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MOHAMED M.M. OSMAN, SHAABAN A. HEMEDA, ABEER A.I. HASSANIN, WALAA A. HUSSEINY

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Polymorphism of Prolactin Gene and Its Association with Egg Production Trait in Four Commercial Chicken Lines

Mohamed, M.M.Osman¹, Shaaban, A.Hemeda², Abeer A.I.Hassanin^{1*}, Walaa A. Husseiny¹

¹ *Department of Animal Wealth Development, Faculty of Veterinary Medicine,
Suez Canal University, Ismailia, Egypt.*

² *Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine,
Alexandria University, Alexandria, Egypt*

ABSTRACT. Broodiness is a behavioral trait observed in most common breeds of domestic fowl and due to its fundamental role in avian reproduction, it has been of great interest to poultry scientists, breeders and producers of hatching eggs. Prolactin gene (PRL) is generally accepted as crucial to the onset and maintenance of broodiness in birds and thus plays a crucial role in egg production. Therefore, the present study aimed to screen the Single Nucleotides Polymorphisms (SNPs) of prolactin gene in four commercial chicken lines namely Hubbard F15, Lohmann, Cobb500, and Avian48 using PCR and direct sequencing. A total number of forty chickens (ten females from each of the four commercial chicken lines) were used. Blood samples were collected aseptically from brachial (wing) vein of the chickens for genomic DNA extraction. PCR reaction was done using five pairs of primers, one sense (F) and one antisense (R) primer for each of the five exons of prolactin gene. Finally, DNA sequencing and Single Nucleotide Polymorphisms (SNPs) analysis was done using Laser gene Megalign program. The results showed three SNPs in Hubbard F15 chicken line; one synonymous SNP at the position 3838 bp (ACC/ACT-transition) in exon 2 while in exon 5, two SNPs were detected; one non-synonymous single nucleotide polymorphism at the position 7921bp (CCT/TCT-transition) which results in amino acid changes at codon positions 169 (P/S), and one synonymous single nucleotide polymorphism at the position 8187 bp T/ C. The study concluded that this SNP in PRL gene could be used as the potential molecular markers for egg production traits in chicken.

Key words: Prolactin gene, Single Nucleotide Polymorphism, Egg production, Chicken.

Corresponding Author:
Abeer A.I.Hassanin,
E-mail: drabeer2000@gmail.com

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INTRODUCTION

Prolactin (PRL) is one of the hormone family that includes growth hormone (GH), placental lactogen (PL), and somatolactin (**Power, 2005**). It is a polypeptide hormone which synthesized and secreted by the animal's anterior pituitary gland (**Jiang et al., 2009**) and playing an essential role in regulating reproduction and lactation in mammals (**Bonomo et al., 2007**).

In birds, PRL affects a wide variety of physiological functions, PRL mRNA expression observed mainly in the cephalic lobe of the anterior pituitary gland whereas the most studied effects of PRL involve crop-sac development in columbiforms, the induction and maintenance of broody behavior, regulation of gonadal function and immune responsiveness in a variety of species (**Kansaku et al., 2008**).

Chicken PRL (cPRL) gene plays an important role in egg production. It is generally known as a crucial gene to the onset and maintenance of broodiness which results in regression of the ovary (**Sharp et al., 1984**) and loss of egg production (**Nestor, 1980**). The occurrence of broodiness is always associated with an increase in plasma prolactin concentration. During incubation, prolactin mRNA reaches its highest level which suppose that prolactin is important in the maintenance of broodiness (**Alipanah et al., 2011**).

The PRL gene has been cloned in a variety of avian species including the domestic chicken, turkey, quail, duck, and pigeon (**Liu et al., 2008**). cPRL gene is located in chromosome number 2. It is a polypeptide with a molecular weight of 21700-26000 Dalton (**Alipanah et al., 2011**). The gene is composed of five exons and four introns. The five exons are 28 bp, 182 bp, 108 bp, 180 bp, and 192 bp long respectively, while, the four introns are 1,520 bp, 408 bp, 1,348 bp, and 1,909 bp long respectively (**Au et al., 2002**). All the exon-intron junctions conform to the GT-AG rule (**Ohkubo et al., 2000**).

Since the avian PRL gene was cloned and sequenced, most studies have concentrated on identifying new polymorphic sites in this gene. It was found that most of the chicken PRL gene sequence polymorphisms were found in 5' flanking region, 3' flanking region, and the coding region of the signal peptide (**Cui et al., 2006**).

Up to date, a large number of Single Nucleotides Polymorphisms (SNPs) have been reported in the 5'-flanking region of chicken PRL gene influencing its function. For example, a 24-bp insertion occurring in the promoter of chicken PRL gene was associated with decrease the expression of PRL, leading to non-broodiness which could be used as a genetic marker in breeding against broodiness in chickens (**Jiang et al., 2005**). Six single nucleotide polymorphisms (C-2402-T, C-2161-G, T-2101-G, C-2062-G, T-2054-A, and G-2040-A) in chicken PRL promoter region and their association with egg production and chicken broodiness traits were investigated by **Cui et al. (2006)**. Furthermore, **Begli et al. (2010)** investigated the polymorphism of prolactin promoter and its association with economic traits in native fowl of Yazd province, A 24 bp indel (insertion or deletion) at nucleotide position (np) 358 was identified showing that association of genes polymorphisms with egg production could be used to improve the performance of native fowl of Yazd province.

Modern poultry production is generally aimed at elevation of egg production and inhibition of incubation behavior (**Xu et al., 2010**). Studies conducted over the past few years indicated that the PRL gene partakes, directly and indirectly, in shaping many production traits in poultry. Thus, these genes are now being considered as candidate markers of these traits (**Wilkanowska et al., 2014**).

Therefore, this study aimed to screen the SNPs of chicken PRL gene in its five exons in four commercial chicken lines and analyze their correlation with egg production trait.

MATERIAL AND METHOD

I. Chicken breeds:

A total number of forty chickens (ten females from each of the four commercial chicken lines namely Lohmann, Avian48, Cobb500 and Hubbard F15 were used in this study. Chicken lines were precisely chosen from four farms; two farms, El Salhia El Gedidah farm, and Hamdy El Masry farm in Sharkia province, and two, Abo Sultana farm, and Abo Hegazy farm, in Dakhliya province. Egg production data were collected from daily farm records maintained at the farms.

II. Blood sample collection:

Blood samples were collected aseptically from

brachial (wing) vein of the forty chickens under the study using 5 ml syringes. Samples were quickly transferred to vacutainer tubes containing Na₂EDTA as an anticoagulant, kept immediately in ice- box containing gel cool packs and transported to the laboratory, stored at -20°C until DNA extraction.

Ethics statement:

The protocol of this study was approved by the scientific research and bio-ethics committee at the Faculty of Veterinary Medicine, Suez Canal University (Permit Number: 201518).

III. Genomic DNA extraction:

Genomic DNA was extracted from blood samples using Thermo Scientific GeneJET Whole Blood Genomic DNA Purification MiniKit (#K0781) according to the manufacture's protocol. DNA concentration and purity were determined spectrophotometrically as described by **Sambrook and Russel (2001)**. A260/A280 ratio was between 1.7 and 1.9.

IV. Amplification of chicken prolactin gene exons using Polymerase chain reaction (PCR):

Five pairs of primers, one sense (F) and one antisense (R) primer for each of the five exons (exon 1, exon 2, exon 3, exon 4, exon 5) of the chicken prolactin gene were designed by the web-based software Primer3Plus (**Andreas et al. 2007**) from the published DNA sequence of chicken prolactin gene (cPRL) deposited in the gene bank under accession number (GeneBank: AF288765; **Au and Leung, 2002**). The primers sequences and the product size are shown in Table 1.

