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Effect of Breed on *cis*-9, *trans*-11 and *trans*-10, *cis*-12 Conjugated Linoleic Acids (CLA) Concentrations in Milk Fat of Dairy Cattle and the Relationship of These CLA with other Unsaturated C_{18} Fatty Acids

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ABSTRACT

Much attention has been given to unsaturated carbon 18 fatty acids in milk, particularly conjugated linoleic acids (CLAs) which have a beneficial effect on human health. This study was undertaken to investigate the effect of breed on *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers in the milk fat of dairy cattle and their relationship with other unsaturated carbon 18 fatty acids. Mafriwal (n=15) and Jersey (n=15) cows were at mid-lactation period, grazed on pasture and given 5.5kg of concentrate per head daily. The composition of milk fatty acid was determined using gas chromatography after the extraction of milk fat using the modified Folch's method. The results showed that breed had an effect on *cis*-9, *trans*-11 CLA deposition in milk fat. The level of *cis*-9, *trans*-11 CLA in milk fat of Mafriwal was significantly higher ($P<0.05$) than that of the Jersey cows, while the levels of *trans*-10, *cis*-12 CLA were not significantly different between the two breeds. The levels of *cis*-9, *trans*-11 CLA were positively correlated with the concentration of *trans* 11-octadecenoic ($C_{18:1}$), *cis* 9-octadecenoic ($C_{18:1}$) and octadecatrienoic ($C_{18:3}$) acids. A positive correlation was also observed between the levels of *trans*-10, *cis*-12 CLA and octadecatrienoic ($C_{18:3}$) acid in milk fat. These results indicated that breed selection could be used to improve the quality of milk for human consumption.

Keywords: Breed, Dairy cattle, Conjugated linoleic acids, milk fat, Unsaturated carbon 18 fatty acids.

INTRODUCTION

Conjugated linoleic acids (CLAs) are a mixture of positional and geometric isomers of linoleic acid (LA) containing conjugated double bonds. Furthermore, each double bond can be in the trans or cis configuration. Therefore, various forms of CLA are possible (Sehat *et al*., 1998; Yurawecz *et al*., 1999). *Cis*-9, *trans*-11 and *trans*-10, *cis*-12 are the more active CLA isomers and they have been found to be predominant in ruminant meat and dairy products. These CLA isomers have been receiving particular attention in the past decade because they are natural food components claimed to possess important health benefits. These beneficial effects include anti-carcinogenic (Ip *et al*., 1994; Guo *et al*., 2007), anti-atherogenic (Lee *et al*., 1994; Valeille *et al*., 2006), anti-obesity (Lee *et al*., 2006), anti-diabetic (Ryder *et al*., 2001; Belury *et al*., 2003) and immune system enhancement (Ntambi *et al*., 2002). *Cis*-9, *trans*-11 CLA is

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produced primarily in the mammary gland from desaturation of vaccenic acid (*trans*-11 $C_{18:1}$) via Δ9-desaturase enzyme. In addition, fatty acid is produced as an intermediate throughout the incomplete biohydrogenation of dietary linoleic acid ($C_{18:2}$) to stearic acid ($C_{18:0}$) in the rumen, while *trans*-10, *cis*-12 CLA in cows' milk fat arise directly from ruminal production and cannot be produced endogenously (AbuGhazaleh *et al*., 2007).

Manipulation of the cow's diet, particularly the type of supplemental fat, is the most popular way to change or improve the fatty acid content in cows' milk fat; however, this particular approach confers certain disadvantages, among which is the fact that it ignores the animal genetic effect on milk fatty acid composition. Indeed, any change or improvement due to diet is not permanent, because if the feed supplementation is changed, the additional nutritional quality will also be changed. Improvement in animal genetic is persistent and has the advantage of generating additional value through selection. Various studies have been conducted on the effects on cow breeds. In addition, the differences in the milk-fat composition in Holstein and Jersey breeds were investigated by White *et al*. (2001) and Mele *et al*. (2007), and another study was carried out by Talpur *et al*. (2006) for White Thari and Red Sindhi breeds. Most of the published studies on CLA were performed on animals kept under temperate climate. Therefore, cattle kept under hot and humid tropical conditions (like in Malaysia) were postulated to contain different CLA concentrations in their milk fat. Indeed, the current compositional information on CLA in milk fat has only been conducted in a few countries, not including Malaysia.

