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# EFFECT OF GREEN FODDER FEEDING ON CONJUGATED LINOLEIC ACID IN MILK AND GHEE (CLARIFIED BUTTER OIL) OF COWS AND BUFFALOES

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Ten multiparous crossbred cows and ten Murrah buffaloes (*Bos Bubalis*) in their early stage of lactation were randomly divided into four group of 5 animals each (Groups1 and 2 for cows and groups 3 and 4 for buffaloes). Animals of groups 1 and 3 were offered *ad lib.* berseem fodder (*Trifolium alexandrinum*) and wheat straw and groups 2 and 4 were fed concentrate mixture and wheat straw *ad lib.* for 12 weeks of experimental period. After an adaptation period of 4 weeks, the milk samples collected at fortnightly intervals were analyzed for milk fat, solid not fat, total solids and milk protein. Milk sample (100 ml) from each animal of all the four groups was collected from the morning and evening milking at fortnightly interval and pooled for fatty acid analysis including CLA. *Ghee* (clarified butter oil) was also prepared at fortnightly intervals by indigenous and creamery (commercial) methods from the total milk of each group. The milk yield in four respective groups averaged 12.06, 11.93, 9.99 and 7.80 kg per day showing a significant ($P<0.01$) increase in the milk yield of buffaloes fed high fodder diet as compared to high concentrate diet, while there was no effect in cows. Milk composition was not affected by dietary treatments in both cows and buffaloes. Milk total CLA content averaged 19.55, 6.44, 16.38 and 6.78 mg/g fat in four respective groups, showing significantly ($P<0.01$) higher values in berseem fed groups of both the species. In cows, total CLA content increased two folds in ghee prepared by indigenous method irrespective of diet i.e.10.40 vs 20.07 (group 1), 6.93 vs 12.13 (group 2), whereas, it increased by 40-50% in buffaloes (10.38 vs 16.87 in group 3 and 5.83 vs 7.59 mg/g in group 4). Feeding of fresh fodder to the animals along with indigenous method of ghee preparation resulted in 310% increase in CLA content.
**Keywords:** Berseem, conjugated linoleic acid, fatty acids, buffalo, cow, ghee

## INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (c-9, c-12 $C_{18:2}$) with two conjugated double bonds at various carbon positions in the fatty acid chain. It is formed as an intermediate during the biohydrogenation of linoleic acid to stearic acid or from the endogenous conversion of t-11, $C_{18:1}$ trans vaccenic acid in mammary gland. CLA occurs in many foods; however, the principle dietary sources are dairy products. More than 82 % of the CLA in dairy products is cis-9, trans-11 isomer (Chin *et et al.*, 1992). Milk fat is the richest natural dietary source of CLA and whole milk contains on an average 4.5mg CLA/g of fat. Diets fed to ruminants exert a major influence on CLA content of milk. Cows grazing natural permanent pastures had 500% higher CLA content in milk compared with cows fed typical dairy cow diet containing preserved forage and grain in a 50:50 ratio (Dhiman *et et al.*,1999). Various other studies have also shown higher CLA content in milk of cows fed predominantly on pasture/forage diet (Jahreis *et et al.*, 1997, Kelly *et et al.*, 1998, Tyagi *et et al.*, 2007). Milk is mainly consumed either as raw or in the form of indigenous milk products. Besides improving CLA content in milk through dietary manipulation, there are reports indicating that processing of milk can also alter CLA content. Ghee is prepared by many commercial methods involving heating of butter or cream at different temperatures. However, in villages, the whole milk is converted to Dahi (yoghurt) by fermentation and butter is removed by churning and is heated to prepare ghee. Such ghee is reported to have considerably higher levels of CLA (Aneja and Murthi, 1990). Buffaloes contribute 52% of total milk production of India, but, no information is available about CLA content in buffalo milk or milk products. It is therefore worthwhile to compare the two species with respect to their CLA content and attempts should be made to enhance the CLA content of buffalo milk through dietary manipulation and processing of milk. The present paper presents the findings of an experiment conducted in cows and buffaloes to find out the effect of dietary forage and method of ghee preparation on their CLA content.

## MATERIAL AND METHODS

### Management of cows and buffaloes

Ten crossbred (Holstein Friesian x Tharparkar) cows and ten multiparous Murrah buffaloes (*Bos Bubalis*) in early lactation, having similar body weight (480 -536 kg) and milk yield (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.

### Feeding

Ten cows and buffaloes were randomly divided into 4 equal groups of 5 animals each (Group I & II for cows and Group III & IV for buffaloes) on the basis of body weight and milk yield. The nutritional requirements of cows and buffaloes were fulfilled by ad lib. feeding of berseem (*Trifolium alexandrinum*) fodder + wheat straw (Groups I & III) and concentrate mixture + wheat straw (Group II & IV) as per Kearl (1982). The ingredient composition of concentrate mixture is given in Table1. Berseem was chosen as a fodder for feeding to experimental cows and buffaloes (Group I & III) since it is commonly used for feeding to dairy animals during winter season in which the experiment was conducted. Berseem was offered four times in a day. The ratio of concentrate and wheat straw was maintained at 60:40 in groups II & IV. After an adaptation period of four weeks, feed intake, orts and milk yield were recorded daily for eight weeks. The buffaloes were milked twice at 600 and 1800 hrs whereas cows were milked three times at 600, 1200 and 1800 hrs daily. The proximate composition (AOAC, 1995) and cell wall constituents (Van Soest *et et al.*, 1994) of various feedstuffs used in the experiment were estimated.

**Table 1. Ingredient composition of concentrate mixture**

| Ingredient | Percentage |
|---|---|
| Maize | 33 |
| Groundnut cake (Expeller) | 22 |
| Mustard cake (expeller) | 11 |
| Wheat bran | 31 |
| Mineral mixture* | 2 |
| Common salt | 1 |

Dicalcium phosphate 1.65 kg; Chalk powder 0.3312kg; 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; Sodiumfluoride 0.0003 kg; Zinc sulphate 0.0075 kg.

