

A STUDY OF ASSOCIATION BETWEEN GENETIC MARKERS AND PRODUCTIVE TRAITS IN LAYER CHICKEN

By

SAADEYA SAAD EL-DEIN MEKKY MOHAMED

B.Sc. Agric. Sc. (Poultry Production), Ain Shams University, 1992

M.Sc. Agric. Sc. (Poultry Breeding), Ain Shams University, 2008

**A thesis submitted in partial fulfillment
of
the requirements for the degree of**

**DOCTOR OF PHILOSOPHY
in
Agricultural Sciences
(Poultry Breeding)**

**Department of Poultry Production
Faculty of Agriculture
Ain Shams University**

2017

Approval Sheet

**A STUDY OF ASSOCIATION BETWEEN GENETIC
MARKERS AND PRODUCTIVE TRAITS
IN LAYER CHICKEN**

By

Saadeya Saad El-Dein Mekky Mohamed

B.Sc. Agric. Sc. (Poultry Production), Ain Shams University, 1992

M.Sc. Agric. Sc. (Poultry Breeding), Ain Shams University, 2008

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

Dr. Hassan Hassan Younis

Prof. of Poultry Breeding, Faculty of Agriculture, Kafr El-Sheikh
University

Dr. Hassan E. Ayoub

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

Dr. Ahmed Galal El-Sayed

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

Dr. Ali Zein El-Deen Hassan

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

Date of Examination: / / 2016

A STUDY OF ASSOCIATION BETWEEN GENETIC MARKERS AND PRODUCTIVE TRAITS IN LAYER CHICKEN

By

SAADEYA SAAD EL-DEIN MEKKY MOHAMED

B.Sc. Agric. Sc. (Poultry Production), Ain Shams University, 1992

M.Sc. Agric. Sc. (Poultry Breeding), Ain Shams University, 2008

Under the supervision of:

Dr. Ali Zein El-Deen Hassan Farrag

Prof. Emeritus of Poultry Breeding, Poultry Production Department,

Faculty of Agriculture, Ain Shams University

(Principal Supervisor)

Dr. Ahmed Galal El-Sayed Gad

Prof. of Poultry Breeding, Poultry Production Department, Faculty of

Agriculture, Ain Shams University

Dr. Hassan Ismael Zaky

Researcher Prof. of Poultry Breeding, Animal and Poultry Breeding

Department, Desert Research Center

ABSTRACT

Saadeya Saad El-Dein Mekky Mohamed: A Study of Association between Genetic Markers and Productive Traits in Layer Chicken. Unpublished Ph. D. Thesis, Poultry Production Department, Faculty of Agriculture, Ain Shams University, 2017.

The aim of this study was to measure the genetic variability in two commercial layer strains Hy-line (brown) and Hy-line (W-36) using five microsatellite markers and evaluation of their productive performance under Egyptian environmental conditions. The present study was carried out using a total of 663 layer chickens to determine egg production characteristics (447 brown Hy-line layers and 216 W-36 Hy-line layers). Egg production traits were recorded throughout the first three months of production period. However, egg quality traits were conducted at 24 weeks of age. Results indicated that brown Hy-line layers had heavier body weight than white ones. Brown layers commenced to lay at an earlier age. Brown eggs were heavier than white eggs. There was insignificant effect of strain on egg number and egg production percentage. However, egg mass was highly significant for brown layers than white ones. Data of egg quality showed that the brown Hy-line eggs were highly significant for egg weight, egg shape index, shell thickness, wet and dry shell weight and shell percentage than w-36 Hy-line eggs. Concerning internal egg quality, results indicated that brown eggs were higher than white eggs for albumen weight. However, Brown layers had higher Haugh units and yolk index than the white ones, but the differences were not statistically significant. Concerning genetic diversity of brown and white Hy-line strains, results showed that there were 18 alleles found at five loci across the two strains. The mean number of alleles per locus for the studied loci was 3.6 alleles ranged from 2 for MCW0246 to 5 for MCW0241. A total of 8 common alleles were detected versus five microsatellite loci overall genotypes. Regarding specific alleles, a total of 10 out of 18 alleles (56 %) were

