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Conjugated linoleic acid: A mixture of bio- active fatty acids in milk fat of ruminants

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Abstract

Conjugated linoleic acid (CLA) present in ruminant milk, have several health benefits including anticarcinogenic, antiatherogenic, immuno-modulating, growth promotion and lean body mass promotion. In the present work we have investigated the content of one dominant (*c9, t11-CLA), one* intermediate *(t10, c*12-CLA) and the three minor CLA isomers (*t11, c13- , t7, c9-* CLA) in ruminant milk from three districts of Sindh. A total of 167 milk samples were collected throughout the year from Dairy Farms in Thatta, Dadu and Hyderabad, Sindh. The results show strong variation in CLA content, depending on ruminant milk and season. The mean CLA concentration was higher in cow's milk fat ranging from 8.81 -10.99 mg /g followed by sheep 8.39 – 9.10 mg /g, goat 5.90 -6.35 mg /g and buffalo milk fat 5.10 – 6.22 mg /g respectively. Summer milk fat contains 7.60 – 24.00 % higher content of total CLA as compared to winter milk in all ruminants. The differences in CLA contents are possibly due to the different activity of desaturase enzymes among ruminants, while seasonal variations in milk CLA concentrations reflect the availability of green pasture and its quality.

Keywords: Conjugated linoleic acid, Seasonal variation, Ruminants, Green pastures

Introduction

Conjugated linoleic acid (CLA) represent a mixture of positional and geometrical isomers of octadecadienoic acid with conjugated double bounds. CLA have been demonstrated to have a range of positive health effects in experimental animals. These include suppression of carcinogenesis [1 – 2], antiobesity effect [3], modulation of the immune system [4] and reduction in atherogenesis [5] and diabetics [6]. Lee et al. (1994) [7] and Nicolas et al. (1993) [8] have also reported positive effects of CLA on cardiovascular risk factors in animal models. CLA occurs naturally in food; however, the principal dietary sources are dairy products and other foods derived from ruminant animals [9]. Milk and dairy products from ruminants (Cow, goat, sheep etc.) are the opulent sources of *c9, t11*-CLA isomer incorporate 90% of total CLA in milk fat, small amounts of *t7, c9-, c11, t13-* and *t10, c12*-CLA isomers may also occur [10]. Naturally occurring *c9, t11* CLA has been shown to reduce the number and incidence of mammary tumours in the rat [11 – 12], suggesting that predominant CLA isomer in bovine milk fat is the active component. The evidence became so convincing that the National Academy of Science advised in 1996 that "Conjugated linoleic acid (CLA) is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals" [13].

In the rumen *c9, t11*-CLA isomer results primarily as a product of endogenous synthesis via the enzyme Δ^9-desaturase, with the subtract being t -11 18:1, an intermediate formed in rumen biohydrogenation of linoleic and linolenic acids [10, 14] .Conjugated linoleic acid is also an intermediate in the rumen biohydrogenation of linoleic acid, and some escapes complete biohydrogenation and provides the reminder of the CLA in milk [15].

The content of CLA in milk can vary widely, about 3 to 25 mg g^{-1} fat [16 -18]. The underlying factor resulting in this variation are predominately related to the ruminant diet, including forage to concentrate ratio [19], level of intake [20] and intake of unsaturated fatty acids, especially plant oils that are high in linoleic acid [21].

Recently Thordottir et al. (2004) [22] investigated the distribution of c9, t11 CLA in milk fat from Nordic countries with comparatively lower content of CLA than European countries [23]. Frequency distribution of conjugated Linoleic acid and *t* fatty acids contents in Europeans bovine milk fats. These differences are attributed due to shorter summers, hence shorter periods of pasture grazing, as the ruminal outflow of c9, t11-CLA is enhanced by pasture intake [24].

There is however, no data available on CLA contents of ruminant milk in Pakistan. Therefore the present study was design to investigate CLA content in the milk of ruminant from Sindh (Pakistan), under varying dietary habitats of ruminants in this region.

