.3 vs. 511.3g/ d) i.e. 16% daily milk fat yield was higher in buffaloes in spite of their lower milk yield. Fatty acid composition fatty acid composition was similar between cow and buffaloes milk at different periods of sampling. C4:0, C14:0, C15:0, C16:0 and C 16:1 9-t fatty acids were higher (P<0.01) in buffalo milk than cow milk, whereas, C8:0, C10:0, C11:0, C12:0, C14:1 were higher in cow milk. Total unsaturated fatty acids were higher (P<0.01) in cow milk as compared to buffalo milk fat (369.0 vs. 337.9 mg/g milk fat), while the saturated fatty acids showed reverse trend (611.5 vs. 662.0 mg/g milk fat). Mono and polyunsaturated fatty acids were higher in cow milk. Total CLA was higher (P<0.01) in cow than buffalo milk fat (13.0 and 11.58 mg/g milk fat). The levels of different fatty acids present in milk of cow and buffalo was similar as reported by Dhiman *et al.* (1999) and Tyagi *et al.* (2007) in their different experiments. The rationale might be the influence of differences in ruminal biohydrogenation, microbial ecology and activity of SCD in the mammary gland which contribute to variation

Table 3. Dry matter intake, milk yield and its composition of cows and buffaloes fed green fodder and concentrate mixture

Particulars	Cow		Buffalo		SEM
	Green fodder	Concentrate	Green fodder	Concentrate	
Body weight (kg)	413.8±26.33	433.8±11.06	545.8±20.19	567.4±29.42	
DM intake (kg/d)*	13.3[b]	12.2 [a]	13.4[b]	11.1[a]	0.56
Fat intake	0.44	0.46	0.45	0.42	0.01
Milk yield/day (kg)*	12.0[c]	11.9[c]	9.9[b]	7.8[a]	1.10
Fat (g/100ml)*	4.2[a]	4.2[a]	7.4[b]	8.2[b]	0.36
Total fat (g)*	511.3[a]	510.8[a]	740.3[c]	637.8[b]	8.87
Protein (g/100ml)	3.0	3.0	3.8	3.8	0.35
SNF (g/100ml)	7.6[a]	7.3[a]	9.3[b]	8.8[b]	0.24
Total solids (g/100ml)*	11.9[a]	11.6[a]	16.7[b]	17.0[b]	0.32

[a,b] Means with different superscript in row differ significantly at (P< 0.01). Mean value represents average from sampling fortnight 2 through 5. SNF, solid not fat; SEM, standard error of means.

in milk t7c9 and c9t11 CLA concentrations (Baumgard *et al.* 2002). Lawless *et al.* (1999) reported variation of CLA content in different breed of cows maintained at similar pasture or TMR respectively. Other authors have also reported differences in lipid profiles among breeds (Garcia *et al.* 2008), or the interaction between breed and feeding system (Nuernberg *et al.* 2005).

Buffalo milk contained significantly lower amounts of polyunsaturated fatty acids. These fatty acids cannot be formed *de novo* in humans and are essential for health and thus are needed to be ingested from the diet. The ω6/ω3 ratio, was significantly (P < 0.001) higher for cow milks. Since the recommended ω6/ω3 ratio is about 4–5 while the current dietary ω6/ω3 ratio is about 10 (Kris-Etherton *et al.* 2000), the lower ω6/ω3 ratio in buffalo milk is significant with regard to improving human nutrition. Blasi *et al.* (2008) reported higher ω6/ω3 ratios of 10 for buffalo milk and 9.5 for cow milk. These higher ratios were explained by a higher amount of 18:2n-6 and a lower amount of linolenic acid, in contrast to the results of present study.

Effect of diets on milk fatty acid composition of cow and buffaloes: Short chain fatty acids (C-4 to C-12) of cow and buffalo milk were similar by feeding two different diets (103 and 99 mg/g fat respectively for green fodder and concentrate), while medium chain fatty acids i.e. C-14 to C-16 were higher (P<0.01) in berseem forage fed animals as compared to concentrate mixture fed animals (Table 4). Total C18:1-trans fatty acid was 51.0 and 40.28 mg/g in the milk fat of berseem forage and concentrate fed animals respectively, whereas total C18:3 were higher (P<0.01) in green fodder groups than concentrate mixture fed group (11.8 vs. 2.6 mg/g of fat in milk). Total CLA (18.0 and 6.6 mg/g fat) and vaccenic acid (C18:1 t-11) (45.3 and 17.3 mg/g milk fat) content were higher (P<0.01) in berseem forage fed than the concentrate fed animals. Diet is the major factor affecting the PUFA content of milk (Chilliard *et al.* 2000, Khanal and Olson 2004), with concentrations typically higher under forage compared with concentrate based diet. Additionally, a variation in fatty acid composition of milk in different breeds owing to differential activity in rumen as well as mammary glands of buffaloes and cows has been reported (Griinari and Bauman 1999).

