Association of Single Nucleotide Polymorphism in *Melanocortin Receptor* Gene with Egg Production Traits in Lohmann Brown Chickens

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Abstract

Melanocortin receptor (MC4R) plays an important role in central melanocortin system and regulation of feed intake in birds. In this study the melanocortin gene (MC4R) was proposed as a candidate gene for egg production traits (yield and weight) in Lohmann Brown hens. DNA from blood samples was extracted to amplify the MC4R gene and the purified PCR products were sequenced. Alignment of sequence data from each group revealed that there is a variation detected in MC4R-1 at nucleotide 22 (T-G) (sense mutation) for high egg weight. Hens with the AB genotype produced significantly higher egg weight compared to hens with the AA genotype. There was no significant effect of this mutation on egg yield. The detected mutation and the analysis of egg production means revealed a significant association between MC4R polymorphism and egg weight. The MC4R-SNP could be considered as a useful marker in chicken selection especially for egg weight.

Keywords: Lohmann Brown, MC4R, Egg weight, SNP, Sense mutation

Lohmann Kahverengi Tavuklarda *Melanocortin Reseptörü* Geninde Tek Nükleotid Polimorfizmi İle Yumurta Verim Özellikleri Arasındaki İlişki

Özet

Melanocortin reseptörü (MC4R) merkezi melanocortin sisteminde ve kuşlarda yem tüketiminin düzenlenmesinde önemli rol oynar. Bu çalışmada melanocortin geninin (MC4R) Lohmann Kahverengi tavuklarda yumurta verim özellikleri (sayı ve kilo) üzerine etkili bir gen olabileceği düşünülmüştür. MC4R genini amplifiye etmek amacıyla kan örneklerinden DNA ekstrakte edildi ve PCR ürünlerinin sekanslaması yapıldı. Her bir grupta sekans verisinin dizilimi MC4R-1'in nükleotid 22'sinde (T-G) (Sense mutasyon) yüksek yumurta ağırlığı için varyasyon bulunduğunu ortaya koydu. AB genotipine sahip tavuklar AA genotipine sahip tavuklar ile karşılaştırıldığında istatistiki olarak daha yüksek yumurta ağırlığına sahiptiler. Bu mutasyonun yumurta sayısı üzerine bir etkisi bulunmamaktaydı. Tespit edilen mutasyon ve ortalama yumurta üretimi analizi MC4R polimorfizmi ile yumurta ağırlığı arasında anlamlı bir ilgi bulunduğunu gösterdi. MC4R-SNP özellikle yumurta ağırlığı bakımından önemli bir belirteç olarak kullanılabilir.

Anahtar sözcükler: Lohmann Kahverengi, MC4R, Yumurta ağırlığı, SNP, Sense mutasyon

INTRODUCTION

Understanding the associations between genotype and phenotype is very important for faster improvement in animal breeding with potential economic benefits. Results inferred from molecular genetic studies play an important role in breeding value prediction systems and in the construction of commercial lines and populations. In the 20th century, strong selection of production traits started when commercial breeds were selected for egg and meat production [1]. Selection programs based on productive traits have been of major importance to the poultry industry. Amie-Marini et al.[2] reported that single nucleotide polymorphism (SNP) is an effective method to detect nucleotide sequence mutation in amplified DNA. The investigation strategy for a specific favorable SNP involves a novel and lengthy process of the identification of the DNA molecular marker for a major effect gene. Holsinger and Weir [3] revealed the importance of discovery a large number of SNPs in the genomes from several species that has enabled exploration of genome-wide signatures in selection via an assessment of variation in marker allele frequencies

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among these populations. Genes associated with productive traits have been identified using single nucleotide polymorphisms of many candidate genes [4,5]. It is recognized that the egg production traits of chickens are controlled by a complicated multiple genes [6].

Melanocortin 4 receptor (MC4R) is a protein expressed in the hypothalamus in humans and it has been found to be involved in feed intake, the regulation of metabolism and body weight [7]. Mutations of the MC4R gene were associated with the appetite and growth in many animal species [8]. According to the results of El-Sabrout [9] on rabbit, MC4R gene has many important behavioral and growth functions. Moreover, the mutations of MC4R gene have been found association with carcass quality in cattle [10], and broiler chickens [11].

