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# ASSOCIATION OF MC4R GENE POLYMORPHISMS WITH FEED INTAKE AND BODY WEIGHT IN LOCAL CHICKEN

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**ABSTRUCT : The Melanocortin 4 receptor gene plays an important role in the control of food intake, body weight and energy balance. The purpose of the present study was to analyze association of the MC4R gene with feed intake and body weight in local chicken. DNA from blood samples was extracted to amplify the MC4R gene and purified by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and investigated its association with economic traits, and statistical data were analyzed using SAS program. There was significantly affect ($p<0.05$) of the MC4R gene polymorphism on mean of the weekly live body weight and weekly feed intake in all weeks, the genotype CC recorder the highest mean followed by genotype GC and GG.**

***Key words :*** MC 4 gene polymorphism, chicken.

## INTRODUCTION

In the last few years, the poultry industry presented a significant increase in the animal production sector. Genetic improvement was the main factor that contributed for this important development of poultry production.In the 20th century, strong selection of production traits started when commercial breeds were selected for egg and meat production (Burt, 2005).

The candidate gene approach studies the relationship between the traits and known genes that may by associated with the physiological pathways underlying the traits (Liu *et al*, 2008). The gene or part of gene, are sequenced in a number of different animals, and any variation found in the DNA sequences, in tested for association with variation in the phenotypic trait (Koopaei and Koshkoiyeh, 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-Ya and Hui,2006). Mutation of the MC4R gene was associated with the appetite and growth in many animal species (Sinha *et al*, 2004). According to the results of El-Sabrout (2017) on rabbit, MC4R gene has many important behavioral and growth functions. Moreover, the mutation of MC4R gene have been found association with carcass quality in cattle (Zhang *et al*, 2009), and broiler chickens (Wang *et al*, 2009).

Melanocortins are peptide hormones derived from proopiomelanocortin (POMC), they have an important role in regulating melanocyte pigmntation and energy homeostasis (Boswell and Takeuchi, 2005). Now, all five melanocortin receptors (MC4Rs) have been isolated in chicken, each of the chicken MCR subtype has a different pattern of tissue expression of function. Recently, several studies in animal models suggest that MC3R and MC4R are essential in the regulation of feeding and energy homeostasis, respectively (Schwartz *et al*,2000).

Null mutations in the pro-opiomelanocortin (POMC) gene and the MC4R gene or overexpression of the melanocortin receptor antagonists agouti and agouti-related protein (AGRP) caused a sever obesity syndrome in mice and humans (Vaisse *et al*, 2000; Butler *et al*, 2000).

There are two basic methods of Quantitative trait loci (QTLs) identification: the candidate gene approach and whole genome linkage-disequilibrium scanning (Ikeobi *et al*, 2002; Kim *et al*, 2005). The candidate gene approach is a powerful method for finding QLTs responsible for genetic variation in the traits of interest in agricultural animal species and determining whether specific genes are related to economic traits in farm animals (Li *et al*, 2003). In Iraq, chicken meat is considered highly

preferable meat in many communities and due to lack of data regarding the genetic maps of local chicken, so this study was conducted to investigate the association of MC4R gene with feed intake and body weight in local chicken.

Many scientists have reported relationships between the MC4R gene and phenotypic traits the results obtained have contradicted each other in many cases (Meng *et al*, 2010; McLean and Schmutz, 2011). Polymorphism of the MC4R gene is associated with economic and growth traits (birth weight, average daily gain) (Zhang *et al*, 2009; Liu *et al*, 2010). An association between economic traits and the MC4R gene has been reported by (Seong *et al*, 2012).

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 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).

## MATERIAL AND METHODS

### Experimental animals

This study was conducted in the Poultry Research Station (20 km west of Baghdad) /State Board for Agricultural Researches/ Ministry of Agriculture. A total of 150 chickens were screened. Blood sample was drawn from wing vein and prepared for DNA isolation by using the procedure suggested by (Hoelzel, 1992). Each accession 2ml blood were taken as source of DNA a stored in K3 EDTA tubes and stored of -20 ºC.

### DNA primers

The primers sequences used in the PCR were presented in Table 1.