Three folds increase of total CLA content berseem forage fed animals than concentrate mixture fed animals was also reported by Dhiman *et al.* (1999) and then by Schroeder *et al.* (2003) by feeding solely pasture or fresh forage. Similar to present experiment Kay *et al.* (2004) and Tyagi *et al.* (2007) also observed 90% cis-9, trans-11 C18:2 of total CLA content in milk irrespective of diet whereas, major impact on cis-9, trans-11 CLA content of diet in milk fat has been reported (Palmquist *et al.* 2005) since it originates from the incomplete biohydrogenation of polyunsaturated fatty acids. The higher trans-11 18:1 fatty acid concentrations in milk of animals fed berseem forage suggest a higher substrate supply for endogenous c9t11 CLA synthesis in the mammary gland. Moreover the ratio of t11 18:1/c9t11 CLA concentrations in milk of cows and buffaloes although differ in different diet groups, they are almost comparable in animals fed similar diet (approximately 2.7 in berseem fodder and approximately 2.5 in concentrate diet) which indicate that the diet has played a major role in this regard. Reduction of trans 18:1 is rate limiting for the complete hydrogenation of 18-carbon unsaturated fatty acids to 18:0, it is thus reasonable to expect that higher intake of C18:3 from the diet increases the amounts of trans 18:1 biohydrogenation intermediates leaving the rumen. The majority of the CLA in milk fat is synthesized via the enzyme "9-desaturase from trans-11 C18:1, an intermediate in the rumen biohydrogenation of linoleic and linolenic acids (Corl *et al.* 2001, Piperova *et al.* 2002) and similar to the present study, their concentration in milk and meat from ruminant was found associated with increased intake of C18 PUFA (Bessa *et al.* 2007).

Effect of method of ghee preparation on fatty acid composition: Indigenous method of ghee preparation increased 93 and 75% CLA content in cows as against 62 and 30% in buffalo ghee on forage and concentrate based diets than commercial preparation of ghee with the same combination of diet (Table 5), indicating higher increase of CLA content in cows than buffaloes. An overall 73% increase in the CLA content was observed indigenous method of ghee preparation as compared to creamery

Table 4. Comparison of fatty acid composition (mg/g of fat) in milk of cows and buffaloes fed green fodder and concentrate based diet (n=3)