### Collection and analysis of milk samples

After adaptation period, milk samples were collected at fortnightly intervals for the analysis of milk fat (BIS, 1989), total solids (BIS, 1989) and milk protein (AOAC, 1995). 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 morning and evening milking at fortnightly intervals and pooled for fatty acid analysis including CLA (Dhiman *et et al.*, 1999) using gas chromatograph fitted with ionization detector and 3396A integrator. Samples containing methyl esters of fatty acids in hexane (1 to 3$\mu$l) were directly injected through the splitless injection port onto a supelco wax 10, fused silica capillary column (60m x 0.32m i.d.0.25 $\mu$m film thickness; Supelco Inc., Belleforte, P.A.). Injector temperature of the gas chromatograph was maintained at 250ºC. Initial oven temperature was 50ºC which was programmed to increase @ 20ºC per min upto 200ºC and was held for 50 min. Thereafter, it was raised to 225ºC @ 10ºC per min. and held for 20 min. Detector temperature of GC was 250ºC. The CLA and other fatty acid standards were procured from Sigma chemicals. 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 fortnightly intervals by two different methods i.e. indigenous and creamery (commercial) from the total milk of each group on that particular day.

### Indigenous method

Milk was boiled at 80$^0$C and cooled to refrigerated temperature and thick creamy layer was collected. Clotted cream was cultured overnight with NCDC 167 culture containing *Lactococcus lactis sp. Lactis.* It was dispersed in water and was churned in a mixer for separating the butter. Butter was heated in a metal pan on an electric heater until all the moisture was removed and ghee was separated. The contents were left undisturbed so that the curd particles are settled down at the bottom of the pan. Then, the clear fat was carefully decanted off into the storage vessel.

### Creamery method

In this method, cream was separated by centrifugation and *ghee* was prepared directly from cream. Cream was heated to 115$^0$C in a stainless steel jacked 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 colour of the ghee residue turned to golden yellow (Rajorhia, 1993).

### Statistical analysis

Statistical analysis of the data for milk production and fatty acid composition of milk and *ghee* of different treatment groups was carried out through SPSS 10.0 software package using general linear model. The effect of different treatment (groups) was determined by analysis of variance (Snedecor and Cochran, 1980). The statistical model used is described below.

**Milk analysis**

$Y_{ijkl} = \mu + B_i + D_i + A_k + e_{ijkl}$

Where,

$Y_{ijkl}$ = The $l^{th}$ observation of $k^{th}$ animal fed with $j^{th}$ diet and belonging to $i^{th}$ breed

$\mu$ = overall mean

$B_i$ = Effect due to $i^{th}$ breed

$D_i$ = Effect due to $j^{th}$ diet

$A_i$ = Effect due to $k^{th}$ animal

$e_{ijkl}$ = Random error

**Ghee analysis**

$Y_{ijk} = \mu + D_i + M_j + e_{ijk}$

Where,

$Y_{ijk}$ = The of $k^{th}$ observation estimated by $j^{th}$ method and fed with $i^{th}$ diet

$\mu$ = overall mean

$D_i$ = Effect due to $i^{th}$ diet

$M_i$ = Effect due to $j^{th}$ method

$e_{ijk}$= Random error

## RESULTS AND DISCUSSION

### Composition of experimental feeds

The proximate and cell wall composition of concentrate mixture, berseem and wheat straw are presented in Table 2. The ether extract content was 4.92, 3.34 and 2.14 percent in concentrate mixture, berseem and wheat straw, respectively. Fatty acid composition of berseem fodder, concentrate mixture and wheat straw (Table 3) indicated that berseem was the major source of C18:3 (14.98mg/g), while concentrate mixture was high in C18:2 (14.22mg/g). Elgersma *et et al.*, (2003a) reported higher concentration of linolenic acid in fresh grass which varies with the plant and environmental factors such as stage of maturity as well as season. Concentrate mixture provided highest content of fat containing more amount of saturated and mono-unsaturated fatty acids to the animals, whereas, berseem fodder provided higher amount of poly-unsaturated fatty acids. (Bu *et al.*, 2007).

**Table 2. Chemical composition of forage and concentrate mixture**

**(% DM basis)**

| Component | Concentrate mixture | Berseem | Wheat Straw |
|---|---|---|---|
| OM | 91.30 | 83.78 | 91.64 |
| CP | 23.32 | 19.20 | 3.24 |
| CF | 7.84 | 25.97 | 45.17 |
| EE | 4.92 | 3.34 | 2.14 |
| Ash | 8.70 | 16.22 | 8.36 |
| NFE | 55.22 | 35.27 | 40.36 |
| NDF | 40.01 | 45.78 | 85.76 |
| ADF | 13.58 | 29.09 | 54.11 |
| Hemicelluose | 26.43 | 16.70 | 31.65 |

### Dry matter intake, milk yield and milk composition

Body weight of cows and buffaloes in the two treatment groups was similar (Table 4). DM intake was significantly higher in berseem fed groups I and III as compared to concentrate fed groups II and IV. Tyagi et. al. (2006) also recorded higher dry matter intake in green fodder fed buffaloes as compared to concentrate fed buffaloes ((12.6 vs10.3 kg). Though the ether extract was low in berseem fodder (3.34 %) than concentrate mixture (4.92%), but, the total fat intake in all the groups of animals was similar (Table 4) due to higher dry matter intake through green fodder. The average milk yield in four respective groups was 12.0, 11.9, 9.9 and 7.8 kg per day showing a significant increase ($P > 0.01$) in milk yield of buffaloes fed fodder diet as compared to concentrate diet. Similar to the present findings, White *et et al.* (2001) did not record any difference in the milk yield of cows due to changes in the dietary proportion of forage and concentrate mixture. Milk fat content was higher ($P > 0.01$) in buffalo milk as compared to cow milk due to species variation which has also been documented earlier by many workers (Kathirvalen, 2007; White *et et al.* 2001; Bargo *et et al.* 2002). The higher fat content in buffalo milk also resulted in higher total solids as well as solid-not fat content (Tyagi et. al., 2006). However, dietary treatments did not affect the milk composition in both the species. In general, milk production and milk composition are characteristic of the species. Crossbred cows produce more milk as compared to buffaloes, while the later produce milk with higher fat content. As a result, daily yield of milk fat was higher in buffaloes to the extent of 16% (740.3 vs 637.8 g/d) inspite of their lower milk yield.