noticed overall loci for the two genotypes studied. 5 specific alleles were observed in HY-line (Brown). Also, 5 ones were obtained in the case of HY-line (White) strains. the microsatellite MCW0241, ADL0273, MCW0246, MCW0258 and ADL0188 gives number of alleles 5, 3, 2, 4 and 4 with a size range from 295-355, 161-183, 250-280, 161-194 and 164-200bp, respectively. The value of expected heterozygosity (H_e) was quite high ranging from 0.5556 to 0.6444 with the average of 0.6121 while, observed heterozygosity (H_o) was 1.000 with the average of 1.000. The mean F_{ST} values of 0.1748 measured the degree of differentiations within and between strains. Where, the F_{ST} value indicated a lack of genetic differentiation between the chicken strains. The highest allele frequency overall loci was (0.50), while the lowest one was (0.100) in brown and (W-36) Hy-line strains. In addition, the highest average of allele frequency estimated was (0.50) in HY-line (W-36) strain, the lowest one was (0.33). (PIC) value for HY-line (W-36) ranged from (0.50) to (0.58) with mean 0.548 for all loci. Meanwhile, it ranged from 0.50 to 0.62 with mean of 0.556 for HY-line (Brown) strain. These differences reflect high genetic variability within HY-line (Brown) strain.

Key words: Microsatellite markers, genetic diversity, Brown Hy-line, W-36 Hy- line strain, productive performance, egg quality.

ACKNOWLEDGEMENTS

In the lead, I would like to express my prayerful thanks to "**ALLAH**" who helped me in everything.

My deepest gratitude is extended to **Prof. Dr. A. Zein El-Dein**, Emeritus Professor of Poultry Breeding, Poultry Production Department, Faculty of Agriculture, Ain Shams University for his supervision, revising the manuscript and continuous support.

I wish to express my deepest appreciation to **Dr. A. Galal**, Professor of Poultry Breeding, Poultry Production Department, Faculty of Agriculture, Ain Shams University for his supervision, interest, encouragement and revising the manuscript.

I want to express the extent of my gratitude and appreciation to **Dr. H. I. Zaky**, Professor of Poultry Breeding, Desert Research Center for his supervision, encouragement and revising the manuscript.

Many thanks for **Dr. M.Y. Mahrous**, Associate Professor of Poultry Breeding, Poultry Production Department, Faculty of Agriculture, Ain Shams University for his encouragement and valuable advices during this study.

Special thanks for **Dr. M.A. El-Sayed**, Researcher of Biotechnology, Animal Genetic Resources Department, National Gene Bank, Agriculture Research Center for his valuable advice and help.

I would like to express thanks to Habiba H. Rezk and all staff members of Poultry Production Department Faculty of Agriculture, Ain Shams University.

I'd like to thank Dr. Afaf A. El-Sheref Researcher of Animal Nutrition, Animal and Poultry Nutrition Department, Desert Research Center.

Finally, I want to thank every member of my family specially my parents, my brother, my sister and their families and my niece Dr. Randa I. Mahmoud for their continuous support and encouragement during this study.

CONTENTS	Page
LIST OF TABLES.....	V
LIST OF FIGURES	VI
LIST OF ABBREVIATIONS	VII
INTRODUCTION	1
REVIEW OF LITERATURE.....	3
1. Productive performance.....	3
1.1. Phenotypic measurements.....	3
1.1.1 Body weight.....	3
1.1.2. Body measurements	4
1.2. Egg production measurements.....	4
1.2.1. Age at sexual maturity.....	5
1.2.2. Egg number	6
1.2.3. Egg weight and egg mass	7
1.3. Egg quality Parameters	8
1.3.1. Egg weight	9
1.3.2. External egg quality measurements	9
1.3.2.1. Egg shape index	10
1.3.2.2. Specific gravity	10
1.3.2.3. Egg volume	11
1.3.2.4. Egg shell thickness	11
1.3.2.5. Egg shell breaking strength	12
1.3.2.6. Egg shell weight and percentage	13
1.3.3. Internal egg quality measurements	14
1.3.3.1. Albumen quality	14
1.3.3.1.1. Albumen weight and percentage	14
1.3.3.1.2. Haugh units	16
1.3.3.2. Yolk quality	16
1.3.3.2.1. Yolk weight and percentage	16
1.3.3.2.2. Yolk index.....	17
2. Molecular genetics	18
2.1. Development of the domestic chicken.....	18
2.2. The chicken genome.....	19