Fatty Acids (mg/g)	Green fodder		Concentrate		SEM	Significance of effects		
	Cow	Buffalo	Cow	Buffalo		Diet	Breed	Diet*Breed
C4:0	24.11^{b}	32.07^{a}	24.43^{b}	30.98^{a}	3.443	0.806	<0.001	0.648
C6:0	16.77	17.47	26.03	14.97	10.394	0.470	0.270	0.211
C8:0	10.387^{ab}	9.40^{b}	10.91^{a}	6.95^{c}	1.092	0.054	<0.001	0.004
C10:0	22.43^{a}	17.61^{b}	23.83^{a}	13.07^{c}	2.563	0.176	<0.001	0.012
C11:0	2.95^{a}	1.10^{b}	2.91^{a}	0.77^{b}	0.419	0.323	<0.001	0.437
C12:0	25.78^{ab}	22.61^{b}	27.13^{a}	17.29^{c}	3.076	0.154	<0.001	0.019
C14:0	101.09^{b}	116.39^{a}	93.83^{b}	98.83^{b}	8.874	0.003	0.013	0.200
C14:1	9.39^{a}	6.60^{bc}	8.37^{ab}	5.84^{c}	1.804	0.275	0.002	0.873
C15:0	18.67^{a}	16.10^{b}	9.43^{d}	11.72^{c}	1.164	<0.001	0.748	0.000
C16:0	283.08^{b}	326.07^{a}	235.39^{c}	293.51^{b}	19.352	<0.001	<0.001	0.386
C16:1 9-t	3.39^{a}	3.00^{ab}	1.01^{b}	1.33^{b}	0.414	0.004	0.313	0.187
C16:1	11.57^{b}	16.71^{a}	9.99^{b}	18.07^{a}	2.645	0.926	<0.001	0.219
C17:1	3.20a	2.89^{ab}	1.55^{c}	2.60^{b}	0.437	<0.001	0.061	0.001
C18:0	133.1	109.69	163.93	164.39	36.844	0.012	0.489	0.472
C18:1 9-t	1.89^{b}	1.37^{c}	2.29^{a}	1.51^{c}	0.286	0.039	<0.001	0.314
C18:1 10–t	3.25^{b}	2.69^{c}	4.25^{a}	3.27^{b}	0.328	<0.001	<0.001	0.164
C18:1 11-t	51.50^{a}	42.19^{b}	18.31^{c}	16.44^{c}	4.587	<0.001	0.153	0.457
C18:1 12-t	2.57^{a}	0.32^{c}	2.63^{a}	1.37^{b}	0.629	0.051	<0.001	0.085
C18:1 13,14-t,6,7,8-c	8.93 ^{a}	5.64^{b}	6.35^{b}	3.44^{c}	0.728	<0.001	<0.001	0.562
Total C18:1-trans	67.58^{a}	52.14^{b}	34.63^{c}	28.42^{d}	4.897	<0.001	<0.001	0.040
C18:1 9-c	203.13^{c}	192.67^{c}	271.22^{a}	236.90^{b}	19.108	<0.001	0.011	0.168
C18:1 11-c	10.04	8.79	10.47	10.05	0.997	0.064	0.066	0.362
C18:2 9-c,12-c	14.57^{a}	13.93^{a}	13.95^{a}	10.33^{b}	1.164	<0.001	<0.001	0.006
C20:0	2.11^{c}	1.99^{c}	6.53^{a}	5.49^{b}	0.722	<0.001	0.078	0.166
C20:1	1.35^{c}	1.02c	4.81^{a}	2.85^{b}	0.378	<0.001	<0.001	<0.001
C18:3 9-12-15–c	12.55^{a}	11.19^{a}	2.30^{b}	2.85^{b}	1.374	<0.001	0.511	0.124
Total C18:3	12.56^{a}	11.19^{a}	2.33^{b}	2.85^{b}	1.379	<0.001	0.492	0.131
C18:2 9-c,11-t	18.73^{a}	15.25^{b}	6.39^{c}	6.45^{c}	1.920	<0.001	0.051	0.044
C18:2 10–t,12-c	1.29^{a}	1.15^{a}	0.04^{b}	0.32^{b}	0.538	<0.001	0.762	0.394
Total CLA	19.55^{a}	16.38^{b}	6.44^{c}	6.78^{c}	2.031	<0.001	0.125	0.058
Total Omega3	15.42^{a}	13.58^{b}	11.11^{c}	10.98^{c}	1.369	<0.001	0.169	0.113
Total Omega6	14.88^{a}	13.84^{a}	14.26^{a}	10.47^{b}	1.207	0.001	<0.001	0.014
Omega-6:Omega-3	9.71^{b}	10.95^{b}	13.11^{a}	9.43^{b}	1.242	0.095	0.032	<0.001
ΣSFA	643.35^{ab}	670.11^{a}	619.65^{b}	653.96^{a}	22.945	0.997	0.023	0.456
ΣUFA	357.67^{ab}	329.89^{b}	380.35^{a}	346.04^{b}	22.869	0.063	0.004	0.751
ΣMUFA	260.4b^{c}	231.05^{c}	308.67^{a}	280.80^{ab}	26.593	<0.001	0.019	0.950
ΣPUFA	26.32	21.18	23.91	21.66	5.750	0.709	0.156	0.577

Means bearing different superscripts (^{a,b,c}) in a row differ significantly. SEM, Standard error of means; CLA, conjugated linoleic acid; SFA, saturated fatty acids; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

method, irrespective of the species. However, total saturated or unsaturated fatty acid content of ghee in 2 groups was similar. There was no difference in short chain fatty acids content due to preparation of ghee whereas C15:0, C16:0, C16:1 9-t. C16:1, C17:1 fatty acids were higher (P<0.01) in indigenous method. These results are in consistence with the results of Joshi (2014), where she found that ghee prepared by traditional method contains higher amount of health beneficial fatty acids.