### Fatty acid composition of cow and buffalo milk

The treatment by fortnight interaction was non-significant for FA in milk; therefore average values for milk FA composition are presented in Table 5. The levels of C4:0, C14:0, C15:0, C16:0 and C16:1 fatty acids were significantly higher in buffalo milk as compared to cow milk, whereas, levels of C8:0, C10:0, C11:0, C12:0, C14:1 were higher in cow milk. Arumugam and Narayanan (1979) reported similar pattern of short and medium chain fatty acids in cow and buffalo milk fat. Dhiman *et al.* (1999) and Tyagi *et et al.* (2007) observed consistent values of fatty acids in the milk of cow and buffalo, respectively. Among long chain fatty acids, the level of all the isomers of C18:1 and C20:1 was higher in cow milk indicating more proportion of unsaturated fat. The total amount of unsaturated fatty acids was more in cow milk as compared to buffalo milk fat (369.0 vs 337.9), while the amount of saturated fatty acids showed reverse trend

**Table 3. Fatty acid composition of forage and concentrate mixture**

| Fatty Acid[1] | 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 |
| Total Omega-3 | 14.884 | 10.320 | 0.193 |
| Total Omega-6 | 3.735 | 14.325 | 0.715 |
| Total Fat (mg) | 25.385 | 45.834 | 3.027 |
| Total Saturated fatty acids | 5.43 | 8.66 | 1.35 |
| Total Unsaturated fatty acids | 94.57 | 91.34 | 98.65 |
| Mono-Unsaturated fatty acids | 1.33 | 21.31 | 0.76 |
| Poly-Unsaturated fatty acids | 18.62 | 15.86 | 0.91 |

[1]Expressed as number of carbons: number of double bonds; *c* = *cis*

**Table 4. Dry matter intake, milk yield and milk composition of cows and buffaloes fed green fodder and concentrate mixture**

| Particulars | Cow (Green fodder) | Cow (concentrate) | Buffalo (Green fodder) | Buffalo (concentrate) | SEM[2] |
|---|---|---|---|---|---|
| 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[a] | 12.2[b] | 13.4[a] | 11.1[b] | 0.56 |
| Fat | 0.44 | 0.46 | 0.45 | 0.42 | 0.01 |
| Milk yield/day (kg) | 12.0[a] | 11.9[a] | 9.9[b] | 7.8[c] | 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[b] | 637.8[c] | 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] Values with different superscript in row differ significantly at ($P< 0.01$). Mean value represents average from sampling fortnight 2 through 5

(611.5 vs 662.0). The content of both mono-and poly-unsaturated fatty acids was higher in cow milk. The differences in the two species may be due to more amount of *de novo* synthesis at the mammary gland level in cows which might also be responsible for more CLA content in cow milk fat as compared to buffalo milk fat (Baumgard *et et al.*, 2002). Irrespective of dietary treatments, total CLA content averaged 13.0±1.56 and 11.58±1.98 mg/g milk fat in cow and buffalo milk, respectively and was significantly higher in cow as compared to buffalo milk fat. Lawless *et et al.* (1999) maintained Irish Holstein/Friesian, Dutch Holstein/Friesian, Montbeliardes, and Normandes breeds on pasture and compared their milk CLA content. They reported that Montbeliardes had 13% greater CLA content in milk fat than the other three breeds. White *et et al.* (2001) compared Holstein and Jersey cows that were either fed a TMR in confinement or grazing pasture and found that Holstein cows had slightly higher milk fat concentrations of CLA than Jersey cows (~18% greater overall). Whitlock *et et al.* (2002) also reported a breed x diet interaction in a study with Brown Swiss and Holstein cows.

**Table 5. Average fatty acid composition* (mg/g fat) in milk of cows and buffaloes**

| Fatty acid | Cow | Buffalo |
|---|---|---|
| C4:0 | $24.2^{b}$±1.18 | $32.5^{a}$±1.85 |
| C6:0 | 21.4±0.84 | 16.9±0.92 |
| C8:0 | $10.6^{a}$ ±0.78 | $8.1^{b}$ ±0.69 |
| C10:0 | $23.1^{a}$ ±1.67 | $15.3^{b}$ ±1.95 |
| C11:0 | $2.9^{a}$ ±0.65 | $0.9^{b}$ ±0.56 |
| C12:0 | $26.4^{a}$ ±1.95 | $19.95^{b}$ ±2.06 |
| C14:0 | $97.4^{b}$ ±6.45 | $107.6^{a}$ ±4.85 |
| C14:1 | $8.8^{a}$ ±0.99 | $6.2^{b}$ ±0.85 |
| C15:0 | $14.0^{a}$ ±0.32 | $13.9^{b}$ ±0.25 |
| C16:0 | $259.2^{b}$ ±17.52 | $309.7^{a}$ ±18.69 |
| C16:1 9-t | 3.1±0.15 | 2.1±0.19 |
| C16:1 | $10.7^{b}$ ±1.52 | $17.3^{a}$ ±1.69 |
| C17:1 | 2.3±0.16 | 2.7±0.20 |
| C18:0 | 148.5±14.63 | 137.0±12.32 |
| C18:1 9-t | $2.1^{a}$ ±0.18 | $1.4^{b}$ ±0.25 |
| C18:1 10-t | $3.7^{a}$ ±0.42 | $2.9^{b}$ ±0.23 |
| C18:1 11-t | 33.3±1.25 | 29.3±1.36 |
| C18:1 12-t | $3.0^{a}$ ±0.23 | $2.1^{b}$ ±0.19 |
| C18:1 13,14-t,6,7,8-c | $7.6^{a}$ ±0.56 | $4.5^{b}$ ±0.52 |
| Total C18:1-trans | $51.1^{a}$ ±3.58 | $40.3^{b}$ ±3.69 |
| C18:1 9-c | $237.1^{a}$ ±10.36. | $214.8^{b}$ ±11.32 |
| C18:1 11-c | $10.2^{a}$ ±0.66 | $9.4^{b}$ ±0.82 |
| C18:2 9-c,12-c | $14.2^{a}$ ±1.25 | $12.1^{b}$ ±1.63 |
| C20:0 | 4.3±0.32 | 3.7±0.29 |
| C20:1 | $3.0^{a}$ ±0.46 | $1.9^{b}$ ±0.32 |
| C18:3 9-12-15-c | 7.4±0.65 | 7.0±0.73 |
| Total C18:3 | 7.4±0.36 | 7.0±0.0.31 |
| C18:2 9-c,11-t | $12.5^{a}$ ±0.86 | $10.8^{b}$ ±0.95 |
| C18:2 10-t,12-c | 1.1±0.09 | 0.9±0.06 |
| **Total CLA** | $13.0^{a}$ ±1.56 | $11.58^{b}$ ±1.98 |
| Total Omega3 | 13.2±1.95 | 12.3±1.09 |
| Total Omega6 | $14.5^{a}$ ±1.30 | $12.1^{b}$ ±1.42 |
| Omega-6:Omega-3 | 11.4±1.41 | 10.1±1.12 |
| Total Saturated | $611.5^{a}$ ±8.10 | $662.0^{b}$ ±9.15 |
| Total Unsaturated | $369.0^{a}$ ±6.15 | $337.9^{b}$ ±7.10 |
| Mono-Unsaturated | $284.5^{a}$ ±6.15 | $255.9^{b}$ ±5.8 |
| Poly-Unsaturated | $25.1^{a}$ ±1.61 | $21.4^{b}$ ±1.90 |