Material and Methods

Milk sampling

Total 167 samples of milk were collected from dairy cows (n = 46), buffaloes (n = 50), goats (36), and sheep (n=35) during one year, twice in winter (December and March) and twice in summer (June and August). Samples were procured from six dairy farms; situated in Dadu, Thatta and Hyderabad region, while milking times at 0500 and 1700 h. Milk samples were composted for each ruminant and divided in to three sub samples in order to get triplicate analysis. Milk samples were packed in ice and dispatched to laboratory, where stored at –20 ^{0}C until analysis.

Fat extraction

Fat in milk (1 ml) was extracted using a procedure for total lipids in human milk as described by Jensen et al. (1991) [25]. Except that 1 ml of an internal standard (1 mg/ml, of methyl nonadecnoic acid [C19: 0]; Sigma chemical Co., St. Louis, MO; dissolved in chloroform/methanol, 2:1, vol/vol) was added to each sample before extraction, and the extracted lipids were evaporated under nitrogen.

The lipids were then esterified in capped screw-top tubes (Teflon lined) with 6 ml of 0.5 *N* sodium methoxide heated at 50°C for 10 min [26]. The fatty acid methyl esters (**FAME**) were then cooled to room temperature, and 2 ml of iso-octane and 3 ml of 10% acetic acid were added. The tubes were recapped to prevent evaporation of the short chain fatty acids. The FAME were centrifuged (2000 × *g*) for 10 min, and a portion of the top layer removed and placed in sealed gas chromatography vials and kept at −20°C until analyzed.

Gas chromatographic analysis

The FAME were analyzed in a gas chromatograph (Perkin Elmer 8700) fitted with SP-2340 fused silica capillary column (60 m × 0.25 mm i.d. × 0.2-μm film thickness: Supelco, Inc., Bellefonte, PA) using manual injection. Nitrogen was the carrier gas, which was set at a 33 psi. The injection volume was 2µl with a split/ splitless ratio (80/20).

The column parameters were as follows: Initial column temperature was held at 70 °C for 2 min; increased 15 °C/ min to 155 °C (held for 25 min), then increased at 3 °C / min to 215 °C (held for 8 min). The total run time was 61 min.

Data was collected automatically using the computer program: ChromPerfect for Windows (Justice Innovations, Mountain View, CA). High purity individual c9, *t11*- and t10, c12- CLA (Matreya, Inc., Pleasant Gap, PA) were used to identify the CLA isomers of interest. Additional CLA standard mixture (Sigma chemical co.) was used to identify CLA isomers in ruminants.

Statistical analysis

Statistical analysis was carried out using Minitab (Minitab, 13.0, PA, USA). Differences between seasonal periods were assessed by ANOVA using GLM model. Differences between periods mean were determined by Tukey's studentized procedure and were accepted as significantly different $P < 0.05$. Data for fatty acid concentrations are presented as least square mean with pooled standard error of mean (SEM).

Result and discussion

The concentration (mg / g) of conjugated linoleic acid (CLA) isomers including *c9, t11-*, *t10,* *c*12- and the sum of *t7, c9- and t11, c13-* CLA in ruminants (i.e. buffalo, cow, goat and sheep) milk fat is reported in Table 1. Depending on ruminant milk and season, the CLA content was subjected to strong variation ($P \leq 0.05$). The concentration of mean CLA was found between 5.35 to 10.42 mg / g of total fatty acids, with higher in cow's milk fat ranging from 8.81 -10.68 mg /g followed by sheep 8.39 – 9.10 mg /g, goat 5.90 -6.35 mg /g and buffalo milk fat 5.10 – 6.22 mg /g respectively.

Variation in CLA content of ruminants has been associated with several factors such as stage of lactation, parity [27], and breed [28 - 29].However, diet is the most important factor influencing CLA concentration [17, 29-30]. In particular, the CLA concentration has been found higher in milk from animals fed pasture than those fed dry diets [31]. Summer milk produce more elevated concentration 7.60 – 22 % (5.93 – 10.99 mg /g) of total CLA in comparison to winter (5.55 – 8.59 mg/g), when less pasture and fresh grass was available and ruminants were also fed dry forages.

