UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF CANDIDATE GENES AND MOLECULAR MARKERS WITH MALE REPRODUCTIVE TRAITS AND MEAT QUALITY PROPERTIES IN BOER AND BOER CROSS GOATS

SAEID NIKBIN

FP 2013 6

ASSOCIATION OF CANDIDATE GENES AND MOLECULAR MARKERS WITH MALE REPRODUCTIVE TRAITS AND MEAT QUALITY PROPERTIES IN BOER AND BOER CROSS GOATS

By

SAEID NIKBIN

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

March 2013

DEDICATION

MY FATHER AND MOTHER

MY WIFE AND SON,

I Love You Forever

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

ASSOCIATION OF CANDIDATE GENES AND MOLECULAR MARKERS WITH MALE REPRODUCTIVE TRAITS AND MEAT QUALITY PROPERTIES IN BOER AND BOER CROSS GOATS

By

SAEID NIKBIN

March 2013

Chairman : Professor Jothi Malar Panandam, PhD

Faculty : Agriculture

Boer goats are popularly reared in Malaysia as pure breed or as crosses with the local goat populations. As such, identification of the factors influencing their meat production and reproduction is necessary in order to design optimal breeding and selection programs. Conventional selection methods are usually costly for traits which are expressed later in life and evaluated in adulthood, and may be inappropriate when animals need to be sacrificed for such evaluation to be carried out. Using candidate genes in the selection criteria for male reproduction traits may allow early selection thus decreasing the rearing cost, decrease generation interval and increase accuracy of selection. It is more important in the selection for meat quality traits as animals need not be slaughtered to confirm their genetic merit for these traits, thus making them available for breeding. This study aimed to identify

and elucidate the effects of functional candidate genes as well as non genetic factors on meat and semen quality traits in Boer goats and Boer crosses. This objective was accomplished through two independent studies.

In the first study, fresh and thawed 1-day and 6-month frozen semen from 36 Boer bucks and 17 Boer crosses were evaluated. The semen quality traits were analyzed for the effects of fixed factors and 17 candidate genes chosen based on their physiological or biological functions. The effects of age, population, cryopreservation period and some of their interactions on the semen quality traits were significant (P<0.05). Sperm motility and average velocity were 44.16 % and 96.35 µm/s, respectively, after cryopreservation for 1 day, and 37.61 % and 90.04 µm/s, respectively, after 6 months. The younger goats showed higher sperm motility (43.32 %) for fresh and (38.45 %) for post-thaw semen after six months than older bucks. Restriction fragment length polymorphism (PCR-RFLP) and single strand conformation polymorphism (SSCP) analysis and comparative sequencing revealed three single nucleotide polymorphisms (SNP) in exon 3 of follicle stimulating hormone beta (*FSHB),* 200A>G (*FSHB3-1*), 226T>C (*FSHB3-2*) and 237A>G (*FSHB3-3*); two SNPs in the coding region of heat shock protein 70 (*HSP70),* 73A>C (*HSP70-1*) and 190C>G (*HSP70-2*); one SNP in exon 2 of Luteinizing hormone beta (*LHB)* (207T>C) (*LHB2*); and one SNP in 5'-UTR of neuropeptide Y (*NPY)* (182G>T) gene. There was also an indel in position 29 of *NPY3.* Analyses of variance revealed significant association of the candidate genes with libido and semen quality traits. The three SNPs of *FSHB3* had significant effect on libido (P<0.05), progressive motility and abnormality of fresh semen ($P < 0.05$), and on motility, velocity and viability traits of post-thaw semen ($P < 0.05$). The SNPs of *HSP70* were associated with libido, semen volume (VOL), sperm concentration

(SCON), motility traits and sperm viability of fresh semen (P<0.05) and with motility and viability traits of post-thaw semen (P<0.05). The two SNPs of *NPY* gene influenced libido (P<0.05), progressive motility (PROG) of fresh semen (P<0.05), and motility traits, velocity traits, amplitude lateral sperm head (ALH), straightness (STR), linearity (LIN) and acrosome integrity (ACI) of post-thaw semen (P<0.05).

In the second study, carcass characteristics and meat quality traits of 30 Boer goats, which were either not transported or transported in low or high stocking density before slaughter, were evaluated. The effects of non genetic factors and 24 candidate genes chosen for their known physiological or biological functions were investigated. The effects of aging, transportation and muscle types and their interactions were significant on meat quality traits (P<0.05). The transported goats showed a significant (P<0.05) decrease in meat pH and tenderness at Day 0. Aging caused a drop of pH and increased meat tenderness. PCR-RFLP (restriction fragment length polymorphism) and sequencing analysis revealed one SNP in growth hormone (*GH)* gene, and two SNPs in the coding region of *HSP70* gene, 73A>C (*HSP70-1)* and 190C<G *(HSP70-2);* and two SNPs in the coding region of *HSP27b*, 119T>C *(HSP27b-1)* and *132C>G (HSP27b-2).* Analyses of variance showed significant association of *GH4-HaeIII* with carcass dressing percentage ($P < 0.05$); the two SNPs of *HSP70* were associated with pH, glycogen content, drip loss, cooking loss, redness (a*) and chroma (P<0.05); while the two SNPs of *HSP27b-1* were associated with calpastatin level and drip loss shear force (P<0.05).