In conclusion, total PUFA and SFA levels of cow and buffalo milk with same feeding regimen were non-significant. However, the total MUFA content significantly increased in cows when fed with green fodder as compared to buffaloes. Total CLA content in ghee prepared using indigenous method was higher as compared to that of creamery method.

Table 5. Fatty acid composition in ghee prepared by indigenous and creamery methods of cow and buffalo milk fed on green fodder and concentrate (mg/g of fat) (n=3)

Fatty Acids (mg/g)	Cow				Buffalo				SEM*	Significance of effect (p)						
	Green fodder		Concentrate		Green fodder		Concentrate									
	Indigenous	Creamery	Indigenous	Creamery	Indigenous	Creamery	Indigenous	Creamery		Breed (B)	Diet (D)	Method (M)	B*D	B*M	D*M	B*D*M
C4:0	28.05^{b}	26.89^{b}	27.83^{b}	25.77^{b}	33.21^{a}	32.66^{a}	34.33^{a}	32.81	1.276	<0.001	0.981	0.162	0.477	0.752	0.609	0.987
C6:0	17.24	17.83	17.42	17.61	17.04	18.05	17.12	15.29	0.816	0.278	0.258	0.988	0.271	0.503	0.182	0.308
C8:0	10.34^{ab}	11.12^{a}	10.46^{ab}	11.04^{a}	8.33^{cd}	9.03^{bc}	7.99^{cd}	7.14^{d}	0.521	<0.001	0.160	0.429	144	0.317	0.252	0.369
C10:0	21.49^{a}	24.69^{a}	22.087^{a}	23.83^{a}	15.78^{bc}	17.17^{b}	14.99^{bc}	13.12^{c}	1.144	<0.001	0.135	0.187	0.175	0.1133	0.164	0.582
C11:0	2.31^{c}	3.036^{a}	2.45^{bc}	2.93^{ab}	0.96^{d}	1.04^{d}	0.87^{d}	0.72^{d}	0.185	<0.001	0.478	0.045	0.399	0.026	0.379	1.000
C12:0	25.06^{ab}	27.92^{a}	25.65^{ab}	27.04^{a}	20.^{35c}	21.79^{bc}	19.45^{c}	17.57^{c}	1.332	<0.001	0.171	0.325	0.217	0.232	0.222	0.629
C14:0	100.75^{ab}	108.67	101.65	95.05	108.48	113.377	108.36	99.85	5.169	0.121	0.090	0.877	0.950	0.740	0.074	0.940
C14:1	7.51^{abc}	9.22^{a}	7.95^{ab}	8.33^{a}	5.94^{dc}	6.29^{bcd}	6.09^{bcd}	4.99^{d}	0.604	<0.001	0.363	0.440	0.692	0.117	0.125	0.950
C15:0	12.76^{cd}	20.08^{a}	13.83^{c}	10.41^{d}	13.40^{c}	16.77^{b}	12.35^{cd}	11.90^{cd}	0.878	0.303	<0.001	0.014	0.296	0.703	<0.001	0.013
C16:0	266.5^{bc}	239.05^{c}	272.48^{bc}	241.25^{c}	345.93^{a}	314.14^{ab}	314.58^{ab}	295.95^{abc}	21.186	0.001	0.500	0.087	0.350	0.892	0.878	0.781
C16:1 9-t	1.81	2.46	2.06	1.067	1.90	3.25	1.52	1.33	0.515	0.681	0.032	0.592	0.434	0.318	0.044	0.948
C16:1	11.63^{b}	11.81^{b}	12.04^{b}	10.29^{b}	16.08^{a}	16.2^{a}	17.52^{a}	16.34^{a}	1.099	<0.001	0.881	0.411	0.398	0.874	0.311	0.844
C17:1	2.17^{bc}	3.2^{a}	2.26^{bc}	1.71^{c}	2.64^{ab}	3.21^{a}	2.54^{ab}	2.69^{ab}	0.207	0.009	0.003	0.057	0.200	0.700	0.004	0.064
C18:0	149.35^{ab}	145.92^{abc}	145.26^{bc}	164.03^{a}	117.87^{bc}	112.72^{c}	128.87^{bc}	147.40^{ab}	10.226	0.004	0.056	0.336	0.290	0.947	0.132	0.960
C18:1 9-t	2.1767^{ab}	1.4548^{bc}	2.06^{ab}	2.36^{a}	1.52^{bc}	1.54^{bc}	1.04^{c}	1.73^{abc}	0.242	0.005	0.480	0.673	0.140	0.118	0.025	0.605
C18:1 10–t	3.48	3.09	3.64	4.06	2.94	2.75	3.47	3.71	0.269	0.085	0.003	0.925	0.625	1.000	0.127	0.625
