

ORIGINAL ARTICLE

Direct administration of high CLA producing *Butyrivibrio fibrisolvens* along with dietary oil on CLA profile of muscles and fat tissue of crossbred kids

Sheikh Asraf Hossain¹, Nilufar Haque², Amrish K. Tyagi³**HOW TO CITE THIS ARTICLE:**

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ABSTRACT

Conjugated linoleic acid (CLA), found in ruminant-derived food products such as meat is one of the functional food components. Manipulating rumen microbial population might be a strategy to develop the value added goat meat. In present experiment, the effect of high CLA producing *Butyrivibrio fibrisolvens* strain 4a (bf4a) administration along with dietary oil contained linoleic acid (LA) was investigated on CLA content in meat of crossbred kids of four different groups (n=20): Control (C; basal diet); T1 (C+ LA source @200 mg/L of rumen fluid), T2 (C+ suspension of bf4a, 10⁹ CFU head⁻¹) and T3 (T1 + suspension of bf4a, 10⁹ CFU head⁻¹). Total CLA content was enhanced by 30.0, 19.1 and 30.0%, respectively, in Longissimus dorsi, Semitendinosus muscle and adipose tissue thereby improving the desaturation index in bf4a along with dietary oil administered group as compared to the control. Administration of *B. fibrisolvens* 4a along with linoleic acid enriched dietary oil is a useful strategy to increase beneficial CLA levels in ruminant's meat by altering the ruminal biohydrogenation pattern.

KEYWORDS

- *Butyrivibrio Fibrisolvens* • Fatty Acid • Conjugated Linoleic Acid • Muscle
- Adipose Tissue • Goat Kids

AUTHOR'S AFFILIATION:

¹ Assistant Director, Veterinary Research & Investigation, Department of Animal Resources Development, West Bengal, India.

² Assistant Director of Extension, West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal, India.

³ Former ADG, ICAR, New Delhi, India.

CORRESPONDING AUTHOR:

Nilufar Haque, Assistant Director of Extension, West Bengal University of Animal & Fishery Sciences, Kolkata, West Bengal, India.

E-mail: haquenilufar@gmail.com

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INTRODUCTION

The prospects for improving the fatty acid profile of meat from ruminant animals represent a growing market for the global livestock sector as a mean to support better human health. Food products obtained from ruminant animals are important source of nutrients, supplying high quality protein, energy and a variety of minerals and vitamins (Mills, Ross, Hill, Fitzgerald, & Stanton, 2011). The greater proportion of saturated fatty acids (SFA) in ruminant meats compared with other protein sources has become a subject of concern because of the potential role of dietary SFA in the etiology of obesity, hypertension, and coronary heart disease in humans (Baghurst, 2004; Givens, 2005). Besides a lower total fat intake, human nutritionists are recommending a higher intake of unsaturated fatty acids (UFA) because of their health-promoting effects (Raes, De Smet, & Demeyer, 2004; Elwood, Pickering, Givens, & Gallacher, 2010; Muka et al., 2016). Among these fatty acids, conjugated linoleic acid (CLA) and vaccenic acid (VA), including other 18:1 monoenes, are the microbial metabolites from rumen. Recent studies have demonstrated potential benefits of CLA for human health through putative anticarcinogenic, antioxidative, and antidiabetic effects (Koba & Yanagita, 2014). Therefore, modifying ruminal microbial metabolism of fatty acid in rumen through animal diet formulation is an effective way to improve the fatty acid composition of ruminant-derived foods such as meat to reduce saturated: unsaturated fatty acid ratio and increase CLA, monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) content may have value to the milk and meat industries (Radunz et al., 2009; Or-Rashid, Wright, & McBride, 2009).

