Journal of Animal and Veterinary Advances 10 (1): 38-42, 2011
ISSN: 1680-5593

Conjugated Linoleic Acids in Cattle Slaughtered for Human Consumption

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Abstract: Conjugated Linoleic Acids (CLAs) are group of positional and geometric isomers of octadecadienoic acid with conjugated double bonds and believed to have many health promoting effects. The present study focused on the quantitative analysis of CLAs in liver, Superficial Pectoral (SP), Longissimus Dorsi (LD) and Semimembranosus (SM) muscles of local Malaysian_Kedah-Kelantan (KK) cattle slaughtered for human consumption. Fatty acids were extracted using Folch method and determined by gas chromatography. The average content of CLAs in the liver, SP, LD and SM muscles were 38.71, 18.24, 11.03 and 13.04 mg/100 g of sample, respectively. The quantity of CLAs in the liver was significantly ($p<0.05$) higher than other samples. The percentages of cis-9, trans-11 CLA isomer were 63.39, 76.04, 90.66 and 82.82% of total CLAs in the liver, SP, LD and SM muscles, respectively. Positive correlations between CLAs and trans-11-octadecenoic acid concentration were observed in all samples. This study confirmed that meat from KK cattle could be the potential source of CLA but still its content has to be improved to make their meat more beneficial for consumers.

Key words: Conjugated linoleic acids, liver, superficial pectoral muscle, longissimus dorsi muscle, semimembranosus muscle, Kedah Kelantan cattle

INTRODUCTION

Fatty acid composition of ruminants' meat has been studied extensively due to its direct or indirect involvement in human health (Wood *et al*., 2008). Particularly; saturated fatty acids have been associated with increased risk of obesity, hypercholesterolemia and cardiovascular diseases (WHO, 2003). Fortunately, it has been reported in recent years that the meat derived from ruminants is the main natural source of Conjugated Linoleic Acids (CLAs) which are believed to have several health promoting effects in cluding anticarcinogenic, antiatherogenic, immuno-modulating and lean body mass promotion (MacDonald, 2000; Bhattacharya *et al*., 2006). Conjugated linoleic acids are group of positional and geometric isomers of octadecadienoic (18:2) acid with conjugated double bonds.

Conjugated double bonds are in either cis or trans configuration and present in positions 7-9, 8-10, 9-11, 10-12 or 11-13 (Bhattacharya *et al*., 2006). Conjugated linoleic acids are formed as the intermediate product of lipid metabolism by rumen bacteria (Bauman *et al*., 2000). In addition, it has been suggested that animal tissues synthesize endogenously cis-9, trans-11 CLA isomer from trans-11-octadecenoic acid using Δ^9-desaturase enzyme (Corl *et al*., 2001; Schmid *et al*., 2006). Most of the published research on quantitative analysis CLAs in meat were performed on animals kept under temperate climate.

It has been reported that sunlight, temperature and seasonal factors (Tsiplakou *et al*., 2006; Alfaia *et al*., 2007) could affect the ultimate CLAs concentration in animal's tissues.

Thus, cattle kept under hot and humid tropical conditions like in Malaysia were postulated to contain different CLAs concentration in their tissues. Indeed, the current compositional information of CLAs in meat is insufficient and has only been conducted in a few countries. Therefore, the objective of this study was to determine the profile and concentration of CLAs in liver, SP, LD and SM muscles of KK cattle slaughtered for human consumption.

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MATERIALS AND METHODS

Eighty-eight samples were collected from liver (caudate lobe), SP, LD and SM muscles when local Malaysian KK cattle were slaughtered at abattoirs from May-June 2007. Approximately, 10 g of tissues were taken and wrapped with aluminium foil. All samples were kept between blocks of ice, transported to the laboratory and stored at -20°C until analysis. The total fatty acids were extracted using chloroform-methanol (2:1, v/v) (Merk kGaA, Darmstadt, Germany) based on a method described by Folch *et al.* (1957). Methylation of the extracted fatty acids to Fatty Acid Methyl Esters (FAME) was carried out using 14% methanolic boron trifluoride (Sigma Chemical Co., St. Louis, Missouri, USA). Heneicosanoic (21:0) acid was used as an internal standard. The FAME was separated on a HP-88 silica capillary column (60 m, 0.25 mm internal diameter, 0.20 μm film thickness) in a 6890N, network GC system (Agilent technology). High purity (99.99%) nitrogen (MOX, Sdn Bhd, Malaysia) was used as the carrier gas at 28.8 mL min^{-1} flow rate. The column head pressure was 22.52 psi. The injector and the detector temperatures were programmed at 250°C. The column temperature was set at the range of 80-200°C with temperature programming at the rate of 2.5°C min^{-1} increment. About One 1 of FAME was injected to the GC manually in splitless mode. The individual FAME's peak was identified using known standards of FAME containing CLAs and other 37 fatty acids (Sigma, Chemical Co., USA).

