CONJUGATED LINOLEIC ACID AND OTHER FATTY ACID CONTENT IN THE MILK FAT OF MAFRIWAL AND JERSEY COWS AND THE ANTIOXIDANT ACTIVITY OF SELECTED CONJUGATED LINOLEIC ACID ISOMERS

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By

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Special attention has been given to the milk fatty acids (FA) such as mono and poly unsaturated fatty acids particularly the conjugated linoleic acids (CLA) that have beneficial effects for human health. The present study was undertaken to investigate the breed effect on CLA and other fatty acid contents in cow milk fat and to assess the antioxidant activity of cis-9, trans-11 and trans-10, cis-12 as a single or mixed CLA isomers. Milk samples were obtained from 30 lactating cows from two breeds, Mafriwal (n=15) and Jersey (n=15) from Institut Haiwan Kluang, Johor, Malaysia. All the cows were grazed on pasture and given 5.5 kg of concentrate per head daily. Milk fatty acid content was determined using standard gas chromatography. The mean value of cis-9, trans-11 CLA in milk fat of Mafriwal (3.5 mg/g of total fatty acids) was significantly higher ($P < 0.05$) than that of Jersey cows (2.3 mg/g of total fatty acids). The mean values of trans-10, cis-12 CLA were 0.3 and 0.25 mg/g of total fatty acids for Mafriwal and Jersey cows, respectively, which were not significantly different ($P > 0.05$). However, the CLA-desaturase index was significantly ($P < 0.05$) higher in Mafriwal than that of Jersey cows. A significant positive correlation ($r = 0.423, P < 0.05$) was observed between cis-9, trans-11 CLA and milk
production. The CLA-desaturase index was also positively correlated \((r = 0.636, P < 0.01)\) with milk production. As for the antioxidant activity, a microplate reader was used to determine the free radical scavenging properties of \textit{trans}-10, \textit{cis}-12 and \textit{cis}-9, \textit{trans}-11 as single or mixed at two ratios, 1:6 and 1:13 \((\textit{trans}-10, \textit{cis}-12/ \textit{cis}-9, \textit{trans}-11)\), against the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) in ethanol. The kinetic reactions of CLA-DPPH• showed that \textit{trans}-10, \textit{cis}-12 and \textit{cis}-9, \textit{trans}-11 as single or mixed CLA isomers have exerted radical scavenging activities in a dose-dependent manner with the lowest concentration of 2.5 mg of CLA/mL in ethanol. The two CLA isomers and the mixtures were observed to immediately react and quench DPPH radicals at all tested levels and no lag phase was noticed in CLA-DPPH• reactions. The median inhibitory concentration (IC\(_{50}\)) value for \textit{cis}-9, \textit{trans}-11 CLA \((11.1 \pm 3.8 \text{ mg/mL})\) was observed to be more effective than \textit{trans}-10, \textit{cis}-12 CLA \((12.6 \pm 3.4 \text{ mg/mL})\) and the mixtures of \textit{trans}-10, \textit{cis}-12 and \textit{cis}-9, \textit{trans}-11 at the ratios of 1:6 and 1:13 \((16.3 \pm 1.1 \text{ mg/mL and } 27.9 \pm 8 \text{ mg/mL})\), respectively. Furthermore, \textit{cis}-9, \textit{trans}-11 CLA quenched significantly more \((P < 0.05)\) DPPH radicals at low concentrations \((5 \text{ and } 10 \text{ mg/mL})\) than that of \textit{trans}-10, \textit{cis}-12 CLA and the two mixtures of \textit{trans}-10, \textit{cis}-12 and \textit{cis}-9, \textit{trans}-11 at the ratios of 1:6 and 1:13 \((\textit{trans}-10, \textit{cis}-12/ \textit{cis}-9, \textit{trans}-11)\). Meanwhile, \textit{trans}-10, \textit{cis}-12 CLA quenched significantly more \((P < 0.05)\) DPPH radicals at high concentrations \((40 \text{ and } 80 \text{ mg/mL})\) than the other tested CLAs. Total antioxidant capacity (TAC) of CLA as single or mixed isomers was estimated and compared with the potent antioxidants such as vitamin E (vit E) and butylated hydroxytoluene (BHT). All tested CLAs were less effective radical scavengers as compared to vit E and BHT at a level of 50 mM, although all tested CLAs quenched a high amount \((P < 0.05)\) of DPPH free radicals. Conversely, TAC of \textit{trans}-10, \textit{cis}-12 CLA was significantly more \((P < 0.05)\) effective than the other tested CLAs followed by \textit{cis}-9, \textit{trans}-11CLA, the mixture of \textit{trans}-
10, cis-12 and cis-9, trans-11 at ratio of 1:6 and the mixture of trans-10, cis-12 and cis-9, trans-11 at ratio of 1:13. In conclusion, the breed factor has a considerable effect on CLA concentration and other FA content in cows’ milk fat. The Mafriwal cows had significantly ($P < 0.05$) higher concentrations of CLA in their milk fat than that of the Jersey cows, which would provide a better benefit to human health. In addition, the CLAs had the ability to directly react and quench DPPH free radicals in ethanol, suggesting that the free radical scavenging activity of the CLA isomers may contribute to their diverse biological activities.