CLA found in ruminants products are mainly originated from two sources (Griinari, & Bauman, 1999). First source is CLA formed during ruminal biohydrogenation of linoleic acid (LA) and second source is CLA synthesized by the animal's tissue from VA. Several species of CLA-producing bacteria have been isolated from the rumen, intestine and starter cultures used in the dairy industry (Ogawa et al., 2005; Punia, Chaitanya, Tyagi, De, & Singh, 2008; Das & Qin, 2012; Zhu, Hang, Mao, & Zhu, 2014; Hussain et al., 2016). According to these reports, the prevalent ruminal bacterium

Butyrivibrio fibrisolvens isomerizes LA to *cis*-9, *trans*-11 CLA exclusively by LA isomerase (LA-I) faster than any other bacterial species (Fukuda, Furuya, Suzuki, Asanuma, & Hino, 2005).

Chevon (goat meat) is one of the most widely eaten red meats in the world and enjoys great popularity in many developing countries, especially in Asia, Africa and the Far East (Talpur, Bhangar, & Sharazi, 2008). India ranked second in goat meat production. Worldwide, the demand for meat and other animal products is increasing at a substantial rate driven by a combination of population growth, urbanisation and rising income. Over the past few years, meat from goat has gained acceptance around the world mainly because it is leaner than beef and mutton and has low cholesterol content (Mahgoub et al., 2002). Regardless of the importance of goat as a source of lean meat, the nutritive value of goat meat has received little attention and consequently there are very few reports on the fatty acid composition of meat and adipose tissues from different goat breeds. The opportunities are there to exploit the diet of meat animals or by any other means. So the present study is envisaged to access the effect of direct administration of high CLA producing *Butyrivibrio fibrisolvens* strain 4a (*bf4a*) with or without dietary oil on performance and CLA content of rumen *Longissimus dorsi*, *Semitendinosus* muscle and adipose tissue of crossbred kids.

MATERIALS AND METHODS

Experimental animals and management

Twenty crossbred (Alpine × Beetal) male goat kids of 7-8 months age group were divided into four groups of 5 each in a completely randomized block design on the basis of average body weight and age as control (C), T1, T2 and T3 groups at National Dairy Research Institute's Farm, Karnal, Haryana, India. The animals were kept in the shade with individual feeding pens under hygienic conditions. They were let loose for exercise in the attached open yard. All the animals were healthy and free from any worm infestation. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of National Dairy Research Institute and were carried out by an experienced researcher.

Feeding of experimental animals

All the experimental kids were individually fed isocaloric and isonitrogenous diet consisting compound concentrate mixture (maize 57 parts, Ground Nut Cake (GNC) 40 parts, mineral mixture 2 parts and common salt 1 part) and maize fodder and the quantity of the same was adjusted at weekly interval according to change in body weight as per NRC (1981) feeding standards for a period of 56 days (8 weeks). The kids under group-I was termed as Control (C), in addition, the kids under the group-II (T1) and group-IV (T3) were given commercially available sunflower oil (procured from M/S. Agro tech Foods Ltd., India), a blend of sunflower and corn oil (60:40) @ 4 - 10 g/d, so that the animals will avail linoleic acid @ 200 mg/L of rumen fluid along with compound concentrate mixture. Moreover, the kids under the group-III (T2) and group-IV (T3) were inoculated with anaerobic culture of *Butyrivibrio fibrisolvens* strains 4a (bf4a), the highest CLA producing bacterial strain isolated from Indian buffalo (*Bubalus bubalis*, Hussain et al., 2016), @ 10^9 CFU/ animal, directly into the rumen through syringe in every alternate day of first and fourth week from the beginning of experiment. Clean, fresh and wholesome tap water was made available ad libitum to all the experimental animals throughout the day (24 h).

Ruminal inoculation with *Butyrivibrio fibrisolvens* 4a

Before the kids were inoculated with the bacteria, samples (2 ml) were removed from the bacterial culture bottles for enumeration of bacterial numbers and determination of the dose of the inoculum. Samples were taken, using a sterile needle and syringe and sealed inside the anaerobic chambers (95% CO₂, 5% H₂) just prior to inoculation. These were placed on ice, transported to the animal pen for inoculation of the kids. A subsample of each was also visually examined (Olympus BH-2 microscope, Olympus Optical Co. Ltd, Japan) to ensure that the bacteria appeared morphologically sound and that no contamination had occurred immediately after the following incubation. Prior to the morning feeding, kids under group III (T2) and group IV (T3) were inoculated with 5 to 25 ml culture so that each animal received 10^9 CFU/head, directly into the rumen through syringe.