PCR reaction contained 2X PCR master mix solution (GeneDireX) (25µl), 3µl template DNA, and 10µM each of degenerate primers in a final volume of 50 µL. The cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 40 amplification cycles of denaturation at 95°C for 30 s, annealing at 63°C for 45sec, and extension at 72°C for 45 sec, and a final extension step for 7 min at 72°C. In order to confirm the amplification of the target sequences of chicken prolactin gene exons, the PCR product was electrophoresed on 1% agarose gel stained with ethidium bromide, visualized on UV trans-illuminator (Slimeline™ series) and the image of the gel was acquired.

V. DNA sequencing and Single Nucleotide Polymorphisms (SNPs) analysis:

A total number of forty PCR products from the four chicken lines under the study were sequenced. For each line, there were a total number of ten PCR products. All loci were directly sequenced in both forward and reverse directions using the same primers used for PCR amplification by dye terminator cycle sequencing technique.

Sequence data obtained from the forward and reverse primers were crosschecked to confirm the polymorphic sites detected. Sequence alignment of the resulted sequences with the corresponding functional chicken prolactin gene sequences available in GeneBank (AF288765) to locate sequence variants in DNA and protein sequences were done by the CLUSTAL W method using Laser gene Megalign program

Table 1. Gene-specific oligonucleotide primers used in PCR amplification of chicken prolactin gene exons.

Exon No.	Primer name	Nucleotide Sequence	Length (bp)	Product size (bp)
Exon 1	PRL1F	5'-ACT CCA CGA CCT GCT GAA TGT A-3'	22	296
	PRL1R	5'-ATA GTG GTG AAG CTG CCT CCA C-3'	22	
Exon 2	PRL2F	5'-CTG CCT CTG ACA GCT ATT TCC A-3'	22	294
	PRL 2R	5'-CAT GTT CTC ACT CCC AGG AAA A-3'	22	
Exon 3	PRL 3F	5'-GAC CAT TGC TAA GAT CCC CAA T-3'	22	257
	PRL 3R	5'-TTC CCC CAC ACT CTA TCT CAC A-3'	22	
Exon 4	PRL 4F	5'-TGT GGA CCA GCA TGA AGA CCT A-3'	22	259
	PRL 4R	5'-AGA TCC AGT CCC TTC ACA TGG T-3'	22	
Exon 5	PRL 5F	5'-CTG TTC TAC ACC CAG ACA GAT TGA-3'	24	609
	PRL 5R	5'-AAG GTA TAA GCC ATC CCA GCT ATT-3'	24	

(Ver 5.52,2003, DNASTAR Inc). All the resulted sequences were submitted to the GeneBank/NCBI data bank to have a unique accession number for each.

VI. Statistical analysis and Egg production records:

Data regarding total egg production, mortality, age at first lay, age at peak of lay for the four chicken lines under the study were collected from the weekly farm records maintained at the farms. Egg per hen per week and egg per hen per day was calculated using the data obtained from the records. First egg laid was considered as age at point of lay while flock obtaining the highest number of eggs on any week was considered as peak and this week was regarded as age at peak of lay.

Dates obtained werestatistically analysed for the effects of chicken breed, peak of egg production, and SNP on the studied egg production traits and mortality rates. The main and interaction effects were analysed using three-way analysis of variance (ANOVA). The general linear model procedures (GLM) of SAS (SAS institute, 1998) and Duncan's Multiple Range test (Duncan, 1955) were carried out to detect the significance differences among means. Least square means and their standard errors were presented for main effects of Breed, Peak and SNP. A probability value (P) of less than 0.05 was considered to be significant.

RESULTS:

I. Sequence analysis of cPRL gene exons in the four commercial chicken lines under the study.

Analysis of the database published Chicken pro-

Hen-day egg production on daily basis was calculated using the following formula given by North (1984):

$$\text{HDEP} = \frac{\text{Number of eggs produced on daily basis}}{\text{Number of birds available in the flock on that day}}$$

While, Hen-housed egg production was worked using the following formula given by North (1984):

$$\text{HHEP} = \frac{\text{Total number of eggs produced by a flock}}{\text{Total number of hens housed}}$$

lactin gene sequence (Accession no. AF288765) shows that cPRL gene consists of five exons and four introns. The coding region of the cPRL gene starts in the first exon and ends within the last exon.

In the four chicken lines under the study, PRL1F and PRL1R primers were annealed successfully at 2103 bp-2124 bp location at 5' upstream region and

2377 bp-2398 bp location at intron one of chicken prolactin gene respectively. These two primers resulted in amplification of approximately 296 bp PCR product at nucleotide position 2103-2398 bp of prolactin gene representing a part of 5' upstream region (85 bp), exon 1 (74 bp) and part of intron I (137 bp) of chicken prolactin gene respectively (Figure 1). PRL2F and PRL2R primers were annealed successfully at 3704 bp-3725 bp location at intron 1 and 3976 bp-3997 bp location at intron 2 of chicken prolactin gene respectively. These two primers resulted in amplification of approximately 294 bp PCR product at nucleotide position 3704-3997 bp of prolactin gene representing a part of intron 1 (78 bp), exon 2 (180 bp) and part of intron 2 (36 bp) of chicken prolactin gene respectively (Figure 2A, B). However, PRL3F and PRL3R primers were annealed successfully at 4270 bp-4291 bp location at intron 2 and 4505 bp-4526 bp location at intron 3 of chicken prolactin gene respectively. These two primers resulted in amplification of approximately 257bp PCR product at nucleotide position 4270-4526 bp of prolactin gene representing a part of intron 2 (100 bp), exon 3 (108 bp) and part of intron 3 (49 bp) of chicken prolactin gene respectively (Figure 3). Moreover, PRL4F and PRL4R primers were annealed successfully at 5816 bp-5825 bp location at intron 3 and 6053bp-6074bp location at intron 4 of chicken prolactin gene respectively. These two primers resulted in amplification of approximately 259bp PCR product at nucleotide position 5816-6074 bp of prolactin gene representing a part of intron 3 (10 bp), exon 4 (183 bp) and part of intron 4 (66 bp) of chicken prolactin gene respectively (Figure 4). Finally, PRL5F and PRL5R primers were annealed successfully at 7856 bp-7879 bp location at intron 4 and 8441bp-8464bp location at 3' upstream region of chicken prolactin gene respectively. These two primers resulted in amplification of approximately 609bp PCR product at nucleotide position 7856-8464 bp of prolactin gene representing a part of intron 4 (62 bp), exon 5 (420 bp) and part of intron 5 (127 bp) of chicken prolactin gene respectively (Figure 5A, B).

The five exons of prolactin gene in each of the four chicken lines under the study has been deposited in the GenBank/NCBI data bank with the following accession numbers KP739310, KP739307, KP739308, and KP739309 in Hubbard F15, Lohmann, Cobb 500, and Avian 48 chicken lines respectively.

Figure 1. Nucleotide sequence of the amplified PCR product (296 bp) representing exon 1 of prolactin gene in Hubbard f15, Lohmann, Cobb 500, and Avian 48 chicken lines. [Uppercase is the target fragment in the amplification process (exon one), lowercase before exon one nucleotide sequence represent the part of 5' upstream region while the lowercase after exon one nucleotide sequence represent the part of intron 1 amplified using PRL1F and PRL1R primer pair . Positions of both primer pair are underlined].