Perhatian khas telah diberi kepada asid lemak susu (AL) seperti mono dan poli asid lemak bukan tepu terutama asid linoleik terkonjugat (ALT) yang bermanfaat untuk kehidupan manusia. Kajian ini dijalankan untuk menyelidik kesan baka terhadap ALT dan asid lemak yang lain dalam lemak susu lembu dan untuk menilai aktiviti antioksidant cis-9, trans-11 dan trans-10, cis-12 secara individu atau campuran isomer ALT. Sampel susu lembu diperolehi daripada 30 ekor lembu tenuku baka Mafriwal (n=15) dan Jersey (n=15) daripada Institut Haiwan Kluang, Johor, Malaysia. Kesemua lembu meragut rumput dan diberi dedak sebanyak 5.5 kg setiap ekor lembu sehari. Kandungan asid lemak susu ditentukan melalui kromatografi gas piawai. Purata kandungan ALT cis-9, trans-11 dalam lemak susu Mafriwal (3.5 daripada jumlah asid lemak) adalah lebih tinggi tererti (P < 0.05) daripada lemak susu Jersey (2.3mg/g daripada jumlah asid lemak). Purata kandungan ALT trans-10, cis-12 ialah 0.3 dan 0.25 mg/g jumlah asid lemak masing-masing pada lembu Mafriwal dan Jersey yang tidak menunjukkan perbezaan tererti (P > 0.05). Namun demikian, indek ALT-desaturase adalah lebih tinggi tererti (P < 0.05) pada lembu Mafriwal berbanding dengan lembu Jersey. Perkaitan positif tererti (r =0.423, P < 0.05) telah dicerap untuk kepekatan ALT cis-9, trans-11 di antara lemak susu dan produksi susu. Indeks ALT-desaturase juga menunjukkan perkaitan positif (r =0.636, P < 0.01) dengan produksi susu. Untuk menilai aktiviti antioksidant, mikroplat digunakan bagi menentukan ciri penghapus radikal bebas trans-10, cis-12 dan cis-9, trans-11 sebagai individu atau campuran pada dua nisbah iaitu 1:6 dan 1:13 (trans-10, cis-12/cis-9, trans-11) terhadap radikal stabil 2, 2-diphenyl-1-picryhydrazyl (DPPH). Tindakbalas kinetik ALT-DPPH menunjukkan bahawa trans-10, cis-12 dan cis-9, trans-11 sebagai individu atau campuran isomer ALT telah menimbulkan aktiviti radikal penghapus dalam situasi dos pautan dengan kepekatan yang paling rendah iaitu 2.5 mg ALT/mL. Dua isomer ALT dan campurannya telah dicerap untuk reaksi spontan dan
menghapuskan radikal DPPH pada semua tahap ujian dan tiada fase pegun kelihatan dalam
tindakbalas ALT-DPPH. Nilai medium kepekatan perencatan (IC\textsubscript{50}) untuk ALT \textit{cis}-9, \textit{trans}-11
(11.1 ±3.8 mg/mL) didapati lebih berkesan daripada ALT \textit{trans}-10, \textit{cis}-12 (12.6 ±3.4 mg/mL)
dan campuran \textit{trans}-10, \textit{cis}-12 dan \textit{cis}-9 \textit{trans}-11 pada kadar nisbah 1:6 dan 1:13 (16.3 ±1.1
mg/mL dan 27.9 ±8 mg/mL). Tambahan pula, ALT \textit{cis}-9, \textit{trans}-11 lebih tererti h sup (\(P < 0.05\))
radikal DPPH pada kepekatan rendah (5 dan 10 mg/ml) berbanding dengan ALT \textit{trans}-10, \textit{cis}-12
Manakala, ALT \textit{trans}-10, \textit{cis}-12 lebih h sup tererti (\(P < 0.05\)) radikal DPPH pada kepekatan
tinggi (40 dan 80 mg/mL) berbanding dengan ALT yang lain. Jumlah kapasiti antioksidant
(JKA) ALT sebagai isomer individu atau isomer campuran berdasarkan anggaran dan bandingan
dengan antioksidant potent seperti vitamin E (vit E) dan butilated hidroxitolui (BHT). Kesemua
ALT yang diuji kurang efektif radikal penghapus berbanding dengan vit E dan BHT pada aras 50
mM, walaupun kesemua ALT yang diuji telah menghapus radikal bebas DPPH pada amaun yang
tinggi (\(P < 0.05\)). Sebaliknya, jumlah kapasiti antioksidant (JKA) ALT \textit{trans}-10, \textit{cis}-12 adalah
lebih efektif tererti (\(P < 0.05\)) berbanding ALT lain diikuti dengan \textit{cis}-9, \textit{trans}-11 campuran
\textit{trans}-10, \textit{cis}-12 dan \textit{cis}-9, \textit{trans}-11 pada nisbah 1:6 dan campuran \textit{trans}-10, \textit{cis}-12 dan \textit{cis}-9,
\textit{trans}-11 pada nisbah 1:13. Kesimpulannya, faktor baka memberi kesan besar pada kepekatan
ALT dan asid lemak yang lain terkandung dalam lemak susu lembu. Lembu Mafriwal
mempunyai kepekatan ALT yang lebih tinggi tererti dalam lemak susu berbanding lembu Jersey
yang mana akan memberi lebih faedah kepada kesihatan manusia. Tambahan pula, ALT
mempunyai kemampuan untuk bertindak terus dan menghapuskan radikal bebas DPPH,
mengusulkan bahawa aktiviti penghapus radikal bebas isomer ALT mungkin penyumbang
tepada kepelbagaian aktiviti biologinya.
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I certify that a Thesis Examination Committee has met on 22 October 2010 to conduct the final examination of Yassir Mohammed Ali on his thesis entitled “Conjugated Linoleic Acid and Other Fatty Acid Content in the Milk Fat of Mafriwal and Jersey Cows and the Antioxidant Activity of Selected Conjugated Linoleic Acid Isomers” in accordance with the Universities and University College Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not currently, submitted for any other degree at Universiti Putra Malaysia or other institution.