C18:1 11-t	29.31^{bc}	53.38^{a}	31.33^{b}	19.98^{bc}	27.19^{bc}	44.1^{2a}	21.32^{bc}	18.72^{c}	3.647	0.043	<0.001	0.018	0.982	0.878	<0.001	0.143
C18:1 12-t	2.09	3.61	2.91	2.83	1.96	1.69	1.73	1.77	0.525	0.011	0.938	0.419	0.896	0.275	0.402	0.216
C18:113, 14-t,6,7,8-c	6.66^{b}	9.50^{a}	6.90^{b}	6.52^{b}	4.69^{cd}	5.76^{bc}	4.12^{d}	3.91^{d}	0.425	<0.001	0.001	0.014	0.800	0.198	0.002	0.125
Total C18: 1-trans	44.11^{bcd}	71.32^{a}	47.25^{bc}	36.29^{cde}	38.43^{cde}	55.91^{b}	31.86^{de}	30.07^{e}	3.918	0.001	<0.001	0.011	0.963	0.961	<0.001	0.107
C18:1 9-c	242.1^{ab}	209.18^{bc}	231.73^{abc}	267.6^{a}	204.85^{bc}	200.5^{c}	232.52^{abc}	253.03^{a}	12.211	0.103	0.002	0.588	0.366	0.707	0.015	0.222
C18:1 11-c	9.68	9.09	9.29	9.49	8.87	9.06	9.23	10.75	0.655	0.959	0.194	0.355	0.407	0.385	0.182	0.962
C18:2 9-c,12-c	13.75^{a}	15.35^{a}	14.31^{a}	14.11^{a}	11.11^{b}	13.78^{a}	10.94^{b}	10.65^{b}	0.692	<0.001	0.059	0.072	0.202	0.627	0.027	0.565
C20:0	4.91^{ab}	2.44^{cd}	4.31^{ab}c	6.30^{a}	2.99^{bcd}	1.78^{d}	5.06^{ab}	6.40^{a}	0.744	0.424	<0.001	0.874	0.123	0.777	0.004	0.374
C20:1	3.32^{b}	1.40^{d}	3.07^{bc}	4.56^{a}	2.02^{cd}	0.98^{d}	2.62^{bc}	3.10^{bc}	0.355	0.002	<0.001	0.344	0.855	0.906	<0.001	0.078
C18:3 9-12-15-c	6.1^{bc}	13.71^{a}	7.14^{b}	2.88^{c}	6.25^{bc}	11.32^{a}	4.18^{bc}	2.19^{c}	1.266	0.120	<0.001	0.091	0.702	0.939	<0.001	0.198
Total C18:3	6.12^{bc}	13.71^{a}	7.22^{b}	2.88^{c}	6.25^{bc}	11.32^{a}	4.18^{bc}	2.19^{c}	1.273	0.115	<0.001	0.098	0.690	0.964	<0.001	0.195
C18:2 9-c,11-t	9.87^{bc}	18.97^{a}	11.36^{b}	6.87^{bc}	9.64^{bc}	15.81^{a}	7.28^{bc}	5.83^{c}	1.369	0.043	<0.001	0.028	0.661	0.978	<0.001	0.142
C18:2 10–t,12-c	0.53^{bc}	1.1^{a}	0.77^{ab}	0.06^{cd}	0.74^{ab}	1.05^{a}	0.31^{bcd}	0.0^{d}	0.154	0.421	<0.001	0.752	0.131	0.741	<0.001	0.153
Total CLA	10.4^{bc}	20.07^{a}	12.13^{b}	6.93^{c}	10.38^{bc}	16.87^{a}	7.58^{bc}	5.83c	1.461	0.047	<0.001	0.041	0.564	0.951	<0.001	0.128
Total Omega3	12.85	16.59	12.84	11.47	11.57	12.58	11.81	11.54	0.696	0.017	0.003	0.053	0.111	0.754	0.002	0.168
Total Omega6	14.15^{a}	15.76^{a}	14.44^{a}	14.41^{a}	11.34^{b}	13.87^{a}	10.94^{b}	10.65^{b}	0.603	<0.001	0.014	0.039	0.153	0.709	0.019	0.498
ω-6:ω-3	11.02^{bc}	9.48^{d}	11.25^{b}	12.58^{a}	9.86^{d}	10.27^{bcd}	9.29^{d}	9.23^{d}	0.379	<0.001	0.128	0.895	<0.001	0.602	0.040	0.007
ΣSFA	636.08	627.69	640.52	620.02	682.69	658.10	660.41	642.94	16.269	0.019	0.390	0.143	0.468	0.778	0.915	0.682
ΣUFA Unsaturated	363.92	372.31	359.48	379.98	317.31	341.90	339.59	357.06	16.269	0.019	0.390	0.143	0.468	0.778	0.915	0.682
ΣMUFA Unsaturated	290.25^{a}	272.68^{ab}	272.47^{ab}	301.84^{a}	246.53^{b}	249.83^{b}	273.60^{ab}	291.34^{a}	11.454	0.032	0.025	0.326	0.097	0.779	0.076	0.331
ΣPUFA Unsaturated	27.76	30.83	20.34	22.80	16.82	16.78	19.49	21.07	3.946	0.025	0.457	0.536	0.062	0.723	0.931	0.845

