

**ASSOCIATION BETWEEN SINGLE NUCLEOTIDE
POLYMORPHISM OF CALPAIN1 GENE WITH CHICKEN
MEAT TENDERNESS IN MALAYSIAN NATIVE AND
COMMERCIAL BROILER LINE**

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AUTHOR DECLARATION

In the Name of Allah, The Most Compassionate, The Most Merciful

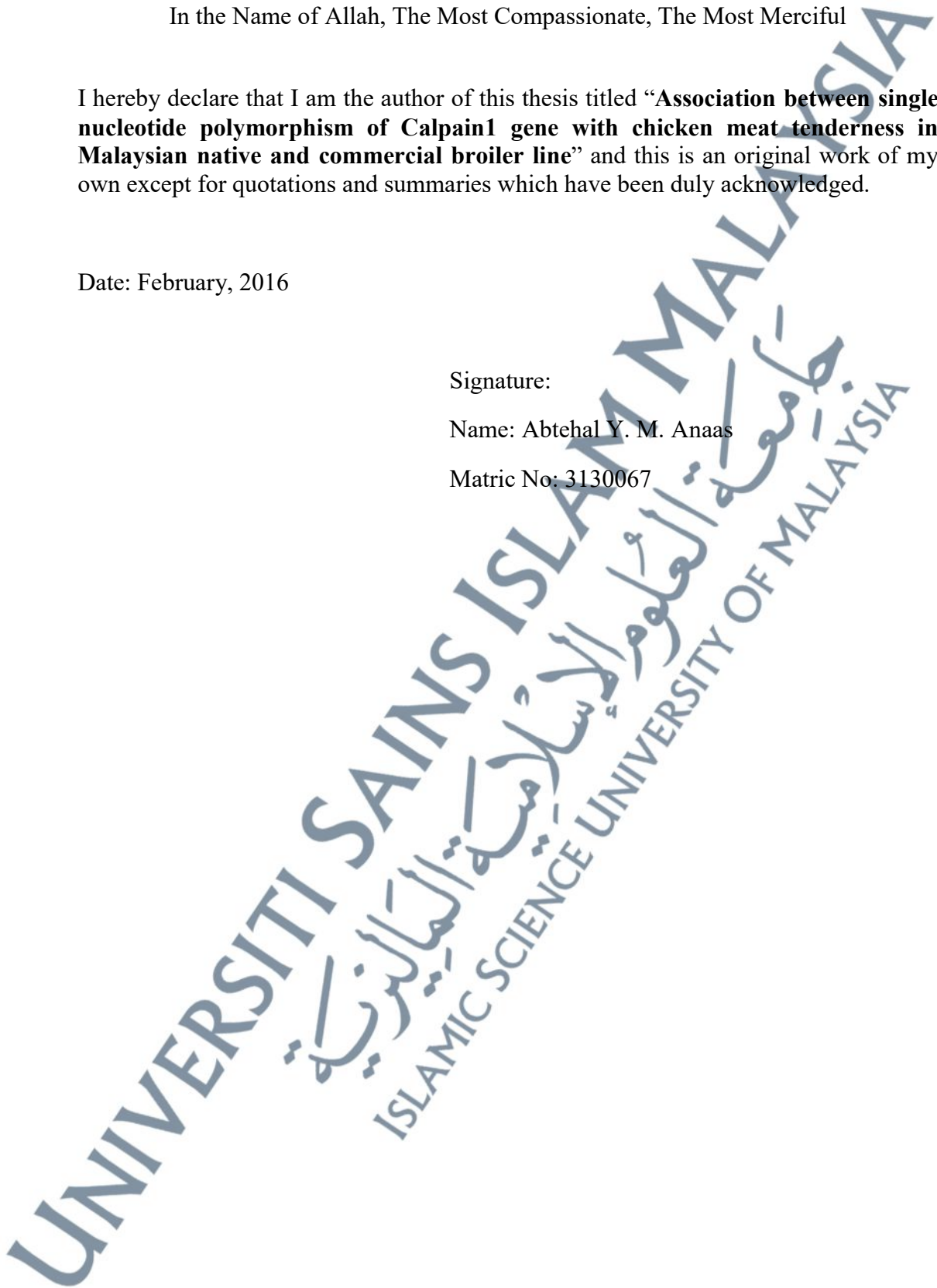
I hereby declare that I am the author of this thesis titled “**Association between single nucleotide polymorphism of Calpain1 gene with chicken meat tenderness in Malaysian native and commercial broiler line**” and this is an original work of my own except for quotations and summaries which have been duly acknowledged.