A lot of attention has also been given to other unsaturated fatty acids (USFAs), such as unsaturated carbon 18 fatty acids in cows' milk fat, which seem to be favourable for human health (McGuire *et al*., 2000). Many studies have investigated the phenotypic correlation of these fatty acids and other milk fatty acid contents in milk fat, suggesting that animal selection based on the fatty acid profile may be possible (Peterson *et al*., 2002; AbuGhazaleh *et al*., 2003; Soyeurt *et al*., 2006). It is also critical to clarify whether CLA isomers have any relationship with other unsaturated carbon 18 fatty acids. Therefore, this study focused on the effect of breed on the levels of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers and their relations with other unsaturated carbon 18 fatty acids in the milk fat of Mafriwal and Jersey cows fed with the same diet.

MATERIALS AND METHODS

Animals and Milk Samples

This study was carried out in one herd of Mafriwal and Jersey dairy cattle at the Institut Haiwan in Kluang, Johor, Malaysia. This farm, which served as a main farm for animal selection and development, belongs to the Department of Veterinary Services (DVS), Ministry of Agriculture and Agro-based industries, Malaysia. Milk samples were obtained from thirty lactating cows of Mafriwal (n=15) and Jersey (n=15) at the mid-lactation period of 123.5±16.4 days in milk, within the same parity (1.7 ±0.7). Each cow was fed on pasture and supplemented with 5.5 kg concentrate per day. The milk samples were obtained after a complete individual milking for each cow and kept at -20ºC prior to analysis for fatty acid composition using gas chromatography (GC) (Agilent technologies Inc., USA).

Total Lipid Extraction

Lipid extraction from the milk samples was performed according to Folch *et al*. (1957). Milk samples (3 mL) were mixed with chloroform-methanol (2:1, v/v) solution (40 mL) with vigorous shaking for 5 minutes and allowed to stand for 12 hours with occasional shaking. The mixture was then filtered into another separating flask, and this was followed by an addition of 10 mL of normal saline. The lower chloroform phase was subsequently recovered and evaporated using rotary evaporator (Heidolph®, Germany). Then, 100 µL of internal standard (4mg/mL of heneicosanoic acid [C_{21}] in chloroform methanol) was added to the extracted

fat that had been mixed previously with 5 mL of chloroform-methanol prior to fatty acid methyl ester (FAME) preparation.

FAME Preparation and Analysis

A method by Wijngaarden (1967) was used to prepare the FAME from the milk sample. The extracted lipid was dried using nitrogen steady flow, and 2 mL of potassium hydrochloride was added. The mixture was then heated for 10 minutes in a boiling water bath with occasional shaking, and 2 mL of 14 % boron-trifluoride in methanol was added after cooling of the mixture. The mixture was reheated again for 20 minutes, and 4 mL of deionized water and 4 mL petroleum ether were added after cooling of the mixture. Finally, 2 µL of FAME was injected into the gas chromatography (GC) fitted with HP-88 silica capillary column (60 m, 0.25 mm id, 0.20 µm film thickness) (Agilent technologies Inc., USA) after separating the petroleum phase containing FAME by centrifugation.

The individual FAME peak was identified according to the similar retention time by using known external standard. The quantitative analysis was carried out based on the proportional comparison of the chromatographic peak areas between an identified fatty acid and the known internal standard.

Statistical Analysis

The differences in the milk parameter between the two breeds were assessed using independent *T*-test after verification of the normal distribution of the data. The correlations between the two CLA isomers and other unsaturated carbon 18 fatty acids were analyzed using Pearson's correlation (SPSS 15 software package). Significant differences were tested at $P < 0.05$ level.