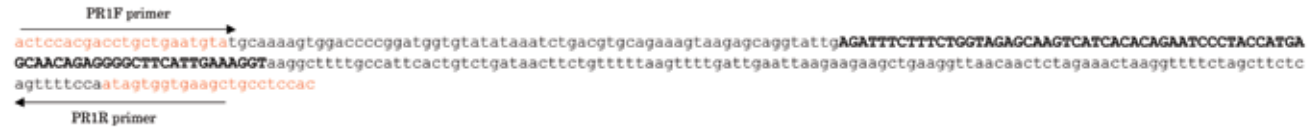


Figure 2A. Nucleotide sequence of the amplified PCR product (294 bp) representing exon 2 of prolactin gene in Hubbard f15 Chicken line. Uppercase is the target fragment in the amplification process (exon two), lowercase before exon 2 nucleotide sequence represent the part of intron 1 while the lowercase after exon 2 nucleotide sequence represent the part of intron 2 amplified using PRL2F and PRL2R primer pair . Positions of both primer pair are underlined.

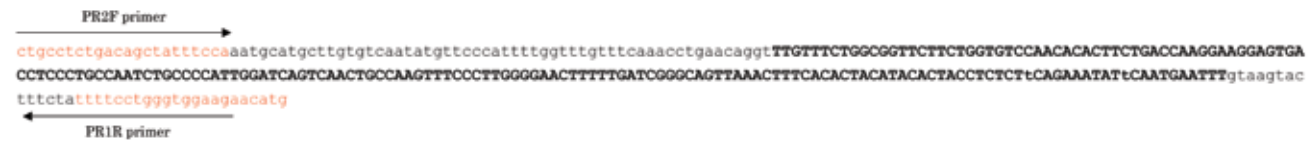


Figure 2B. Nucleotide sequence of the amplified PCR product (294 bp) representing exon 2 of prolactin gene in Lohmann, Cobb 500, and Avian 48 chicken lines. Uppercase is the target fragment in the amplification process (exon two), lowercase before exon 2 nucleotide sequence represent the part of intron 1 while the lowercase after exon 2 nucleotide sequence represent the part of intron 2 amplified using PRL2F and PRL2R primer pair . Positions of both primer pair are underlined.

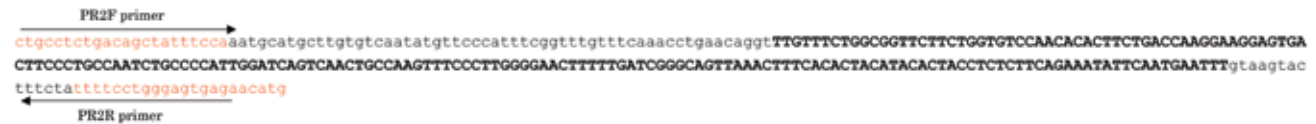


Figure 3. Nucleotide sequence of the amplified PCR product (257 bp) representing exon 3 of prolactin gene in Hubbard f15, Lohmann, Cobb 500, and Avian 48 chicken lines. Uppercase is the target fragment in the amplification process (exon three), lowercase before exon 3 nucleotide sequence represent the part of intron 2 while the lowercase after exon 3 nucleotide sequence represent the part of intron 3 amplified using PRL1F and PRL1R primer pair . Positions of both primer pair are underlined.



II. Sequence alignment results of cPRL gene exons in the four commercial chicken lines under the study.

Nucleotides and amino acid Sequences alignment of chicken prolactin (cPRL) gene exons in four commercial chicken lines was performed by the CLUSTAL W method using Laser gene Megalign program (Ver 5.52,2003, DNASTAR Inc). The aligned nucleotide and amino acid sequences of exons 1 (Figures 6, 7), exon 3 (Figures 10, 11), and exon 4 (Figures 12, 13) shows complete homogeneity between the four chicken lines under the study. No SNPs were identified. The nucleotide sequence alignment of cPRL gene exons 2 shows one synonymous SNP at the position 3838 (ACC/ACT-transition) in Hubbard F15 chicken line (Figures 8) with no effect on the coded amino acid sequences (Figures 9). Moreover, in exon 5 the aligned sequences shows two SNPs in Hubbard F15

chicken line; one non-synonymous single nucleotide polymorphism at the position 7921 (CCT/TCT-transition) (Figure 14) which result in amino acid changes at positions 169 (P/S), (Figure 14) and one synonymous single nucleotide polymorphism at the position 8187 T/ C (Figure 15).

III.Effects of chicken breed, Peak of lay and SNP on egg production traits and mortality rate:

Table 2 shows Least square means and their standard errors for main effects of Breed, Peak and SNP on egg production traits (including total egg production, egg production per hen per week, egg production per hen per day, hen housed, hen day) and mortality rate in the four chicken lines under the study.

Concerning with the effect of chicken breed on egg production traits and mortality rates, results

Figure 4. Nucleotide sequence of the amplified PCR product (259 bp) representing exon 4 of prolactin gene in Hubbard f15, Lohmann, Cobb 500, and Avian 48 chicken lines. Uppercase is the target fragment in the amplification process (exon four), lowercase before exon 4 nucleotide sequence represent the part of intron 3 while the lowercase after exon 4 nucleotide sequence represent the part of intron 4 amplified using PRL4F and PRL4R primer pair. Positions of both primer pair are underlined.

PR4F primer
 tgtggaccagCATGAAGACCTA CTGAATTTAGTAGTGGGAGTGTCTGCTTCTGGAATGATCCCTGATCCATCTGGCCCTCTGAAGTGCAAAGAATCAAAGAAGCTCCAGATACCACTCTCTGGAAGGCTGTA
 GAGATTGAGGAGCAAAACAAGAGGCTTCTAGAAGGAATGGAGAAAATAGTTGGGCGGGTA agtattaggctccttacagtccttccagcctattggtgcaaaagaa ccatgtgaaggactggatct
 PR4R primer

Figure 5A. Nucleotide sequence of the amplified PCR product (609 bp) representing exon 5 of prolactin gene in Hubbard f15 Chicken line. Uppercase is the target fragment in the amplification process (exon five), lowercase before exon 5 nucleotide sequence represent the part of intron 4 while the lowercase after exon 5 nucleotide sequence represent the part of 3' upstream region amplified using PRL5F and PRL5R primer pair. Positions of both primer pair are underlined.

PR5F primer
 ctgttctacaccagacagattgactatcctctcttactgtatgattatgtctctctaggttCATCCTGGTGATGCTGGAAATGAAATTTACTCTCACTGGGACGGCCTCCATCCCTGCAACTCGCTGATGA
 GGACTCCAGACTCTTTGCTTTTTATAACCTGCTGCATTGCCCTCCGAGAGATTCACCAAAAATTGACAACATATCTTAAAGTTTTGAAGTGCCGCTAATCCATGATAGCAATGCTAAGTACCTGTGGGCTGC
 ATTACTCACTGAAACCAATTCATCATGGTGTCTTGTGCTTTGCCACTTTTCACTGOGAACTTTATGAAAAACCAATTGTACAGCACCAGGATCATCAGTAACCTTCAGGCATGCTTGTGTAATCTGGCT
 GCAACTATTAGTGCCATTTATCTTGGTGTAAAAATGCTGTAATACACTTGTGTAACAAGAATAAACCTTTCTGCTAATTTCCtacttattagcatttcattgataagtagatcatgtagatacaaaaa
 atattattaattcaaaaccagtaacaacaagtggtatcatgaacaaaaaatatagctgggatggctttatacctt
 PR5R primer

Figure 5B. Nucleotide sequence of the amplified PCR product (609 bp) representing exon 5 of prolactin gene in Lohmann, Cobb 500, and Avian 48 chicken lines. Uppercase is the target fragment in the amplification process (exon five), lowercase before exon 5 nucleotide sequence represent the part of intron 4 while the lowercase after exon 5 nucleotide sequence represent the part of 3' upstream region amplified using PRL5F and PRL5R primer pair. Positions of both primer pair are underlined.