*Mean values of different fatty acids in milk of cow and buffalo irrespective of their diet. i.e. green fodder and concentrate mixture

[a,b] Values with different superscript in row differ significantly at (P<0.01). Mean value represents average from sampling fortnight 2 through 5

**Effect of diet on milk fatty acid composition**

There was no variation in short chain fatty acid content (C-4 to C-12) of milk due to variation in the dietary composition (Table 6). The content of medium chain fatty acids i.e. C-14 to C-17 was significantly increased due to feeding of green fodder as compared to concentrate mixture. Total C18:1- trans fatty acid averaged 59.8±4.45 and 31.5±2.56 mg/g in the milk fat of green fodder and concentrate fed animals, whereas total C18:3 was significantly higher in green fodder groups (11.8 vs 2.6mg/g). In the present study, cis-9, trans-11 C18:2 fraction constituted 90 percent of total CLA content of milk irrespective of diet similar to the findings of Kay et al (2004) and Tyagi et al (2006). Total CLA content averaged 18.0 and 6.6 mg/g in berseem and concentrate fed animals, respectively which showed 3 fold increase in total CLA by green fodder feeding. Dhiman et al (1999) also reported highest CLA content in the milk of cows fed solely on pasture than those fed on one third and two third pasture. Increasing the forage content in the diet either through fresh chopped fodder or pasture grazing resulted in similar increase in CLA content (Ward *et et al.* 2003). Schroeder *et et al.* (2003) recorded 2-3 fold higher milk CLA concentration in dairy cows fed pasture as compared with TMR. Bauman *et et al.* (2001) and Chilliard *et et al.* (2001) reported that diet can have a major impact on cis-9, trans-11CLA content in milk fat since it originates from the incomplete biohydrogenation of polyunsaturated fatty acids. The majority of the CLA in milk fat is synthesized via the enzyme $\Delta^{9}$-desaturase from trans-11,C18:1, an intermediate in the rumen biohydrogenation of linoleic and linolenic acids (Griinari *et et al.*, 2000; Corl *et et al.*, 2001; Lock and Garnsworthy, 2002; Piperova *et et al.*, 2002). This enzyme also plays a critical role in maintaining fluidity of cellular membranes and milk fat (Chilliard *et et al.*, 2000). The level of vaccinic acid (C18:1 t-11) was significantly (P<0.01) higher (45.3 mg/g) in berseem fed than the concentrate fed animals (17.3 mg/g, Table- 6). The high content of C18:3 in the green fodder might have increased the ruminal production of vaccinic acid. In addition, C18:3 may reduce the conversion of vaccinic acid to C18:0 which is the rate limiting step in biohydrogenation of fatty acids (Griinari and Bauman 1999) resulting in large amount of vaccinic acid absorbed post ruminally in green fodder fed animals. These results revealed that feeds, rich in $C_{18:3}$ fatty acids, increased the CLA content in the milk possibly due to conversion of TVA to CLA at mammary gland level. The ratio of omega3 and omega 6 fatty acids was 1:1 in both the diets which has been reported to be beneficial for the cardiac health (Gandig *et et al.*, 2001).