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ABSTRAK

Kelembutan daging adalah salah satu faktor yang paling penting yang mempengaruhi penilaian pengguna terhadap kualiti daging. Perubahan dalam kelembutan daging dikawal secara genetik dan berbeza antara baka, dan ia juga dipengaruhi oleh faktor persekitaran yang boleh menjejaskan penciptaannya semasa 'rigor mortis' dan semasa 'postmortem'. Pada fasa akhir 'postmortem', kelembutan daging bergantung kepada sejauh mana proteolisis protein myofibrillar disebabkan oleh aktiviti dalaman sistem proteolitik calpain. Sistem calpain merangkumi pelbagai cystein protease yang bergantung kepada kalsium dan juga perencat calpastatin. Ia diterima secara meluas bahawa dalam haiwan ternakan termasuk ayam, gen μ -calpain (CAPN1) adalah gen calon fisiologi yang mempengaruhi kelembutan daging. Tujuan kajian ini adalah untuk mengenal pasti hubungan antara penanda nukleotida polymorfisma tunggal (SNP) CAPN1 dengan kelembutan pada daging dada ayam kampung dan juga ayam jenis belaan untuk tujuan komersil. Ayam kampung berusia sepuluh dan lima bulan serta ayam belaan komersil berusia sepuluh dan 42 hari dikumpulkan dari pasaran dan tisu otot pada bahagian dada telah dikeluarkan dua jam selepas disembelih, diletakkan dalam beg plastik berasingan dan disimpan pada suhu -20°C selama 24 jam. Fenotip kelembutan untuk dada semua ayam ditentukan oleh daya ricih Warner-Bratzler. Kapasiti pegangan air (WHC) diukur bersama kadar pencairan dan kadar kehilangan memasak (kaedah mendidih). Tindak balas rantaian polimerase (PCR) telah digunakan untuk mengenal pasti SNP CAPN1 G3535A, C7198A dan G9950A yang dilaporkan sebelum ini, dan menilai hubungan mereka dengan sifat-sifat kualiti daging pada kedua-dua baka. Dada ayam komersil menunjukkan nilai daya ricih dan kadar kehilangan pencairan lebih rendah daripada ayam kampung ($p < 0.05$), manakala kadar kehilangan memasak adalah sama bagi keduanya. Keputusan mengesahkan beberapa keputusan sebelum ini bahawa penanda CAPN1 C7198A dan G9950A didapati tidak mempunyai perkaitan dengan sifat-sifat kelembutan daging antara baka ayam. Walau bagaimanapun, keputusan ini tidak bersetuju dengan hubungan yang signifikan yang dilaporkan daripada polymorfisma penanda G3535A dengan sifat-sifat kualiti daging dan kesan perbezaan genotip antara baka. Oleh itu, kajian lanjut diperlukan untuk mengesahkan fungsi mekanisme molekular SNP ini dan menilai perkaitan mereka dalam populasi ayam yang berbeza.

Kata kunci: ayam, calpain (CAPN1), SNP, tindak balas rantai polimerase, kelembutan daging.

ABSTRACT

Meat tenderness is one of the most important factors affecting consumers' assessment of meat quality. Variation in meat tenderness among chickens is genetically controlled and it is also influenced by environmental factors. There has been an increased interest in genetic polymorphisms associated with the variation in meat tenderness among chicken breeds. Understanding and identifying the polymorphisms of candidate genes will facilitate selection programs for genetic improvement in tenderness. Calpain1 (CAPN1), a gene encoding the enzyme μ -calpain which degrades myofibrillar proteins post-mortem, is a physiological candidate gene for meat tenderness. In Malaysia there are two commercially available chicken lines, native and commercial broilers. Native breeds are known to have lower meat tenderness compared to the broilers. The aim of this study is to investigate the reported association between the single nucleotide polymorphisms (SNPs) G3535A, C7198A and G9950A markers in the CAPN1 gene with the tenderness of chicken breast meat from the two Malaysian breeds. Ten, five months old native chickens and ten, 42 days old broilers were collected from the local market. The tenderness for all chickens' breast meats were determined by Warner-Bratzler shear force. Thawing and cooking losses were also measured. Polymerase chain reaction (PCR) was used to amplify the previously identified regions located within the CAPN1 gene. The broiler samples showed significantly ($P < 0.05$) smaller shear force values and lower thawing loss rates than the native chicken samples, whereas they were similar in the rates of cooking loss. Polymorphisms were identified for all markers. There were no significant ($p > 0.05$) differences in the allelic frequencies of the three loci SNPs between the two chicken populations. For SNP G3535A the allele G was less frequent than the allele A. In the loci SNP G9950A the allele G was dominant in both populations where the frequency of allele A was zero in broilers and was much less frequent in the native population. For the SNP C7198A, the allele C was more polymorphic than the allele A in the native population. Thus, the markers C7198A, G3535A and G9950A in the CAPN1 gene were not significantly associated with variation in meat tenderness between the studied populations. Therefore, further study is needed to confirm the functional molecular mechanism of these SNPs and evaluate their associations in different Malaysian chicken breeds.

