USE OF MOLECULAR GENETICS TECHNIQUE FOR DIFFERENTIATION OF MEAT SPECIES AND DETECTION OF MEAT ADULTERATION

BY

MOHAMMED ALI MOHAMMED ABDELFATTAH
B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003
M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY
in

Agriculture Sciences
(Animal Breeding)

Department of Animal Production
Faculty of Agriculture
Ain Shams University

2016

Approval Sheet

USE OF MOLECULAR GENETICS TECHNIQUE FOR DIFFERENTIATION OF MEAT SPECIES AND DETECTION OF MEAT ADULTERATION

BY

MOHAMMED ALI MOHAMMED ABDELFATTAH

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003
M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

This thesis for Ph. D. degree has been approved by:

Dr. Samy Abou-bakr Mahmoud ……..……

Prof. of Animal Breeding, Faculty of Agriculture, Cairo University

Dr. Ahmed Ragheb Shemeis …...…….

Prof. of Animal Breeding, Faculty of Agriculture, Ain Shams University

Dr. Mohammed Abdelsalam Rashed ……...

Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Omar Yousry Abdallah ……..

Prof. Emeritus of Animal Breeding, Faculty of Agriculture, Ain Shams University

Date of Examination: 17 / 7 / 2016

USE OF MOLECULAR GENETICS TECHNIQUE FOR DIFFERENTIATION OF MEAT SPECIES AND DETECTION OF MEAT ADULTERATION

BY

MOHAMMED ALI MOHAMMED ABDELFATTAH
B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2003
M. Sc. Agric. Sc. (Animal Breeding), Ain Shams University, 2010

Under the supervision of:

Dr. Omar Yousry Abdallah
Prof. Emeritus of Animal Breeding, Department of Animal Production, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Mohammed Abdelsalam Rashed
Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

ABSTRACT

Mohammed Ali Mohammed Abdelfattah. The Use of Molecular Genetics Technique for Differentiation of Meat Species and Detection of Meat Adulteration. Unpublished Doctor of Philosophy Dissertation, University of Ain Shams, Faculty of Agriculture, Department of Animal Production, 2016.

This work described a simplex and a multiplex polymerase chain reaction (PCR) assays for the accurate identification of two meat kinds forbidden in Islamic foods (pig and donkey meats) and five meat kinds commonly marketed in Egypt (goat, sheep, cattle, camel and buffalo meats). Meat samples from the seven investigated species were used for molecular analysis of each species as per standard method. *Cytochrome-b* gene was amplified by PCR using a common forward oligonucleotide primer. By mixing species specific reverse oligonucleotide primers in the appropriate ratio, DNA-fragments could be identified by only one multiplex PCR. PCR products were resolved by agarose gel electrophoresis and characteristic band pattern was observed for each species. The PCR products showed amplicons of 290, 370, 480, 580, 700, 800, 1000 bp from goat, sheep, pig, cattle, camel, buffalo and donkey meats, respectively. Simplex PCR assay was applied to detect adulteration in luncheon, burger and hotdog manufactured by three reputed companies. Following genomic DNA extraction from raw products which were claimed to be made of beef, PCR was performed and detected buffalo (*Bubalus bubalis*) meat as an adulterant. The oligonucleotide primers amplified mitochondrial DNA sequences and revealed specific 580 and 800 bp for cattle and buffalo, respectively. The sequel of this study suggests that the method of detection used can be applied by quality control laboratories and inspection services to determine adulteration of different kinds of meats and meat products. Linear and nonlinear types of regression were developed to

construct a curve that has the best fit to a series of data points of Y= DNA percentage versus X= PCR amplicon concentration (ng/µl). With coefficients of determination ranging between 0.78 and 0.96, the linear model appeared the most statistically appropriate to be used for estimating DNA percentage for all the seven investigated species, since only for the linear model the regression coefficient was significantly different from zero ($P<0.05$) for all the investigated species.

Keywords: Meat adulteration, Meat origin species, Species identification, *Cyt-b* gene, Simplex and multiplex PCR assays.

ACKNOWLEDGEMENT

I dedicate this work to the spirit of the deceased Dr. Karima A. Shahin, Professor of Animal Breeding, Faculty of Agriculture, Ain Shams University, who inspired me to work on this subject under her supervision.

I would like to express my sincere gratitude to my advisor Dr. Omar Y. Abdallah, Professor of Animal Breeding, Department of Animal Production, Faculty of Agriculture, Ain Shams University for the continuous support of my Ph.D study and related research, for his patience, motivation and immense knowledge. His guidance helped me in all the time of research and writing of the thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. My sincere gratitude also goes to my co-advisor Dr. Mohammed A. Rashed, Professor of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University who provided me an opportunity to join his team, and also gave me access to his laboratory and research facilities. Without his precious support it would not be possible to conduct this research.

Dr. Mahmoud Magdy, Lecturer of genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University has been always there to listen and give advice. I am deeply grateful to him for the long discussions that helped me sort out the technical details of my work. My deepest gratitude is to Dr. Gouda F. Gouda, Lecturer of Animal Breeding, Department of Animal Production, Faculty of Agriculture, Ain Shams University. His insightful comments, constructive criticisms and valuable suggestions at different stages of my research were thought-provoking and they helped me focus my ideas. I am also indebted to the members of Department of Animal Production and Department of Genetics, Faculty of Agriculture, Ain Shams Universty with whom I have interacted during the course of my graduate studies.

My immediate family, to whom this dissertation is dedicated to, has been a constant source of love, support and strength all these years.

CONTENTS

LIST OF TABLES

LIST OF FIGURES