**Table 6. Average Fatty acid composition* of milk fat (mg/g) on green fodder and concentrate diet**

| Fatty acid | Green fodder | Concentrate mixture |
|---|---|---|
| C4:0 | 29.1±1.06 | 27.7±1.14 |
| C6:0 | 17.8±0.78 | 20.5±0.85 |
| C8:0 | 9.9±0.35 | 8.9±0.42 |
| C10:0 | 20.0±1.22 | 18.4±1.31 |
| C11:0 | 2.0±0.29 | 0.9±0.46 |
| C12:0 | 24.2±1.32 | 22.2±1.42 |
| C14:0 | $108.7^{a} \pm 5.08$ | $96.3^{b} \pm 7.12$ |
| C14:1 | 8.0±0.65 | 7.1±0.45 |
| C15:0 | $17.4^{a} \pm 0.45$ | $10.5^{b} \pm 0.68$ |
| C16:0 | $304.5^{a} \pm 14.58$ | $264.4^{b} \pm 15.52$ |
| C16:1 9-t | $4.2^{a} \pm 0.25$ | $1.2^{b} \pm 0.16$ |
| C16:1 | 14.1±1.02 | 14.0±0.98 |
| C17:1 | $3.0^{a} \pm 0.19$ | $2.0^{b} \pm 0.25$ |
| C18:0 | $121.4^{b} \pm 17.25$ | $164.1^{a} \pm 18.56$ |
| C18:1 9-t | 1.7±0.12 | 1.9±0.16 |
| C18:1 10-t | $2.9^{b} \pm 0.21$ | $3.7^{a} \pm 0.22$ |
| C18:1 11-t | $45.3^{a} \pm 4.21$ | $17.3^{b} \pm 2.65$ |
| C18:1 12-t | $2.7^{b} \pm 0.16$ | $3.3^{a} \pm 0.23$ |
| C18:1 13,14-t,6,7,8-c | $7.3^{a} \pm 0.69$ | $4.9^{b} \pm 0.58$ |
| Total C18:1-trans | $59.8^{a} \pm 4.45$ | $31.5^{b} \pm 2.56$ |
| C18:1 9-c | $197.9^{b} \pm 9.56$ | $254.0^{a} \pm 8.26$ |
| C18:1 11-c | $9.4^{b} \pm 0.95$ | $10.2^{a} \pm 0.86$ |
| C18:2 9-c,12-c | $14.2^{a} \pm 1.20$ | $12.1^{b} \pm 0.86$ |
| C20:0 | $2.0^{b} \pm 0.26$ | $6.0^{a} \pm 0.58$ |
| C20:1 | $1.2^{b} \pm 0.12$ | $3.8^{a} \pm 0.26$ |
| C18:3 9-12-15-c | $11.8^{a} \pm 1.26$ | $2.5^{b} \pm 0.18$ |
| Total C18:3 | $11.8^{a} \pm 1.60$ | $2.6^{b} \pm 0.24$ |
| C18:2 9-c,11-t | $17.0^{a} \pm 0.98$ | $6.4^{b} \pm 0.13$ |
| C18:2 10-t,12-c | $1.4^{a} \pm 0.10$ | $0.5^{b} \pm 0.02$ |
| Total CLA | $18.0^{a} \pm 0.95$ | $6.6^{b} \pm 0.16$ |
| Total Omega3 | $14.5^{a} \pm 0.65$ | $11.0^{b} \pm 0.85$ |
| Total Omega6 | $14.3^{a} \pm 0.98$ | $12.3^{b} \pm 0.67$ |
| Omega-6:Omega-3 | 10.3±0.65 | 11.2±0.68 |
| Total Saturated | 636.7±7.45 | 636.8±6.45 |
| Total Unsaturated | 343.7±6.54 | 346.2±5.58 |
| Mono-Unsaturated | $245.7^{b} \pm 4.58$ | $294.7^{a} \pm 5.02$ |
| Poly-Unsaturated | 23.1±1.12 | 22.4±1.68 |

$^{a,b}$ Values with different superscript in row differ significantly at ($P \leq 0.01$)
Mean value represents average from sampling fortnight 2 through 5.
*Mean values of different fatty acids in milk of green fodder and concentrate mixture fed animals irrespective of the species. i.e. cow and buffalo

**Table 7. Average fatty acid composition* in ghee prepared by indigenous and creamery methods (mg/g of fat)**

| Fatty acid | Creamery method | Indigenous Method |
|---|---|---|
| C4:0 | 29.9±1.12 | 30.4±1.07 |
| C6:0 | 16.7±0.53 | 17.6±0.22 |
| C8:0 | 9.2±0.54 | 9.6±0.40 |
| C10:0 | 18.5±1.41 | 19.7±1.23 |
| C11:0 | 1.7±0.29 | 1.8±0.28 |
| C12:0 | 22.5±1.29 | 23.7±1.12 |
| C14:0 | 101.0±3.07 | 108.0±2.00 |
| C14:1 | 6.7±0.51 | 7.4±0.43 |
| C15:0 | $12.1^{b} \pm 0.47$ | $15.7^{a} \pm 0.98$ |
| C16:0 | 287.4±13.00 | 285.0±14.96 |
| C16:1 9-t | $1.5^{b} \pm 0.15$ | $2.3^{a} \pm 0.35$ |
| C16:1 | 13.6±0.96 | 14.4±0.86 |
| C17:1 | $2.3^{b} \pm 0.15$ | $2.8^{a} \pm 0.15$ |
| C18:0 | $144.7^{a} \pm 7.04$ | $133.2^{b} \pm 5.58$ |
| C18:1 9-t | 1.9±0.12 | 1.5±0.17 |
| C18:1 10-t | $3.5^{a} \pm 0.17$ | $3.2^{b} \pm 0.15$ |
| C18:1 11-t | $23.8^{b} \pm 1.92$ | $37.5^{a} \pm 4.08$ |
| C18:1 12-t | $2.2^{b} \pm 0.26$ | $2.5^{a} \pm 0.33$ |
| C18:1 13,14-t,6,7,8-c | $5.4^{b} \pm 0.38$ | $6.6^{a} \pm 0.63$ |
| Total C18:1-trans | $37.2^{b} \pm 2.05$ | $51.6^{a} \pm 4.72$ |
| C18:1 9-c | $241.9^{a} \pm 8.63$ | $218.5^{b} \pm 6.84$ |
| C18:1 11-c | 9.8±0.34 | 9.2±0.29 |
| C18:2 9-c,12-c | 12.4±0.49 | 13.6±0.62 |
| C20:0 | $5.1^{a} \pm 0.58$ | $3.4^{b} \pm 0.44$ |
| C20:1 | $3.2^{a} \pm 0.33$ | $2.0^{b} \pm 0.27$ |
| C18:3 9-12-15-c | $4.3^{b} \pm 0.77$ | $9.1^{a} \pm 1.24$ |
| Total C18:3 | $4.3^{b} \pm 0.77$ | $9.1^{a} \pm 1.24$ |
| C18:2 9-c,11-t | $8.0^{b} \pm 0.74$ | $13.3^{a} \pm 1.48$ |
| C18:2 10-t,12-c | 0.3±0.11 | 0.8±0.12 |
| Total CLA | $8.4^{b} \pm 0.84$ | $14.5^{a} \pm 1.58$ |
| Total Omega3 | $11.9^{b} \pm 0.26$ | $13.7^{a} \pm 0.66$ |
| Total Omega6 | $12.6^{b} \pm 0.52$ | $13.7^{a} \pm 0.63$ |
| Omega-6:Omega-3 | 10.7±0.43 | 10.0±0.27 |
| Total Saturated | 645.4±8.49 | 646.7±9.45 |
| Total Unsaturated | 354.5±8.49 | 353.3±9.45 |
| Mono-Unsaturated | $282.5^{a} \pm 7.74$ | $267.1^{b} \pm 6.18$ |
| Poly-Unsaturated | 22.1±1.75 | 21.9±2.57 |

