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Comparison of Conjugated Linoleic Acid and Other Fatty Acid Content of Milk Fat of Mafriwal and Jersey Cows

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Abstract: Special attention has been given to the milk Fatty Acids (FA) that have a beneficial effect for human health such as mono and poly unsaturated fatty acids in particularly the Conjugated Linoleic Acids (CLA). This study was undertaken to investigate the milk fat contents of CLA variables (CLA and CLA-desaturase index) and other FA composition of Mafriwal and Jersey cows under same feeding system. In addition, the relationship between these two CLA variables with milk production and milk fat percent was determined. All the cows were grazed on pasture and given 5.5 kg of concentrate per head daily. Milk FA composition was determined using gas chromatography after extraction of milk fat using modified Folch's method. The results showed a significant variation ($p<0.05$) in the FA contents of the two breeds. The *cis*-9, *trans*-11 CLA and CLA-desaturase index in milk fat of Mafriwal were significantly higher ($p<0.05$) than that of Jersey cows. Mafriwal cows produced significantly ($p<0.05$) higher concentrations of $C_{18:0}$, $C_{18:1}$ *cis*-9, $C_{18:3}$ and $C_{20:1}$ than that of Jersey, while Jersey cows produced significantly ($p<0.05$) higher concentrations of $C_{12:0}$ and $C_{14:0}$ than Mafriwal cows. Additionally, significant positive correlations were observed between CLA variables and milk production. This study indicates that the breed of cows has an effect on CLA and other FA composition of milk fat and Mafriwal cows produced significantly higher percentages of CLA than Jersey cows which would provide better benefits for human health. Furthermore, the milk fat content of CLA and CLA-desaturase index were positively related to the milk production.

Key words: Breed, conjugated linoleic acid, cow milk fat, milk production, desaturase index, FA composition

INTRODUCTION

Due to the growth of consumer demands, great attention has been given to features related to food safety, health and nutritional value. In this framework, animal products have an important role due to their Fatty Acids (FA) content that may influence human health. Among the fatty acids, much interest is given to the mono and poly unsaturated fatty acids and in particularly Conjugated Linoleic Acids (CLA) (McGuire and McGuire, 2000). The major sources of CLA are meat and milk from ruminant animals.

The growing interest in CLA attributed to a number of positive health effects such as anti-carcinogenic (Soel *et al*., 2007), anti-diabetic (Moloney *et al*., 2007), anti-atherogenic (Munday *et al*., 2007), anti-obesity (Kang *et al*., 2004) and immune system enhancement (Lai *et al*., 2005). The *cis*-9, *trans*-11 CLA in milk fat is the most prevalent comprising 75-90% of the total CLA in cow milk fat (Tsiplakou *et al*., 2006). Furthermore, it has been well known as having anti-carcinogenic effect when consumed as a natural ingredient in foods or included as a dietary supplement (Lawless *et al*., 1999). This CLA isomer is produced primarily in the mammary gland from desaturation of vaccenic acid (*trans*-11 $C_{18:1}$) via Δ9-desaturase enzyme. In addition, it's produced as an intermediate throughout the incomplete biohydrogenation of dietary linoleic acid to stearic acid in the rumen (Abu Ghazaleh and Jacobson, 2007).

A number of factors have been shown to influence the levels of CLA and other FA composition in cow milk fat including exogenous factors such as feed allowance, oil supplementation (Chilliard *et al*., 2001) and seasonal variation (Lock and Garnsworthy, 2003), meanwhile endogenous factors like breed, lactation period and parity (Dhiman *et al*., 2005). The exogenous factors have been extensively investigated the effectiveness on FA composition. In contrast, the endogenous factors have received little attention.

In Malaysia, there is an increasing of total milk consumption in recent years (Palani, 2004). The Mafriwal,

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a crossbred of Friesian and Sahiwal is still subjected to various studies on its performance in comparison to other breeds. Therefore, this study focused on the breed effect of CLA and other FA composition in milk fat of Mafriwal and Jersey cows fed with the same diet.

