



STUDY ON PROTEIN PROFILING OF
SLAUGHTERED AND STUNNED CHICKEN IN
PREPARATION FOR RAPID TEST KIT
DEVELOPMENT

BY

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for the degree of Master of Science
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ABSTRACT

Stunning is a process where chickens are paralyzed by mild electrical treatment prior to exsanguinations. Such treatment is economic wise and efficient especially in mass production of slaughtered chicken. However, the stunning process needs to be monitored in order to confirm that the chickens are still alive when slaughtering takes place. In Malaysia, The National Fatwa Committee has decided that water bath stunning is allowed in poultry slaughtering provided that the current used must be in the range of 0.25 A to 0.5 A. In this study, the relationship between stunning parameters and protein expression was analyzed by Design Expert Software Version 6.0.8. The stunning parameters studied are current (0.25 A, 0.5 A, 0.75 A) and voltage (10 V, 40 V, 70 V). A simple and reliable method using proteomics approach was employed. Two-dimensional (2D) protein profiles were analyzed by ImageMaster 2D Platinum 6.0 software to identify potential biomarkers in differentiating the over stunned chicken from the standard stunned and religious slaughtered chicken. This study is in line with the understanding on the nature of proteins which are dynamic and responsive to both internal and external stimuli. Thus, electrical treatment of stunning is considered as one type of external stimuli to the chicken, thereby, expecting it to cause changes in the level of protein expression. It was observed that the protein intensity is increasing as the current and voltage increased. The potential biomarkers discovered through 2D gel electrophoresis were identified by MALDI-TOF mass spectrometer and further verified at the transcriptional level by Real Time PCR. The potential biomarkers identified in this study are Troponin I, Actin Alpha Cardiac Muscle 1, Muscle Creatine Kinase (M-CK), Aldolase A, Voltage-dependent Anion Channel 2, Actin Related Protein 2 Homolog (ARP2), Aldolase C and Enolase 1. Three of these proteins were only detected in over stunning treatment of 0.75 A, 70 V which are Aldolase A, ARP2 and Enolase 1. The rest of the proteins could be detected at current of 0.5 A and some even at 0.25 A, but they are completely absent in 0 A, 0 V non stunned chickens. These proteins could be developed as biomarkers after optimization of antigen-antibody specific reaction by determining a specific threshold for the over stunned expression. Upregulation of genes for the protein biomarkers confirmed that electrical treatment in stunning alters the expression and protein constituents of the chickens. The highest gene upregulation was detected in ARP2 with 1086 while the lowest is M-CK with a fold change of 4.7. These identified proteins are highly beneficial for development of detection kit for halal slaughtered chicken. Then, over stunned chicken will be identified even if they are already in market.

خلاصة البحث

الصعق هو عملية يتم شل الدجاج فيها بواسطة معالج كهربائي خفيف قبل استنزاف الدم. مثل هذه المعاملة هي اقتصادية وفعالة وخاصة في انتاج كميات كبيرة من الدجاج المذبوح. غير أن عملية الصعق يجب أن يتم رصدها من أجل التأكد من أن الدجاج لا تزال على قيد الحياة عند وقت الذبح. في ماليزيا، قررت اللجنة الوطنية للإفتاء أن يسمح استخدام حمام ماء لصعق الدواجن الذبح شريطة أن يكون المحول التيار المستخدمة في نطاق أمبير 0.25 الى 0.5 أمبير. في هذه الدراسة، تم تحليل العلاقة بين معايير الصعق وبروتين تعبير بواسطة البرمجيات Design Expert النسخة 6.0.8. درست هنا معايير الصعق وهي التيار (0.25، 0.5، 0.75 أمبير) الجهد الكهربائي (10، 40، 70 فولت). وهناك طريقة بسيطة وموثق بها باستخدام نهج البروتيوميكا تم استخدامها. وقد تم تحليل ملامح بروتين ثنائية الأبعاد بواسطة البرمجيات ImageMaster 2D platinum 6،0 لتحديد العلامات البيولوجية المحتملة في التمييز بين الدجاج فوق المصعوق من الدجاج مستوى الصعق و الذبح الديني. هذه الدراسة وفقا للتفاهم حول طبيعة البروتينات التي هي دينامية ومستجابة لمؤثرات كل من الداخلية والخارجية. وبالتالي، يعتبر معالجة الكهربائي للصعق هو أحد أنواع المحفزات الخارجية للدجاج، وبالتالي، متوقعا أن يسبب تغيرات في مستوى بروتين تعبير. لوحظ كثافة البروتين في تزايد عند زيادة التيار و الجهد الكهربائي. العلامات الحيوية المحتملة اكتشفت من خلال ثنائية الأبعاد الكهربائي الهلامي وقد تم تحديد بواسطة مطياف الكتلة MALDI-TOF والتحقق منها بصورة أكبر على مستوى الترانسكريبت بواسطة Real Time PCR. المؤشرات الحيوية المحتملة التي تم تحديدها في هذه الدراسة هي Troponin I ، Actin ، Aldolase A، Muscle Creatine Kinase (M-CK) ، Alpha Cardiac Muscle 1 Voltage-dependent Anion Channel 2، Actin Related Protein 2 (ARP2)، Enolase 1 و Aldolase C. تم الكشف عن ثلاثة فقط من هذه البروتينات فيما يزيد علاج الصعق في 0.75 أمبير و 70 فولت والتي هي Aldolase A و ARP2، و Enolase 1. ويمكن الكشف عن ما تبقى من البروتينات في التيار البالغ 0.5 أمبير وبعض حتى في 0.25 أمبير، لكنها غائبة تماما في 0 أمبير، 0 فولت في الدجاج الغير مصعوق. ويمكن تطوير هذه البروتينات في المؤشرات الحيوية بعد العملية التحسين تفاعل ضدي مستضدي معين من خلال تحديد الحد أقصى محددة للتعبني عن أكثر من مصعوق. التنظيم الأعلى للجينات لعلامات البيولوجية من البروتين أكدت أن العلاج الصعق الكهربائي بغير مكونات التعبير والبروتين في الدجاج. تم الكشف عن أعلى الجينات في ARP2 مع 1086 بينما كانت أقل في M-CK مع وجود تغيير أضعاف من 4.7. هذه البروتينات التي تم تحديدها هي مفيدة للغاية لتطوير جهاز لكشف الدجاج المذبوحة حلالا أو غير ذلك. بعد ذلك، الدجاج فوق المصعوق يتم تحديدها حتى لو كانت موجودة بالفعل في السوق.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology Engineering)

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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*In memory of Mohd Aminuddin bin Mohd Rosli,
who had returned to the Eternal Life on March 4, 2009,
May Allah bestow His Mercy, His Forgiveness, and His Gardens.
Al Fatihah*

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