The results of the present study shows there are candidate genes associated with semen quality as well as carcass and meat quality traits of Boer goats and Boer crosses. These genes may be targeted and used in marker assisted selection for the

respective traits to improve the production and reproduction performance of the animals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERHUBUNGAN GEN CALON DAN PENANDA MOLEKUL DENGAN TRAIT PEMBIAKAN JANTAN DAN CIRI KUALITI DAGING DALAM KAMBING BOER DAN KACUKAN BOER

Oleh

SAEID NIKBIN

Mac 2013

Pengerusi : Profesor Jothi Malar Panandam, PhD

Fakulti: Pertanian

Kambing Boer diternak secara popular di Malaysia sebagai baka tulen atau kacukan dengan populasi kambing tempatan. Oleh kerana itu, pengenaplastian faktor yang mempengaruhi pengeluaran daging dan reproduksi kambing Boer adalah perlu untuk merancang program pembiakbakaan dan pemilihan yang optima. Kaedah pemilihan konvensional biasanya menyebabkan kos yang lebih untuk trait yang dipernyatakan lambat dalam hidup dan dinilai pada yang dewasa, dan mungkin tidak bersesuaian apabila ternakan perlu dikorban untuk membuat pernilaian berkenaan. Menggunakan gen calon dalam kriteria pemilihan untuk trait pembiakan jantan boleh membenarkan pemilihan awal dan maka mengurangkan kos penternakan, mengurangkan selang generasi dan meningkatkan ketepatan pemilihan. Ia adalah lebih penting dalam pemilihan untuk trait kualiti daging oleh kerana ternakan tidak perlu disembelih untuk mengesahkan merit genetik mereka untuk trait tersebut, dengan itu membolehkan mereka digunakan untuk pembiakan. Kajian ini adalah bertujuan

untuk mengenal pasti dan menjelaskan kesan gen calon berfungsi serta faktor bukan genetik ke atas trait kualiti daging dan air mani bagi kambing Boer dan kacukan Boer. Objektif tersebut tercapai melalui dua kajian bebas.

Dalam kajian pertama, air mani segar dan yang dicairkan selepas beku 1-hari dan 6 bulan daripada 36 ekor kambing jantan Boer dan 17 ekor kacukan Boer telah dinilai. Trait kualiti air mani dianalisa untuk kesan faktor-faktor tetap dan 17 gen calon yang dipilih berdasarkan fungsi fisiologi atau biologi mereka. Kesan umur, populasi, tempoh krioawetan dan beberapa kesan interaksi ke atas ciri-ciri kualiti air mani adalah signifikan ($P<0.05$). Motiliti dan halaju min sperma adalah 44.16% dan 96.35 μm/s, masing-masing, selepas krioawetan untuk 1 hari, dan 37.61% dan 90.04 μm/s, masing-masing, selepas 6 bulan. Kambing muda menunjukkan motiliti sperma yang lebih tinggi dalam air mani segar (43.32%) dan yang dicairkan selepas enam bulan (38.45%) daripada jantan yang lebih tua. Analisis *restriction fragment length polymorphism* (PCR-RFLP) dan *single strand conformation polymorphism* (SSCP) dan penjujukan perbandingan mendedahkan tiga *single nucleotide polymorphisms* (SNP) di exon 3 gen *follicle stimulating hormone beta* (*FSHB*), 200A>G (FSHB3-1), 226T>C (*FSHB3-2*) dan 237A>G (*FSHB3-3*); dua SNP di rantau pengekodan gen *heat shock protein 70* (*HSP70*), 73A>C (HSP70-1) dan 190C>G (*HSP70-2*); satu SNP di exon 2 gen *luteinizing hormone beta* (*LHB*) (207T>C) (*LHB2*); dan satu SNP di *5'-UTR* gen *neuropeptide Y* (*NPY*) (182G>T). Terdapat juga *indel* dalam kedudukan 29 *NPY3*. Analisis varians menunjukkan hubungan yang signifikan antara gen calon dengan ciri-ciri libido dan kualiti air mani. Tiga SNP *FSHB3* mempunyai kesan ketara ke atas libido ($P <0.05$), motiliti progresif dan keabnormalan air mani segar ($P<0.05$), dan motiliti, halaju dan daya hidup ciri-ciri air mani terbeku pasca-cair ($P <0.05$). SNPs daripada HSP70 berhubungan dengan libido, isipadu air mani,

kepekatan sperma, trait motiliti dan daya hidup sperma air mani segar (P<0.05), dan dengan trait motiliti dan viability air mani pasca-cair (P<0.05). Dua SNP gen *NPY* mempengaruhi libido (P<0.05), motility progresif air mani segar (P<0.05), dan trait motiliti, trait halaju dan amplitud sisi kepala sperma air mani pasca-cair (P<0.05).

