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Estimation of Conjugated Linoleic Acid (CLA) Content in Ruminant Meat and Fats

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ABSTRACT

Conjugated linoleic acid (CLA) is a naturally occurring bioactive fatty acid in meat and fats of ruminant animals. CLA is gaining much attention due to its nutritional and therapeutic properties. In this study, the presence of CLA in fat and muscle tissues of sheep, goat, and buffalo was established. The CLA content of a large number of fat samples (N=140) was analyzed by measuring the UV-VIS absorbance at 233 nm and comparing with the standard CLA absorbance curve. GC/FID and GC/MS were used for further used for quantification and confirmation. Results showed a CLA concentration of 2.5 to 8.5 mg/g fat and 1.4 to 3.7 mg/g of meat in different ruminant species. Further, rendered fat had a significantly ($P<0.05$) higher CLA than meat and fat samples. Gas chromatography and mass-spectrometry studies confirmed the presence of CLA in the samples analyzed.

Keywords: *Conjugated linoleic acid, Ruminant meat, GC-MS, Bioactive compound, Rendered fat*

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INTRODUCTION

Conjugated Linoleic Acid (CLA) is a naturally occurring bioactive compound found mainly in ruminant meat and dairy products. CLA is a group of positional and geometrical isomers of linoleic acid(cis-9, cis-12, 18:2).The ruminal bacterium Butyrivibrio fibrisolvensis responsible for the synthesis of the CLA as an intermediate in the biohydrogenation of linoleic acid to vaccenic acid (Kepleret al. 1966). Scientific research suggests that CLA helps to build muscle and reduce body fat, and possesses potential anticarcinogenic, anticholestrolemic and immuno-modulatory health benefits (Park and Pariza 2007). It has been well established that that grass fedruminant's fat and meat are the best natural source of CLA in foods (French et al. 2000).

In India, ruminant meat production generates significant economic benefits but the full economic gains are not realized due to the low utilization of the animal by-products. Currently, considerable quantity of by-products such as fat, offal, and trimmings are underutilized by producing at best, low value rendered products. Valuable bioactivecompounds such as CLA could be produced from by-product streams for use as dietary supplements, nutraceuticals, and functional ingredients. Earlier research findings further, indicate that meat and milk products from grass fed ruminant animals contain higher amount of CLA as compared to grain and stall fed (French et al 2000; Ponnampalam 2006). Since most of the Indian meat animals are reared on free range, and fed on natural grass, it can be hypothesized that meats and fats of these animals could be significant sources of CLA. However, there are not many studies havebeen conducted to show the presence of significant amount of CLA in meat and fatty tissues of ruminants in India. Recently,Mandalet al. (2014)reported that feeding of essential oils at 1.5 g/kg of dry matter improved the concentrations of CLA in meat in Black Bengal goats. Similarly, Royet al. (2013) observed that feedingof vegetable oils (soy and sunflower) at a concentration of 45 g/kg of total dietsincreased the PUFA and CLA content inmuscle and adipose tissues of Black Bengal goats. Current compositional information on the CLA content in meat or fats of sheep, goat and buffalo isvery limitedand no studies in have been conducted in India to know the same.Therefore, the aim of the present study was to establish the presence of significant amount of CLA and provide data on the CLA content in fat obtained from ruminant meats.

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MATERIALS AND METHODS

Sample collection and storage: The fat/meat samples used in this study were collected over a period of one year from different animal carcasses (sheep, goat and buffalo) sold in the local retail markets. Rendered fat was obtained from the nearby modern abattoirs. Samples were processed immediately after arrival to laboratories. Otherwise, samples were stored at -18 °C under hygienic conditions.

Reagents and chemicals: An analytical grade, standard CLA (cis- and trans-9, 11- and -10, 12-octadecadienoic acids) in 1 g ampoule was obtained from Sigma-Aldrich, India. All other solvents used were of reagent grade.

Extraction of CLA from fat and muscle tissues for uv-vis absorbance measurement: The extraction of fatty acids in fat tissue obtained from different meat was done using reagent alcohol (90% ethanol, 5% methanol/acetone, and 5 % isopropanol).One gram fat samples were homogenized for 1 min in 10 ml reagent alcohol. Samples were centrifuged (4000 rpm, 5 min) and filtrate of an aliquot of the supernatant was diluted to 1:5 ratios with reagent alcohol prior to reading the absorbance at 233 nm(Aldai et al. 2007).

Preparation of standard CLA curve: A standard CLA curve was developed using analytical grade 95% pure CLA standard. The CLA powder was diluted in reagent alcohol over a linear range of 0.002 to 0.025 mg CLA/ml reagent alcohol which encompassed the range of sample concentrations in this study. Sample absorbance was measured at 233 nm usinga uv-vis spectrophotometer and CLA concentration in the samples was determined using the

molar absorption coefficient calculated from the curve. Several experiments were carried out to obtain a standard curves with regression coefficient (R2) value above 0.90.