Means bearing different superscripts (^{a,b,c,d}) in a row differ significantly. SEM, standard error of means.

ACKNOWLEDGEMENT

The present research work was funded by Department of Biotechnology (DBT, Government of India) under the project entitled "Increasing the anti-carcinogenic potency of buffalo milk by enhancing its CLA content through dietary modification". Authors thankfully acknowledge National Dairy Research Institute (NDRI), Karnal to carry out this work.

REFRENCES

Aneja R P and Murthi T N. 1990. Conjugated 18:2 n-6 contents of Indian curds and ghee. *Indian Journal of Dairy Science* **43**: 231–38.

AOAC. 1995: Dairy products. *Official Methods of Analysis*. 16th edn. Association of Official Analytical Chemists Inc., Virginia, USA.

AOAC. 2005: *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington DC, USA.

Baumgard L H, Matitashvili E, Corl B A, Dwyer D A and Bauman D E. 2002. Trans-10,cis-12 conjugated 18:2n-6 decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *Journal of Dairy Science* **85**: 2155–63.

Bessa R J B, Alves S P, Jeronimo E C, Alfaia M M, Prates J A M and Santos-Silva J. 2007. Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lambs. *European Journal of Lipid Science and Technology* **109**: 868–78.

Bu D P, Wang J Q, Dhiman T R and Liu S J. 2007. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated 18:2n-6 in milk from Dairy Cows. *Journal of Dairy Science* **90**: 998–1007.

Chilliard Y, Ferlay A, Mansbridge R M and Doreau M. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales De Zootechnie* **49**: 181–205.

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J and Doreau M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology* **109**: 828–55.

Corl B A, Baumgard L, Dwyer H, Briinari D A, Phillps J M and Bauman E. 2001. The role of delta (9) desaturase in the production of cis9, trans11. *Journal of Nutritional Biochem*istry **12**: 622–30.

Dewhurst R J, Shingfield K J, Lee M R F and Scolan N D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology* **131**: 168–206.

Dhiman T R, Anand G R and Pariza M W. 1999. Conjugated 18:2n-6 content of milk from cows fed different diets. *Journal of Dairy Science* **82**: 2146–56.

Elgersma A, Ellen G, Dekker P R, Van der Horst H, Boer H and Tamminga S. 2003. Effect of perennial rye grass (*Lolium perenne*) cultivars with different 18:2n-6 contents on milk fatty acid composition. *Aspects of Applied Biology* **70**: 107–14.

Elgersma A, Tamminga S and Ellen G. 2006. Modifying milk composition through forage. *Animal Feed Science and Technology* **131**: 207–25.