RESULTS

Conjugated Linoleic Acids

Conjugated linoleic acid concentrations in milk fat from the two breeds of cattle are expressed in *Fig. 1*. Total CLA (*trans*-10, *cis*-12 + *cis*-9, *trans*-11 CLA) content in milk fat of Mafriwal was significantly higher ($P<0.05$) than that of the Jersey cows (3.87 and 2.55 mg/g of total fatty acids, respectively). However, the mean values of *trans*-10, *cis*-12 CLA of 0.3 and 0.25 mg/g of the total fatty acids for the Mafriwal and Jersey cows, were respectively not significantly ($P>0.05$) different. Meanwhile, the mean value of *cis*-9, *trans*-11 CLA in the milk fat of Mafriwal was significantly higher ($P<0.05$) than that of the Jersey cows (3.5 and 2.3 mg/g of the total fatty acids, respectively). The possible ratios between *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA isomers were estimated to be 1:6 to 1:13 (*trans*-10, *cis*-12/ *cis*-9, *trans*-11) in the milk fat from the two breeds.

Unsaturated Carbon 18 Fatty Acids

The mean values of unsaturated carbon 18 fatty acid compositions of the milk fat from the two breeds are summarized in Table 1. The dominant fatty acid observed among the unsaturated carbon 18 fatty acid in the milk fat of Mafriwal and Jersey cows was *cis*-9 octadecenoic acid ($C_{18:1}$) (17.03 g/100g), followed by *trans*-11 octadecenoic ($C_{18:1}$) (0.55 g/100g), *cis*-9, *trans*-11 CLA (0.29 g/100g), octadecatrienoic ($C_{18:3}$) (0.1 g/100g) and *trans*-10, *cis*-12 CLA (0.03 g/100g).

TABLE 1
The mean values conjugated linoleic acids and other unsaturated carbon 18 fatty acids (g/100g) in the milk fat of mid lactation Mafriwal and Jersey cows

Fatty acids		Mean ±SD	Range
$C_{18:1}$	*cis*-9 octadecenoic	17.03 ±5.9	6.48 - 32.6
$C_{18:1}$	*trans*-11 octadecenoic	0.55 ±0.2	0.15 - 1.04
$C_{18:2}$	*cis*-9, *trans*-11 CLA	0.29 ±0.1	0.18 - 0.58
$C_{18:2}$	*trans*-10, *cis*-12 CLA	0.03±0.008	0.015 - 0.051
$C_{18:3}$	Octadecatrienoic	0.1 ±0.05	0.05 - 0.28

Values represent mean ± SD (n=30). SD= standard deviation

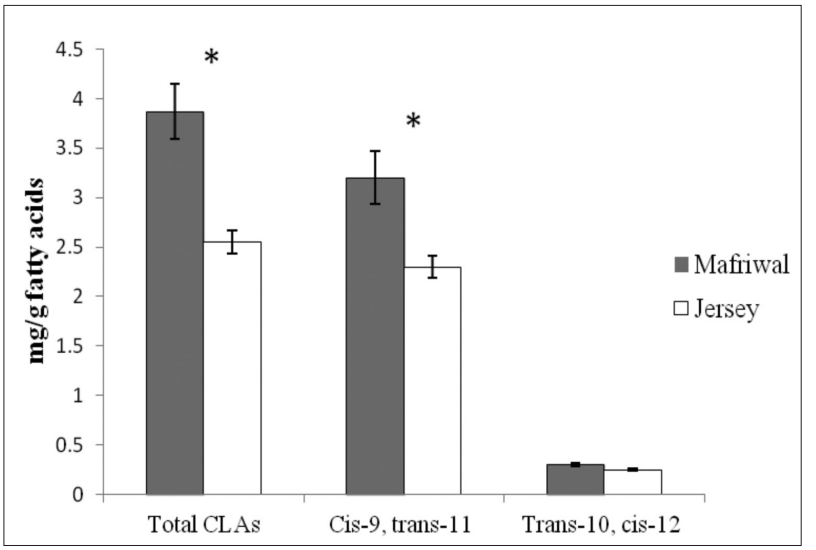

Fig. 1: Conjugated linoleic acid contents of milk fat for mid lactation Mafriwal and Jersey cows. Values are mean (n=15) ± SEM. An asterisk (*) at the top of a column indicates that a significant difference at P < 0.05 level was detected between the two breeds. Total CLAs= cis-9, trans-11 CLA + trans-10, cis-12 CLA.

