UNIVERSITI PUTRA MALAYSIA

MOLECULAR AND QUANTITATIVE ANALYSIS OF BEEF CATTLE BREEDS IN MALAYSIA

ALIREZA MAJIDI

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BY

ALIREZA MAJIDI

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

August 2014

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DEDICATION

MY FATHER AND MOTHER, MY WIFE AND MY CHILDREN

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

MOLECULAR AND QUANTITATIVE ANALYSIS OF BEEF CATTLE BREEDS IN MALAYSIA

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ALIREZA MAJIDI

August 2014

Chairman: Professor Jothi Malar Panandam, PhD.

Faculty: Agriculture

The Malaysian beef industry is an important component of the livestock sector, producing animal food protein for the population. Growth and carcass traits are economically important traits of beef cattle and are under the control of multiple genes. The estimation of genetic parameters is an integral part of livestock breeding to predict possible outcomes based on selection objectives, but this requires proper record keeping. Genetic markers in candidate genes for traits of interest in beef animals may be useful in marker-assisted selection. The objective of the present project was to evaluate some beef cattle breeds in Malaysia using molecular markers and to evaluate the Brakmas breed using quantitative genetics method.

In the first study, 20 single nucleotide polymorphism loci (SNP) DNA markers at candidate genes for growth and meat quality traits were investigated for seven cattle breeds, namely the Kedah Kelantan (KK), Brakmas, Brangus, Cheroke, Nellore, and two KK non descript crosses (KKC1 and KKC2). The candidate genes assessed were leptin (1,2,3), Diglyceride aciltransfrase (DGAT1), Calpastatin, Calpastatin promoter, Myostatin, Growth hormone (GH), Growth hormone receptor (GHR), Insulin-like growth factor binding protein (IGFBP1, IGFBP2), Growth hormone releasing hormone (GHRH), Beta-Lactoglobulin (LGB), Tyroglobulin (TG), Lipoprotein lipase (LPL), Heat shock protein70 (HSP70), Calpain (1,2), Growth hormone three (GH3) and Neurotensin. DNA extracted from blood samples of 50 animals of each breed (except for Brangus n=17) were analysed for the targeted loci using PCR-RFLP. Of the total of 20 loci, 15 were polymorphic and five monomorphic in all breeds. Significant differences were found between the KK, KK crosses and exotic breeds at *CAST*, with the KK and KK crosses showing a higher frequency of the favorable *A* allele, which has been reported to be positively correlated with meat tenderness. High polymorphism was observed in Brangus (75%), KK and Charoke (70%), Brakmas and KKC1 (60%), and Nellore (55%); the lowest was in KKC2 (45%). High number of fixed alleles were obtained for KKC2 (11 alleles), KKC1, Brakmas and Nellore (8 alleles), and KK and Charoke (6 alleles); Brangus has fixation of five alleles.

The highest total heterozygosity is exhibited by Brakmas (0.39±0.42) and lowest heterozygosity by KKC1 (0.20±0.28). The highest heterozygosity was observed for *CAST* in Brakmas and KK (0.88 and 0.86, respectively), CALP1 in Brakmas (0.60), *GHR* in Nellore (0.88), *IGFBP2* in KK and Charoke (0.92 and 0.90, respectively), *LEP1* in Charoke (0.94), *LEP2* in KKC2 (0.58), *LEP3* in Brakmas and KKC1 (0.70 and 0.62), *LGB* in KKC1 (0.82) and *TG* in KK (0.88).

High gene flow was found between Brakmas and KKC2 (Fst=0.26), while lowest gene flow was found between KKC1 and KKC2 (Fst=0.023). Despite this, the KKC1 was found to be very similar to KKC2 (genetic distances (GD)=0.01), and Charoke was similar to Brakmas (GD=0.06). Population structure analysis of the seven breeds inferred to five clusters, with KKC1 and KKC2 being pooled together and Brangus and Cheroke, both breeds with *B. taurus* genes, being pooled together.

The second study evaluated the Brakmas breed for birth weight (BW), 3-month weight (W3), weaning weight at 180 days of age (WW), and average daily gain from birth to 90 days of age (ADG3) and to 180 days (ADG6) using available and validated records from the year 2000 to 2009. Effect of season on all investigated traits was non-significant. The effect of sex was significant ($p<0.05$) for BW, W3, ADG3, ADG6, but there was no significant effect of sex on W6. Parity had a significant ($p<0.01$) effect only on BW. Year showed significant ($p<0.01$) effect on BW, W3 and ADG3. The year 2004 showed higher levels of BW and ADG in comparison of 2006 and 2007. The BW showed moderate heritability (0.30) that was higher than other traits (0.18-0.26).