Statistical analysis: Fatty acid profiles of samples were expressed as mean mg/100 g of tissue with their respective standard deviation, percent total fatty acids and mg g^{-1} of fat. The difference in CLAs content among tissue samples was assessed using one way analysis of variance followed by Bonferroni's post hoc multiple comparisons. The relationships between the amount of CLAs and other fatty acids were analyzed using Pearson's correlation.

RESULTS AND DISCUSSION

The data on fatty acid profiles of liver, SP, LD and SM muscles from KK cattle slaughtered for meat consumption are shown in Table 1 while CLAs concentration and isomeric composition are as shown in Table 2. The average amount of CLAs in the liver, SP, LD and SM muscles were 38.71, 18.24, 11.03 and 13.04 mg/100 g of tissue, respectively. Proportionally, CLAs made up 1.36, 1.20, 0.79 and 0.69% of the total fatty acids in the liver, SP, LD and SM muscles, respectively. The amount of CLAs in the liver was significantly ($p<0.05$) higher than that of muscles. There was no significant difference among muscles on their CLAs content in mg/100 g but on the percent fatty acid basis, the SP muscle had significantly ($p<0.05$) higher proportion of CLAs than other muscles. There was no significant difference between LD and SM muscles. The average CLAs content in the KK cattle was calculated as 3.03 mg g^{-1} of fat in tissue samples.

The percentages of cis-9, trans-11 CLA isomer were 63.39, 76.04, 90.66 and 82.82% of total CLAs in the liver, SP, LD and SM muscles, respectively. The proportions of trans-10, cis-12 CLA isomer were 20.77, 21.00, 7.62 and 10.43% in liver, SP, LD and SM muscles of the total CLAs, respectively. The remaining percentage was comprised of other CLA isomers. Positive correlations between CLAs and trans-11-octadecenoic acid concentration were observed in liver ($r = 0.556$, $p<0.05$), SP ($r = 0.642$, $p<0.05$), LD ($r = 0.489$, $p<0.05$) and SM ($r = 0.520$, $p<0.05$) muscles.

Ruminants' meat is an important food, providing protein with good balance of amino acids, vitamins and essential minerals (Wood *et al.*, 2008). It is also associated with relatively higher proportion of Saturated Fatty Acids (SFA) and low proportion of Polyunsaturated Fatty Acids (PUFA) than meat from other animals (Alfaia *et al.*, 2007; Wood *et al.*, 2008). Consumption of diet with such proportion has been associated with increased risk of some metabolic problems like obesity, hypercholest erolemia and cardiovascular diseases (WHO, 2003). In contrary to this meat derived from ruminants is the main source of Conjugated Linoleic Acids (CLAs) which are believed to have several health promoting effects in cluding anticarcinogenic, antiatherogenic, immuno-modulating and lean body mass promotion (MacDonald, 2000; Bhattacharya *et al.*, 2006). In this study, CLAs were detected and quantified in liver and other tissue samples.

Beef liver could also be the potential source of CLAs as it was evidenced in this study since amount of CLAs in the liver was significantly ($p<0.05$) higher than other tissue samples. This may be attributed to the function of liver as an organ of chain extension and desaturation of fatty acids. The amount and activity of enzymes like Δ^9-desaturase may also be the reason why CLA is higher in the liver than other samples.

The CLAs present in the muscle are either locally synthesized within the muscle itself or from the rumen microbial fermentation (Corl *et al.*, 2001; Schmid *et al.*, 2006). The average amount of CLAs in the SP, LD and SM muscles were 18.24, 11.03 and 13.04 mg/100 g of tissue, respectively. These results were comparable to the amount of CLAs (10.5-19.5 mg/100 g of beef) reported by Mir *et al.* (2004). The amount of CLAs varies not only

Table 1: Fatty acid profile of the liver, Semimembranosus (SM), Longissimus Dorsi (LD) and Superficial Pectoral (SP) muscles

