MYOSTATIN POLYMORPHISM IN BALI CATTLE AND THE GAUR

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FACULTY OF AGRICULTURE

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MYOSTATIN POLYMORPHISM IN BALI CATTLE AND THE GAUR

BY

KHAIRUN NADWA BINTI RADZUAN

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CERTIFICATION FORM

This project report entitled **"MYOSTATIN POLYMORPHISM IN BALI CATTLE AND THE GAUR"** is prepared by **Khairun Nadwa Binti Radzuan** and submitted to the Faculty of Agriculture 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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LIST OF ABBREVIATIONS

kg	-	kilogram
g	-	gram
µl	-	microliter
ml	-	milliliter
ng	-	nanogram
bp	-	base pair
mM	-	micromolar
nm	-	nanometer
U	-	unit
ºC	-	degree of celsius
T_m	-	melting temperature
C	-	cytosine
T	-	thymine
G	-	guanine
A	-	adenine
MSTN	-	myostatin gene
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleotide triphosphate
PCR	-	polymerase chain reaction
GDF-8	-	growth/differentiation factor-8 gene
Indel	-	insertion and deletion

IUCN	-	international union for conservation of nature
PBS	-	phosphate buffered saline
rpm	-	revolution per minute
$MgCl_2$	-	magnesium chloride
TBE	-	tris borate EDTA
UV	-	ultraviolet
SNP	-	single nucleotide polymorphism
TD PCR	-	touchdown polymerase chain reaction

ABSTRACT

MYOSTATIN POLYMORPHISM IN BALI CATTLE AND GAUR

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SUPERVISOR: PROF. DR. JOTHI MALAR PANANDAM

Species, gender, breed, nutrition, physical activities and growth promoting agent are among the factors that influence the growth rate of muscles. Genes are one of the contributing factors in muscle development. Myostatin gene (*MSTN*) is a regulatory gene that has a distinct effect in increasing the number and size of muscle fibre. Mutations of *MSTN* result in muscle hyperplasia, an increase in number of muscle fibre. Mutation of this gene is responsible for the double muscle trait in cattle. The Bali cattle and gaur are more muscular compared to normal cattle. There may be some forms of mutation present in *MSTN* in these species which may be similar to the double muscled cattle breeds.

The objective of this study was to determine the presence of mutation in *MSTN* in the highly muscled Bali cattle and gaur which may be responsible for their high muscular trait. The study was carried out (i) to optimise the PCR amplification protocol for *MSTN* for Bali cattle and the gaur using published protocols for cattle, (ii) to determine the partial nucleotide sequences of *MSTN* in the Bali cattle and gaur, and (iii) to compare the partial *MSTN* sequences of the Bali cattle and gaur with those of normal cattle, the

Belgian Blue and Piedmontese. DNA were extracted from meat tissue samples of two Bali cattle and faecal samples of two Gaurs. DNA and $MgCl_2$ concentrations as well as PCR amplification protocols were varied to optimise the amplification of three regions of *MSTN*. The success of PCR amplification was confirmed using agarose gel electrophoresis, and the purified PCR products sequenced. Although successful PCR amplification was achieved for two of the regions (*MSTN 1* and *MSTN 2*), sequence data was obtained only for *MSTN 1*.

The results showed 15 single nucleotide polymorphisms (SNPs) in *MSTN 1* of one Bali cattle (Bali 1) when compared to cattle (*Bos taurus*). The base substitutions were 4885T>A, 5024T>A, 4889G>C, 4888-4890T>G, 4908G>T and 4947A>C. Three insertions were found: 4887_4888insC, 5172_5173insA and 5173insA. Six deletions were observed: 4896delA, 4906delA, 5168delA, 4900delT, 4917delT and 4898delG. In Bali 2 there were substitutions (4890A>G, 4891T>G, 4891T>G), 4949T>A), insertion (5172_5173insC) and deletions (4889delG, 4898delG). In *MSTN 1* of Gaur 1 three insertions occurred (4867insC, 4871insC, 5172insC). In addition to five single base deletions (4865delT, 4873delT, 4874delT, 4907delT, 4928delA), there was a 28 bp deletion (4876_4903del). *MSTN 1 l*of gaur 2 had a deletion of 24 bp (4880_4923del), and six single base deletions (4865delT, 4968delT, 4970delT, 4873delT, 4874delT, 4869delG). The presence of SNPs in *MSTN* of Bali cattle and the gaur suggests that their muscular nature may be related to the mutations in this gene. However, further study has to be done using more animals.

Keyword: Double muscled trait, MSTN, GDF-8, Gene sequencing, SNP, indel

ABSTRAK

POLIMORFISMA MYOSTATIN DALAM LEMBU BALI DAN SELADANG

NAMA: KHAIRUN NADWA BINTI RADZUAN (1665992)

PENYELIA: PROF. DR. JOTHI MALAR PANANDAM

Spesies, jantina, baka, pemakanan, aktiviti fizikal dan ejen penggalakkan pertumbuhan adalah antara faktor yang mempengaruhi kadar pertumbuhan otot. Gen adalah salah satu faktor yang menyumbang dalam pertumbuhan otot. Gen myostatin (*MSTN*) adalah gen pengawalseliaan yang memberi kesan yang jelas dalam meningkatkan bilangan dan saiz gentian otot. Mutasi *MSTN* menyebabkan hiperplasia otot, iaitu peningkatan jumlah gentian otot. Mutasi gen ini bertanggungjawab ke atas sifat otot berganda dalam lembu. Lembu Bali dan seladang lebih otot berbanding lembu biasa. Mungkin ada beberapa jenis mutasi dalam *MSTN* spesis ini yang mungkin sama dengan lembu baka otot berganda.

