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TLR4 GENE POLYMORPHISM AND MILK TRAITS IN HEALTHY AND MASTITIS DAIRY COWS

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TLR4 GENE POLYMORPHISM AND MILK TRAITS IN HEALTHY AND MASTITIS DAIRY COWS

BY

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A project report submitted to the Faculty of Agriculture, Universiti Putra Malaysia in fulfillment of the requirement of SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science)

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CERTIFICATION

This project entitle "**TLR4 Gene Polymorphism and Milk Traits in Healthy and Mastitis Cows**" is prepared by Lim Su Ching and submitted to the Faculty of Agriculture in fulfillment of the requirement of the course SHW 4999 (Final Year Project) for the award of the degree of Bachelor Agriculture (Animal Science).

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LIST OF ABBREVIATIONS AND SYMBOLS

bp	base pair
cm	centimeter
DC	direct current
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
EtBr	ethidium bromide
g	gram
M	molarity
mg	milligram
$MgCl_2$	magnesium chloride
min	minute
ml	milliliter
mM	millimole
ng	nanogram
nm	nanomole
PBS	phosphate buffer salime
rpm	revolutions per minute

SV	spin or vacuum
TBE	tris borate EDTA
U	unit
UV	ultraviolet
V	volt
%	percentage
°C	degree Celcius
µg	microgram
µL	microliter
µM	micromole
µm	micrometer

ABSTRACT

TLR4 GENE POLYMORPHISM AND MILK TRAITS IN HEALTHY AND MASTITIS DAIRY COWS

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LIM SU CHING

Bovine mastitis, an inflammatory disease of the mammary gland generally caused by intramammary infections, is the most frequently occurring disease in Malaysia dairy industry. During mastitis infection, cells of the innate immune system become activated through pattern recognition receptors that recognize conserved molecular signatures associated with the invading pathogen. Toll-like receptor 4 gene (*Tlr4*) is an important pattern recognition receptor that recognizes endotoxins associated with gram-negative bacterial infections. Its role in pathogen recognition and subsequent initiation of the inflammatory and immune response makes it a suitable candidate gene for enhanced mastitis resistance. There is limited molecular information on the *Tlr4* single nucleotide polymorphisms (SNPs), especially for differentiation of mastitis resistance or susceptibility. This study was conducted to determine the DNA sequence variations of the *Tlr4* of the mastitis and non-mastitis dairy cows. Milk was collected from 23 Friesian and 7 Friesian-Jersey crossbred lactating cows at the Redagri Farm, Linggi, Negeri Sembilan. Four of the Friesians suffered from mastitis. DNA was extracted from the milk samples and a region of *Tlr4* was amplified using polymerase chain reaction (PCR) and the amplified DNA fragment sizes were estimated using gel electrophoresis. The somatic cell

count (SCC), somatic cell score (SCS) and the composition traits of the milk were also determined. The PCR amplicons from two infected and two healthy samples were purified and sequenced and their associations with somatic cell score and milk composition traits were analyzed. The result showed that there was no significant variation in the PCR products from milk samples from the mastitis and non-mastitis cows; a single DNA band of 248 bp was exhibited. The sequence alignment of the purified PCR amplicons too showed 100% nucleotide similarity. There were no variations in the sequences of *Tlr4* in mastitis and non-mastitis cows. Milk parameters recorded for mastitis and non mastitis animals were 14.72±2.17 vs. 8.86 ± 0.24 for SCS, 0.65±0.40 vs. 1.09±0.08 for milk fat percentage, 3.76±1.70 vs. 3.02±0.05 for protein percentage, 2.21±1.32 vs. 4.97±0.05 for lactose percentage, 6.42±2.14 vs. 8.59±0.08 for solid non-fat percentage and 7.21±2.42 vs. 9.68±0.12 for total solid percentage respectively. For all parameters there were no significant difference between the mastitis and non mastitis cows. The partial region of *Tlr4* investigated in the present study may not be suitable to differentiate susceptibility of Friesian cows to mastitis. Other regions of the gene should also be investigated. The small number of mastitis animals in the study may also be the reason why SNPs in the *Tlr4* region and differences in the milk parameters were not detected. A larger sample size of both healthy and mastitis animals should be investigated.