CLA estimation using chromatography (GC-FID) analysis: The method described by Aldai et al.(2007) with minor modifications was used. The lipids were extracted with a chloroform methanol mixture (2:1, by 200 ml). Four 10 ml aliquots were saved for the next steps.Aliquots of thelipid extract were esterified with boron trifluoride-methanol. The CLA composition of each aliquotwas determined by gas chromatography on a 100 m fusedcapillary column with an internal diameter of 0.25 mm(HP 88, 100m ×0.25×0.2 µm). The analysis was performed on an Agilent 7890 gas chromatograph equipped with aflame ionization detector. Nitrogen was used as carrier gasat flow rate of 1 ml/ min. The injection porttemperature was 200 ºC and the detector temperature was280 ºC. Oven temperature was ramped to 140 ºC for 5min and increased to 240 ºC at 4 ºC/min; it was thenheld at 240 ºC for 15 min. A software calculated retention times and peak area percentages. Fatty acidswere identified by comparing sample retention times withstandard retention times (CLA standard, Sigma-Aldrich India).Quantification was carried out bynormalization and transformation of the area percentageto mg per g of meat using the lipidconversion factor.

GC-MS Data Acquisition and Analysis: GC-MS analysis of the extracts of CLA was performed using an Agilent 7890A series system comprising an Agilent 7683 auto-sampler and a gaschromatograph interfaced to a mass spectrometer. The samples

l) were injected via an auto sampler (split less, split open after 90 sec). A fused silica capillary column (100m x 0.25 mm ID, 0.2µm film thickness; Agilent J&W HP-88) was used for separation of the target compounds. Temperatures of the GC oven throughout the run were as follows: initial temperature of 140 °C, held for 5 min, a ramp in temperature of 2 °C per minute to 240 °C,for a total run time. Source fragmentation was done by electron ionization (EI) with a scan range of 40amu to 400 amu (atomic mass units). In the GC/EI-MS full scan mode, m/z was recorded. For GC/ EI-MS in the SIM mode, fragment ions including m/z 237.1, m/z 366.2and m/z 472.2 for CLA were recorded throughout the run. CLA extracted from fat were identified by comparing the mass spectra of the analytes with those of pure standard and from MS-libraries.

Statistical analysis: The mean values and standard deviations of the samples were calculated. Analysis of variance test was used to find out the significance difference (P<0.05) among the species and tissues. IBM-SPSS-10 software was used for the data analysis.

RESULTS AND DISCUSSION

A typical standard curve regressing absorbance at 233 nm and CLA concentrations is presented in Fig 1. (R2 =0.99) indicating linearity between absorbance and the CLA concentration across the standard range. Aldai et al.(2007) reported that direct extraction using reagent alcohol followed by measuring absorbance at 233 nm is by far the simplest and viable method for the rapid sorting of carcasses with different CLA content. In their study, the accuracy of this CLA determination is shown to be acceptable compared with reliable GC method. Hence, in this study, uv-vis absorbance at 233 nm was used to quantify the CLA in test samples

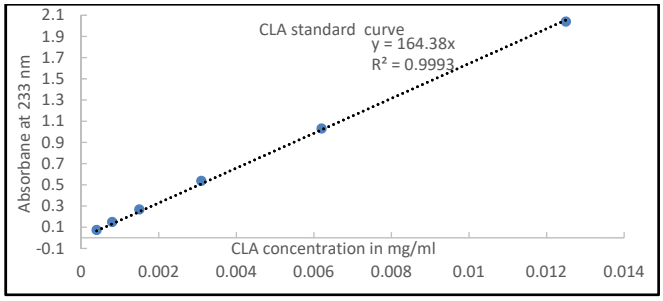

Fig. 1 CLA Standard curve

Table 1: Mean±S.E of CLA (mg/g) as measured by peak absorbance at 233 nm (N=40)

Species	Fat	Meat
Buffalo	2.52±0.08a	1.43±0.03a
Goat	3.13±0.02a	3.78±0.06b
Sheep	3.04±0.04a	2.61±0.01a
Rendered fat	8.62± 0.06b	-

a-c Means within a Colum with different superscripts differ significantly. (P<0.05)

Table 2: Mean±S.E Mean CLA (mg/g) in fat as measured by Gas chromatography (n=4)

Species	CLA content
Goat	4.36 ±0.12a
Sheep	3.91 ±0.27a
Rendered fat	9.14 ±0.32b

a-c Means within a Colum with different superscripts differ significantly. (P<0.05).

The confirmation of presence of CLA was further investigated by GC-MS analysis. The data acquired by GC-MS is shown in Fig 2.The standard CLA solution was analyzed by GC-MS to reduce the risk of incorrectly identifying the target molecule of CLA. The data of mass to charge (m/z) for the standard CLA and sample extracts were similar. For GC/EI-MS in the SIM mode, fragment ions including m/z 237.1, m/z 366.2and m/z 472.2 for CLA was recorded throughout the run for all samples. These results confirmed the presence of CLA in the tested samples.