Conjugated Linoleic Acid Correlations

Significant positive correlations were observed between *cis*-9*, trans*-11 CLA and *trans* 11-octadecenoic ($C_{18:1}$) acid (r=0.540, *P*<0.05), *cis* 9-octadecenoic ($C_{18:1}$) acid (r=0.554, *P*<0.05), and octadecatrienoic ($C_{18:3}$) acid (r=0. 808, *P*<0.05) (*Fig. 2*). Meanwhile, positive correlations were also observed between *trans*-10, *cis*-12 CLA and *trans* 11-octadecenoic ($C_{18:1}$) acid (r=0.299), *cis* 9-octadecenoic ($C_{18:1}$) acid (r=0.198), and octadecatrienoic ($C_{18:3}$) acid (r=0. 537, *P*<0.05), but the correlation was significant only with octadecatrienoic ($C_{18:3}$) acid (*Fig. 3*).

DISCUSSION

In this study, the total amount of CLAs in the milk fat of Mafriwal was significantly higher than that of the Jersey cows, although the mean values of *trans*-10, *cis*-12 CLA which had been shown to be effective in various cancer models (Hubbard *et al*., 2003; Masso-Welch *et al*., 2004), were not significantly different (0.3 and 0.25 mg/g of total fatty acids, respectively). Meanwhile, Mafriwal cows had significantly higher (*P*<0.05) level of *cis*-9, *trans*-11 CLA in their milk fat than that of the Jersey cows (3.5 and 2.3 mg/g of total fatty acids, respectively). The values of *cis*-9, *trans*-11 CLA were lower than those reported by Talpur *et al*. (2006) for White Thari and Red Sindhi cows, but were comparable to the figures reported earlier by White et al. (2001) for Holstein and Jersey cows. The disparity of the CLA deposition in the milk fat between Mafriwal and Jersey cows probably attributed to the tissue quantity and activity of Δ9-desaturase enzyme, which converted vaccenic acid (*trans*-11 $C_{18:1}$) to *cis*-9, *trans*-11 CLA by desaturation process in the mammary gland. The possible ratios of *trans*-10, *cis*-12/ *cis*-9, *trans*-11 CLA isomers were estimated to be 1:6 to 1:13 in milk fat from the two breeds. In other words, the *cis*-9, *trans*-11 CLA concentration in the milk fat of cows in this study was approximately 6 to 13 times more than the *trans*-10, *cis*-12 CLA concentration. *Trans*-10, *cis*-12 CLA in cow milk fat arises directly from ruminal production