ABSTRAK

GEN TLR4 POLYMORPHISM DAN CIRI-CIRI KOMPOSISI SUSU DALAM LEMBU TENUSU YANG SIHAT DAN MASTITIS

Oleh

LIM SU CHING

Penyakit radang kelenjar susu yang berlaku pada lembu tenusu umumnya disebabkan oleh jangkitan antara kelenjar susu. Ia merupakan penyakit yang paling kerap berlaku dalam industri tenusu di Malaysia. Semasa jangkitan berlaku, sel-sel sistem imun semula jadi menjadi aktif melalui reseptor pengenalan pola yang mengenali tanda-tanda molekul lestari yang berkaitan dengan pathogen yang menyerang masuk dalam badan lembu tenusu. Gen Toll-like receptor 4 *(Tlr4)* adalah pengenalan pola reseptor yang penting untuk mengenal endotoksin yang berkaitan dengan jangkitan bakteria gram negatif. Peranannya dalam pengiktirafan patogen dan permulaan tindak balas keradangan dan kebal membuatnya menjadi calon gen yang sesuai untuk meningkatkan rintangan bagi penyakit radang kelenjar susu. Maklumat molekul bagi *Tlr4* adalah terhad, terutamanya untuk membezakan kesusahan atau kesenangan untuk mengalami penyakit radang kelenjar susu. Kajian ini telah dijalankan untuk menentukan variasi jujukan DNA dalam *Tlr4* daripada haiwan yang mengalami penyakit radang kelenjar susu dan haiwan yang tidak mengalami penyakit radang kelenjar susu. Sampel susu telah dikumpulkan daripada 23 ekor Friesian dan 7 ekor kacukan Friesian-Jersey dari Redagri

Farm, Linggi, Negeri Sembilan. Antaranya terdapat empat ekor Friesian yang mengalami penyakit radang kelenjar susu. DNA telah diekstrak daripada sampel susu dan sebahagian daripada *Tlr4* telah diamplifikasi dengan menggunakan *polymerase chain reaction* (PCR) serta saiz fragmen DNA yang telah diamplifikasi dianggarkan dengan menggunakan elektroforesis gel. Hubungan antara kiraan sel somatik, skor sel somatik dan ciri-ciri komposisi susu juga ditentukan. Amplikon PCR daripada dua sampel susu yang dijangkiti dan dua sampel susu yang sihat telah ditulenkan dan disekuens dan hubungan mereka dengan skor sel somatik dan ciri-ciri komposisi susu telah dianalisis. Hasil kajian menunjukkan bahawa tiada perbezaan yang ketara antara produk-produk PCR daripada sampel susu yang dijangkiti dan sampel susu yang sihat. Keempat-empat sampel susu mempamerkan satu jalur DNA, iaitu 248 bp. Penjajaran sekuens untuk amplikon PCR menunjukkan 100% persamaan nukleotid. Tiada perbezaan ditunjukkan dalam jujukan nukleotid *Tlr4* untuk sampel susu yang dijangkiti dan sample susu yang sihat. Parameter susu dicatat untuk haiwan yang mengalami penyakit radang kelenjar susu dan haiwan yang tidak mengalami penyakit radang kelenjar susu adalah 14.72 ± 2.17 vs 8.86 ± 0.24 bagi skor sel somatik, 0.65 ± 0.40 vs 1.09 ± 0.08 bagi peratusan lemak susu, 3.76 ± 1.70 vs 3.02 ± 0.05 bagi peratusan protein, 2.21 ± 1.32 vs . 4.97 ± 0.05 bagi peratusan laktosa, 6.42 ± 2.14 vs 8.59 ± 0.08 bagi peratusan pepejal bukan lemak dan 7.21 ± 2.42 vs 9.68 ± 0.12 bagi jumlah peratusan pepejal masing-masing. Bagi semua parameter didapati tiada perbezaan yang signifikan antara satu sama lain. Sebahagian daripada *Tlr4* yang disiasat dalam kajian ini mungkin tidak sesuai untuk membezakan kerentanan lembu Friesian yang mengalami penyakit

radang kelenjar susu. Bahagian lain gen tersebut juga perlu disiasat. Jumlah kecil haiwan yang mengalami penyakit radang kelenjar susu dalam kajian ini juga mungkin menjadi penyebab kenapa polimorfisme nukleotida tunggal dalam bahagian *Tlr4* dan perbezaan dalam parameter susu tidak dapat dikesan. Satu sampel saiz yang lebih besar daripada kedua-dua haiwan yang sihat dan tidak sihat harus diselidiki.

CHAPTER 1

INTRODUCTION

Mastitis, as one of the most prevalent and severest disease of dairy cattle, contributes to great economic losses in the dairy cattle industry worldwide. The prevalence of mastitis ranges from 10-15% (Neelesh, 2007). It leads to reduced milk yield and quality, premature culling of highly susceptible cows, and increased production cost due to treatment and herd management strategies. Mastitis is a mammary gland inflammation generally caused by pathogens from the environment or other lactating cows suffering from epidemic mastitis (Harmon, 1994). Approximately 30% of the cows carry the pathogens and about one third of all cows suffer from mastitis once a year (Tenhagen *et al.,* 2006).