PR5F primer
 ctgttctacaccagacagattgactatcctctcttactgtatgattatgtctctctaggttCATCCTGGTGATGCTGGAAATGAAATTTACTCTCACTGGGACGGCCTCCATCCCTGCAACTCGCTGATGA
 GGACTCCAGACTCTTTGCTTTTTATAACCTGCTGCATTGCCCTCCGAGAGATTCACCAAAAATTGACAACATATCTTAAAGTTTTGAAGTGCCGCTAATCCATGATAGCAATGCTAAGTACCTGTGGGCTGC
 ATTACTCACTGAAACCAATTCATCATGGTGTCTTGTGCTTTGCCACTTTTCACTGOGAACTTTATGAAAAACCAATTGTACAGCACCAGGATCATCAGTAACCTTCAGGCATGCTTGTGTAATCTGGCT
 GCAACTATTAGTGCCATTTATCTTGGTGTAAAAATGCTGTAATACACTTGTGTAACAAGAATAAACCTTTCTGCTAATTTCCtacttattagcatttcattgataagtagatcatgtagatacaaaaa
 atattattaattcaaaaccagtaacaacaagtggtatccatgaacaaaaaatatagctgggatggctttatacctt
 PR5R primer

showed that there were highly significant differences between the four breeds under the study in all the above mentioned traits ($p \leq 0.0001$). The highest significant values of total egg production, egg production per hen per week, egg production per hen per day, hen housed, hen day and mortality rate were obtained in Lohmann breed ($434095^a \pm 2885$, $6.21^a \pm 0.03$, $0.89^a \pm 0.01$, $6.08^a \pm 0.04$, $0.87^a \pm 0.01$, and $113.11^a \pm 2.94$) respectively.

Moreover, results showed that there were highly significant differences between the four breeds under the study in all egg production traits and mortality rates during peak of lay, before peak of lay, and after peak of lay. The highest significant values for all egg production traits under the study were obtained during the peak of egg production. These values were as follows; ($176406^a \pm 18496$, $5.50^a \pm 0.08$, $0.79^a \pm 0.01$, $5.34^a \pm 0.08$, and $0.76^a \pm 0.01$) for total egg production, egg production per hen per week, egg production per hen per day, hen housed, and hen day traits re-

spectively. Concerning with the effect of peak of lay on the mortality rate traits, results showed that the highest significant values were obtained both during ($67.90^a \pm 10.82$) and after ($60.40^a \pm 9.39$) the peak of lay.

Regarding the effect of SNPs, results showed that there were significant differences ($P \leq 0.012$) between the three breeds which contain no SNPs (Lohmann, Cobb 500, and Avian 48) and Hubbard F15 breed which contain three SNPs in PRL gene in total egg production trait. The highest significant value ($197071^a \pm 18859$) was obtained in the three chicken breeds contain no SNPs. However, there was a highly significant difference in mortality rate ($P \leq 0.0002$). The highest significant value ($76.49^a \pm 9.51$) was obtained in the three chicken breeds contain no SNPs. On the other hand, there were no significant differences between the three breeds which contain no SNPs (Lohmann, Cobb 500, and Avian 48) and Hubbard F15 breed in egg production per hen per week, egg production per hen per day, hen housed, and hen day traits.

Figure 6. Nucleotide sequences alignment of Prolactin gene, exon 1 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

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                *      20      *      40      *      60      *
Hubbard : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACCATGAGCAACAGAGGGGCTTCATTGAAAGGT : 74
Lohmann : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACCATGAGCAACAGAGGGGCTTCATTGAAAGGT : 74
Cobb 500 : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACCATGAGCAACAGAGGGGCTTCATTGAAAGGT : 74
Avian 48 : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACCATGAGCAACAGAGGGGCTTCATTGAAAGGT : 74
    
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Figure 7. Amino acid sequences alignment of Prolactin gene, exon 1 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

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Hubbard : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACC ATG AGC AAC AGA GGG GCT : 62
                                                M S N R G A : 4
Lohmann : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACC ATG AGC AAC AGA GGG GCT : 62
                                                M S N R G A : 4
Cobb 500 : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACC ATG AGC AAC AGA GGG GCT : 62
                                                M S N R G A : 4
Avian 48 : AGATTTCTTTCTGGTAGAGCAAGTCATCACACAGAATCCCTACC ATG AGC AAC AGA GGG GCT : 62
                                                M S N R G A

                *
Hubbard : TCA TTG AAA GGT : 74
                S L K G : 8
Lohmann : TCA TTG AAA GGT : 74
                S L K G : 8
Cobb 500 : TCA TTG AAA GGT : 74
                S L K G : 8
Avian 48 : TCA TTG AAA GGT : 74
                S L K G
    
```

Figure 8. Nucleotide sequences alignment of Prolactin gene, exon 2 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines shows one synonymous SNP at position 3838 (ACC/ACT-transition) in Hubbard F15 chicken line, where identical bases highlighted in black and polymorphic variations highlighted in gray.

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                *      20      *      40      *      60      *      80
Hubbard : TTGTTTCFGGCGGTTCTTCTGGTGCCAACACACTTCTGACCAAGGAAGGAGTGACTCCCTGCCAATCTGCCCCATTGGA : 81
Lohman : TTGTTTCFGGCGGTTCTTCTGGTGCCAACACACTTCTGACCAAGGAAGGAGTGACTCCCTGCCAATCTGCCCCATTGGA : 81
Cobb 500 : TTGTTTCFGGCGGTTCTTCTGGTGCCAACACACTTCTGACCAAGGAAGGAGTGACTCCCTGCCAATCTGCCCCATTGGA : 81
Avian 48 : TTGTTTCFGGCGGTTCTTCTGGTGCCAACACACTTCTGACCAAGGAAGGAGTGACTCCCTGCCAATCTGCCCCATTGGA : 81
                T

                *      100      *      120      *      140      *      160
Hubbard : TCAGTCAACTGCCAAGTTTCCCTTGGGGAACTTTTGATCGGGCAGTTAAACTTTCACACTACATACACTACCTCTCTTCA : 162
Lohman : TCAGTCAACTGCCAAGTTTCCCTTGGGGAACTTTTGATCGGGCAGTTAAACTTTCACACTACATACACTACCTCTCTTCA : 162
Cobb 500 : TCAGTCAACTGCCAAGTTTCCCTTGGGGAACTTTTGATCGGGCAGTTAAACTTTCACACTACATACACTACCTCTCTTCA : 162
Avian 48 : TCAGTCAACTGCCAAGTTTCCCTTGGGGAACTTTTGATCGGGCAGTTAAACTTTCACACTACATACACTACCTCTCTTCA : 162

                *      180
Hubbard : GAAATATFCAATGAATTT : 180
Lohman : GAAATATFCAATGAATTT : 180
Cobb 500 : GAAATATFCAATGAATTT : 180
Avian 48 : GAAATATFCAATGAATTT : 180
    
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Figure 9. Amino acid sequences alignment of Prolactin gene, exon 2 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

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Hubbard : TTG TTT CTG GCG GTT CTT CTG GTG TCC AAC ACA CTT CTG ACC AAG GAA GGA GTG ACC TCC : 60
          L F L A V L L V S N T L L T K E G V T S
Lohman : TTG TTT CTG GCG GTT CTT CTG GTG TCC AAC ACA CTT CTG ACC AAG GAA GGA GTG ACT TCC : 60
          L F L A V L L V S N T L L T K E G V T S
Cobb 500 : TTG TTT CTG GCG GTT CTT CTG GTG TCC AAC ACA CTT CTG ACC AAG GAA GGA GTG ACT TCC : 60
          L F L A V L L V S N T L L T K E G V T S
Avian 48 : TTG TTT CTG GCG GTT CTT CTG GTG TCC AAC ACA CTT CTG ACC AAG GAA GGA GTG ACT TCC : 60
          L F L A V L L V S N T L L T K E G V T S

Hubbard : CTG CCA ATC TGC CCC ATT GGA TCA GTC AAC TGC CAA GTT TCC CTT GGG GAA CTT TTT GAT : 120
          L P I C P I G S V N C Q V S L G E L F D
Lohman : CTG CCA ATC TGC CCC ATT GGA TCA GTC AAC TGC CAA GTT TCC CTT GGG GAA CTT TTT GAT : 120
          L P I C P I G S V N C Q V S L G E L F D
Cobb 500 : CTG CCA ATC TGC CCC ATT GGA TCA GTC AAC TGC CAA GTT TCC CTT GGG GAA CTT TTT GAT : 120
          L P I C P I G S V N C Q V S L G E L F D
Avian 48 : CTG CCA ATC TGC CCC ATT GGA TCA GTC AAC TGC CAA GTT TCC CTT GGG GAA CTT TTT GAT : 120
          L P I C P I G S V N C Q V S L G E L F D

Hubbard : CGG GCA GTT AAA CTT TCA CAC TAC ATA CAC TAC CTC TCT TCA GAA ATA TTC AAT GAA TTT : 180
          R A V K L S H Y I H Y L S S E I F N E F
Lohman : CGG GCA GTT AAA CTT TCA CAC TAC ATA CAC TAC CTC TCT TCA GAA ATA TTC AAT GAA TTT : 180
          R A V K L S H Y I H Y L S S E I F N E F
Cobb 500 : CGG GCA GTT AAA CTT TCA CAC TAC ATA CAC TAC CTC TCT TCA GAA ATA TTC AAT GAA TTT : 180
          R A V K L S H Y I H Y L S S E I F N E F
Avian 48 : CGG GCA GTT AAA CTT TCA CAC TAC ATA CAC TAC CTC TCT TCA GAA ATA TTC AAT GAA TTT : 180
          R A V K L S H Y I H Y L S S E I F N E F
    
```