$^{a,b}$ Values with different superscript in row differ significantly at ($P \leq 0.01$)
Mean value represents average from sampling fortnight 2 through 5.
*Mean values of different fatty acids in ghee of indigenous and creamery method preparation irrespective of the species (cow and buffalo) and diets (green fodder and concentrate)

## Effect of method of ghee preparation on its fatty acid composition

Indigenous method of preparation of ghee resulted in 93 and 75 % increased CLA content in cows and 62

**Table 8. Fatty acid composition in milk of cows and buffalo fed on green fodder and concentrate diet (mg/g fat)**

| Fatty Acids (mg/g) | COW | | Buffalo | |
|---|---|---|---|---|
| | GF | Concentrate | GF | Concentrate |
| C4:0 | 24.12±108 | 24.43±1.07 | 30.86±1.49 | 6.14±1.62 |
| C6:0 | 16.99±0.89 | 26.03±8.94 | 16.39±1.61 | 14.9±0.66 |
| C8:0 | 10.54±0.73 | 10.92±0.65 | 8.72±0.51 | 6.91±0.25 |
| C10:0 | 23.45±1.87 | 23.82±2.04 | 16.46±1.13 | 12.99±0.46 |
| C11:0 | 2.99±0.44 | 2.91±3.03 | 1.01±0.07 | 0.78±0.10 |
| C12:0 | 26.73±2.37 | 27.12±2.56 | 21.34±1.16 | 17.20±0.62 |
| C14:0 | 103.24±6.75 | 93.84±5.52 | 115.52±2.43 | 98.53±3.99 |
| C14:1 | 9.55±1.83 | 8.37±0.99 | 6.27±0.29 | 6.12±1.26 |
| C15:0 | 18.20±0.98 | 9.43±0.19 | 15.84±0.54 | 11.86±0.29 |
| C16:0 | 288.03±13.05 | 235.40±6.91 | 331.79±9.56 | 291.50±10.37 |
| C16:1 9-t | 3.22±0.43 | 1.01±0.06 | 2.89±0.20 | 1.33±0.05 |
| C16:1 | 11.61±1.49 | 9.99±0.80 | 16.31±1.01 | 18.61±3.10 |
| C17:1 | 3.02±0.37 | 1.55±0.13 | 2.87±0.25 | 2.72±0.15 |
| C18:0 | 131.07±15.94 | 163.92±8.64 | 110.62±3.15 | 165.69±27.43 |
| C18:1 9-t | 1.86±0.32 | 2.28±0.16 | 1.34±0.03 | 1.47±0.04 |
| C18:1 10-t | 3.17±0.24 | 4.24±0.15 | 2.69±0.13 | 3.22±0.15 |
| C18:1 11-t | 49.62±3.47 | 18.44±0.68 | 41.30±2.49 | 18.47±1.98 |
| C18:1 12-t | 2.54±0.59 | 2.64±0.33 | 0.38±0.19 | 1.15±0.23 |
| C18:1 13,14-t,6,7,8-c | 8.89±0.78 | 6.34±0.36 | 5.54±0.48 | 3.21±0.25 |
| Total C18:1-trans | 66.26±66.31 | 34.63±1.25 | 51.31±2.93 | 27.87±2.15 |
| C18:1 9-c | 195.29±14.13 | 271.23±5.39 | 195.20±8.65 | 236.89±6.53 |
| C18:1 11-c | 9.66±0.79 | 10.47±0.35 | 8.74±0.45 | 17.01±5.83 |
| C18:2 9-c,12-c | 14.43±0.56 | 13.96±0.93 | 13.55±0.49 | 10.22±0.65 |
| C20:0 | 2.11±0.33 | 6.52±0.38 | 2.04±0.27 | 5.17±0.47 |
| C20:1 | 1.29±0.09 | 4.61±0.17 | 1.03±0.14 | 2.84±0.25 |
| C18:3 9-12-15-c | 12.67±0.57 | 2.30±0.21 | 11.01±1.05 | 2.98±0.57 |
| Total C18:3 | 12.70±0.57 | 2.34±0.22 | 11.01±1.05 | 2.98±0.57 |
| C18:2 9-c,11-t | 18.11±2.16 | 6.38±0.17 | 14.78±1.06 | 6.65±0.53 |
| C18:2 10-t,12-c | 0.94±0.16 | 0.04±0.02 | 1.13±0.11 | 0.35±0.09 |
| **Total CLA** | 19.05±2.26 | 6.42±0.17 | 15.91±1.11 | 7.01±0.55 |
| Total Omega3 | 15.50±0.67 | 10.98±0.76 | 13.34±1.00 | 10.93±0.36 |
| Total Omega6 | 14.70±0.68 | 14.27±1.06 | 13.68±0.45 | 10.37±0.65 |
| Omega-6:Omega-3 | 9.55±0.45 | 13.11±0.42 | 11.14±1.03 | 9.48±0.29 |
| Total Saturated | 648.53±20.02 | 619.64±6.60 | 670.25±5.48 | 653.09±10.41 |
| Total Unsaturated | 351.46±20.02 | 380.35±6.60 | 329.74±5.48 | 346.90±10.41 |
| Mono-Unsaturated | 253.67±20.08 | 308.67±5.35 | 249.05±5.43 | 282.23±8.47 |
| Poly-Unsaturated | 26.50±7.18 | 23.91±1.74 | 21.72±4.44 | 21.58±1.76 |

Mean Value represents average from sampling fortnight 2 through 5

and 30% increase in buffalo ghee on forage and concentrate based diets, respectively (Table-9) showing comparatively more increase in CLA content due to method of preparation of ghee in cows. Aneja and Murthi (1990) also recorded reduced response in CLA content due to indigenous method of ghee preparation in buffaloes as compared to cows (66 vs 30%). An overall 73 % increase in the CLA content was observed due to indigenous method of ghee