Clinical mastitis (CM) has low heritability, ranging from 0.01 to 0.10, with the mean value of 0.03 (Mrode & Swanson, 1996). Therefore, direct selection for resistance to CM may be a difficult approach in dairy breeding (Chen *et al.,* 2011). The strong positive genetic correlation between somatic cell count (SCC) or somatic cell score (SCS) and mastitis, ranging from 0.30 to 0.98 (Philipsson *et al.,* 1995; Emanuelson *et al.,* 1988), has resulted in the acceptance of these as indicator traits for CM. The measurement of SCC has become one of the most reliable indicators for determining milk quality and the price of raw milk within the dairy industry (Jayarao *et al.,* 2004). In a review, Schutz (1994) reported that indirect selection, genetic evaluation and

selection of sires for lower SCC or SCS may reduce the occurrence of mastitis.

The innate immune response is the first line of defense against microbial pathogens (Janeway & Medzhitov, 2002). The genes coding for immune factors that detect and eliminate the pathogens are strong potential markers for enhancing resistance against infectious diseases like mastitis (Beecher *et al.,* 2010; Oviedo-Boyso *et al.,* 2007). Toll-like receptor 4 (Tlr4) is a protein on cell surface that has been implicated as receptors mediating cellular activation in response to bacterial lipopolysaccharide (LPS), a component of the ectoblast of Gram-negative bacteria, which is the pathogen-associated molecular patterns (PAMPs) for Tlr4 (McGuire *et al.,* 2006; Akira & Takeda, 2004; Werling & Jungi, 2003). These genes are liable for the initiation of the inflammatory response to foreign contagious pathogen, for example bacteria, fungi and viruses (Steven *et al.,* 2008).

The bovine Tlr4 gene has been mapped to the distal end of *Bos taurus* autosome 8 (BTA8) (McGuire *et al.,* 2006; White *et al.,* 2003b) and is located within or nearby the genomic region of SCS quantitative trait loci (Sharma *et al.,* 2006). Given that *Tlr4* is involved in PAMP recognition, mutations in *Tlr4* can compromise the host immune response to certain pathogens. Since *Tlr4* is highly polymorphic in the bovine species (White *et al.,* 2003a) and the *Tlr4* expression is associated with inframammary infection (IMI), this gene may be a functional and potential candidate gene for use in marker-assisted selection (MAS) to enhance mastitis resistance in dairy cattle. The polymorphisms at *Tlr4* were reported as associated with SCS (Wang *et al.,* 2008). Wang *et al*. (2007) reported that cows with AA genotype at this locus showed lower SCS

and were not easily infected with mastitis in comparison to cows with the BB genotype. They suggested that allele A might be the beneficial allele for mastitis resistance.

Owing to the effect of mastitis on milk yield and composition (Leitner *et al*., 2004) and the existence of a genetic correlation between mastitis and milk yield and composition (Hinrichs *et al*., 2005), it is possible that *Tlr4,* as a candidate gene influencing mastitis resistance, also affects milk production and components. Literature that addresses the association of this gene with milk-related traits is limited (Beecher *et al*., 2010).

1.1 Problem Statement

The association between the *Tlr4* locus and mastitis in cows has to be further investigated and validated. There is no reported study on investigation for genetic markers for mastitis in cows in Malaysia.

1.2 Research Hypothesis

Cows with AA genotype at *Tlr4* would show lower SCS in comparison to the cows with the BB genotype. This genotype would also have higher fat concentration in milk samples.

1.3 Objectives

General objective of the proposed study was to evaluate the association between variations within the *Tlr4* with milk SCC which reflects mastitis resistance in dairy cattle.

The specific objectives of the study were:

a) To estimate the milk SCC, SCS and fat percentage for the lactating Friesian (F) and Friesian-Jersey (FJ) cows.

b) To identify the single nucleotide polymorphisms (SNPs) in the bovine *Tlr4* of the above cows.

c) To analyze the association of *Tlr4* polymorphism with milk SCC, SCS and fat percentage.

1.4 Significance of Study

Findings from this study will indicate whether the *Tlr4* SNPs are useful molecular markers for mastitis resistance or susceptibility. If suitable marker is identified, it will provide some reference for implementing additional selection criteria in breeding programs for achieving a sustainable reduction in clinical mastitis and thereby improving the productivity of the dairy cattle.

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