MATERIALS AND METHODS

Animals and milk samples: 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) from Institut Haiwan Kluang, Johor, Malaysia. Each cow was fed on pasture and supplemented with 5.5 kg concentrate per day (Table 1). Milk samples were obtained after complete individual milking and two composite milk samples were formed for each cow. One of them was subjected to the chemical analysis to determine fat and protein percent using Milkoscan (134 A/B series equipment, Foss Electric, Denmark), while the other milk sample was kept in -20°C prior to analysis for fatty acid composition using Gas Chromatography (GC) (Agilent technologies Inc., USA).

Milk fat extraction: Milk fat was extracted by using a modified Folch *et al.* (1957) method. About 3 mL of milk samples were mixed with 40 mL chloroform-methanol (2:1, v/v) solution. The mixture was filtered after standing for 12 h and 10 mL of normal saline were added. The lower chloroform phase was subsequently recovered and evaporated using rotary evaporator (Heidolph®, Germany). Then, the extracted fat was mixed again with 5 mL of chloroform-methanol and 100 μL of internal standard (4 mg mL^{-1} of heneicosanoic acid (C_{21}) in chloroform methanol) were added prior to Fatty Acid Methyl Ester (FAME) preparation.

FAME preparation and analysis: The FAME was prepared according to the method of Wijngaarden (1967). About 2 mL of potassium hydrochloride were added to the extracted fat after the drying under nitrogen and 2 mL of 14% boron-trifluoride in methanol were added to the mixture after reheating. About 4 mL of deionized water and 4 mL petroleum ether were added after reheating the mixture. Finally, petroleum phase containing FAME was separated by centrifugation and 2 μL of FAME were injected into the GC fitted with HP-88 silica capillary column (60 m, 0.25 mm id, 0.20 μm film thickness) (Agilent technologies Inc., USA).

Table 1: The nutritional composition of concentrate diet and pasture, fed to Mafriwal and Jersey cows

Composition (%)	Concentrate	Pasture
DM	93.3	25.20
CP	15.0	7.50
EE	2.8	1.60
CF	13.5	35.20
As	6.0	8.00
Ca	0.5	0.25
P	0.6	0.17
NFE	62.7	47.50
TDN	77.1	53.40
ME MJ kg^{-1}	11.8	8.20

DM = Dry Matter, CP = Crude Protein, NFE = Nitrogen Free Extract, TDN = Total Digestible Nutrition, ME = Metabolic Energy, CF = Crude Fiber, EE = Ether Extract; The concentrate diet consists of 67% palm kernel

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

The ratio between products and substrate for Δ9-desaturase were used as indirect measurement for desaturase activities and CLA-desaturase index was calculated as [*cis*-9, *trans*-11 CLA]/[*cis*-9, *trans*-11 CLA+ *trans*-11 18:1] (Nudda *et al.*, 2008).

Statistical analysis: The difference between the two breeds was assessed using independent T-test after verification of the normal distribution of the data. The relationship between CLA and milk production, milk fat percent was analyzed using Pearson's correlation (SPSS 15 software package). Significant differences were tested at $p<0.05$ level.

RESULTS AND DISCUSSION

The milk production, percentage of protein and fat content for Mafriwal and Jersey cows were shown in Table 2. The daily milk production of Mafriwal was significantly ($p<0.01$) higher than that of Jersey cows (10.24 and 6.23 kg day^{-1}, respectively). Mafriwal cows may have higher adaptability to the local climate, which may explain their higher milk production than Jersey cows. However, Jersey cows had significantly ($p<0.05$) higher milk protein percentage than that of Mafriwal (3.78 and 3.27% of milk, respectively). The milk fat percentage was not significantly different between Mafriwal and Jersey cows (3.46 and 3.77% of milk, respectively). The values of milk protein and fat percent were comparable as reported by White *et al.* (2001) for Holstein and Jersey cows.