Feed intake and body weight

The feeds offered and residue left were recorded daily to find out the total DM intake of the animals. Body weight of the animals was recorded at weekly interval for two consecutive days before offering feed and water using electronic weighing balance.

Analysis of feed samples and their fatty acid profile

The concentrate mixture and fodder samples were analyzed for proximate principles as per AOAC (2005). Fatty acids composition were analysed as per direct *trans*-esterification method of O'Fallon, Busboom, Nelson, & Gaskins (2007). In brief, fatty acids in the feed were obtained by hydrolysis with 10 N KOH at 55°C for 1.5 h with vigorous shaking for 5 sec at 20 min. interval to properly permeate. After adding internal standard, the sample was acidified with 24 N H₂SO₄. The tubes were mixed by inversion and incubated at the same time and temperature combination. 3 ml hexane was then added in cooled tubes and each tube was vortex mixed for 5 min. Tubes were then centrifuged for 5 min at 3500 rpm and the hexane layer containing fatty acids methyl esters (FAME) was extracted. The same was concentrated under nitrogen and stored at -20°C until analysis. Methyl esters were separated by using a gas chromatography (GC; 450-GC, Bruker, USA) and equipped with a SGE Forte GC capillary column (60m x 0.25mm x 70 µm- BPX70). Helium was used as carrier gas at constant inlet pressure (205 kPa). 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 minutes. The identification of individual fatty acid was based on a commercial standard mixture (Supelco, Belfonte, USA) and published isomeric profiles.

Determination of FA profile of muscles and adipose tissue.

Slaughter procedure and sampling

After 56 days of experimental period, all the experimental kids were slaughtered by conventional *Jhatka* method. Animals were kept off feed for 12 hrs before slaughter, though water was provided. After slaughtering, the carcasses were split along the spine into two halves and about 100 g each samples were taken from *Longissimus dorsi* muscle (between

3rd and 4th lumber vertebrae, loin portion), *Semitendinosus* muscle (distal region) and adipose tissue (AT; (subcutaneous, marbling fat) from right and left halves within 20 min after slaughter. Immediately after collection, the muscle sample was immersed in liquid nitrogen for 90 sec and then wrapped tightly in aluminum foil and was stored at -80°C till further analysis for intramuscular fatty acid composition.

Analysis of fatty acids profile

Estimation of CLA in muscle and adipose tissue were analyzed as per direct transesterification method of O'Fallon, Busboom, Nelson, & Gaskins (2007) with slight modification. For the methyl ester formation, 1 g of each muscle and 0.5 g adipose tissue samples was taken. Lipids were extracted by the method of Kramer et al. (1997) with purification of samples with 20% of HCl. Extracted lipids in tubes were dissolved in 1 ml of hexane and 1 ml of internal standard (tridecanoic acid; Fluka, Sigma-Aldrich, USA). 2 ml of transesterification reagent (1N methanolic sodium methoxide) were added to the mixture. The mixture was held in a water bath at 50°C for 30 min. After addition of 3 ml of 3N methanolic HCl (Supelco, USA), the mixture was incubated in the water bath at 50°C for 1 h. To separate the hexane layer in the mixture, 1 ml of hexane and 1 ml of distilled water was added. Finally, the mixture was centrifuged at 200 g for 5 min. The hexane layer was used for the determination of fatty acid methyl esters by gas chromatography [450-GC, Bruker, USA; equipped with a SGE Forte GC capillary column (60m x 0.25mm x 70 µm- BPX70)]. 1 µl sample was injected in GC and GC analysis parameters and conditions had already been described in section 2.5.