	Liver		SM		LD		SP	
Fatty acid*	Mean±SD	TFA (%)	Mean±SD	TFA (%)	Mean±SD	TFA (%)	Mean±SD	TFA (%)
10:0	6.72±2.94	0.24	7.21±7.37	0.38	2.24±0.65	0.16	ND	0.00
12:0	11.00±4.85	0.39	16.05±8.02	0.85	7.67±6.72	0.55	ND	0.00
14:0	40.23±26.75	1.41	48.90±28.45	2.59	35.98±24.82	2.59	30.78±23.89	2.03
14:1	14.30±6.03	0.50	19.88±14.82	1.05	11.27±5.34	0.81	13.10±8.77	0.86
15:0	29.5±14.18	1.04	34.41±13.95	1.82	21.45±10.35	1.54	16.90±7.35	1.11
15:1	31.97±27.46	1.12	12.44±4.01	0.66	11.59±4.54	0.83	13.45±13.45	0.89
16:0	344.74±285.31	12.11	406.76±228.40	21.53	311.37±143.96	22.39	321.50±202.70	21.19
16:1	38.92±35.92	1.37	62.95±43.36	3.33	25.23±12.64	1.81	43.30±27.10	2.85
17:0	26.46±10.99	0.93	26.33±12.52	1.39	18.13±11.43	1.30	14.43±5.83	0.95
17:1	23.12±7.02	0.81	22.14±14.93	1.17	10.49±1.95	0.75	18.81±12.52	1.24
18:0	730.37±369.94	25.65	278.25±124.12	14.73	216.11±84.83	15.55	231.65±126.42	15.27
18:1trans	35.13±3.12	1.23	37.84±17.53	2.00	30.65±15.89	2.20	52.59±11.03	3.47
18:1cis	508.41±353.76	17.85	333.61±213.49	17.66	256.90±230.24	18.48	362.51±184.09	23.88
18:2 trans, trans	17.00±14.25	0.60	14.19±2.78	0.75	19.13±14.80	1.38	11.56±3.72	0.76
18:2 cis, cis	203.41±149.57	7.14	172.29±69.71	9.12	126.95±62.91	9.13	117.20±66.07	7.72
18:3	64.54±36.21	2.27	44.99±18.77	2.38	37.73±19.90	2.71	35.92±14.93	2.37
20:0	15.15±7.31	0.53	12.96±8.64	0.69	9.56±2.90	0.69	14.00±10.22	0.92
18:2 CLA_1	24.54±11.15	0.86	10.80±4.93	0.57	10.00±5.90	0.72	13.87±7.26	0.91
18:2 CLA_2	8.04±7.13	0.28	1.36±0.36	0.07	0.84±2.05	0.06	3.83±2.93	0.25
18:2 CLA_3	6.13±6.13	0.22	0.88±1.72	0.05	0.19±0.64	0.01	0.54±1.38	0.04
20:2	74.87±43.07	2.63	34.99±7.99	1.85	23.4±4.81	1.68	31.85±26.07	2.10
22:0	22.42±3.27	0.79	32.14±13.12	1.70	73.22±7.19	5.27	80.61±29.50	5.31
20:4	274.46±72.12	9.64	83.34±43.85	4.41	26.64±13.94	1.92	25.14±24.85	1.66
22:1	24.62±13.82	0.86	ND	0.00	19.33±18.92	1.39	ND	0.00
20:5	62.11±37.57	2.18	37.91±23.71	2.01	23.63±12.03	1.70	24.24±10.34	1.60
24:0	13.55±5.65	0.48	ND	0.00	14.41±4.80	1.04	ND	0.00
24:1	37.66±12.51	1.32	83.86±0.00	4.44	ND	0.00	ND	0.00
22:5	129.87±6.97	4.55	15.27±5.12	0.81	18.62±5.62	1.34	ND	0.00
22:6	28.56±86.01	1.00	37.59±11.91	1.99	27.81±11.95	2.00	39.70±21.09	2.62
Total fatty acid	2847.80	100.00	1889.34	100.00	1390.50	100.00	1517.50	100.00
Total saturated	1240.14±381.74	43.55	863.01±421.61	45.68	710.14±235.14	51.07	709.87±311.94	46.78
Total unsaturated	1607.66±418.01	56.45	1026.33±328.45	54.32	680.40±200.43	48.93	807.61±180.92	53.22
Total MUFA	714.13±383.13	25.08	572.72±322.43	30.31	365.46±222.46	26.28	958.55±209.47	63.17
Total PUFA n-3	285.08±161.52	10.01	135.76±48.80	7.19	107.79±39.19	7.75	77.94±30.46	5.14
Total PUFA n-6	577.78±251.71	20.29	306.17±91.41	16.21	196.96±67.70	14.16	210.65±86.75	13.88
Ratio UFA/SFA	1.30	-	1.19	-	0.96	-	1.14	-
Ratio PUFA/SFA	0.72	-	0.52	-	0.44	-	0.41	-
Ratio n-6/n-3	2.03	-	2.26	-	1.83	-	2.70	-
Ratio CLAs/saturated	0.031	-	0.015	-	0.015	-	0.025	-

*Fatty acids are represented as number of carbon atoms (10-24) and number of carbon-carbon double bond(s) (0-6); CLA_1: contains mainly cis-9, trans-11 CLA isomer; CLA_2: contains mainly trans-10, cis-12 CLA isomer; CLA_3: contains other isomers; Values were expressed in mean±SD (standard deviation) mg/100 g of sample and percent total fatty acid (%TFA); ND: Not Detected; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; UFA = Unsaturated Fatty Acids; SFA = Saturated Fatty Acids