In conclusion result of studies confirmed the existence of polymorphism at candidate genes for traits of interest in beef cattle breeds in Malaysia. This has potential for use in the improvement of the local cattle breeds. However, the effects of these SNPs should be validated by conducting association studies. The second study showed that the Brakmas cattle has potential for improvement. With proper record keeping and utilization of estimated genetic parameters and exploitation of SNPs at candidate genes, the performance of this beef breed may be improved.

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

ANALISIS MOLEKUL DAN KUANTITATIF BAKA LEMBU PEDAGING DI MALAYSIA

Oleh

ALIREZA MAJIDI

Ogos 2014

Pengerusi: Profesor Jothi Malar Panandam, PhD.

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Industri daging lembu Malaysia merupakan komponen penting dalam sektor ternakan, menghasilkan makanan protein haiwan untuk penduduk. Ciri-ciri pertumbuhan dan karkas adalah ciri-ciri penting dari segi ekonomi lembu daging lembu dan adalah di bawah kawalan gen berbilang. Anggaran parameter genetik adalah merupakan sebahagian penting pembiakbakaan ternakan untuk meramalkan hasil yang mungkin berdasarkan objektif pemilihan, tetapi ini memerlukan penyimpanan rekod yang betul. Penanda genetik dalam gen calon untuk ciri-ciri yang menarik pada lembu pedaging mungkin berguna dalam pemilihan dibantu penanda. Objektif projek ini adalah untuk menilai beberapa baka lembu pedaging di Malaysia dengan menggunakan penanda molekul dan untuk menilai baka Brakmas dengan menggunakan kaedah genetik kuantitatif.

Dalam kajian pertama, 20 penanda lokus polimorfik nukleotid tunggal (SNP) DNA pada gen calon untuk sifat pertumbuhan dan kualiti daging telah disiasat bagi tujuh baka lembu, iaitu Kelantan Kedah (KK), Brakmas , Brangus , Cheroke , Nellore , dan dua kacuakn *non descript* KK (KKC1 dan KKC2). Gen calon yang dinilai adalah leptin (1,2,3), Diglyceride aciltransfrase (DGAT1), Calpastatin, Calpastatin promoter, Myostatin, Growth hormone (GH) , Growth hormone receptor (GHR), Insulin-like growth factor binding protein (IGFBP1, IGFBP2), Growth hormone releasing hormone (GHRH), Beta-lactoglobulin (LGB), Tyroglobulin (TG), Lipoprotein lipase (LPL) , Heat shock protein 70 (HSP70), Calpain (1,2), Growth hormone 3 (GH3) dan Neurotensin. DNA yang diekstrak daripada 50 sampel darah setiap ekor baka (kecuali untuk Brangus n=17) telah dianalisis bagi lokus yang disasarkan dengan menggunakan PCR- RFLP. Daripada jumlah 20 lokus, 15 adalah polimorfik dan lima monomorfik dalam semua baka. Perbezaan yang signifikan didapati antara KK , kacukan KK dan baka eksotik dalam CAST, dengan KK dan kacukan KK mempamerkan kekerapan lebih tinggi alel A yang digemari, yang telah dilaporkan mempunyai korelasi positif dengan kelembutan daging. Polymorphism tinggi diperhatikan dalam Brangus (75%), KK dan Charoke (70%), Brakmas dan KKC1 (60%), dan Nellore (55%); yang terendah adalah pada KKC2 (45%). Bilangan tinggi alel tetap diperolehi bagi KKC2 (11 alel), KKC1, Brakmas dan Nellore (8 alel), dan KK dan Charoke (6 alel); Brangus mempunyai penetapan lima alel.