2.3. Genetic diversity.....	20
2.4. Molecular genetic markers in the chicken	22
2.4.1. Microsatellites (SSR)	23
2.5. Applications of Microsatellites	25
2.6. Z chromosome.....	28
MATERIAL AND METHODS.....	29
1. Location and study period.....	29
2. Experimental breeds	29
3. Flock Management	29
3.1. Housing system	29
3.2. Feeding system.....	29
3.3. Lighting system.....	29
4. Productive performance.....	30
4.1. Phenotypic parameters.....	30
4.1.1. Body weight	30
4.1.2. Body measurements	30
4.1.2.1. Shank length	30
4.1.2.2. Comb and wattle lengths.....	30
4.2. Egg production parameters.....	30
4.2.1. Age at sexual maturity	30
4.2.2. Egg number and egg production rate (%).....	30
4.2.3. Egg weight	30
4.2.4. Egg mass	31
4.3. Egg quality measurements	31
4.3.1. Egg weight.....	31
4.3.2. External egg quality measurements.....	31
4.3.2.1. Egg shape index.....	31
4.3.2.2. Specific gravity (Gradational densities salt solutions method).....	31
4.3.2.3. Egg volume.....	31
4.3.2.4. Eggshell breaking strength	32
4.3.2.5. Shell weight.....	32
4.3.2.5.1. Wet Shell weight.....	32
4.3.2.5.2. Dry Shell weight	32

III

4.3.2.6. Shell percentage	32
4.3.2.7. Shell thickness with shell membranes	33
4.3.3. Internal egg quality measurements	33
4.3.3.1. Albumen quality	33
4.3.3.1.1. Albumen weight	33
4.3.3.1.2. Albumen percentage	33
4.3.3.1.3. Haugh units	33
4.3.3.2. Yolk quality	33
4.3.3.2.1. Yolk weight	33
4.3.3.2.2. Yolk percentage	34
4.3.3.2.3. Yolk index	34
5. Molecular DNA marker	34
5.1. Blood samples	34
5.2. The laboratory procedures	34
5.3. Microsatellite markers	36
5.4. PCR program	38
5.5. Statistical analysis	39
RESULTS AND DISCUSSION	43
1. Productive performance	43
1.1. Phenotypic parameters	43
1.1.1. Body weight	43
1.1.2. Body measurements	43
1.2. Egg production measurements	45
1.2.1. Age at sexual maturity	45
1.2.2. Egg number	45
1.2.3. Egg weight	45
1.2.4. Egg mass	46
1.2.5. Egg production percentage	46
1.3. Egg quality measurements	47
1.3.1. Egg weight	48
1.3.2. External egg quality measurements	48
1.3.2.1. Egg shape index	49
1.3.2.2. Egg Volume	49
1.3.2.3. Specific gravity	49

1.3.2.4. Egg shell breaking strength	50
1.3.2.5. Egg shell thickness	50
1.3.2.6. Egg shell weight	51
1.3.2.6.1. Wet shell weight	51
1.3.2.6.2. Dry shell weight	51
1.3.2.7. Egg shell percentage	52
1.3.3. Internal egg quality measurements	52
1.3.3.1. Albumen quality	52
1.3.3.1.1. Albumen weight	52
1.3.3.1.2. Albumen percentage.....	53
1.3.3.1.3. Haugh units	53
1.3.3.2. Yolk quality	53
1.3.3.2.1. Yolk weight	53
1.3.3.2.2. Yolk percentage.....	54
1.3.3.2.3. Yolk index	54
2. Genetic diversity	57
SUMMARY AND CONCLUSION	67
REFERENCES	74
ARABIC SUMMARY	