Few researches have been published to improve the effect of MC4R mutations on egg production traits in chickens. Therefore, the present study was carried out to investigate the association between MC4R gene and the egg production traits (yield and weight) in Lohmann Brown chickens.

MATERIAL and METHODS

Animal, Housing and Feeding Management

This experiment was carried out on 200 Lohmann Brown hens (11 months of age). Hens were divided into two groups according to their egg weight (120 hens for control egg weight (AA avg. 57.7 g), 70 hens for high egg weight (AB avg. 60.4 g) and 10 hens for low egg weight (BB avg. 55.1 g)). Birds were housed in single cages with an intensive system and were offered *ad libitum* access to fed commercial pelleted diet (18% protein and 2800 Kcal/kg). The study was approved by Alexandria University Animal Ethics Committee (2016).

Molecular Analysis

DNA was extracted from whole blood by taking random blood samples (20 samples per group) from the wing vein in centrifuge tubes containing EDTA as anticoagulant using DNA isolation kit (Zymo® Research, USA) following the manufacturer's protocol and stored at -20°C until used. Two different primers from MC4R gene: MC4R-1 (500 bp) and MC4R-2 (492 bp) [12] were investigate from Biosearch Technologies (USA) to generate PCR profiles from DNA samples *(Table 1)*. The total PCR reaction volume was 25

Table 1. List of the PCR primers used in this study and their sequences	
Primer Code	**Nucleotide Sequence (5'-3')**
MC4R-1	F 5'- CAACCCCAGTTACCAGCACT-3' R 5'- GCATTGCTGTGCAGTCCATA-3'
MC4R-2	F 5'- CCATTGCAGTGGACAGGTATT-3' R 5'- TCCGGAGTGCATAAATGAGA-3'

μL, included 3 μL of genomic DNA of each group, 1 μL of each primer, 15 μL of 2× Thermo Multiplex PCR Master Mix and 6 μL of RNase-free water. PCR program was performed using a thermal cycler (Thermo® Scientific Corporation, EU) included three main steps: initial denaturation at 95°C for 5 min, followed by 35 cycles, denaturation at 95°C for 30 sec; annealing at 45°C for 30 sec and lasted by extension at 72°C for 1 min then an extension cycle at 72°C for 8 min. Amplicones were separated on 1.5% agarose gel, stained with Ethidium Bromide and visualized under UV Transilluminator. GelAnalyzer application [13] was used to analysis the determined DNA bands on the agarose gel. The amplified DNA fragments of growth genes were digested with *MSP1*. The RFLP was carried out on PCR product according to Zhou et al.[14]. The purified DNA was sequenced using the automated sequencer by Macrogene Company (South Korea). Sequence analysis and alignment were carried out using the CodonCode Aligner software (http:// www.codoncode.com/aligner).

Egg Production Traits

Data of egg yield (%) and egg weight (g) (n=50 samples per group) were recorded daily during the experiment period (6 months). Egg weight of all eggs was determined with digital balance (g).

Statistical Analysis

Statistics of means differences in egg and weight production between the two groups were determined by ANOVA followed by Duncan´s multiple range test using SPSS [15]. The association between the genotypes of MC4R gene and the egg production traits were analyzed by the means and standard errors method as applied in the General Linear Model (GLM) procedure of SPSS [15] according to the following statistical model:

$$Y_{ijm} = \mu + G_i + EY_j + e_{ijm}$$

Where Y is the dependent variable (egg weight), μ is the overall mean of observations, G is the fixed genotype effect, EY (egg yield) is the covariate, and e is the residual error.

RESULTS

All primers were amplified and yielded distinct polymorphic PCR profiles at molecular weight ranged from 210 to 370 bp. RFLP analysis of PCR product using *MSP1* did not produce restriction fragments. The results of MC4R-1 electrophoresis showed three genotypes (AA, AB and BB) with frequencies of 0.60, 0.35 and 0.05, respectively *(Table 2)*. The frequency of allele A was 0.78 while the frequency of allele B was 0.22. It means that allele A was dominant in Lohmann Brown hens. The purified PCR products were sequenced in those had the highest and lowest egg weight. Alignment of sequence data of 15 samples from