**Table 9. Fatty acid composition in ghee prepared by indigenous and creamery methods of cow and buffalo milk fed on green fodder and concentrate (mg/g fat)**

| Fatty acids (mg/g) | Cow | | | | buffalo | | | |
|---|---|---|---|---|---|---|---|---|
| | GF | | Conc. | | GF | | Conc. | |
| | creamery | Indigenous | creamery | Indigenous | creamery | Indigenous | creamery | Indigenous |
| C4:0 | 28.05±1.50 | 26.90±1.44 | 27.83±1.82 | 25.77±0.42 | 33.21±1.91 | 3.2.66±9.61 | 34.33±0.48 | 32.81±9.71 |
| C6:0 | 17.24±0.76 | 17.83±0.46 | 17.42±0.74 | 17.61±0.94 | 17.04±0.99 | 18.04±5.31 | 17.12±0.18 | 15.29±4.67 |
| C8:0 | 10.34±0.35 | 11.12±0.06 | 10.46±0.77 | 11.04±0.73 | 8.33±0.51 | 9.03±2.66 | 8.00±0.25 | 7.14±2.20 |
| C10:0 | 21.49±0.77 | 24.68±0.82 | 22.09±1.72 | 23.83±1.90 | 15.78±0.88 | 17.17±5.05 | 14.99±0.49 | 13.12±4.03 |
| C11:0 | 2.31±0.17 | 3.04±0.07 | 2.45±0.30 | 2.93±0.38 | 0.96±0.03 | 1.05±0.31 | 0.87±0.03 | 0.72±0.23 |
| C12:0 | 25.06±1.05 | 27.91±1.11 | 25.65±2.07 | 27.04±2.12 | 20.35±1.33 | 21.80±6.41 | 19.45±0.65 | 17.57±5.29 |
| C14:0 | 100.75±7.79 | 108.67±1.77 | 101.65±6.91 | 95.05±5.27 | 105.14±36.12 | 113.38±33.35 | 108.36±1.87 | 99.85±29.80 |
| C14:1 | 7.51±0.54 | 9.22±0.49 | 7.95±0.69 | 8.33±1.18 | 5.94±0.58 | 6.29±1.85 | 6.09±0.13 | 5.00±1.54 |
| C15:0 | 12.76±1.02 | 20.08±1.44 | 13.84±0.64 | 10.41±0.25 | 13.40±1.17 | 16.77±4.98 | 12.35±0.82 | 11.90±3.51 |
| C16:0 | 266.50±19.69 | 239.05±52.93 | 272.48±14.83 | 241.25±7.57 | 345.93±11.68 | 314.14±92.40 | 314.58±1.91 | 295.96±87.51 |
| C16:1 9-t | 1.81±0.32 | 2.46±1.34 | 2.06±0.14 | 1.07±0.01 | 1.90±0.35 | 3.25±0.98 | 1.52±0.30 | 1.33±0.40 |
| C16:1 | 11.63±1.54 | 11.81±1.06 | 12.04±1.22 | 10.30±0.89 | 16.08±1.57 | 16.20±4.77 | 17.52±1.03 | 16.34±4.85 |
| C17:1 | 2.17±0.27 | 3.20±0.35 | 2.26±0.01 | 1.71±0.07 | 2.64±0.25 | 3.21±0.95 | 2.54±0.11 | 2.69±0.82 |
| C18:0 | 149.35±17.33 | 145.92±12.21 | 145.26±12.84 | 164.03±12.17 | 117.87±4.46 | 112.72±33.15 | 128.87±3.15 | 147.40±43.94 |
| C18:1 9-t | 2.18±0.21 | 1.45±0.34 | 2.06±0.28 | 2.36±0.14 | 1.52±0.06 | 1.54±0.47 | 1.04±0.44 | 1.73±0.51 |
| C18:1 10-t | 3.48±0.49 | 3.10±0.42 | 3.64±0.18 | 4.06±0.18 | 2.94±0.14 | 2.75±0.82 | 3.48±0.26 | 3.71±1.10 |
| C18:1 11-t | 29.31±3.67 | 53.39±5.58 | 31.34±2.41 | 19.98±0.76 | 27.19±4.97 | 44.12±13.27 | 21.32±4.59 | 18.72±5.64 |
| C18:1 12-t | 2.09±1.07 | 3.61±0.43 | 2.91±0.26 | 2.84±0.17 | 1.96±0.08 | 1.69±0.90 | 1.73±0.09 | 1.77±0.53 |
| C18:1 13,14-t,6,7,8-c | 6.66±0.32 | 9.50±0.56 | 6.90±0.52 | 6.52±0.26 | 4.70±0.46 | 5.76±1.71 | 4.13±0.61 | 3.91±1.17 |
| Total C18:1-trans | 44.11±3.04 | 71.32±6.71 | 47.25±3.47 | 36.29±1.50 | 38.43±5.26 | 55.91±16.58 | 31.86±4.58 | 30.07±8.98 |
| C18:1 9-c | 242.10±19.38 | 209.18±22.88 | 231.73±9.36 | 267.60±6.67 | 204.85±5.50 | 200.50±59.02 | 232.52±5.89 | 253.03±74.99 |
| C18:1 11-c | 9.69±0.4 | 9.09±1.02 | 9.29±0.24 | 9.95±0.19 | 8.80±0.23 | 9.07±2.73 | 9.23±0.47 | 10.76±3.31 |
| C18:2 9-c,12-c | 13.75±0.10 | 15.36±1.41 | 14.31±0.36 | 14.11±0.37 | 11.11±0.62 | 13.78±4.07 | 10.94±1.00 | 10.65±3.14 |
| C20:0 | 4.91±1.23 | 2.44±0.18 | 4.31±0.47 | 6.30±0.53 | 2.99±1.40 | 1.78±0.53 | 5.06±0.74 | 6.40±1.89 |
| C20:1 | 3.32±0.81 | 1.40±0.13 | 3.07±0.20 | 4.56±0.25 | 2.02±0.35 | 0.98±0.29 | 2.62±0.37 | 3.10±0.91 |
| C18:3 9-12-15-c | 6.10±1.72 | 13.71±1.25 | 7.14±0.52 | 2.88±0.29 | 6.25±1.83 | 11.32±3.39 | 4.18±2.11 | 2.20±0.66 |
| Total C18:3 | 6.12±1.73 | 13.71±1.25 | 7.22±0.60 | 2.88±0.29 | 6.25±1.83 | 11.32±3.39 | 4.18±2.11 | 2.20±0.66 |
| C18:2 9-c,11-t | 9.87±1.55 | 18.97±2.26 | 11.36±0.57 | 6.84±0.88 | 9.64±1.92 | 15.82±4.75 | 7.28±1.60 | 5.83±1.73 |
| C18:2 10-t,12-c | 0.53±0.20 | 1.10±0.01 | 0.77±0.06 | 0.06±0.06 | 0.74±0.19 | 1.05±0.32 | 0.31±0.31 | 0.00± |
| **Total CLA** | 10.40±1.75 | 20.07±2.26 | 12.13±0.53 | 6.93±0.02 | 10.38±2.05 | 16.87±5.05 | 7.59±1.91 | 5.83±1.73 |
| Total Omega3 | 12.85±0.25 | 16.60±0.84 | 12.84±0.37 | 11.47±0.47 | 11.58±0.57 | 13.58±4.07 | 11.81±1.22 | 11.54±3.42 |
| Total Omega6 | 14.15±0.19 | 15.77±1.18 | 14.44±0.30 | 14.41±0.29 | 11.35±0.40 | 13.87±4.09 | 10.94±1.00 | 10.65±3.14 |
| Omega-6:Omega-3 | 11.02±0.36 | 9.48±0.30 | 11.26±0.34 | 12.58±0.37 | 9.86±0.70 | 10.27±3.04 | 9.29±0.11 | 9.23±2.72 |
| Total Saturated | 636.08±16.08 | 627.69±38.40 | 640.52±13.55 | 620.02±7.83 | 644.29±39.31 | 658.10±193.56 | 660.41±1.24 | 642.94±189.46 |
| Total Unsaturated | 363.92±16.08 | 372.31±38.40 | 359.48±13.55 | 379.98±7.83 | 355.71±39.31 | 241.90±100.57 | 339.59±1.24 | 357.06±105.67 |
| Mono-Unsaturated | 290.25±15.95 | 272.68±23.68 | 272.47±8.28 | 301.84±5.40 | 246.53±1.13 | 249.83± | 273.60±4.11 | 291.34±86.25 |
| Poly-Unsaturated | 27.76±5.57 | 30.84±9.44 | 20.34±0.74 | 22.80±1.36 | 16.82±2.14 | 16.78±5.05 | 19.49±0.11 | 21.05±6.22 |