LIST OF TABLES

No.	TITLE	Page
1	Microsatellite loci used, their traits, chromosome number, location on genome, primer sequence, annealing temperature, accession no. and size range.....	37
2	The amounts of the reagents for gel preparations.....	38
3	Means $\pm$ SE of some body measurements (cm) for Hy-line strains.....	44
4	Means $\pm$ SE of egg production characteristics for Hy-line strains.....	47
5	Means $\pm$ SE of external egg quality measurements for Hy-line strains.....	55
6	Means $\pm$ SE of external egg quality measurements for Hy-line strains.....	55
7	Means $\pm$ SE of Albumen quality measurements for Hy-line strains.....	56
8	Means $\pm$ SE of yolk quality measurements for Hy-line strains.....	56
9	Specific alleles, common alleles and total number of alleles for Brown and (W-36) Hy-line strains.....	57
10	Characteristics of microsatellite loci used, chromosomal location, fragment size and number of alleles observed for each locus.....	59
11	Microsatellite loci used, chromosomal location, observed (HO) and expected (HE) heterozygosities and the fixation index (F_{ST}) for the chicken strains.....	60
12	Alleles size in base pair, their frequencies for each locus and strain average of allele frequencies observed in two strains.....	62
13	Exact P-Values of the Hardy-Weinberg probability test for each of 5 microsatellite loci in two Hy-line strains.....	64
14	AMOVA analysis of two strains based on microsatellite DNA variation.....	65

LIST OF FIGURES

No.	TITLE	Page
1	Body weight (g) for Brown and (W-36) Hy-line layer strains.....	44
2	Age at sexual maturity (d) for Brown and (W-36) Hy-line layer strains.....	47
3	Egg weight (g) for Brown and (W-36) Hy-line layer strains.....	54

LIST OF ABBREVIATIONS

AFE	Age at First Egg
cM	CentiMorgan
DNA	Deoxyribonucleic acid
EN	Egg number
ES	Egg shell strength
EW	Egg weight
FiS	Fixation indices(Among populations)
FiT	Fixation indices(Within individuals)
FST	Fixation indices(Among individuals within populations)
HE	Expected Heterozygosity
Ho	Observed Heterozygosity
HU	Haugh-unit
Ng	Nano gram
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphisms
SSR(s)	Simple sequence repeat(s)
STRs	Simple tandem repeats

INTRODUCTION

Poultry industry is considered to be one of the most important economic industries, which contribute significantly the national income of Egypt. Poultry breeding also provides a source of high protein and high nutritional value costs less compared to other meat. Where, the total number of consumed chicken eggs in the year is estimated by 55 million metric tons (**Muir *et al.*, 2008**).

Chickens are good converters of feed into useable protein both in meat and egg. Eggs of the chicken are considered to be one of the finest foods which provide balance nutrients from proteins, vitamins, minerals and fatty acids with a great biological value. Layers of commercial strains commence to lay eggs at 20 weeks of age. Furthermore, commercial hens produced about 0.9 eggs per day (**Kekeocha, 1985**).

Egg quality is important characteristics for consumers and producers; the internal egg quality is very important for consumers but for producers the external egg quality is very important. The economic success of poultry production is measured by the total number of produced eggs that have a good quality (**Monira *et al.*, 2003**). Egg shell quality is an important factor to poultry industry; it has direct effects on prices in poultry industry of commercial strains. Where, the percentage of egg shell breakage reached to 7- 8% during transport from producers to consumers. Consequently, the total number of broken and cracked eggs causes dangerous economic problems for both producers and traders (**Hamilton, 1982**). Hence, egg shell breakage result in annual losses of millions of dollars, therefore it is very important to evaluate the egg quality traits.

There is a strong relationship between poultry breeding and poultry genetics, where poultry genetics provide the biological base for poultry breeding. Poultry genetics has a proud history of achievements, in providing modern Knowledge as in facing the global need of food. The genetic variation which exists in the basic economic traits may due