The mean value of *cis*-9, *trans*-11 CLA which has been shown numerous beneficial physiological effects such as anti-cancer, anti-atherogenic and anti-diabetic (Pariza *et al.*, 2001) was significantly ($p<0.05$) higher in milk fat of Mafriwal than that of Jersey cows (3.5 and 2.3 mg g^{-1} of total fatty acids, respectively). These values are comparable as reported earlier by White *et al.* (2001) for Holstein and Jersey cows and lower than reported by

Table 2: The milk production, percentage of protein and fat measured from milk samples of mid lactation Mafriwal and Jersey cows

	Mafriwal		Jersey		
Variables	Mean±SEM	Range	Mean±SEM	Range	p-value
Milk production (kg day)	10.24±0.40	7.4-13.50	6.23±0.35	3.3-8.900	**
Milk protein (%)	3.27±0.13	2.44-4.58	3.78±0.16	3.06-4.97	*
Milk fat (%)	3.46±0.15	2.64-4.78	3.77±0.19	2.12-4.90	NS

NS = Non Significant;* =Significant at p<0.05; ** = Significant at p<0.01

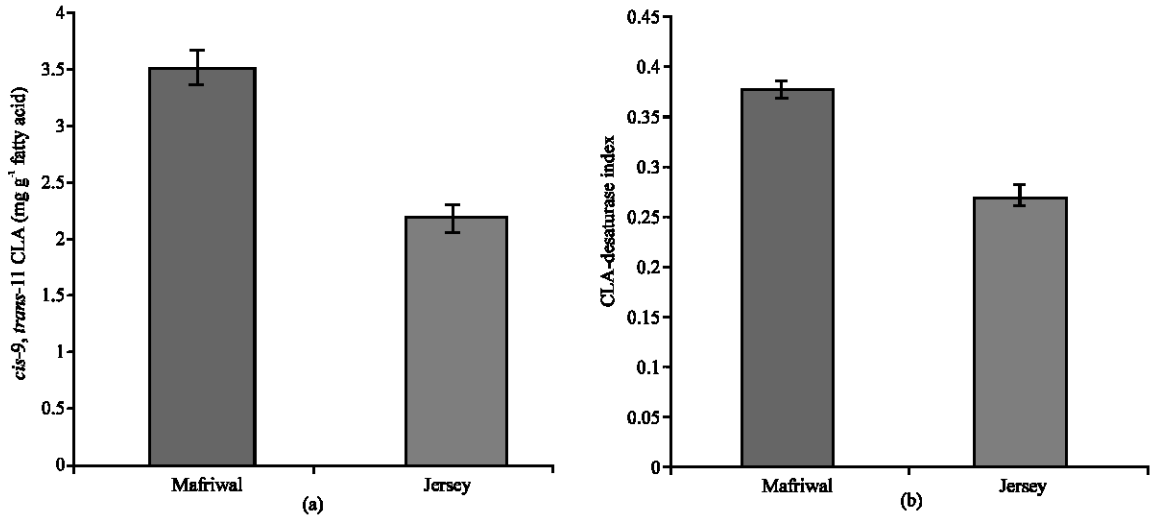

Fig. 1: Milk fat content of CLA (a) and CLA-desaturase index (b) for mid lactation Mafriwal and Jersey cows

Talpur *et al.* (2006) for Red Sindhi and White Thari cows. The larger portion of *cis*-9, *trans*11 CLA in milk fat is endogenous origin, synthesized by Δ-9 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). The relationship between *cis*-9, *trans*-11 CLA, and *trans*-11 $C_{18:1}$ reflects Δ9-desaturase activity and can be used to calculate a CLA-desaturase index.

The comparison of CLA concentration and CLA-desaturase index in milk fat of Mafriwal and Jersey dairy breeds is shown in Fig. 1. CLA-desaturase index was also significantly ($p<0.05$) higher in Mafriwal than that of Jersey. Likewise, Kelsey *et al.* (2003) reported a higher value of CLA-desaturase index for Brown Swiss than Holstein cows.

Most of the differences of CLA concentration in milk fat of Mafriwal and Jersey cows probably attributed to the rumen output of *trans*-11 $C_{18:1}$ and to a smaller extent *cis*-9, *trans*-11 CLA and to the tissue quantity and activity of Δ9-desaturase enzyme. Fatty acids composition of milk from two breeds was reported as g/100 g of total fatty acids as shown in Table 3.

The Saturated Fatty Acids (SFA) contents were significantly higher ($p<0.05$) in milk fat of Jersey than that of Mafriwal (78.4 vs. 74.5 g/100 g, respectively). These values were higher than Holstein (63.38 g/100 g) and Jersey (66.64 g/100 g) cows on pasture and concentrate feeding which reported previously by White *et al.* (2001). Within the group of SFAs, palmitic acid ($C_{16:0}$) found to be highest proportion in milk fat of both breeds with mean values of 30.26 and 29.37 g/100 g for Mafriwal and Jersey breeds, respectively.