Elwood P C, Pickering J E, Givens D I and Gallacher J E. 2010. The consumption of milk and dairy foods and the incidence of vascular disease and diabetes: an overview of the evidence. *Lipids* **45**: 925–39.

Garcia P T, Pensel N A, Sancho A M, Kloster N J L, Amigone M A and Casal J J. 2008. Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science* **79**: 500–08.

Griinari J M and Bauman D E. 1999. Biosynthesis of conjugated 18:2n-6 and its composition, incorporation into meat and milk in ruminants. *Advances in CLA Research*. Champaign(IL), AOCS Press, pp 180–200.

Joshi K S. 2014. Docosahexaenoic acid content is significantly higher in ghrita prepared by traditional Ayurvedic method. *Journal of Ayurveda and Integrative Medicine* **5**: 85–88.

Kay J K, Mackle T R, Aduldist M J, Thomson N A and Bauman D E. 2004. Endogenous synthesis of cis-9, trans-11 conjugated 18:2n-6 in pasture fed dairy cows. *Journal of Dairy Science* **87**: 369–78.

Kearl L C. 1982. *Nutrient Requirements of Ruminants in Developing Countries*. International Feed Stuffs Institute, Utah State University, Logan, Utah, USA.

Khanal R C and Olson K C. 2004. Factors affecting conjugated 18:2n-6 (CLA) content in milk, meat, and egg: a review. *Pakistan Journal of Nutrition* **3**: 82–98.

Lawless F, Stanton C., Escop P, Devery R, Dillon P and Murphy J J. 1999. Influence of breed on bovine milk cis-9, trans-11 conjugated linoleic acid content. *Livestock Production Science* **62**: 43–49.

NRC, 2001. *Nutrient Requirements for Dairy Cattle.* 7th Revised edn. National Research Council, National Academy of Science, Washington, DC 20418, USA

Nuernberg K, Dannenberger D, Nuernberg G, Ender K, Voigt J, Scollan N D, Wood J D, Nute G R and Richardson R I. 2005. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of different cattle breeds. *Livestock Production Science* **94**: 137–47.

O'Fallon J V, Busboom J R, Nelson M L and Gaskins C T. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils and feedstuffs. *Journal of Animal Science* **85**: 1511–21.

Palmquist D L, Lock A L, Shingfield K J and Bauman D E. 2005. Biosynthesis of Conjugated 18:2n-6 in ruminants and humans. *Advances in Food and Nutrition Research* **50** 180–217.

Parodi P. 1994. Conjugated 18:2n-6: an anticarcinogenic fatty acid present in milk. *Australian Journal of Dairy Technology* **49**: 93–97.

Piperova L S, Sampugna J, Teter B B, Kalscheur K F, Yurawecz M P and Erdman R A. 2002. Duodenal and milk trans octadecenoic acid and conjugated 18:2n-6 isomers indicate that post absorptive synthesis is the predominant source of cis-9 containing CLA in lactating dairy cows. *Journal of Nutrition* **132**: 1235–41.

Rajorhia G S. 1993. Ghee: Encyclopedia of Food Science. *Food Technology and Food Nutrition.* pp 2186–92. Academic press, London.

Rangappa K S, Achaya K T. 1974. *Indian Dairy Products*. pp 119–24.Asia Publishing house.

Schroeder G F, Delahoy J E, Vidaurrete I, Bargo F, Gagliostro G A and Muller L D. 2003. Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *Journal of Dairy Science,* **86**: 3237–48.

Snedecor C W and Cochran W G. 1994. *Statistical Method*. Iowa state university press. Ames, Iowa, USA.

Tremblay A and Gilbert J A. 2009. Milk products, insulin resistance syndrome and type 2 diabetes. *Journal of American College of Nutrition* **28**: 1–91.

Tyagi A K, Kewalramani N, Dhiman T R, Kaur H, Kanwajia S K and Signhal K K. 2006. Conjugated 18:2n-6 content of milk and milk products as influenced by dietary fat sources in buffaloes. *Indian Journal of Animal Sciences* **76**: 742–46.

Tyagi A K, Kewalramani N, Dhiman T R, Kaur H, Singhal K K and Kanawjia S K. 2007. Enhancement of CLA content in buffalo milk through green berseem feeding. *Animal Feed Science and Technology* **133**: 351–58.

Van Soest P J, Robertson P J and Lewis B A. 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.