Lawless *et al*. (1998) [32] and Dhiman *et al* (1999) [33] have also established that animals grazing fresh grass have higher levels of CLA in their milk and meat than those consuming conserved forages. Pasture is a richer source of linoleic and α -linolenic acids; the latter comprise more than 50% of total FA, whereas the preservation of grass, particularly ensilage, causes the loss of these fatty acids [34]. Further more main fatty acid in grass lipids is α- Linoleic acid (C- 18:3) that decreases both in absolute and relative values as grass matures [30, 35].

The majority of CLA content was comprised of *c 9, t11-* isomer contributing 89- 90 % of CLA, while *t10, c12-* consisted of 3.2 – 5.90 % of CLA in all ruminants investigated in present study. These results are consistent with earlier reports by Parodi (1999) [36] and Bauman et al., (2003) [37].

The mechanism behind the variation of CLA levels among ruminants would primarily be related to two factors, a) rumen production of its *t*11-18:1 isomer *trans* vaccenic acid (TVA) and CLA, and b) the activity of Δ^9 - desaturase. However rumen production of TVA and CLA are related to the biohydrogenation of substrates available from the diet, and the type and species of bacteria that biohydrogenate the available substrate to produce CLA and TVA. Considering the fact that rumen out put of CLA contributes only marginally to the overall CLA content in milk and possibly meat fat, the activity of Δ^9- desaturase is important to describe at least some of the animal to animal variation [37].

Table 2 demonstrates the comparison of total CLA content in ruminant with earlier reported data. It is evident from the table that CLA concentrations obtained in present investigation are consistent with previous studies in ruminants under varying conditions.

However, the mean CLA content in milk samples obtained in present work is higher than reported earlier [22] for Nordic countries (0.76 vs. 0.58 %). Similarly Precht and Molketin (2000) [23] have also detected higher CLA content in European milk in comparison with Nordic countries (0.89 vs. 0.58%). According to authors this variation can be explained by the different feeding periods in winter and summer, as it is well known that pasture feeding (summer) increases the content of CLA in milk fat compared to high concentrate allocations in winter. This is due to the high concentrations of omega-3 fatty acids in grass, which are a source of CLA. Shorter summers and longer winters in Nordic countries could explain these indicated CLA differences between the Nordic and Pakistan as well as other European countries.

Table – 1. Conjugated linoleic acid isomers content mg / g in milk fat of different ruminants

Conjugated linoleic acid (CLA) isomers content in different ruminants (mg / g)					
Summer (May - July)					
Ruminant	***c9,t11*-CLA**	***t10, c*12-CLA**	**∑ (t7,c9-, t11, c13-) CLA**	**Mean**	**SEM**
Buffalo	5.55	0.31	0.36	6.22	0.02
Cow	10.00	0.49	0.50	10.99	0.03
Goat	5.76	0.27	0.32	6.35	0.02
Sheep	8.22	0.38	0.50	9.10	0.01
Winter (December – February)					
Ruminant	***C9, t11*-CLA**	***t10, c*12-CLA**	**∑ (t7, c9-, t11, c13-) CLA**	**Mean**	**SEM**
Buffalo	4.65	0.19	0.26	5.10	0.01
Cow	8.01	0.37	0.43	8.81	0.01
Goat	5.30	0.25	0.35	5.90	0.02
Sheep	7.55	0.38	0.46	8.39	0.01

Table - 2. Comparison of total CLA (mg / g) in ruminant milk fat obtained in present study with literature values

Ruminant	**Present study (mean of both seasons)**	**Literature values**	**Reference**
Buffalo	5.75	5.00 – 8.40	39, 40
Cow	9.43	4.4 0 – 17.00	41, 42
Goat	6.23	2.77 – 6.90	43, 44
Sheep	8.46	8.00 - 20.00	44, 45

Conclusion

Present study reveals that the CLA content in ruminant's milk is subjected to variation depending on ruminant and season. The CLA content is higher in summer than in winter, all ruminant studied showing the similar changes. The availability of pasture and fresh grass explain the seasonal differences. The results indicate a higher CLA content in Sindh (Pakistan) than in Nordic countries ruminant milk in average, which may be related to longer summers and hence, longer periods of pasture feeding in the Pakistan. The implications for varying CLA concentration on health have to be studied

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