Similar results of palmitic acid were reported by Kelsey *et al.* (2003) and Talpur *et al.* (2006). Meanwhile, myristic acid ($C_{14:0}$) has also high proportion of SFAs and accounts for 17.37 and 19.53 g/100 g in the milk fat of Mafriwal and Jersey breeds, respectively ($p<0.01$). Significant difference in the proportion of myristic acid ($C_{14:0}$) between Holstein and Jersey cows were reported by White *et al.* (2001) and Kelly *et al.* (1998).

Short and medium chain length fatty acids (<16 carbons) were significantly higher ($p<0.05$) in milk fat of Jersey than that of Mafriwal cows (39 and 45.1 g/100 g, respectively).

These values were lower than reported results by White *et al.* (2001) for Holstein (63 g/100 g) and Jersey (66 g/100 g) cows. While, the longer-chain fatty acids (<16 carbons) were significantly higher ($p<0.05$) in Mafriwal than Jersey cows and represented 31.1/100 g and 22.5 g/100g, respectively.

Table 3: The mean conjugated linoleic acid and other fatty acids (g/100 g) in milk fat of mid lactation Mafriwal and Jersey cows

	Mafriwal		Jersey		
Variables	Mean±SEM	Range	Mean±SEM	Range	p-value
Fatty acid g/100 g					
$C_{4:0}$ (Butyric)	1.6±0.09	0.97-2.1	1.4±0.08	0.87-1.89	NS
$C_{6:0}$ (Caproic)	2.46±0.18	1.41-3.75	2.41±0.27	1.27-5.4	NS
$C_{8:0}$ (Caprylic)	1.73±0.15	0.81-2.84	1.56±0.16	0.93-3.4	NS
$C_{10:0}$ (Capric)	3.66±0.268	1.68-5.31	3.749±0.31	2.26-6.83	NS
$C_{12:0}$ (Lauric)	8.74±1.00	5.1-16.5	13.97±078	7.6-19.6	**
$C_{14:0}$ (Myristic)	17.37±0.38	11.7-19.2	19.53±0.36	17.1-21.9	**
$C_{16:0}$ (Palmatic)	30.26±0.56	26.5-33.2	29.37±0.99	21.8-36.3	NS
$C_{18:0}$ (Stearic)	9.18±0.78	3.91-14.46	6.68±0.4	4.2-10.0	**
$C_{20:0}$ (Arachidic)	0.157±0.01	0.08-0.28	0.13±0.009	0.08-0.21	NS
∑SFA	74.5±1.37	67-86	78.4±0.8	73-85	*
$C_{14:1}$ *cis*-9 (Myristoleic)	1.01±0.05	0.72-1.62	0.96±0.06	0.69-1.67	NS
$C_{16:1}$ *cis*-9 (Palmitoleic)	1.16±0.0	0.66-1.84	1.32±0.09	0.74-1.9	NS
$C_{18:1}$ *cis*-9 (Oleic)	18.93±1.72	14.9-32.6	14.98±1.163	6.48-23.3	*
$C_{18:1}$ *trans*-11(Vaccenic)	0.55±0.06	0.15-1.04	0.55±0.04	0.15-0.89	NS
$C_{20:1}$	0.103±0.006	0.07-0.1	0.07±0.005	0.03-0.11	**
∑MUFA	21.1±0.38	16.3-31.2	17.26±0.32	13.23-30.62	*
$C_{18:2}$ (Linoleic)	1.61±0.08	1.27-2.22	1.73±0.05	1.56-2.15	NS
$C_{18:2}$ *cis*-9, *trans*-11 (CLA)	0.351±0.02	0.2-0.58	0.23±0.01	0.18-0.32	*
$C_{18:3}$ (α-linolenic)	0.138±0.01	0.07- 0.28	0.07±0.004	0.05-0.12	**
$C_{20:2}$	0.091±0.008	0.05-0.15	0.078±0.009	0.04-0.19	NS
∑PUFA	2.12±0.31	1.52-3.73	2.05±0.28	1.39-4.92	NS
∑USFA	23.3±0.41	18.3-33.4	19.3±0.33	14.22-28.52	*
∑Short and medium	39±1.91	23.9-54.2	45.1 ± 1.72	36.6-62.3	*
∑Long	31.1±1.7	23.4-48	22.5±1.6	10.5-33	*
CLA desaturase index	0.407±0.01	0.25-0.47	0.307±0.01	0.16-0.4	**