Keywords: chicken, calpain (CAPN1), SNPs, polymerase chain reaction, meat tenderness.

الملخص

طراوة اللحوم تعتبر واحدة من أهم العوامل التي تؤثر على تقييم المستهلكين لجودة اللحوم، الاختلاف في طراوة اللحوم يتم التحكم بها وراثيا وهي تختلف بين السلالات، كما أنها تتأثر أيضا بالعوامل البيئية التي يمكن أن تؤثر على إنشائها عند تكون اللحم بعد الوفاة. تعتمد الطراوة النهائية للحوم على مدى التحلل البروتيني الناتج عن النشاط الذاتي لنظام كالبين. يشمل نظام كالبين على محفزات تحلل بروتيني مختلفة والتي يعتمد نشاطها على وجود أيون الكالسيوم، وكذلك على المانع البروتيني "كالبستاتين". من المعروف أن الجين كالبين واحد هو الجين المرشح لفسولوجية طراوة اللحوم. تهدف هذه الدراسة إلى التعرف على علامات رابطة تعدد الأشكال النوكليوتيدات في جينات كالبين واحد وربطها مع طراوة اللحم لنوعين مختلفين من الدجاج وهما دجاج الفراريج والدجاج البلدي الماليزي. تم جمع عشرة دجاجات من كل نوع من هاذين النوعين وتم استئصال لحوم الصدر من كل منهما بعد ساعتين الذبح. عينات لحم الصدر تم الاحتفاظ بها في أكياس بلاستيكية في درجة حرارة ٢٠ تحت الصفر لمدة ٢٤ ساعة. تم تحديد خصائص طراوة لهذه العينات باستخدام جهاز فحص القوة القص. كما تم قياس القدرة على الاحتفاظ بالماء وأيضا خسائر الذوبان أثناء الطبخ (طريقة الغليان). وقد استخدم تفاعل البلمرة المتسلسل (PCR) لتحديد تعدد الأشكال الجينية G3535A، C7198A و G9950A المذكورة سابقاً في الجين كالبين واحد وكذلك تم تقييم مدى ارتباط تعدد الأشكال الجينية مع تغير في جودة اللحوم لكل السلالتين. أظهرت النتائج أن لحوم الفروج أكثر طراوة وأقل من الدجاج البلدي في معدلات فقد الماء، في حين كانتا متماثلتين في معدلات فقدان الطهي. هذه الدراسة تؤكد بعض النتائج السابقة في أن علامات CAPN1 C7198A و G9950A لم ترتبط بشكل كبير مع الاختلافات في طراوة اللحوم في هذين النوعين من الدجاج. ولكن هذه الدراسة اختلفت عن الدراسات السابقة في أن هناك رابطة هامة بين تعدد الأشكال للعلامة G3535A وتغير صفات اللحوم بين سلالات الدجاج. لذلك، هناك حاجة إلى مزيد من الدراسات لتأكيد الآلية الجزيئية الوظيفية لهذه الأشكال المتعددة وتقييم مدى ارتباطها بتغير صفات لحوم الدجاج بمختلف أنواعها.

الكلمات المفتاحية: الدجاج، كالبين واحد، الأشكال الجينية، تفاعل البلمرة المتسلسل، طراوة اللحوم.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	analysis of variance
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Bp	base pair
Ca ⁺²	calcium ion
CAPN1	micro-molar calcium activated neutral protease
cDNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DPI	Department of Environment and Primary Industries
kb	kilobase
m- calpain	milli-calpain
mg	milligram
MgCl ₂	magnesium chloride
ml	millilitre
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NCBI	National Centre Biotechnology Institute
nm	nanometer
PCR	Polymerase Chain Reaction
QTL	quantitative trait locus
RE	restriction enzyme
RNA	ribonucleic acid
rpm	rotation per minute
SNP	single nucleotide polymorphism
TAE	tris acetate EDTA
WBSF	Warner-Bratzler shear force
μ-calpain	micço-calpain
μl	microliter