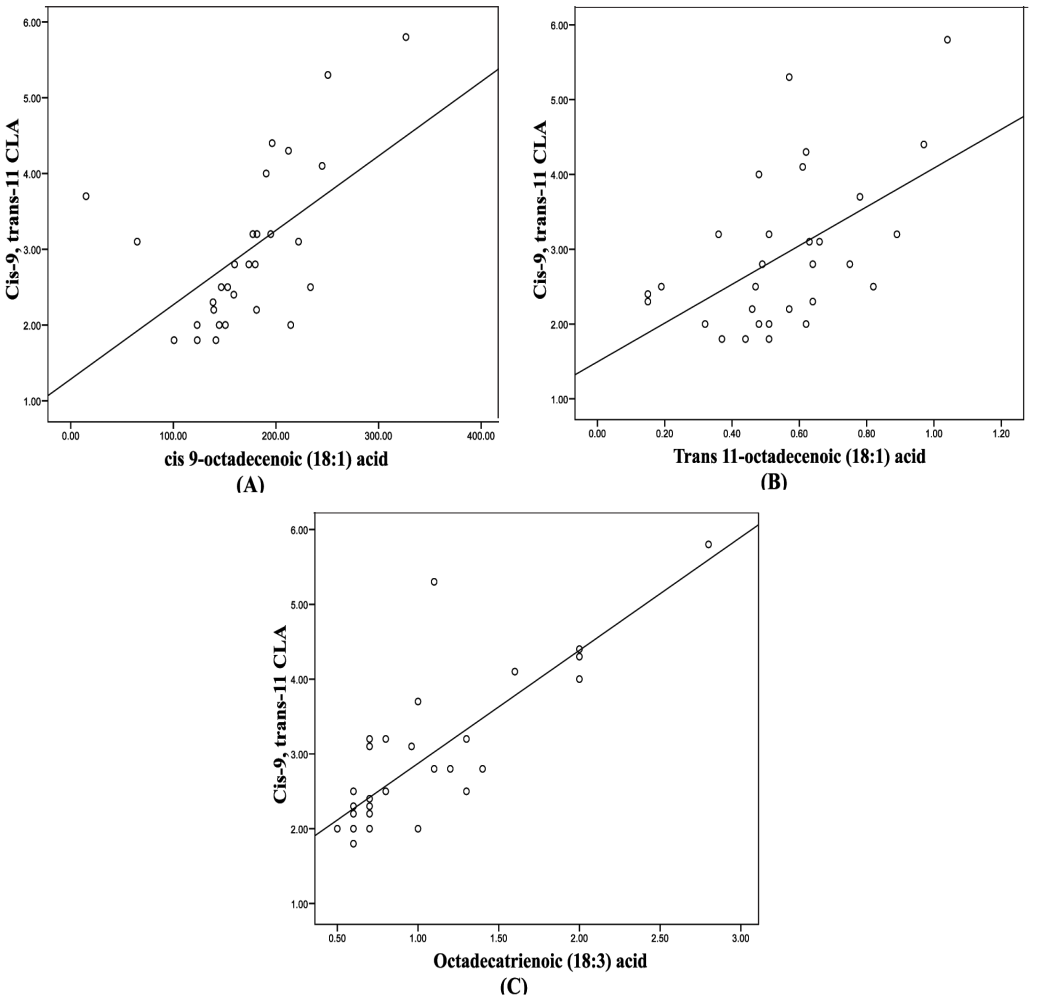

Fig. 2: The relationship between cis-9, trans-11 CLA isomer and cis 9-octadecenoic ($C_{18:1}$) acid (A), trans 11-octadecenoic ($C_{18:1}$) acid (B), and octadecatrienoic ($C_{18:3}$) acid (C) in milk fat of mid lactation Mafriwal and Jersey cows (n=30) as depicted by scattered plot and the best fitting line.

and could be produced endogenously while larger portions of *cis*-9, *trans*-11 CLA are of endogenous origin, synthesized by Δ9-desaturase enzyme from trans-11 $C_{18:1}$ (vaccenic acid), an intermediate in the rumen biohydrogenation of linoleic and linolenic acids (Corl *et al*., 2001). This could be the reason for the lower proportion of *trans*-10, *cis*-12 in cow milk fat as compared to *cis*-9, *trans*-11 CLA isomer.

Among the other unsaturated carbon 18 fatty acids in the present study, a higher proportion of *cis*-9 octadecenoic acid ($C_{18:1}$) was observed in the milk fat of Mafriwal and Jersey cows. The value of *cis*-9 octadecenoic acid ($C_{18:1}$) observed was comparable to that reported earlier by Mele *et al*. (2007) for Holstein cows, but lower than the values reported by White *et al*. (2001) and Kelly *et al*. (1998) for Holstein and Jersey cows.