Values represent means for respective breeds; SEM = Standard Error Mean, NS = Non Significant; *= $p<0.05$; ** = $p<0.01$; ∑Short and medium = ($C_{4:0}$–$C_{14:0}$), ∑Long = (>C_{16})

Sum of Unsaturated Fatty Acids (USFA) was significantly higher ($p<0.05$) in milk fat of Mafriwal than that of Jersey cows (23.3 and 19.3 g/100 g, respectively). These values were lower than Talpur *et al.* (2006) for USFAs in the milk fat of Red Sindhi and White Thari cows. Within the group of USFAs, sum of Mono Unsaturated Fatty Acids (MUFA) was significantly higher ($p<0.05$) in milk fat of Mafriwal than that of Jersey cows (21.1 and 17.2 g/100 g, respectively). In addition, significantly higher ($p<0.05$) proportion of oleic acid ($C_{18:1}$) of MUFA was obtained in Mafriwal than Jersey cows with mean values of 18.93 and 14.98 g/100 g, respectively.

These values were lower than that of White *et al.* (2001) and Kelly *et al.* (1998) for oleic acid in the milk fat of Jersey and Holstein cows. Most of FA variations among different cow breeds probably attributed to the activity of mammary enzyme stearoyl coenzyme. A desaturase, which reported previously by Mele *et al.* (2007). It has ability to oxidize the medium chain of SFAs to corresponding USF. As such as, oxidizing myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic acids ($C_{18:0}$) to myristoleic ($C_{14:1}$), palmitoleic ($C_{16:1}$) and oleic acids ($C_{18:1}$), respectively. It is also involved in *cis*-9, *tans*-11 CLA production. In addition, the different diet composition, seasonal variation and management system may explain the difference in FA composition between breeds of cow in Malaysia and breeds of cow in other countries. The mean values of linoleic acid ($C_{18:2}$) in milk fat of both breeds (1.61 and 1.73 g/100 g for Mafriwal and Jersey, respectively) were not significantly different. White *et al.* (2001) reported the same proportion of linoleic acid for Holstein and Jersey cows. The mean proportion of α-linolenic acid ($C_{18:3}$) was significantly higher ($p<0.05$) in milk fat of Mafriwal than that of Jersey cows (0.138 and 0.07 g/100 g, respectively). This proportion of α-linolenic acid was lower than reported earlier by White *et al.* (2001) and Talpur *et al.* (2006). Sum of Polyunsaturated Fatty Acids (PUFA) were 2.12 and 2.05 g/100 g in Mafriwal and Jersey breeds, respectively. A lower proportion of PUFA in Mafriwal and Jersey cows was obtained as compared to Talpur *et al.* (2006) and Bobe *et al.* (2003).

In this study, the correlations of CLA concentration in milk fat with milk production and milk fat percent were shown in Fig. 2. While the same relationships with CLA-desaturase index shown in Fig. 3.

Significantly positive correlation ($r = 0.423$, $p<0.05$) was observed for CLA concentration in milk fat and milk production (Fig. 2a). Whereas, CLA concentration in milk fat was negatively correlated ($r = -0.164$, $p>0.05$) with milk fat percent but it was not significant (Fig. 2b). Meanwhile, the CLA-desaturase index was also positively correlated ($r = 0.636$, $p<0.01$) with milk production (Fig. 3a) and negatively correlated ($r = -0.269$, $p>0.05$) with milk fat percent but also it was not significant (Fig. 3b). While, weak correlations of two CLA variables with milk production and milk fat percent were reported by Kelsey *et al.* (2003) for Holsteins and Brown Swiss dairy cows.