Figure 10. Nucleotide sequences alignment of Prolactin gene, exon 3 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

```

Hubbard : GATGAACGTTATGCTCAGGGTCGGGGTTTCATTACAAAAGCTGTTAATGGCTGCCACACTTCCTCCTTAACCACTCCTGAA : 81
Lohman : GATGAACGTTATGCTCAGGGTCGGGGTTTCATTACAAAAGCTGTTAATGGCTGCCACACTTCCTCCTTAACCACTCCTGAA : 81
Cobb 500 : GATGAACGTTATGCTCAGGGTCGGGGTTTCATTACAAAAGCTGTTAATGGCTGCCACACTTCCTCCTTAACCACTCCTGAA : 81
Avian 48 : GATGAACGTTATGCTCAGGGTCGGGGTTTCATTACAAAAGCTGTTAATGGCTGCCACACTTCCTCCTTAACCACTCCTGAA : 81

Hubbard : GATAAGGAGCAAGCTCAGCAGATTCAT : 108
Lohman : GATAAGGAGCAAGCTCAGCAGATTCAT : 108
Cobb 500 : GATAAGGAGCAAGCTCAGCAGATTCAT : 108
Avian 48 : GATAAGGAGCAAGCTCAGCAGATTCAT : 108
    
```

Figure 11. Amino acid sequences alignment of Prolactin gene, exon 3 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

```

Hubbard : GAT GAA CGT TAT GCT CAG GGT CGG GGT TTC ATT ACA AAA GCT GTT AAT GGC TGC CAC ACT : 60
          D E R Y A Q G R G F I T K A V N G C H T
Lohman : GAT GAA CGT TAT GCT CAG GGT CGG GGT TTC ATT ACA AAA GCT GTT AAT GGC TGC CAC ACT : 60
          D E R Y A Q G R G F I T K A V N G C H T
Cobb 500 : GAT GAA CGT TAT GCT CAG GGT CGG GGT TTC ATT ACA AAA GCT GTT AAT GGC TGC CAC ACT : 60
          D E R Y A Q G R G F I T K A V N G C H T
Avian 48 : GAT GAA CGT TAT GCT CAG GGT CGG GGT TTC ATT ACA AAA GCT GTT AAT GGC TGC CAC ACT : 60
          D E R Y A Q G R G F I T K A V N G C H T

Hubbard : TCC TCC TTA ACC ACT CCT GAA GAT AAG GAG CAA GCT CAG CAG ATT CAT : 108
          S S L T T P E D K E Q A Q Q I H
Lohman : TCC TCC TTA ACC ACT CCT GAA GAT AAG GAG CAA GCT CAG CAG ATT CAT : 108
          S S L T T P E D K E Q A Q Q I H
Cobb 500 : TCC TCC TTA ACC ACT CCT GAA GAT AAG GAG CAA GCT CAG CAG ATT CAT : 108
          S S L T T P E D K E Q A Q Q I H
Avian 48 : TCC TCC TTA ACC ACT CCT GAA GAT AAG GAG CAA GCT CAG CAG ATT CAT : 108
          S S L T T P E D K E Q A Q Q I H
    
```

Figure 12. Nucleotide sequences alignment of Prolactin gene, exon 4 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

```

                *      20      *      40      *      60      *      80
Hubbard : CATGAAGACCTACTGAATTTAGTAGTGGGAGTGCCTGCGTTCTGGAATGATCCCCTGATCCATCTGGCCTCTGAAGTGCAA : 81
Lohman : CATGAAGACCTACTGAATTTAGTAGTGGGAGTGCCTGCGTTCTGGAATGATCCCCTGATCCATCTGGCCTCTGAAGTGCAA : 81
Cobb 500 : CATGAAGACCTACTGAATTTAGTAGTGGGAGTGCCTGCGTTCTGGAATGATCCCCTGATCCATCTGGCCTCTGAAGTGCAA : 81
Avian 48 : CATGAAGACCTACTGAATTTAGTAGTGGGAGTGCCTGCGTTCTGGAATGATCCCCTGATCCATCTGGCCTCTGAAGTGCAA : 81

                *      100      *      120      *      140      *      160
Hubbard : AGAATCAAAGAAGCTCCAGATACCATTCTCTGGAAGGCTGTAGAGATTGAGGAGCAAAACAAGAGGCTTCTAGAAGGAATG : 162
Lohman : AGAATCAAAGAAGCTCCAGATACCATTCTCTGGAAGGCTGTAGAGATTGAGGAGCAAAACAAGAGGCTTCTAGAAGGAATG : 162
Cobb 500 : AGAATCAAAGAAGCTCCAGATACCATTCTCTGGAAGGCTGTAGAGATTGAGGAGCAAAACAAGAGGCTTCTAGAAGGAATG : 162
Avian 48 : AGAATCAAAGAAGCTCCAGATACCATTCTCTGGAAGGCTGTAGAGATTGAGGAGCAAAACAAGAGGCTTCTAGAAGGAATG : 162

                *      180
Hubbard : GAGAAAATAGTTGGGCGGGTA : 183
Lohman : GAGAAAATAGTTGGGCGGGTA : 183
Cobb 500 : GAGAAAATAGTTGGGCGGGTA : 183
Avian 48 : GAGAAAATAGTTGGGCGGGTA : 183
    
```

