UNIVERSITI PUTRA MALAYSIA

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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Chairman: Assoc. Prof. Arifah Bt Abdul Kadir, PhD

Faculty: Veterinary Medicine

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 *trans*-10, *cis*-12 and *cis*-9, *trans*-11 as single or mixed at two ratios, 1:6 and 1:13 (*trans*-10, *cis*-12/ *cis*-9, *trans*-11), against the stable 2, 2-diphenyl-1-picryhydrazyl radical (DPPH) in ethanol. The kinetic reactions of CLA-DPPH$^{\bullet}$ showed that *trans*-10, *cis*-12 and *cis*-9, *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$^{\bullet}$ reactions. The median inhibitory concentration (IC_{50}) value for *cis*-9, *trans*-11 CLA (11.1 ±3.8 mg/mL) was observed to be more effective than *trans*-10, *cis*-12 CLA (12.6 ±3.4 mg/mL) and the mixtures of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 at the ratios of 1:6 and 1:13 (16.3 ±1.1 mg/mL and 27.9 ±8 mg/mL), respectively. Furthermore, *cis*-9, *trans*-11 CLA quenched significantly more ($P < 0.05$) DPPH radicals at low concentrations (5 and 10 mg/mL) than that of *trans*-10, *cis*-12 CLA and the two mixtures of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 at the ratios of 1:6 and 1:13 (*trans*-10, *cis*-12/ *cis*-9, *trans*-11). Meanwhile, *trans*-10, *cis*-12 CLA quenched significantly more ($P < 0.05$) DPPH radicals at high concentrations (40 and 80mg/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 *trans*-10, *cis*-12 CLA was significantly more ($P < 0.05$) effective than the other tested CLAs followed by *cis*-9, *trans*-11CLA, the mixture of *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.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

ASID LINOLEIK TERKONJUGAT DAN KANDUNGAN ASID LEMAK DALAM LEMAK SUSU LEMBU MAFRIWAL DAN JERSEY DAN AKTIVITI ANTIOKSIDAN ISOMER ASID LINOLEIK TERKONJUGAT PILIHAN

Oleh

YASSIR MOHAMMED ALI

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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 kesihatan 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 tenusu 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$^{\bullet}$ 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 fasa pegun kelihatan dalam tindakbalas ALT-DPPH$^{\bullet}$. Nilai medium kepekatan perencatan (IC_{50}) untuk ALT *cis*-9, *trans*-11 (11.1 ±3.8 mg/mL) didapati lebih berkesan daripada ALT *trans*-10, *cis*-12 (12.6 ±3.4 mg/mL) dan campuran *trans*-10, *cis*-12 dan *cis*-9 *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 *cis*-9, *trans*-11 lebih tererti hapus ($P < 0.05$) radikal DPPH pada kepekatan rendah (5 dan 10 mg/ml) berbanding dengan ALT *trans*-10, *cis*-12 dan campuran kedua-duanya *trans*-10, *cis*-12 dan *cis*-9 *trans*-11 pada nisbah 1:6 dan 1:13. Manakala, ALT *trans*-10, *cis*-12 lebih hapus 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 hidroxitoluin (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 *trans*-10, *cis*-12 adalah lebih efektif tererti ($P < 0.05$) berbanding ALT lain diikuti dengan *cis*-9, *trans*-11 campuran *trans*-10, *cis*-12 dan *cis*-9, *trans*-11 pada nisbah 1:6 dan campuran *trans*-10, *cis*-12 dan *cis*-9, *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 kepada 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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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

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