Statistical analysis

All data were subjected to an two way ANOVA according to a completely randomized

design, with dietary treatments as a fixed factor, parameters observed as dependent variables and pre-experimental parameters observed (day 0) as covariates, in order to correct for variability of different beginning variables. Where the influence of dietary treatment was significant, multiple comparisons using least significant differences were used to compare diets with different effects on the variable involved. Whole data statistical calculations were performed as a general linear model using the univariate procedure of SPSS version 20.0 (2011; IBM Corporation, Armonk, NY, USA) software package. Duncan's method was used for multiple comparisons among means. Overall differences between treatment means were considered significant when $P < 0.05$.

RESULTS

Chemical and fatty acid composition of diets

The chemical composition and fatty acids composition of feed, fodder and dietary oil has been presented in Table 1 and 2, respectively. Maize fodder was rich source of linoleic acid (C18:2 55.12%) followed by oleic acid (C18:1, 25.49%) and linolenic acid (C18:3; 4.70%). Whereas, the concentrate mixture was rich in C18:1 (36.54%) and C18:2(32.33%) as compared to C18:3(3.10%). The blend of sunflower and corn oil (60:40) contained 64.04% C18:2, 21.87% C18:1 and 1.10% C18:3.

Table 1: Chemical composition of feed and fodder (% DMB)

Particulars	Concentrate mix.	Maize fodder
DM	89.00	18.46
OM	92.84	90.88
CP	20.44	12.95
EE	4.22	1.94
NDF	53.30	54.09
ADF	10.31	41.56
Hemicellulose	42.99	12.53
Total Ash	7.16	9.12

Table 2 Fatty acids composition of feedstuffs

Fatty Acids	Total fatty acids (g/100 g FA)			Total fatty acids (mg/g DM)		
	Conc.	Maize fodder	Sunflower + Corn oil (60:40)	Conc.	Maize fodder	Sunflower + corn oil (60:40)
C12:0	0.00	0.00	0.00	0.00	0.00	0.00
C14:0	0.13	0.05	0.72	0.06	0.01	7.20
C14:1 cis	0.00	0.00	0.00	0.00	0.00	0.00
C16:0	13.26	11.97	7.93	5.97	1.80	79.25
C16:1 cis	0.40	0.36	0.65	0.18	0.05	6.55

table cont....

C18:0	2.74	1.72	2.28	1.23	0.26	22.84
C18:1 c-9	36.54	25.49	21.87	16.44	3.82	218.74
C18:2	32.33	55.12	64.05	14.55	8.27	640.45
C20:0	0.97	0.39	0.66	0.44	0.06	6.61
C18:3	0.10	0.06	0.00	0.05	0.01	0.00
C18:3	3.00	4.64	0.00	1.35	0.70	0.00
Total C18:3	3.10	4.70	1.10	1.40	0.71	10.97
C20:1	0.00	0.20	0.31	0.00	0.03	3.13
C22:0	1.46	0.00	0.32	0.66	0.00	3.17
C22:1	8.05	0.00	0.00	3.62	0.00	0.00
C24:0	1.02	0.00	0.11	0.46	0.00	1.07
ΣFat (mg)				45.00	15.00	1000
ΣSFA	8.62	7.62	12.01	9.62	2.12	120.14
ΣUFA	91.38	92.38	87.99	90.38	12.88	879.85
ΣMUFA	20.33	20.33	22.84	20.33	3.91	228.43
ΣPUFA	15.59	15.59	64.05	15.59	8.97	640.45

Table 3 Fatty acids intake (g/d) by experimental kids

Fatty Acids	Total Fatty Acids Intake (g/d)			
	C	T1	T2	T3
C12:0	0.000	0.000	0.000	0.000
C14:0	0.035	0.105	0.035	0.105
C14:1 cis	0.000	0.000	0.000	0.000
C16:0	3.731	4.391	3.714	4.389
C16:1 cis	0.112	0.173	0.111	0.173
C18:0	0.739	0.941	0.737	0.941
C18:1 c-9	9.962	11.79	9.928	11.79
C18:2	10.20	16.25	10.12	16.24
C20:0	0.252	0.309	0.251	0.309
C18:3	0.027	0.026	0.027	0.026
C18:3	0.926	0.894	0.920	0.893
Total C18:3	0.953	1.030	0.947	1.029
C20:1	0.008	0.040	0.008	0.040
C22:0	0.363	0.373	0.355	0.373
C22:1	1.955	1.883	1.955	1.883
C24:0	0.247	0.249	0.247	0.249
ΣFat	28.55	37.54	28.41	37.53
ΣSFA	5.367	6.37	5.339	6.366
ΣUFA	23.19	31.17	23.07	31.16
ΣMUFA	12.04	13.89	12.00	13.89
ΣPUFA	11.15	17.17	11.07	17.16

