UNIVERSITI PUTRA MALAYSIA

QUALIFICATION OF GENETICALLY MODIFIED MAIZE IN ANIMAL FEED USING REAL-TIME POLYMERASE CHAIN REACTION

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By

JASBEER KAUR A/P KESAR SINGH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2011

DEDICATION

This work is dedicated to those in the scientific community

who believe sincerely

in sharing their knowledge and expertise

for the advancement of science

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

QUANTIFICATION OF GENETICALLY MODIFIED MAIZE IN ANIMAL FEED USING REAL-TIME POLYMERASE CHAIN REACTION

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Chair: **Professor Son Radu, PhD**

Faculty: **Food Science and Technology**

More genetically modified organisms (GMOs) are being developed and approved worldwide resulting in more GM crops being incorporated into livestock production systems. The presence of a foreign gene in the crop has raised concerns of safe feed and safe foods of animal origin such as meat, milk and eggs which are significant sources of high-quality food for humans. Therefore, identifying rapid and economical DNA-based GMO analytical method for screening feed and feed ingredients is imperative. In Malaysia, there is no comprehensive data on the testing and presence of GMOs in feed which are currently not regulated. The objective of the study is to identify suitable DNA

extraction method from feed as well as detect and quantify GM maize events. In Malaysia, such a study has never been undertaken before.

In attempting to identify suitable DNA extraction method for feeds (coarse mix, pellet or extruded feed) from seven different DNA extraction methods (Roche, Qiagen, NucleoSpin, Epicentre, Wizard, CTAB and modified CTAB methods), DNA quality in terms of the amplification ability of single copy maize endogenous *hmg* (high mobility group) gene was compared using real-time polymerase chain reaction (PCR) technique. Relative levels of *hmg* were used to evaluate the DNA quality. DNA was also assessed in terms of yield, purity and integrity. Further in the study, four GM maize events (event MON810, MON863, NK603 and GA21) were analyzed in 103 feed and 20 maize samples extracted using the Roche automated system which uses the proprietary glass magnetic particles to bind DNA. Except for GA21 which is not approved for import in Malaysia, events MON810, MON863 and NK603 are approved.

The findings illustrate that the genomic DNA extraction methods have significant influence on DNA yield, purity, integrity and quality. The Epicentre method extracted significantly the highest DNA yield followed by the modified CTAB method. The Roche and Wizard methods had significantly low DNA

yields but high DNA purity. All six methods, except Wizard produced low molecular weight DNA indicating highly fragmented DNA due to feed processing. The Wizard method was the only method that produced high molecular weight DNA despite having the lowest yield. Finally, the Wizard method also recovered the most amplifiable DNA per reaction, indicating highest DNA quality. For the first time in Malaysia, a total of 103 feed and 20 maize samples were used to obtain an overview of the incidence of GMO presence in feeds and raw maize. GM material was present in 26.2% feeds and 65% maize samples. Single-event and multiple-events were identified in the GM samples with 50% of the GM samples containing multiple-events. All GM samples contained MON810 (100%) followed by NK603 (47.5%), GA21 (25%) and MON863 (2.5%). Surprisingly, the non-approved GA21 was detected more than the approved event MON863. Concentrations of GMOs were also significantly higher for the unprocessed maize compared to the processed feeds. This study which represents a fast and reliable methodology provides an insight on Malaysia scenario and will serve as important baseline data in future policies to regulate GMOs.

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

KUANTIFIKASI JAGUNG YANG DIUBAHSUAI GENETIK DALAM MAKANAN BINATANG MENGGUNAKAN KAEDAH REAL-TIME POLYMERASE CHAIN REACTION

Oleh

JASBEER KAUR A/P KESAR SINGH

Jun 2011

Pengerusi: **Professor Son Radu, PhD**

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Lebih banyak organism diubahsuai genetik (GMO) sedang dibangunkan di dunia, menyebabkan lebih banyak tumbuhan GMO digunakan dalam industri penternakan. Kehadiran gen asing dalam tumbuhan telah membangkitkan kerisauan berkenaan keselamatan makanan ternakan (*animal feed*) dan keselamatan makanan berasaskan haiwan seperti susu, daging dan telur yang merupakan sumber makanan berkualiti tinggi untuk manusia. Oleh kerana itu, adalah penting untuk mengenalpasti kaedah analisis GMO berasaskan DNA

untuk menyaring makanan ternakan serta ramuan-ramuannya. Di Malaysia, pada ketika ini tiada penguatkuasaan GMO dilakukan dan tiada sebarang data komprehensif wujud berkenaan pengujian serta kehadiran GMO dalam makanan ternakan. Objektif kajian ini adalah untuk mengenalpasti kaedah pengekstrakan DNA dalam makanan ternakan disamping mengesan dan mengkuantifikasi event jagung GM. Kajian seumpama ini belum pernah dikendalikan di Malaysia sebelum ini.