Jumlah heterozigositi tertinggi dipamerkan oleh Brakmas (0.39±0.42) dan heterozigositi terendah oleh KKC1 (0.20±0.28). Heterozigositi tertinggi diperoleh untuk CAST di Brakmas dan KK (0.88 dan 0.86, masing-masing), CALP1 dalam Brakmas (0.60), GHR di Nellore (0.88), IGFBP2 di KK dan Charoke (0.92 dan 0.90, masing-masing), LEP1 dalam Charoke (0.94), LEP2 dalam KKC2 (0.58), LEP3 dalam Brakmas dan KKC1 (0.70 dan 0.62), LGB di KKC1 (0.82) dan TG di KK (0.88). Aliran gen yang tinggi didapati di antara Brakmas dan KKC2 (F_{ST}=0.26), sementara aliran gen terendah didapati antara KKC1 dan KKC2 (F_{ST}=0,023). Walaupun begitu, KKC1 didapati hampir sama dengan KKC2 (jarak genetik (GD) = 0.01), dan Charoke adalah serupa dengan Brakmas (GD = 0.06). Analisis struktur tujuh baka disimpulkan kepada lima kelompok, dengan KKC1 dan KKC2 dikumpulkan bersama dan Brangus dan Cheroke, kedua-dua baka dengan gen *B. taurus*, dikumpulkan bersama.

Kajian kedua menilai baka Brakmas untuk berat lahir (BW), berat badan 3-bulan (W3), berat badan semasa disapih pada umur 180 hari (WW), dan purata penambahan harian dari lahir hingga umur 90 hari (ADG3) dan hingga umur 180 hari (ADG6) dengan menggunakan rekod yang ada dan sahih dari tahun 2000 hingga 2009. Kesan musim pada semua ciri yang dikaji adalah tidak penting. Kesan seks adalah signifikan ($p<0.05$) untuk BW, W3, ADG3, ADG6, tetapi seks tidak ada kesan yang ketara ke atas W6. Pariti mempunyai kesan ketara ($p<0.01$) hanya ke atas BW. Tahun menunjukkan kesan yang signifikan ($p<0.01$) ke atas BW, W3 dan ADG3. Tahun 2004 menunjukkan tahap yang lebih tinggi bagi BW dan ADG berbanding dengan tahun 2006 dan 2007. BW menunjukkan heritabiliti yang tinggi (0.30) dan sifat-sifat yang lain menunjukkan heritabiliti yang sederhana (0.18-0.26).

Sebagai kesimpulan, keputusan kajian mengesahkan kewujudan polimorfisma pada gen calon untuk ciri-ciri kepentingan dalam baka lembu pedaging di Malaysia. Ini mempunyai potensi untuk digunakan dalam peningkatan baka lembu tempatan. Walau bagaimanapun, kesan SNP ini perlu disahkan dengan menjalankan kajian berkaitan. Kajian kedua menunjukkan bahawa lembu Brakmas berpotensi untuk penambahbaikan. Dengan penyimpanan rekod yang betul dan penggunaan anggaran parameter genetik dan eksploitasi SNP di gen calon, prestasi baka lembu pedaging ini boleh dipertingkatkan.

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I certify that a Thesis Examination Committee has met on 21 August 2014 to conduct the final examination of Alireza Majidi on his thesis entitled "Molecular and Quantitative Analysis of Beef Cattle Breeds in Malaysia" 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

A	Adenine
µl	Microliter
µM	Micromole
°C	Degree Celsius
1X	Once
ADG	Average daily gain
BLUP	Best linear unbiased prediction
bp	Base pair
BW	Birth weight
C	Cytosine
DF-REML	Derivative-free restricted maximum likelihood
DNA	Deoxyribonucleic acid
DGAT1	Diacylglycerol Acyltransferase
dNTP	Dinucleotide triphosphate
DVS	Department of veterinary services
EBV	Estimated breeding value
EDTA	Ethylenediaminetera acetic acid
EtBr	Ethidium bromide
FAO	Food and Agriculture Organization
GD	Genetic distance
Ho	Observed heterozygosity
h^2	Heritability
He	Expected heterozygosity
HWE	Hardy-Weinberg equilibrium
HWT	Hardy-Weinberg Equilibrium Test
KKC1	Kedah Kelantan cross 1
KKC2	Kedah Kelantan cross 2
LEP	Leptin
MARDI	Malaysian Agriculture Research and Development Institute
$MgCl_2$	Magnesium chloride
ml	Millilitre
mRNA	Messenger ribonucleic acid
Na	Observed number of alleles
Ne	Effective number of alleles
ng	Nanogram
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
rpm	Revolution per minute
SNP	Single nucleotide polymorphism
Tm	Melting temperature
U	Unit
UPGMA	Un-weighted pair group method with arithmetic mean
UPM	Universiti Putra Malaysia
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