C = Control, basal diet as per NRC-1981 feeding standard

T1 = C + Linoleic acid supplementation @400 mg/L of rumen fluid in terms of dietary oil

T2 = C + suspension of *Butyrivibrio fibrisolvens* 4a strain @ 10⁹ CFU/head

T3 = T1 + suspension of *Butyrivibrio fibrisolvens* 4a strain @ 10⁹ CFU/head

SFA = Saturated fatty acid; UFA = Unsaturated FA; MUFA = Mono-unsaturated FA;

PUFA = Poly-unsaturated FA

Effect on DM intake, FA intake and growth performance of kids

The average daily DM intake in terms of kg per day in kids was similar ($P>0.05$) in all the groups with the diet of 65:35 concentrate and roughage ratio (Fig. 1). The average daily fatty acids intake of experimental animals is presented in Table 3. The daily intake of oleic acid (C 18:1), linoleic acid (C 18:2) as well as total fat was significantly higher ($P>0.05$) in both the dietary oil supplemented groups i.e., T1 and T3 groups. The average daily linoleic

acid intake was 10.20, 16.25, 10.12 and 16.24 g and total fat intake (g/d) was 28.55, 37.54, 28.41 and 37.53 in control, T1, T2 and T3 groups, respectively. The initial and final body weight did not differ ($P<0.05$) among themselves (Fig. 2). The effect for treatment and period were statistically similar, though, the interaction effect between treatment and period (t x p) differed significantly ($P<0.05$) from each other. The average daily gain was 44.64, 42.86, 43.75 and 44.64 g, indicating statistically similar gain in all the four groups.