Objektif kajian ini adalah untuk menentukan kewujudan mutasi dalam *MSTN* pada lembu Bali dan seladang yang sangat berotot yang mungkin bertanggungjawab bagi sifat banyaknya otot mereka. Kajian ini telah dijalankan untuk (i) mengoptimumkan PCR protokol amplifikasi untuk *MSTN* untuk lembu Bali dan seladang menggunakan protokol terbitan untuk lembu, (ii) untuk menentukan jujukan nukleotida partial bagi *MSTN* pada lembu Bali dan seladang, dan (iii) untuk membandingkan jujukan nukleotida separa

MSTN lembu Bali dan seladang dengan lembu normal, Blue Belgium dan Piedmontese. Akhir skali membandingkan separa jujukan dengan lembu (*B.taurus*) dan lembu Belgian Blue dan Piedmontese. DNA diekstrasi daripada sampel tisu daging dua ekor lembu Bali dan sampel najis dua ekor seladang. Kepekatan DNA dan $MgCl_2$ serta protokol amplifikasi PCR telah diubahsuai untuk mengoptimumkan amplifikasi tiga kawasan *MSTN*. Kejayaan amplifikasi PCR telah disahkan menggunakan agarose gel elektroforesis, dan produk PCR dipiawaikan dan disekuans. Walaupun berjaya amplifikasi PCR telah dicapai untuk dua daripada kawasan (*MSTN 1* dan *MSTN 2*), data jujukan telah diperolehi hanya untuk *MSTN 1*.

Hasil kajian menunjukkan 15 polimorfisme nukleotida tunggal (SNP) dalam *MSTN 1* seekor lembu Bali (Bali 1) berbanding lembu (*Bos taurus*). Penggantian bes adalah 4885T>A, 5024T>A, 4889G>C, 4888-4890T>G, 4908G>T dan 4947A>C. Tiga insersi didapati: 4887_4888insC, 5172_5173insA dan 5173insA. Enam delesi diperhatikan: 4896delA, 4906delA, 5168delA, 4900delT, 4917delT dan 4898delG. Pada Bali 2 terdapat penggantian (4890A>G, 4891T>G, 4891T>G), 4949T>A), insersi (5172_5173insC) dan delesi (4889delG, 4898delG). Dalam *MSTN 1* daripada seladang 1 tiga insersi berlaku (4867insC, 4871insC, 5172insC). Tambahan pada lima delesi bes tunggal (4865delT, 4873delT, 4874delT, 4907delT, 4928delA), terdapat delesi 28 bp (4876_4903del). *MSTN 1* seladang 2 mempunyai delesi 24 bp (4880_4923del), dan enam delesi bes tunggal (4865delT, 4968delT, 4970delT, 4873delT, 4874delT, 4869delG). Kehadiran SNP dalam *MSTN* lembu Bali dan seladang menunjukkan bahawa sifat berotot mereka mungkin berkaitan dengan mutasi dalam jujukan gen tersebut. Walau bagaimanapun, kajian lanjut perlu dilakukan dengan menggunakan lebih haiwan.

Kata kunci: Sifat berotot berganda, MSTN, *GDF-8*, Penjujukan gen, SNP, indel

CHAPTER 1

INTRODUCTION

Muscle development is a stage when muscle fibres grow in number and size. Muscle development starts myogenesis, the formation of muscle tissues particularly during embryonic stage. During the embryonic stage, the myogenic progenitor cells develop into myoblasts, which then proliferate and divide, and finally differentiate into myotubes. Myotubes then mature into muscle fibres for further development after birth. Species, gender, breed, nutrition, physical activities and growth promoting agent are several factor that influence the growth rate of muscles, and thus the quality of the meat. According to Rehfeldt *et al*. (2000), postnatal muscle fibre hypertrophy is inversely correlated with the total number of muscle fibres within a muscle.

Genes too influence the size and number of muscle fibres in animals. One of the genes that has a distinct effect in increasing the number and size of muscle fibres is the myostatin gene (*MSTN*), also known as growth/differentiation factor-8 gene (*GDF-8*). *MSTN* affects myogenesis during embryonic development by regulating the myoblast proliferation and differentiation. Mutation of *MSTN* results in double muscling condition of cattle, which is due to muscle hyperplasia, an increase in number of muscle fibre, and muscle hypertrophy, enlargement of muscle fibres. In cattle, these conditions are exhibited more in the proximal fore- and hindquarters (Arthur, 1995). Due to mutation of *MSTN*, cattle breeds with the double muscled trait are known to have superior carcass

characteristics, that is, higher carcass percentage, a higher proportion of muscle and a lower proportion of fat and bone (Shahin and Berg, 1985; Arthur, 1995). These characteristics are important and desired in beef production.

1.1 Objective of Study

The general objective of this study was to determine the presence of mutation in *MSTN* in the highly muscled Bali cattle and gaur which may be responsible for their high muscled trait.

The specific objectives of this study were:

i. To optimise the PCR amplification protocol for *MSTN* for Bali cattle and gaur using published protocols for cattle.

ii. To determine the nucleotide sequence of two region of *MSTN* in the Bali cattle and gaur.

iii. To compare the partial sequences of *MSTN* of the Bali cattle and the gaur with each other and with those of cattle (*B. taurus*) and Belgian Blue and Piedmontese.

1.2 Research Hypothesis

The Bali cattle and gaur are more muscular compared to normal cattle. There is probably some form of mutation present in *MSTN* in these species compared to the gene sequence of normal cattle that makes them more muscular.

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