Figure 13. Amino acid sequences alignment of Prolactin gene, exon 4 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines showing complete homogeneity.

```

                *      20      *      40      *      60
Hubbard : CAT GAA GAC CTA CTG AAT TTA GTA GTG GGA GTG CTG CGT TCC TGG AAT GAT CCC CTG ATC : 60
           H E D L L N L V V G V L R S W N D P L I
Lohman : CAT GAA GAC CTA CTG AAT TTA GTA GTG GGA GTG CTG CGT TCC TGG AAT GAT CCC CTG ATC : 60
           H E D L L N L V V G V L R S W N D P L I
Cobb 500 : CAT GAA GAC CTA CTG AAT TTA GTA GTG GGA GTG CTG CGT TCC TGG AAT GAT CCC CTG ATC : 60
           H E D L L N L V V G V L R S W N D P L I
Avian 48 : CAT GAA GAC CTA CTG AAT TTA GTA GTG GGA GTG CTG CGT TCC TGG AAT GAT CCC CTG ATC : 60
           H E D L L N L V V G V L R S W N D P L I

                *      80      *      100      *      120
Hubbard : CAT CTG GCC TCT GAA GTG CAA AGA ATC AAA GAA GCT CCA GAT ACC ATT CTC TGG AAG GCT : 120
           H L A S E V Q R I K E A P D T I L W K A
Lohman : CAT CTG GCC TCT GAA GTG CAA AGA ATC AAA GAA GCT CCA GAT ACC ATT CTC TGG AAG GCT : 120
           H L A S E V Q R I K E A P D T I L W K A
Cobb 500 : CAT CTG GCC TCT GAA GTG CAA AGA ATC AAA GAA GCT CCA GAT ACC ATT CTC TGG AAG GCT : 120
           H L A S E V Q R I K E A P D T I L W K A
Avian 48 : CAT CTG GCC TCT GAA GTG CAA AGA ATC AAA GAA GCT CCA GAT ACC ATT CTC TGG AAG GCT : 120
           H L A S E V Q R I K E A P D T I L W K A

                *      140      *      160      *      180
Hubbard : GTA GAG ATT GAG GAG CAA AAC AAG AGG CTT CTA GAA GGA ATG GAG AAA ATA GTT GGG CGG : 180
           V E I E E Q N K R L L E G M E K I V G R
Lohman : GTA GAG ATT GAG GAG CAA AAC AAG AGG CTT CTA GAA GGA ATG GAG AAA ATA GTT GGG CGG : 180
           V E I E E Q N K R L L E G M E K I V G R
Cobb 500 : GTA GAG ATT GAG GAG CAA AAC AAG AGG CTT CTA GAA GGA ATG GAG AAA ATA GTT GGG CGG : 180
           V E I E E Q N K R L L E G M E K I V G R
Avian 48 : GTA GAG ATT GAG GAG CAA AAC AAG AGG CTT CTA GAA GGA ATG GAG AAA ATA GTT GGG CGG : 180
           V E I E E Q N K R L L E G M E K I V G R

Hubbard : GTA : 183
           V
Lohman : GTA : 183
           V
Cobb 500 : GTA : 183
           V
Avian 48 : GTA : 183
           V
    
```