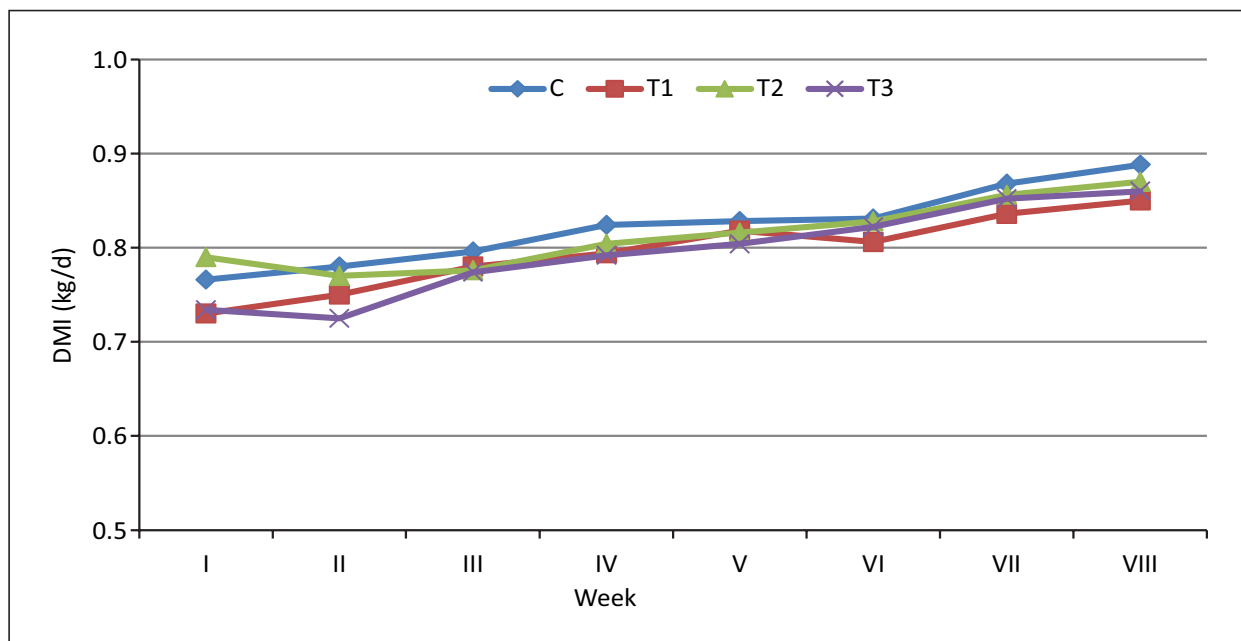


Figure 1: Administration of *Butyrivibrio fibrisolvens* 4a with or without dietary oil on DM intake (kg/d) of crossbred kids

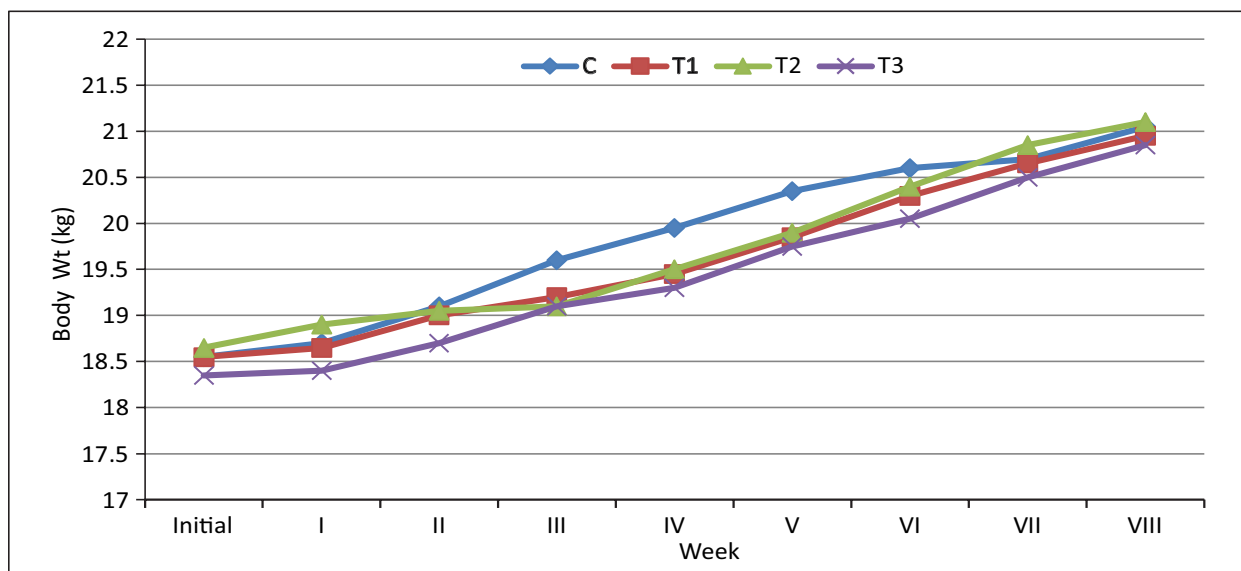


Figure 2: Administration of *Butyrivibrio fibrisolvens* 4a with or without dietary oil on changes in body weight (kg) of experimental kids in weekly intervals