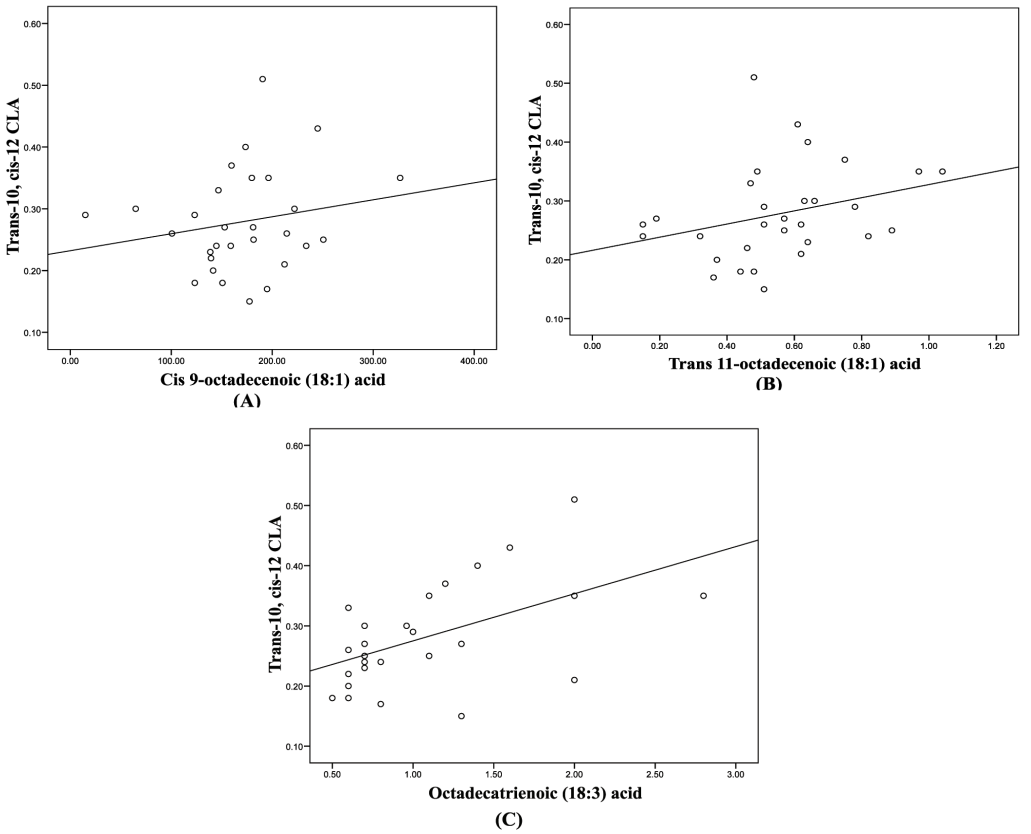

Fig. 3: The relationship between trans-10, cis-12 CLA isomer and cis 9-octadecenoic ($C_{18:1}$) acid (A), trans 11-octadecenoic ($C_{18:1}$) acid (B), and octadecatrienoic ($C_{18:3}$) acid (C) in milk fat of mid lactation Mafriwal and Jersey cows (n=30) as depicted by scattered plot and the best fitting line

The mean values of *trans*-11 octadecenoic ($C_{18:1}$) and octadecatrienoic ($C_{18:3}$) in the milk fat of Mafriwal and Jersey cows were lower than those reported earlier by White *et al*. (2001) for Holstein and Jersey cows, and Talpur *et al*. (2006) for White Thari and Red Sindhi cows. All the fatty acids, with 18 carbon chains in bovine milk fat, were derived from circulating preformed fatty acids (Bauman *et al*., 1974). These fatty acids originate from dietary and microbial lipids absorbed via the digestive tract and incorporated into chylomicrons and very low-density lipoproteins (VLDL) in the intestine and liver, respectively, and the mobilized body fat storage. In the mammary capillaries, triglycerides in VLDL and chylomicrons are hydrolyzed into free fatty acids and glycerol by lipoprotein lipase (LPL), along with adipose-derived non-esterified fatty acids (NEFA), are taken up by the mammary epithelial tissue (Davies *et al*., 1983), and this process may control the deposition of these fatty acids in cow milk fat.

There is an interest in the relationships of CLA isomers to other unsaturated carbon 18 fatty acids in cows' milk fat. In this study, *cis*-9*, trans*-11CLA, was positively correlated with *trans* 11-octadecenoic ($C_{18:1}$) acid, and this finding is in agreement with the work by Peterson *et al*. (2002). Significantly positive correlations were also observed between *cis*-9*, trans*-11 CLA and *cis* 9-octadecenoic ($C_{18:1}$) acid, *cis*-9*, trans*-11

CLA, and octadecatrienoic ($C_{18:3}$). These correlations are consistent with the findings of a previous work by AbuGhazaleh *et al.* (2003). Similarly, a significant positive correlation was also observed between *trans*-10, *cis*-12 CLA and octadecatrienoic ($C_{18:3}$) acid. These various correlations indicated that the concentrations of CLA isomers and other unsaturated carbon 18 fatty acids in cows' milk fat are related, and these relationships are important because they suggest that based on fatty acid profile, animal selection may be possible.

CONCLUSIONS

Within the limits of the conditions of this study, the results indicated that for dairy cattle, breed had an effect on CLA deposition in milk fat, implying that breed selection could be used as a tool to get better milk quality in terms of CLA content for human consumption. Furthermore, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers were correlated positively with some other unsaturated carbon 18 fatty acids, and this indicated that the levels of CLA in cows' milk fat could be increased by increasing the contents of these specific C_{18} fatty acids, possibly through nutritional manipulation.

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