Figure 14. Nucleotide sequences alignment of Prolactin gene, exon 5 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines shows two SNPs in Hubbard F15 chicken line; one non-synonymous single nucleotide polymorphism at the position 7921 (CCT/TCT-transition) and one synonymous single nucleotide polymorphism at the position 8187 T/C, where identical bases highlighted in black and polymorphic variations highlighted in gray.

```

*          20          *          40          *          60          *          80
Hubbard : CATCTGGTGTGCTGGAAATGAAATTTACTCTCCTGACGGCCCTCCATCCCTGCAACTCGCTGATGAGGACTCCAGA : 81
Lohman  : CATCTGGTGTGCTGGAAATGAAATTTACTCTCCTGACGGCCCTCCATCCCTGCAACTCGCTGATGAGGACTCCAGA : 81
Cobb 500 : CATCTGGTGTGCTGGAAATGAAATTTACTCTCCTGACGGCCCTCCATCCCTGCAACTCGCTGATGAGGACTCCAGA : 81
Avian 48 : CATCTGGTGTGCTGGAAATGAAATTTACTCTCCTGACGGCCCTCCATCCCTGCAACTCGCTGATGAGGACTCCAGA : 81

*          100         *          120         *          140         *          160
Hubbard : CTCTTTGCTTTTATAACCTGCTGCATTGCCTCCGCAGAGATFCCACAAAAATTGACAACATCTTTAAAGTTTGAAGTGC : 162
Lohman  : CTCTTTGCTTTTATAACCTGCTGCATTGCCTCCGCAGAGATFCCACAAAAATTGACAACATCTTTAAAGTTTGAAGTGC : 162
Cobb 500 : CTCTTTGCTTTTATAACCTGCTGCATTGCCTCCGCAGAGATFCCACAAAAATTGACAACATCTTTAAAGTTTGAAGTGC : 162
Avian 48 : CTCTTTGCTTTTATAACCTGCTGCATTGCCTCCGCAGAGATFCCACAAAAATTGACAACATCTTTAAAGTTTGAAGTGC : 162

*          180         *          200         *          220         *          240
Hubbard : CGCCTAATCCATGATAGCAATTGCTAAGTACCTGTGGGCTGCATTACTCAGTAAACCATTTCATCATGGTGTCTTGTGTC : 243
Lohman  : CGCCTAATCCATGATAGCAATTGCTAAGTACCTGTGGGCTGCATTACTCAGTAAACCATTTCATCATGGTGTCTTGTGTC : 243
Cobb 500 : CGCCTAATCCATGATAGCAATTGCTAAGTACCTGTGGGCTGCATTACTCAGTAAACCATTTCATCATGGTGTCTTGTGTC : 243
Avian 48 : CGCCTAATCCATGATAGCAATTGCTAAGTACCTGTGGGCTGCATTACTCAGTAAACCATTTCATCATGGTGTCTTGTGTC : 243

*          260         *          280         *          300         *          320
Hubbard : TTGCCACTTTTCACTGCGAACTTTAGGAAAAACCACATTGTACAGCACCAGGATCATCAGTAACTTTCAGGCATGCTTGT : 324
Lohman  : TTGCCACTTTTCACTGCGAACTTTAGGAAAAACCACATTGTACAGCACCAGGATCATCAGTAACTTTCAGGCATGCTTGT : 324
Cobb 500 : TTGCCACTTTTCACTGCGAACTTTAGGAAAAACCACATTGTACAGCACCAGGATCATCAGTAACTTTCAGGCATGCTTGT : 324
Avian 48 : TTGCCACTTTTCACTGCGAACTTTAGGAAAAACCACATTGTACAGCACCAGGATCATCAGTAACTTTCAGGCATGCTTGT : 324

*          340         *          360         *          380         *          400
Hubbard : GTGAATCTGGCTGCAACTATTAGTGCCATTTATCTTGGTGTTTAAATGCTGTAATCACTTGTGTAACAAGAATAAACTT : 405
Lohman  : GTGAATCTGGCTGCAACTATTAGTGCCATTTATCTTGGTGTTTAAATGCTGTAATCACTTGTGTAACAAGAATAAACTT : 405
Cobb 500 : GTGAATCTGGCTGCAACTATTAGTGCCATTTATCTTGGTGTTTAAATGCTGTAATCACTTGTGTAACAAGAATAAACTT : 405
Avian 48 : GTGAATCTGGCTGCAACTATTAGTGCCATTTATCTTGGTGTTTAAATGCTGTAATCACTTGTGTAACAAGAATAAACTT : 405

T

*          420
Hubbard : TTCTGTCTAATTCC : 420
Lohman  : TTCTGTCTAATTCC : 420
Cobb 500 : TTCTGTCTAATTCC : 420
Avian 48 : TTCTGTCTAATTCC : 420

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DISCUSSION:

Among the genes that have significant effects on egg production is the PRL gene (Reddy *et al.*, 2002 and Wang *et al.*, 2011). Consistent research on this gene, which is involved in many physiological pathways, contributes to understanding the molecular basis of useful production traits. Thus, the aim of this study was to correlate the polymorphism of prolactin gene with egg production trait in chickens. The use of this molecular genetic marker potentially will greatly increase the intensity of selection and will most effectively uncover the productive potential of birds. Additionally, studies of candidate genes and their effect on the phenotypic manifestations of interest to researchers are the basis for MAS (Kulibaba and Podstreshnyi, 2012).

The avian PRL gene consists of five exons and four introns (Au and Leung, 2002), results of this

study revealed that molecular size of the five exons in the four chicken lines under the study was 74bp, 180bp, 108bp, 183bp, and 420bp respectively.

The avian PRL gene is highly conserved and most sequence polymorphisms in the cPRL gene occur in 5' flanking region, 3' flanking region, and the coding region of the signal peptide (Kansaku *et al.*, 2008). Most of the scientific researchers focused on polymorphisms in 5' flanking region (promoter region) of cPRL gene as the 5' flanking region has been considered as an excellent experimental model for studying both tissue-specific and hormonally regulated activation of gene transcription (Elsholtz *et al.*, 1991). However, in this study, the SNPs of the five exons of cPRL gene in four commercial chicken lines namely Hubbard F15, Lohman, Cobb 500, and Avian 48 were screened. The aligned nucleotide and amino acid sequences of exons

Figure 15. Amino acid sequences alignment of Prolactin gene, exon 5 in Hubbard, Lohman, Cobb 500, and Avian 48 chicken lines shows one amino acid change at positions 169 (P/S).

			*		20		*		40		*		60										
Hubbard	:	CAT	CCT	GGT	GAT	GCT	GGA	AAT	GAA	ATT	TAC	TCT	CAC	TGG	GAC	GGC	CTT	CCA	TCC	CTG	CAA	:	60
		H	P	G	D	A	G	N	E	I	Y	S	H	W	D	G	L	P	S	L	Q		
Lohman	:	CAT	TCT	GGT	GAT	GCT	GGA	AAT	GAA	ATT	TAC	TCT	CAC	TGG	GAC	GGC	CTT	CCA	TCC	CTG	CAA	:	60
		H	S	G	D	A	G	N	E	I	Y	S	H	W	D	G	L	P	S	L	Q		
Cobb 500	:	CAT	TCT	GGT	GAT	GCT	GGA	AAT	GAA	ATT	TAC	TCT	CAC	TGG	GAC	GGC	CTT	CCA	TCC	CTG	CAA	:	60
		H	S	G	D	A	G	N	E	I	Y	S	H	W	D	G	L	P	S	L	Q		
Avian 48	:	CAT	TCT	GGT	GAT	GCT	GGA	AAT	GAA	ATT	TAC	TCT	CAC	TGG	GAC	GGC	CTT	CCA	TCC	CTG	CAA	:	60
		H	S	G	D	A	G	N	E	I	Y	S	H	W	D	G	L	P	S	L	Q		
			*		80		*		100		*		120										
Hubbard	:	CTC	GCT	GAT	GAG	GAC	TCC	AGA	CTC	TTT	GCT	TTT	TAT	AAC	CTG	CTG	CAT	TGC	CTC	CGC	AGA	:	120
		L	A	D	E	D	S	R	L	F	A	F	Y	N	L	L	H	C	L	R	R		
Lohman	:	CTC	GCT	GAT	GAG	GAC	TCC	AGA	CTC	TTT	GCT	TTT	TAT	AAC	CTG	CTG	CAT	TGC	CTC	CGC	AGA	:	120
		L	A	D	E	D	S	R	L	F	A	F	Y	N	L	L	H	C	L	R	R		
Cobb 500	:	CTC	GCT	GAT	GAG	GAC	TCC	AGA	CTC	TTT	GCT	TTT	TAT	AAC	CTG	CTG	CAT	TGC	CTC	CGC	AGA	:	120
		L	A	D	E	D	S	R	L	F	A	F	Y	N	L	L	H	C	L	R	R		
Avian 48	:	CTC	GCT	GAT	GAG	GAC	TCC	AGA	CTC	TTT	GCT	TTT	TAT	AAC	CTG	CTG	CAT	TGC	CTC	CGC	AGA	:	120
		L	A	D	E	D	S	R	L	F	A	F	Y	N	L	L	H	C	L	R	R		
			*		140		*		160		*		180										
Hubbard	:	GAT	TCC	CAC	AAA	ATT	GAC	AAC	TAT	CTT	AAA	GTT	TTG	AAG	TGC	CGC	CTA	ATC	CAT	GAT	AGC	:	180
		D	S	H	K	I	D	N	Y	L	K	V	L	K	C	R	L	I	H	D	S		
Lohman	:	GAT	TCC	CAC	AAA	ATT	GAC	AAC	TAT	CTT	AAA	GTT	TTG	AAG	TGC	CGC	CTA	ATC	CAT	GAT	AGC	:	180
		D	S	H	K	I	D	N	Y	L	K	V	L	K	C	R	L	I	H	D	S		
Cobb 500	:	GAT	TCC	CAC	AAA	ATT	GAC	AAC	TAT	CTT	AAA	GTT	TTG	AAG	TGC	CGC	CTA	ATC	CAT	GAT	AGC	:	180
		D	S	H	K	I	D	N	Y	L	K	V	L	K	C	R	L	I	H	D	S		
Avian 48	:	GAT	TCC	CAC	AAA	ATT	GAC	AAC	TAT	CTT	AAA	GTT	TTG	AAG	TGC	CGC	CTA	ATC	CAT	GAT	AGC	:	180
		D	S	H	K	I	D	N	Y	L	K	V	L	K	C	R	L	I	H	D	S		
			*		200		*		220		*		240										
Hubbard	:	AAT	TGC	TAA	GTACCTGTGGGCTGCATTACTCACTGAAACCATT	CATCATGGTGTTC	TGT	:	240														
		N	C	*																			
Lohman	:	AAT	TGC	TAA	GTACCTGTGGGCTGCATTACTCACTGAAACCATT	CATCATGGTGTTC	TGT	:	240														
		N	C	*																			
Cobb 500	:	AAT	TGC	TAA	GTACCTGTGGGCTGCATTACTCACTGAAACCATT	CATCATGGTGTTC	TGT	:	240														
		N	C	*																			
Avian 48	:	AAT	TGC	TAA	GTACCTGTGGGCTGCATTACTCACTGAAACCATT	CATCATGGTGTTC	TGT	:	240														
		N	C	*																			
			*		260		*		280		*		300										