The agriculture sector in Malaysia plays significant roles in the country's economic development. Livestock farming is an important part of agriculture in Malaysia providing many products and protein sources. Livestock contributes to 18 percent of the overall food requirement for the country (Loh, 2004). Beef consumption is higher compared to other red meat such as mutton. Although the self-sufficiency level for beef shows an upward trend from 16.06% in 2001 to 29.02% in 20012 (DVS, 2012), the country is dependent on imported beef and live animal for slaughter. The objective of the Malaysian livestock policy is to encourage local production of meat to reduce dependence on imports and save and foreign exchange. The sources of beef in the country are exotic breeds and the indigenous breed. The indigenous cattle breed, the Kedah-Kelantan has an estimated population of 593,299 heads and constitute about 85% of the total beef cattle population in Peninsula Malaysia (DVS, 2006). Exotic cattle breeds in the country are Brahman, Brangus, Nellore, Droughtmaster, Bali cattle and Chinese Yellow cattle. Except for the Brahman and Nellore cattle most of the imported exotic breeds have contributed very little to the development of the beef industry. The poor reproductive performance and the low survival rates are the two main factors for the failure of the exotic breeds in Malaysia (Johari & Jasmi, 2009).

The indigenous Kedah Kelantan (KK) and the imported cattle breeds have been subjected to fast genetic degradation and dilution because of unplanned breeding and crossbreeding. There is a lack of genetic evaluation and improvement of the breeds. Crossbreeding, industrial livestock farming and animal reproduction technologies threaten to rapidly deplete the genetic diversity. The Kedah Kelantan breed is well adopted to survive the local climatic conditions. The crossbred cattle are unable to adapt to the tropical climate and are more prone to many diseases. (FAO/IAEA, 2009). The KK is mainly kept for meat production and is popular among the smaller farm owners because of its small compact body and low maintenance requirement (Payne and Hodges, 1997). However the larger farm owners prefer the larger KK crossbreds or the imported exotic breeds. Genetic improvement of the KK breed has been based on controlled crossbreeding strategies adopted by the government of Malaysia through the Department of Veterinary Services Malaysia (DVS) and Malaysia Agriculture Research and Development Institute (MARDI). The KK has been crossed with Brahman to produce Brakmas, a synthetic cattle breed by MARDI in 1999 as part of its beef cattle research. Brakmas are easy to manage with minimum health problems; these characteristics making the breed suitable for beef production under oil palm plantations (Johari and Jasmi, 2009). Genetic characteristics and performances evaluation of the breeds are lacking. Conventional breeding program in cattle is based on phenotype performance of breeds and requires proper data recording program. Furthermore, traditional selection has been shown to result in slow genetic progress, especially for species with long generation intervals, such as the cattle. In addition, recording of carcass and growth traits may be laborious and costly. DNA marker technology is good tool to complement conventional breeding program. Current technologies enable the use of genetic markers to improve the accuracy and efficiency of selection program through marker assisted selection (MAS), which combines information on molecular genetic polymorphisms (marker loci) with data on phenotypic variation among individuals (and their relatives). Cattle breeds have to be studied in order to identify the genetic differences among the breeds. Establishing estimates of the genetic variability within breeds allows breeders and farmers determine how suitable are their cattle for a variety of breeding goals.

1.1 Research problem

In beef cattle production, growth, meat quality and carcass traits are important. SNPs at candidate genes for production traits and meat quality in beef cattle have been identified. It is therefore useful to evaluate the genetic variability between and within breeds for these SNPs. The polymorphism detected may be analyzed for association with the phenotypic traits, so that informative markers for use in selection programs may be identified.

1.2 Objectives

The general objective of this study was to evaluate some beef cattle breeds in Malaysia using quantitative genetics and biotechnology methods so that appropriate strategies using phenotypic data and molecular markers may be adopted for their futher development.

The specific objectives of this study were:

- To evaluate the polymorphism at 20 candidate genes for growth and meat quality in seven beef cattle breeds in Malaysia.
- To estimate the level of genetic diversity and differentiation within and between these breeds.
- To estimate non genetic factors influencing growth in the Brakmas cattle;
- To estimate some genetic parameters for the Brakmas cattle.

1.3 Hypothesis

Since the Brakmas cattle is from a research station, therefore, the animals should be subjected to proper breeding design. Therefore, it is hypothesized that the quantitative genetics study would show that this bree4d’s performance would be better than that report previously in literature.

As for the molecular genetics study, it is hypothesized that the KK and its crosses would be more genetically similarity compared to the other breeds. Since the herds in Malaysia are not very large, we expect to find low genetic diversity and even inbreeding within the breeds; but there wouold be higher genetic diversity among breeds.

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