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Original scientific paper

POLYMORPHISM OF THE MELANOCORTIN RECEPTOR GENE AND ITS ASSOCIATION WITH EGG PRODUCTION TRAITS IN LOHMANN BROWN CHICKENS

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The melanocortin gene (MC4R) was proposed as a candidate gene in this study for egg production traits (yield and weight) in Lohmann Brown hens. Two different primers from MC4R gene (MC4R-1 and MC4R-2) were investigated. 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 in MC4R-1 at nucleotide 22 (T-G) (sense mutation) for high egg weight. Hens with the AB genotype produced significantly ($P<0.05$) higher egg weight compared to hens with the AA and BB genotypes. There was no significant ($P>0.05$) effect of this mutation on egg yield. There was no variation detected in MC4R-2. 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: egg weight, Lohmann chickens, MC4R, sense mutation, SNP

INTRODUCTION

The egg production is considered as one of the most important economic traits in the poultry industry. Association studies of the identified single nucleotide polymorphism (SNP) in the genes correlated with productive traits have resulted in understanding the normal differences between the individuals and this knowledge will be helpful for improvement of animal breeding

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with potential economic benefits (ATEYA *et al*., 2016). 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 (BURT, 2005). Selection programs based on productive traits have been of major importance to the poultry industry. AMIE-MARINI *et al*. (2012) 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 (2009) revealed that 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 among these populations. Genes associated with productive traits have been identified using single nucleotide polymorphisms of many candidate genes (ZHANG *et al*., 2013; WU *et al*., 2015). It is recognized that the egg production traits of chickens are controlled by a complicated multiple genes (GU *et al*., 2011).

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 (CHUN-YU and HUI, 2006). Mutations of the MC4R gene were associated with the appetite and growth in many animal species (SINHA *et al*., 2004). EL-SABROUT (2017) reported that MC4R gene has many important behavioral and growth functions on rabbit. Moreover, the mutations of MC4R gene have been found to be associated with carcass quality in cattle (ZHANG *et al*., 2009), and broiler chickens (WANG *et al*., 2009).

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.

MATERIALS AND METHODS

Animal, housing and feeding management

This experiment was carried out on 200 Lohmann Brown hens (11 months of age). The study was approved by Alexandria University Animal Ethics Committee (2016). 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).

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) (FONTANESI *et al*., 2013) were investigated from Biosearch Technologies (USA) to generate PCR profiles from DNA samples (Table 1). The total PCR reaction volume was 25 µl, including 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) and included three main steps: initial denaturation at 95°C for 5 minutes, followed by 35 cycles, denaturation at 95°C for 30 seconds; annealing at 45°C for 30 seconds and lasted by extension at 72°C for 1 minutes then an extension cycle at 72 °C for 8 minutes. Amplicons were separated on 1.5 % agarose gel, stained with Ethidium Bromide and visualized under UV Transilluminator. GelAnalyzer application (2010) 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*. (2005). 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).

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′

Egg production traits

Data on egg yield and egg weight (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 (2011). 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 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 SNP 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* didn't produce restriction fragments. The results of MC4R-1 electrophoresis showed three genotypes (AA, AB and BB) (Figure 1) 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 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 were shown in Table 3. AB genotype had significantly (P<0.05) higher egg weight compared with AA and BB genotypes. On the other hand, there was no significant (P>0.05) 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.

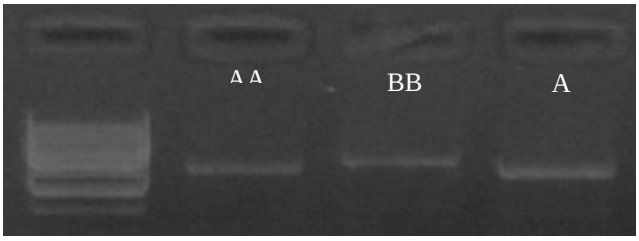

Figure 1. Gel profile of PCR amplification in *MC4R-1* gene.

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	*

[abc]Means 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.

DISCUSSION

Many previous studies were interested to investigate the relationship between the melanocortin-4 receptor gene (MC4R) and the body weights of animals. To determine whether there was an association of MC4R polymorphism with egg production traits in chickens, this study was carried out to test two parts of MC4R gene (MC4R-1 and 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).

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). [ab]Means in the same row with different superscripts are significantly different ($P<0.05$).

DAVIES *et al*. (2002) reported that sense mutation can change the gene expression, which in turn leads to a different protein with different characteristics as a result of amino acid change. This protein may lose its function or become activated or exhibit a new function. It is possible that this variation 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 affects the egg weight. This finding is in agreement with EL-SABROUT and AGGAG (2017), who found that MC4R plays an area responsible for controlling feed intake behavior, which in turn affects 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 heterozygous genotype (AB) at the MC4R gene locus 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 augment the efficient genetic improvement in these quantitative traits (ROTHSCHILD and PLASTOW, 1999). Moreover, MC4R SNPs located at candidate genes for economic traits allow prediction of the genetic merit of individuals and guarantee consumer protection.

CONCLUSION

The significant association of MC4R-1 polymorphism and egg weight in Lohmann Brown chickens was observed by this study. The results of single nucleotide polymorphism demonstrated the efficiency of the 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-assisted selection (MAS) breeding in relation to egg weight trait 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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POLIMORFIZAM MELANOKORTIN RECEPTOR GENA I NJEGOVA POVEZANOST SA PRODUKCIJOM JAJA KOD LOHMAN BRAON PILIĆA

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Izvod

Melanokortin gen (MC4R) je kandidat gen za osobine produkcije jaja (prinos i težina) kod Lohman braon pilića. Dva različita prajmera za MC4R gen (MC4R-1 i MC4R-2) su ispitana. DNK iz krvi je izolovana za amplifikaciju MC4R gena i prečišćeni PCR produkti su sekvencionirani. Podaci sekvencioniranja od svake grupe otkrivaju da postoji variranje u MC4R-1 u nukleotidu 22 (T-G) (sense mutacija) za veću težinu jaja. Pilići sa AB genotipom proizvode značajno teža jaja u odnosu na piliće sa AA i BB genotipom. Nema značajnog efekta (P>0.05) ove mutacije na prinos jaja. Nije detektovano variranje u MC4R-2. Otkrivena mutacija I analiza proseka produkcije jaja otkriva značajnu vezu između MC4R polimorfizma i težine jaja. MC4R-SNP se može smatrati korisnim markerom za selekciju pilića naročito za težinu jaja.

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