Hubbard	:	TGCTTTGCCACTTTTCACTGCGAACTTTTACGAAAAACCACATTGTACAGCACCAGGATCA	:	300																			
Lohman	:	TGCTTTGCCACTTTTCACTGCGAACTTTTACGAAAAACCACATTGTACAGCACCAGGATCA	:	300																			
Cobb 500	:	TGCTTTGCCACTTTTCACTGCGAACTTTTACGAAAAACCACATTGTACAGCACCAGGATCA	:	300																			
Avian 48	:	TGCTTTGCCACTTTTCACTGCGAACTTTTACGAAAAACCACATTGTACAGCACCAGGATCA	:	300																			
			*		320		*		340		*		360										
Hubbard	:	TCAGTAACTTTTCAGGCATGCTTGTGTGAATTCTGGCTGCAACTATTAGTGCCATTTATCT	:	360																			
Lohman	:	TCAGTAACTTTTCAGGCATGCTTGTGTGAATTCTGGCTGCAACTATTAGTGCCATTTATCT	:	360																			
Cobb 500	:	TCAGTAACTTTTCAGGCATGCTTGTGTGAATTCTGGCTGCAACTATTAGTGCCATTTATCT	:	360																			
Avian 48	:	TCAGTAACTTTTCAGGCATGCTTGTGTGAATTCTGGCTGCAACTATTAGTGCCATTTATCT	:	360																			
			*		380		*		400		*		420										
Hubbard	:	TGGTGTTTAAATGCTGTAAATCACTTGTGTAAACAAGAATAAACTTTTCTGTCTAATTTCC	:	420																			
Lohman	:	TGGTGTTTAAATGCTGTAAATCACTTGTGTAAACAAGAATAAACTTTTCTGTCTAATTTCC	:	420																			
Cobb 500	:	TGGTGTTTAAATGCTGTAAATCACTTGTGTAAACAAGAATAAACTTTTCTGTCTAATTTCC	:	420																			
Avian 48	:	TGGTGTTTAAATGCTGTAAATCACTTGTGTAAACAAGAATAAACTTTTCTGTCTAATTTCC	:	420																			

1, exon 3, and exon 4 shows complete homogeneity, no SNPs were located. On the other hand, **Yan *et al.* (2011)** reported one silent polymorphic site (a G→A mutation at the position of the 6017 bp) in exon 4 of Shiqiza Chicken with no change in amino acid sequence. Furthermore, **Wu *et al.* (2008)** detected two SNPs (T-3777-C and A-3785-G) in exon 4 of Muscovy duck which have a significant effect on broodiness behavior.

The nucleotide Sequences alignment of cPRL gene exons 2 shows one synonymous SNP at the position 3838 (ACC/ACT-transition) in Hubbard F15 chicken line with no effect on the coded amino acid sequences and the location far from splicing sites (donor, acceptor, and branch site). The same results were recorded by both **Ding-guo *et al.* (2010)** who studied the correlation analysis between the polymorphism of the exon 2 and egg-laying and broodiness traits in Shiqiza Chicken, and **Fu-wei *et al.* (2014)** who studied the Exon 2 SNP Detection of PRL Gene and its Associations with Egg Production Traits in Wenshang Barred Chicken. Both results indicate the presence of a C/T mutation at the position of the 3838 bp of exon 2. However, in their study, this SNP was non-synonymous and lead to an amino acid changed from Leucine (Leu) to Phenylalanine, but with no significant difference in egg production. In spite of that, the results of other experiments on chickens showed a significant association between SNPs in exon 2 and body weight at hatch, age at sexual maturity (**Rashidi *et al.*, 2012**).

Concerning with SNPs in exon 5, the aligned nucleotide sequences of exon 5 in the four chicken lines under this study shows two SNPs in Hubbard F15 chicken line; one non-synonymous SNP at the position 7921 bp (CCT/TCT-transition) which result in substitution of amino acid Ser with amino acid Pro that differs in chemical and physical characters from serine. Ser is one of the Polar amino acids that may participate in hydrogen bonds while Pro belongs to hydrophobic amino acids that normally buried inside the protein core (**Salam Al Karadaghi, 2015**).

The results of **Rashidi *et al.* (2012)** showed a significant association between SNPs in exon 5 and egg number. Furthermore, **Erehehuara (2003)** reported two SNPs in exon 5 of cPRL gene which were located at 8052 bp (G/C) and 8113 bp (T/C), the 8052 bp SNP (T/C) located in the coding region of the gene, but does not change the amino acid sequence. The geno-

typing results of these two SNPs in Recessive White and Qingyuan Partridge chicken breeds done by **Li *et al.* (2013)** suggested that there were significant associations between T8052C and G8113C genotypes of PRL gene and the egg production traits of AFE and EN 300 concluding that there may be a relationship between these two SNP sites and egg production traits in chickens

The other SNP detected in Exon 5 of Hubbard F15 chicken line was a synonymous SNP at the position 8187 which result in C-T transition. Adenine-Thymine in DNA conformation has two hydrogen bridges (**Muladno, 2010**). Hydrogen bond is non-covalent interaction which has small free energy 2-6 kJ/mole in water so the strength of bridge is weak and easy to cleavage and reunited (**Petsko and Ringe, 2004**). It gives weaker bond dissociation than Cytosin - Guanine which has three hydrogen bridges.

In conclusion, genetic improvement of farm animals, including poultry, is generally aimed at maximizing economic performance and production traits, especially in poultry breeders and table egg producing hens. The rapid development of molecular genetics has enabled scientists to obtain vast amounts of new genomic information that allows for improved estimation of an animal's genetic and breeding value with greater accuracy. A special role is played by individual genes, in particular the PRL gene which considered as a candidate marker for the egg production trait in poultry.

Compliance with Ethical Standards:

Author Mohamed, M. M. Osman declares that he has no conflict of interest. Author Shaaban A. Hameda declares that he has no conflict of interest, Author Abeer A.I. Hassanin declares that she has no conflict of interest, Author Walaa A. Hussein declares that she has no conflict of interest.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. And the protocol of this study was approved by the scientific research and bio-ethics committee at the Faculty of Veterinary Medicine, Suez Canal University (Permit Number: 201518). ■

Table 2. Least square means and their standard errors for main effects of Breed, Peak and SNP on egg production traits (total egg production, egg production per hen per week, egg production per hen per day, hen housed, hen day) and mortality rate in the four chicken lines under the study.

Mortality rate	Hen day	Hen housed	Egg/ hen/ D	Egg/ hen/ wk	Total egg production	Effects
P value = 0.008	P value = 0.0001	P value = 0.0001	P value = 0.0001	P value = 0.0001	P value = 0.0001	Breed
17.85 ^c ±0.42	0.74 ^b ±0.02	5.20 ^b ±0.11	0.76 ^b ±0.02	5.29 ^b ±0.11	74597 ^c ±1633	Hubbard
113.11 ^a ±2.94	0.87 ^a ±0.01	6.08 ^a ±0.04	0.89 ^a ±0.01	6.21 ^a ±0.03	434095 ^a ±2885	Lohmann
51.19 ^b ±2.87	0.68 ^c ±0.03	4.74 ^c ±0.24	0.71 ^b ±0.04	4.96 ^b ±0.25	68519 ^c ±3520	Cobb
65.19 ^b ±27.16	0.58 ^d ±0.02	4.06 ^d ±0.17	0.61 ^c ±0.02	4.27 ^c ±0.17	88597 ^b ±3687	Avian
P value = 0.030	P value = 0.0001	P value = 0.0001	P value = 0.0001	P value = 0.0001	P value = 0.0001	Peak of lay
36.31 ^b ±7.02	0.61 ^b ±0.07	4.25 ^b ±0.49	0.61 ^c ±0.07	4.26 ^c ±0.49	136644 ^c ±39893	Before
67.90 ^a ±10.82	0.76 ^a ±0.01	5.34 ^a ±0.08	0.79 ^a ±0.01	5.50 ^a ±0.08	176406 ^a ±18496	At
60.40 ^a ±9.39	0.64 ^b ±0.03	4.50 ^b ±0.19	0.68 ^b ±0.03	4.77 ^b ±0.18	154467 ^b ±34618	After
P value = 0.0002	P value = 0.10	P value = 0.07	P value = 0.06	P value = 0.41	P value = 0.012	SNP
76.49 ^a ±9.51	0.71 ^a ±0.02	4.96 ^a ±0.14	0.74 ^a ±0.02	5.15 ^a ±0.13	197071 ^a ±18859	0
17.85 ^b ±0.42	0.74 ^a ±0.02	5.20 ^a ±0.11	0.76 ^a ±0.02	5.29 ^a ±0.11	74597 ^b ±1633	1

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