Mean Value represents average from sampling fortnight 2 through 5

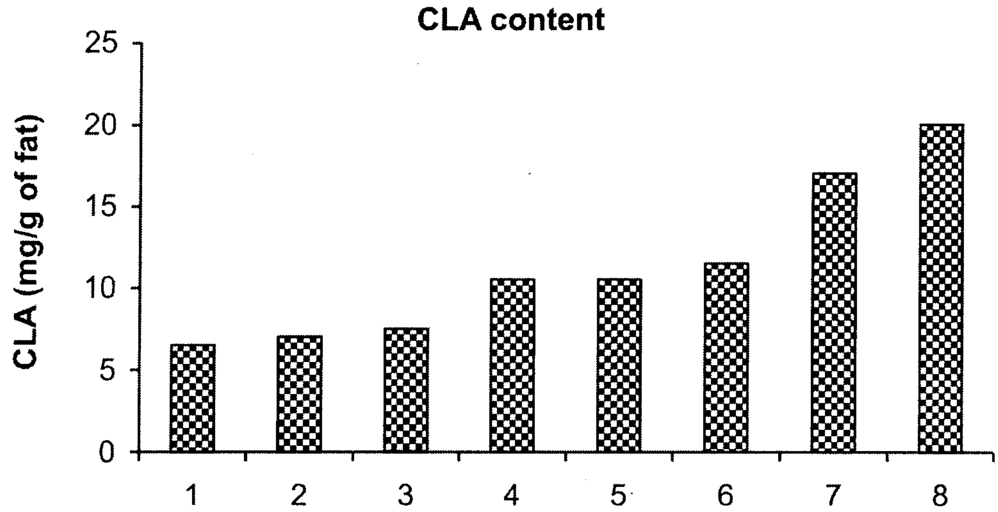


**Fig. 1. Total CLA content in ghee prepared by indigenous and creamery method from cow and buffalo fed with green fodder and concentrate mixture**

1. Buffalo-concentrate-creamery
2. Cow -concentrate-creamery
3. Buffalo-concentrate-indigenous
4. Cow-concentrate- indigenous
5. Buffalo-green fodder-creamery
6. Buffalo-green fodder-creamery
7. Buffalo-green fodder-indigenous
8. Cow-green fodder-indigenous

preparation as compared to creamery method, irrespective of the species (Table-7). The natural microbial fermentation during dahi (curd) formation increased the CLA content in ghee of cows and buffaloes (Aneja and Murthi, 1990). Increased CLA content of milk fat in indigenous method is due to increased production of free fatty acids by lipolysis of milk fat which provided more linoleic acid for conversion to CLA to the culture (Yadav *et et al.*, 2007). In the present study, the amount of omega3 and omega 6 fatty acids was also increased by indigenous method; however, there was no difference in the total saturated or unsaturated fatty acid content of ghee. There was no difference in C- 4 to C-14 fatty acids content due to type of method used for ghee preparation. The amount of C-15 to C-17 fatty acids was higher in indigenous method and content of total C-18 was more in creamery method.

## CONCLUSION

Feeding of green fodder increased CLA content of milk in cows and buffaloes. Cows had higher level of milk CLA as compared to buffaloes. Indigenous method of ghee preparation followed in rural areas increased the CLA content in both cows and buffaloes, however, the increase was more in cows.

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