Untuk mengenalpasti kaedah pengekstrakan DNA yang paling sesuai dari tujuh kaedah (Roche, Qiagen, NucleoSpin, Epicentre, Wizard, CTAB dan modified CTAB) dalam makanan ternakan (coarse mix, pellet or extruded feed), perbandingan kualiti DNA dari segi keupayaan amplifikasi satu salinan (single copy) gen *hmg (*high mobility group*)* dalam jagung telah dijalankan melalui teknik real-time PCR. Kepekatan gen *hmg* telah digunakan untuk menilai kualiti DNA. Penilaian DNA juga dilakukan untuk membandingkan kuantiti, ketulenan serta integriti DNA. Tambahan lagi, empat event jagung GM (event MON810, MON863, NK603 and GA21) telah dianalisa menggunakan 103 sampel makanan ternakan dan 20 sampel jagung mentah. Kaedah pengekstrakan DNA telah menggunakan sistem otomatik Roche berasaskan butiran magnetik glas (proprietary glass magnetic particles) untuk mengasingkan DNA. Event GA21

tidak dibenarkan untuk diimport ke Malaysia manakala event MON810, MON863 and NK603 sudah pun mendapat kelulusan.

Hasil kajian menunjukkan bahawa kaedah pengekstrakan DNA mempunyai pengaruh kuat terhadap kuantiti, ketulenan, integriti serta kualiti DNA. Kaedah Epicentre telah menjana kuantiti DNA yang paling banyak diikuti dengan kaedah CTAB modified. Kaedah Roche dan Wizard pula menghasilkan kuantiti DNA yang rendah tetapi berkualiti tinggi. Selain dari Wizard, kesemua enam kaedah lain hanya berupaya menghasilkan cebisan-cebisan DNA kerana telah melalui proses-proses pembuatan makanan ternakan. Kaedah Wizard adalah satu-satunya kaedah yang telah menghasilkan DNA dengan berat molekul tinggi walaupun menjana kuantiti DNA yang paling rendah. Tambahan pula, kaedah Wizard telah menghasilkan kualiti DNA yang paling baik kerana berupaya mengamplifikasi DNA dalam jumlah tertinggi. Julung kali di Malaysia, sejumlah 103 sampel makanan ternakan dan 20 sampel jagung mentah telah digunakan untuk mendapatkan gambaran berkaitan kehadiran GMO secara amnya. Kehadiran GMO telah dikesan dalam 26.2% sampel makanan ternakan dan 65% sampel jagung. Single event serta multiple events telah dikesan dalam sampel-sampel tersebut, dengan 50% sampel mengandungi *multiple events.* Kesemua sampel GM mengandungi MON810 (100%) diikuti dengan NK603 (47.5%), GA21

(25%) and MON863 (2.5%). Tidak dijangka bahawa event GA21 yang tidak dibenarkan untuk diimport ke Malaysia dikesan dalam jumlah yang jauh lebih tinggi berbanding event MON863 yang dibenarkan pengimportannya. Jagung mentah memperlihatkan kandungan GMO yang lebih tinggi berbanding makanan binatang berproses. Kajian ini yang merupakan metodologi yang cepat serta boleh dipercayai berupaya memberi gambaran yang baik terhadap kehadiran GMO dalam makanan ternakan di Malaysia. Data kajian ini akan menjadi data asas untuk membangunkan perundangan GMO dalam makanan ternakan di masa hadapan.

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APPROVAL

I certify that a Thesis Examination Committee has met on 21 June 2011 to conduct the final examination of **Jasbeer Kaur a/p Kesar Singh** on her thesis entitled **"Quantification of Genetically Modified Maize in Animal Feed by Real-time PCR"** in accordance with the Universities and University Colleges 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 degree.
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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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DECLARATION

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

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JASBEER KAUR A/P KESAR SINGH

Date: 21 June 2011

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