Table 2: Conjugated linoleic acid concentration and isomeric composition

	CLA_1				CLA_2				CLA_3				Total CLAs		
Ugans	Min.	Max.	Mean±D	Percentage	Min.	Max.	Mean±D	Percentage	Min.	Max.	Mean±D	Percentage	Min.	Max.	Mean±SD
Liver	6.01	59.276	24.54±11.45	63.39	0.00	31.99	8.04±7.13	20.77	0.00	17.93	6.13±5.24	15.84	15.98	73.42	38.71±15.27
SP	5.46	32.730	13.87±7.260	76.04	0.00	13.26	3.83±2.93	21.00	0.00	8.48	0.54±1.38	2.96	5.46	45.99	18.24±10.12
LD	2.97	23.670	10.00±5.900	90.66	0.00	8.70	0.84±2.05	7.62	0.00	2.75	0.19±0.65	1.72	2.97	23.67	11.03±5.960
SM	4.08	22.730	10.8±4.9000	82.82	0.00	9.21	1.36±0.31	10.43	0.00	5.44	0.88±3.10	6.75	4.08	22.73	13.04±5.560

CLA_1: Contains mainly cis-9, trans-11 CLA isomer; •CLA_2: Contains mainly trans-10, cis-12 CLA isomer; CLA_3: Contains other isomers; SP: Superficial Pectoral muscle; LD: Longissimus Dorsi muscle; SM: Semimembranosus muscle; %: percent of total CLAs; Min: minimum; Max: maximum; SD: Standard deviation

between animals but also among different sites of each individual animal where samples were taken from. In the present study, the SP muscle contained significantly ($p<0.05$) higher proportion of CLAs than the other muscles. Significant differences in the quantity of CLAs among muscles at different site of the animal were also reported by Lorenzen *et al.* (2007).

On the mg g^{-1} of fat basis, the average CLAs content in the KK cattle (3.03 mg g^{-1}) was greater than the average amount of CLAs reported in American beef (1.7 mg g^{-1} fat) (Mulvihill, 2001) and within the same range (1.2-6.2 mg g^{-1} of fat) as in the Canadian milk and beef products. On the other hand, the average CLAs content in KK cattle was less than the average CLAs

content in Australian beef (7.6 mg g^{-1} of fat) (Mulvihill, 2001). Both extrinsic (diet, season) and intrinsic (breed, sex, age) factors play their role in these variations (Torre *et al*., 2006).

A higher CLAs content in beef from pasture than grain or silage fed cattle was reported by Mir *et al.* (2004). The effect of diet on CLAs content in beef of Angus crossbred steers was studied by Poulson *et al.* (2004) who reported as high as 466% increments in steers fed forage and pasture as compared to those fed with typically high grain diets. Linseed supplementation has been suggested as an efficient way to increase CLAs level in meat (Torre *et al*., 2006). Therefore, the type of feed given by the farmers to the KK cattle could be the main contributing factor for such variation.

Identification of individual CLA isomers is very important since each isomer may have separate physiological functions. In this study, cis-9, trans-11 CLA was found to be the most abundant isomer in all tissue samples. High proportion of cis-9, trans-11 CLA isomer in samples supports the suggestion of endogenous synthesis by Δ^9-desaturase enzyme using trans-11-octadecenoic acid as substrate (Corl *et al*., 2001; Schmid *et al*., 2006). The positive correlation between CLAs and trans-11-octadecenoic acid concentration in the samples were in agreement with the findings of Knight *et al.* (2003). On the other hand, animal tissues lack Δ^{12}-desaturase enzyme (Watkins and German, 2002) which pointed to a very low probability to synthesize trans-10, cis-12 CLA isomer endogenously from trans-10-octadecenoic acid. Therefore, the rumen microbial lipid metabolism remains as the only source for this isomer. This could be the main reason for lower proportion of trans-10, cis-12 CLA in tissue samples.

CONCLUSION

In the study, the present study generated valuable data on CLAs and other fatty acids in the liver, SP, LD and SM muscles of KK cattle slaughtered for human consumption. It is confirmed that KK cattle could be a potential source of CLAs but still their level has to be improved to make the meat from these animals more beneficial for consumers. Better understanding of the biosynthesis of CLAs is essential in devising means for further improvement.

ACKNOWLEDGEMENTS

The researchers would like to thank the staff of Department of Veterinary Services, Putrajaya State Veterinary Department, Kuantan and Banting abattoirs (Malaysia) and laboratory technicians for their contributions to this project which was funded by a research grant number 05-01-04-SF0373, awarded by Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

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