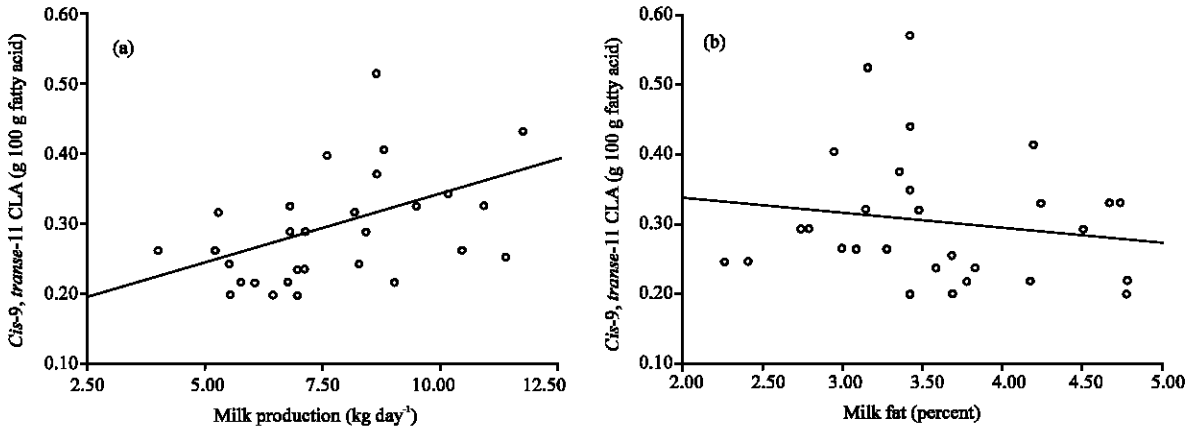

Fig. 2: The relationship between Conjugated Linoleic Acid (CLA) of; (a) milk fat with milk production and (b) milk fat percent of mid lactation Mafriwal and Jersey cows

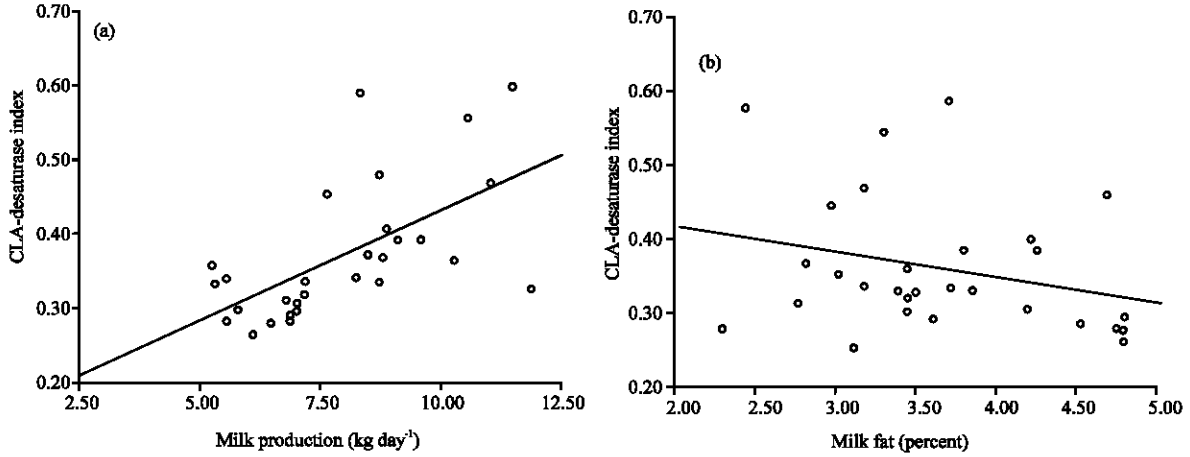

Fig. 3: The relationship between CLA-desaturase index with; (a) milk production and (b) milk fat percent of mid lactation Mafriwal and Jersey cows

CONCLUSION

The study demonstrates that CLA concentration in milk fat of Mafriwal was higher than that of Jersey cows, which would provide a better benefit to human health. In addition, the FA composition in milk fat of Mafriwal contained higher amount of USFAs and lower amount of SFAs than that of Jersey which seems to be favourable for human health. Also in this study, there was significant positive correlation between milk production and two CLA variables (CLA and CLA-desaturase index). The increasing of milk production may cause rising the amount and activity of Δ9-desaturase, which finally cause increasing of CLA concentration in milk fat.

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