Dalam kajian kedua, ciri-ciri karkas dan trait kualiti daging 30 ekor kambing Boer, yang sama ada tidak diangkut atau diangkut dalam stok kepadatan rendah atau tinggi sebelum disembelih, telah dinilai. Kesan faktor bukan genetik dan 24 gen calon yang dipilih kerana fungsi fisiologi atau biologi mereka telah disiasat. Kesan *aging*, pengangkutan dan jenis otot dan interaksi antara mereka ke atas ciri-ciri kualiti daging adalah ketara (P<0.05). Kambing yang diangkut menunjukkan penurunan yang ketara (P<0.05) dalam pH daging dan kelembutan pada Hari 0. *Aging* menyebabkan penurunan pH dan peningkatan kelembutan daging. PCR-RFLP dan analisis penjujukan mendedahkan satu SNP dalam gen growth hormone (*GH*), dan dua SNP di rantau pengekodan gen *HSP70*, 73A>C (*HSP70-1*) dan 190C<G (*HSP70-2*); dan dua SNP di rantau pengekodan *HSP27b*, 119T>C (*HSP27b-1*) dan 132C>G (*HSP27b-2*). Analisis varians menunjukkan perhubungan yang ketara *GH4-HaeIII* dengan *carcass dressing percentage* (P<0.05); dua SNP *HSP70* dengan pH, kandungan glikogen, kehilangan titisan, kehilangan memasak, a* dan kroma (P<0.05); dan dua SNP *HSP27b-1* dengan tahap *calpastatin*, titisan kehilangan dan *shear force* (P<0.05).

Keputusan kajian ini menunjukkan terdapat gen calon yang dikaitkan dengan kualiti air mani serta ciri-ciri kualiti karkas dan daging kambing Boer dan kacukan Boer. Gen-gen tersebut boleh digunakan dalam pemilihan dibantu penanda untuk trait tertentu untuk meningkatkan pengeluaran dan prestasi pembiakan haiwan.

ACKNOWLEDGEMENTS

First, I must bow to Almighty Allah, the most gracious and beneficent, whose bounteous blessings enabled me to perceive life and provided me the opportunity to undertake my PhD study.

My honest appreciation to my supervisory committee members, who were involved in my training towards obtaining this degree. I am most grateful to Professor Dr. Jothi Malar Panandam, the chairperson for her patience, tireless support, willingness to help, encouragement, kindness and guidance throughout the research and during the preparation of the thesis. I am very much indebted to the other members of my supervisory committee, namely Associate Professor Dr. Halimatun Yaakub, Dr. Awis Qurni Sazili and Dr. Murugaiyah Marimuthu, for their encouragement, constructive discussions, excellent advices, comments and suggestions throughout the project.

I would like to extend my deepest and sincere appreciation to Universiti Putra Malaysia (UPM) which supported my candidature, and to the Ministry of Higher Education Malaysia for the research grant which supported the study. I also thank Prof. Dr. Zulkifli Idrus for allowing me to take samples from the animals he had purchased.

I wish to express my sincere gratitude to the management and staff of the Faculty of Agriculture, UPM, for the use of their facilities and the unlimited assistance during the course of this study. I would also like to thank staff members of the Genetics Laboratory, Meat Science Laboratory and Theriogenology Laboratory, in particular Miss. Kamariah Jamhari and Mr. Yap

Keng Chee, for their technical assistance. I would like to thank Mr. Virayah and Mr. Rajan for allowing me to carry out my study in their goat farms. Thanks are also due to the staff on the farms for their help and cooperation.

I will always cherish the friendship and help from Mr. Mehdi Ebrahimi and Dr. Hassan Moeini . Thanks is due to Mehdi Pasabani, Reza Tohidi, Alireza Majidi, Hamidah Binti Ali Kamarzaman, Yow Weng Kit, Hathyam Hago Abdelwahid, Abdoreza Solimani Farjam for all their friendship and help.

I would like to express my appreciation to my parents, who have been a constant source of unconditional support – emotional, moral and of course financial – throughout all my studies in University.

I would also like to thank my beloved wife, who has been, always, my pillar, my joy and my guiding light, and I thank her. She has strengthened me during the times when I thought I could go no further. Without her, none of this would have been possible. I also thank my son Sepehr who was a source of energy and hope for me through my PhD period.

I certify that a Thesis Examination Committee has met on 19 March 2013 to conduct the final examination of Saeid Nikbin on his Doctor of Philosophy thesis entitled "Association of Candidate Genes and Molecular Markers with Male Reproductive Traits and Meat Quality Properties in Boer and Boer Cross Goats" in accordance with 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 degree of Doctor of Philosophy (PhD).

Members of the Examination Committee are as follows:

Abd. Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia (Chairman)

Abd. Wahid b. Haron, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Ismail Idris, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Okeyo, A. Mwai, PhD
Professor
Department of Animal Production
University of Nairobi,
Kenya
(External Examiner)

SEOW HENG FONG, PHD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Jothi Malar Panandam, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Halimatun Yaakub, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Murugaiyah Marimuthu, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Awis Qurni Sazili, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby 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 other institutions.

SAEID NIKBIN

Date: 19 March 2013

TABLE OF CONTENTS