Effect on fatty acids profile in different muscle

In longissimus dorsi muscle, the isomers and total CLA content was significantly increased by 30.0 and 19.3% ($P>0.05$) in T3 group as compared to the control and T1, respectively, whereas, in T2 muscle, it was higher than control only. In semitendinosus muscle, CLA isomers and total CLA content were significantly higher ($P>0.05$) in both the *Butyrivibrio* inoculated groups (T2 and T3) as compared to the control. In adipose tissue, total CLA content were significantly higher ($P>0.05$)

in both the *Butyrivibrio* inoculated groups i.e., T2 (20.5%) and T3 (30%) as compared to the control. (Table 4). CLA accounted for 0.40 to 0.57 percent of total FA in L. dorsi muscle, 0.49 to 0.62 percent of total FA in Semitendinosus muscle and 0.70 to 1.00 percent of total FA in adipose tissue, while, *cis*-9, *trans*-11 CLA isomer contributed to 87 - 93 percent of total CLA followed by *trans*-10, *cis*-12 CLA isomer in both the muscles and intermuscular fat of the kids. However, AT of experimental kids contained significantly higher ($P>0.001$) *cis*-9, *trans*-11 CLA and total CLA than both the muscles of kids.

Table 4: Comparisons of CLA profile (g/ 100 g FA) of experimental kids in different muscles and fat tissue

Attributes	Longissimus dorsi				Semitendinosous				Adipose tissue				SEM	Significance of effects (p)		
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3		Treatment (t)	Muscle (m)	t x m
CLA (c-9,t-11)	0.37	0.41	0.48	0.50	0.44	0.45	0.53	0.56	0.64	0.80	0.81	0.93	0.01	<0.001	<0.001	0.047
CLA (t-10,c-12)	0.04	0.04	0.07	0.06	0.04	0.05	0.06	0.06	0.07	0.06	0.07	0.07	0.00	<0.001	<0.001	0.180
ΣCLA	0.40	0.46	0.55	0.57	0.49	0.51	0.59	0.62	0.70	0.86	0.88	1.00	0.01	<0.001	<0.001	0.147

cis-9, *trans*-11 CLA isomer is the predominant production pathway in the mammary gland and also occurs in the subcutaneous or intramuscular fat by the action of Δ^9 -desaturase enzyme on *trans*-11 VA (C18:1), which is also produced during ruminal biohydrogenation (Griinari, Corl, Lacy, Chouinard, & Nurmela 2000). Genetic differences in Δ^9 -desaturase activity might thus lead to differences in *cis*-9, *trans*-11 CLA content (Smet, Raes, & Demeyer, 2004).

CONCLUSIONS

Direct administration of *Butyrivibrio fibrisolvens* 4a strain (*bf4a*) into the rumen of growing crossbred kids @ 10^9 CFU/head for a week with linoleic acid @ 200 mg/ liter of rumen volume for two months resulted higher CLA accumulation in muscles and fat of kids through altering the rumen metabolic pathways via modulation of the absorbed fatty acids. The results in present study greatly extend the possibilities for the use of *Butyrivibrio fibrisolvens* 4a as a potent feed additive to increase CLA in ruminant derived food product and a fatty acid profiles more compatible with current human dietary recommendations including the possibility of meat being recognized as a functional food.

DISCUSSION

As *B. fibrisolvens* usually resides in the rumen of goats, cattle and buffaloes, it may not be harmful to host animals (Asanuma, Kawato, & Hino, 2001). Furthermore, no difference in DMI or body weight or outward appearance of the kids was observed. When *B. fibrisolvens* 4a strain was administered, neither any visual abnormalities were observed in faeces nor were any visible lesions in organ throughout the experimental period. It indicates that administration of *bf4a* had no apparent toxic effect on goat kids.

The higher CLA value in fat tissue might be due to more Δ^9 -desaturase activity to convert *trans*-11 VA into CLA in adipose tissue as compared to muscles. Comparable values for the CLA have been reported by Todaro et al. (2004) for Nebraska kids and for *L. dorsi* and *Semitendinosus* muscles of Kundi steers (Talpur, Bhanger, & Khuhawar, 2007) and Pateri goats (Talpur, Bhanger, & Sharazi, 2008) in Pakistan. However Lee, Kannan, & Kouakou (2006) have found lesser amount of *cis*-9, *trans*-11 CLA (0.17 g/ 100 g) for Boer goats. Since both *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers are naturally formed as intermediates during the biohydrogenation of linoleic acid in the rumen. Nevertheless, endogenous synthesis of

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