Table 2. *Genotype and allele frequencies of MC4R-1 in Lohmann Brown*

Number of Hens	Genotype Frequency (n)			Allele Frequency	
	AA	AB	BB	A	B
200	0.60 (120)	0.35 (70)	0.05 (10)	0.78	0.22

Table 3. *Means and standard errors (Mean± SEM) of egg yield and egg weight for Lohmann Brown*

Genotypes	N	Egg Yield (%)	Egg Weight (g)
AA	120	87.02±1.72	57.70±0.41[b]
AB	70	86.65±1.61	60.43±0.38[a]
BB	10	87.40±1.33	55.10±0.30[c]
Significance	-	NS	*

a,b,cMeans in the same column with different superscripts are significantly different (P<0.05). NS= not significant at (P<0.05); AA = control egg weight hens; AB = high egg weight hens; BB = low egg weight hens

Table 4. *Association between MC4R SNP and egg weight in Lohmann Brown*

SNPs	Trait	Genotypes		P-value
		AA	AB	
MC4R-1	Egg weight (g)	57.70±0.41[b]	60.43±0.38[a]	0.03

Values are presented by the means and standard errors (Mean ± SEM); a,bMeans in the same row with different superscripts are significantly different (P<0.05)

each group revealed that there is a mutation detected in MC4R-1 at nucleotide 22 (T-G) (sense mutation) for high egg weight, while there was no variation detected in MC4R-2. Means and standard errors (Mean±SEM) of egg yield and egg weight shown in *Table 3*. AB genotype had significantly (P<0.05) higher egg weight compared with AA. On the other hand, there was no significant difference detected between the two groups in egg yield. MC4R gene showed significant (P<0.05) association with egg weight in Lohmann strain *(Table 4)*. The results of SNP polymorphisms demonstrate the possibility to detect association between egg weight in Lohmann chickens and the efficiency of the used primers to predict through the genetic specificity using the single nucleotide polymorphism of MC4R.

DISCUSSION

Many previous studies were interested to investigate the relationship between the melanocortin-4 receptor gene (MC4R) and the body weight of animal. To determine whether there was an association of MC4R polymorphism with egg production traits in chickens, this study was carried to test two parts of MC4R gene (MC4R-1, MC4R-2) as candidate gene for Lohmann Brown hens (commercial layers strain). The frequency of heterozygous genotype (AB) has higher egg weight compared to homozygous (AA) and (BB) genotypes. Also, one sense mutation was identified in high egg weight group (AB). Therefore, it may be assumed that the MC4R-1 gene affected egg weight by regulating of appetite of hens. Analyses of MC4R SNPs and egg weight records showed significant association of MC4R genotypes with egg weight *(Table 4)*.

Davies et al.[9] reported that sense mutation can change the gene expression, which in turn a different protein with different characterizes is created as a result of amino acids change. This protein may lose its function or become activated or exhibit a new function. It is possible that the variation happened in amino acids due to the MC4R mutations causes a significant change of the MC4R function. Amino acids change may also affect the biosynthesis of other nutrients. It can stimulate the feed intake, metabolism and growth of egg, which in turn affect the egg weight. This finding is in agreement with El-Sabrout and Aggag [17], who found that MC4R plays an area responsible for controlling feed intake behavior, which in turn affect the body weight.

In addition, the effect of MC4R in chicken's egg weight suggests it may be an important genetic marker for the production-related traits. The hens within hetero-zygous genotype (AB) at the MC4R gene loci had superior egg production traits. This finding is useful to get commercial egg production chickens with superior production traits. Therefore, this study aims also to enhance selection efficiency on hen productive performance. The use of marker-assisted selection can augmented the efficient genetic improvement in these quantitative traits [18]. More-over, MC4R SNPs located at candidate genes for economic traits allow prediction of the genetic merit of individuals and combined with guarantee consumer protection.

According to the results of this study, there is a significant association between MC4R gene polymorphism and egg weight in Lohmann Brown chickens. The results of single nucleotide polymorphism demonstrate the efficiency of used associated genes to predict through the genetic specificity. The results are also effective in chicken selection for high egg weight without affecting the egg yield. MC4R-1 SNP was potential useful DNA marker for selecting excellent individuals in marker-assist selection (MAS) breeding in relation to egg production traits in chickens. Further studies through expanded and different sampling with more details under various molecular levels will be required to provide clearer explanations.

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