Generally, CLA is obtained in meat, milk and fats of ruminant animals. Differences in the CLA content between different animal tissues, between animals of different breeds/ species were reported. (Shantha et al.1994; Raes et al.2003). In costalis diaphragmatic muscles of Wagyu crossbred and European and British crossbred cattle,Mir et al. (2000) reported a CLA concentrationof 1.7–1.8 mg/g intramuscular

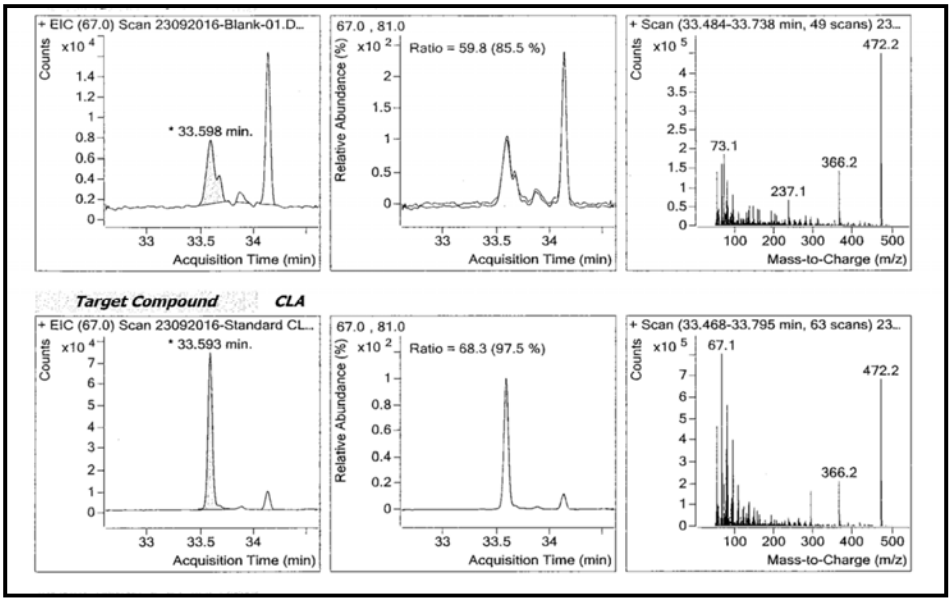

Fig. 2. Mass spectroscopic confirmation of CLA in ruminant fats

lipids. Shantha et al. (1997)observed about 7.7 mg/g intramuscularlipids in the semimembranosusmuscle of grass-fed cattle from North America. De Mendoza (2005) reported a CLA of 1.83 and 1.47 mg/g lipid extracts from longissimus dorsi in buffaloes and cattle respectively. Other researchers have reported a range of 5.6 to 12.0 mg/ g fat of CLA in lamb meat (Chinet al. 1992)and a range of 2.9 to 6/8 mg / g fat in cattle (Chin et al., 1992. Dufeyet al. 1999).In goat meat, Mandal et al. (2014)observed the CLA concentration of 0.97 g/100 g fatty acids and they further found that feed supplemented with essential oil improved this CLA concentration to 1.20 g/ 100 g fatty acids. Similarly, Royet al.(2013) reported in goat meat, a CLA concentration of 0.4 g/100 g fatty acids and feed supplemented with vegetable oils improved this concentration of CLA to 1.2 g/100g fatty acids. Dufey (1999)observed that with cattle from Argentine and Brazil showed highest CLA and that from the US showed the lowest CLA levels. These findings were ascribed to differences in feeding regime between countries. Similarly, French et al. (2000)observed in the intramuscular fat of steers (longissimus dorsi muscle) increasing CLA contents consistent with increasing intakes of grass. These studies clearly indicate that animals fed on grass and pastures will have higher concentration of CLA in meat and fat as compared to grain fed and stall fed. This is relevant to our Indian conditions, as most of the sheep, goat, and buffaloes are reared in open extensive system and are fed with natural grass. Therefore, the CLA concentration and it range observed in this study are higher than earlier studies.Research conducted in India detected CLA in concentrationsranging from 5.0 to 6.0 mg/g lipids in milklipids of grass-fed cows (Kelly et al. 1998) and femalebuffaloes (Aneja and Murthy, 1990). The increased CLA content in meat from animals grazing on pasture is attributed to the high PUFA content of grass, especially n-3 18:3 as PUFA determines the generation of trans fatty acids by rumen bacteria (Lawson et al.2001). Adding oilseeds to the diet has been proven to be an efficient method to increase the CLA content in the muscle lipids. In addition to sunflower seed and linseed, safflower seed was also shown to increase the relative CLA content in the muscle tissues of lambs (Kott et al.2003; Roy et al. 2013).

CONCLUSION

The present study suggests that conjugated linoleic acid is present in a significant quantity in fats and meats of sheep, goat and buffalo. Further, current Indian ruminant meat production system favours the deposition of higher CLA in fatty tissues. Rendered fat is also a significant source of bioactive compound CLA. Hence, further efforts should be made to develop feeding strategies to improve the content of CLA in meats. Development of large scale separation and purification technologies for CLA from meat by-products will enhance the income generation from the meat industry by-products.

COMPETING INTERESTS: The authors have no known competing interests either financial or personal between themselves or others that might bias the work.

ETHICS STATEMENT: Not applicable

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