YASSIR MOHAMMED ALI

Date: 22 October 2010

TABLES OF CONTENTS
CHAPTER

I GENERAL INTRODUCTION 1

II LITERATURE REVIEW 5

2.1 Dairy Cattle in Malaysia 5

2.2 Lipids and Fatty Acids 6

2.2.1 Bovine Milk Lipids 6

2.2.2 Lipid Metabolism 8

2.2.3 Milk Fatty Acids Synthesis 12

2.2.4 Conjugated Linoleic Acid (CLA) 16

2.3 Antioxidant Defence System and Oxidative Stress 26

2.3.1 Antioxidants 26

2.3.2 Oxidative Stress 29

2.3.3 Oxidants 31

2.3.4 Conjugated Linoleic Acid as Antioxidant 37

2.3.5 Evaluation of Antioxidant Activity 38

III BREED EFFECT ON CONJUGATED LINOLEIC ACID AND SELECTED FATTY ACIDS IN COW MILK FAT 42

3.1 Introduction 42

3.2 Materials and Methods 44

3.2.1 Animals and Milk Samples 44

3.2.2 Total Lipid Extraction 46

3.2.3 Fatty Acids Methyl Ester Preparation 47

3.2.4 Gas Chromatography 48

3.2.5 Statistical Analysis 49

3.3 Results 50

3.3.1 Composition and Production of Milk From Mafriwal and Jersey Cows 50
3.3.2 Conjugated linoleic Acid in Milk Fat of Mafriwal and Jersey Cows 51
3.3.3 The Relationship Between CLA Isomers and Other Milk Variables 54
3.3.4 Fatty Acids in Milk Fat of Mafriwal and Jersey Cows 58

3.4 Discussion 66
3.4.1 Composition and Production of Milk From Mafriwal and Jersey Cows 66
3.4.2 Conjugated linoleic Acid in Milk Fat of Mafriwal and Jersey Cows 66
3.4.3 The Relationship Between CLA Isomers and Other Milk Variables 68
3.4.4 Other Fatty Acids Composition in Milk Fat of Mafriwal and Jersey Cows 69

IV ANTIOXIDANT ACTIVITY OF CONJUGATED LINOLEIC ACID AS SINGLE OR MIXED ISOMERS 74
4.1 Introduction 74
4.2 Materials and Methods 77
4.2.1 DPPH Radical Scavenging Assay 77
4.2.2 Statistical Analysis 81
4.3 Results 82
4.3.1 Kinetics of CLA-Radical Reaction 82
4.3.2 Median Inhibitory Concentration (IC\textsubscript{50}) of CLAs 85
4.3.3 Comparison of Total Antioxidant Capacity 87
4.4 Discussion 89

V GENERAL DISCUSSION 94

VI CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH 100
6.1 Conclusions 100
6.2 Recommendations for Future Research 101

REFERENCES 103
APPENDICES 109
BIODATA OF STUDENT 121