INtRON'sMaxime PCR Pre Mix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. Maxime PCR Pre Mix Kit (i- Taq) is the product what is mixed every component: i-Taq DNA polymerase, Dntpmixture, reaction buffer, and so on-in one tube for one rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has very components for PCR, so we can do PCR just add a template DNA, primer set, and D.W... The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

### Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to effect of Spot 14 and MC4R genes genotype in production and physiological parameters of Locale chickens. Least square means (General Linear Model procedure) and Duncan's (1955) Multiple Range test was used to significant compare between means, as well as extracting the distribution ratios of the herd and the frequency of the alleles obtained Chi-Square test of genes based on Hardy and Weinberg low; (Edwards, 2008). The statistical model was as follows:

$$Yil = \mu + Gi + eij$$

Where, Y*ij*: dependent variable; μ : overall mean; G*i*: Effect of genotype (AA-AB - BB of Spot 14 gene and GG- GC - CC of MC4R gene); and e*ij*: Error term.

## RESULTS AND DISCUSSION

### Amplification of the target region of Spot14 and MC4R genes

The target region of the target region of MC4R gene (741 bp), were extracted by using PCR technique by PCR kit, primers, samples of DNA and turn PCR program , and by using DNA marker (100-2000 bp) in first pore of gel, and put the gel in 1ãTBE buffer. The product was electrophoresis on agarose gel 2% at 5 volt/cm². 1ãTBE

**Table 1 :** The specific primer of MC4R gene.

| Primer | Sequence | Tm (ºC) | GC (%) | Product size |
|---|---|---|---|---|
| Forward | 5'-TGA CTC GGT GAT CTG TAG C-3' | 53.8 | 52.6 | 741 BP |
| Reverse | 5'-TTC ACT CCA TGC CCT ACA - 3' | 53.1 | 50 | |

buffer for 1:30 hours. N: DNA ladder 100.

**Distribution of genotype and allele frequency of MC4R gene inlocal chickens**

The results in Table 3 refer to the distribution of genotype of spot 14 gene in local chicken that showed significant differences (P<0.01) in three genotypes (GG, GC and CC). The genotype GG showed a high percentage reached to 66.95% following by the genotype CC in 23.73% percentage, while genotype GC showed a least percentage 9.32%, with frequencies of 0.72 and 0.28 respectively. The frequency of allele G was 0.72 while the frequency of allele C was 0.28. As the law of (Hardy Weinberg equilibrium). It means that the allele G was dominant in local chicken.

**Table 2 :** Distribution of genotype and allele frequency of MC4R gene in local chickens

| Genotype | No | Percentage (%) |
|---|---|---|
| GG (Wild) | 79 | 66.95 |
| GC (Heterozygous) | 11 | 9.32 |
| CC (Mutant) | 28 | 23.73 |
| Total | 118 | 100% |
| Chi-Square ($\div^2$) | —- | 122.186 ** |
| **Allele** | **Frequency** | |
| G | 0.72 | |
| C | 0.28 | |
| ** (P<0.01) | | |

Effect of Spot 14 gene on body weight showed (Table 3) significant differences (p<0.05) by GG genotype in all weeks of rearing that dominant on other genotypes, follow by GC genotype

The effect of Spot 14 gene on feed intake in local chicken (Table 4) showed significant differences (p<0.05) in all weeks as showed in Table 4.

Daviesetal (2002) 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

**Table 3 :** Relationship of the Genotype of MC4R gene with body weight.

| Body weight | Mean ± SE of body weight (gm) | | | Level of sig. |
|---|---|---|---|---|
| **Weeks** | **GG(No. = 79)** | **GC(No. = 11)** | **CC(No. = 28)** | |
| **Wt. at hatching** | 34.74 ± 0.67 a | 31.09 ± 1.65 b | 35.36 ± 1.16 a | * |
| **Week 1** | 54.01 ± 0.84 ab | 50.00 ± 2.37 b | 56.21 ± 1.39 a | * |
| **Week 2** | 96.44 ± 1.34 a | 87.45 ± 3.45 b | 99.46 ± 2.48 a | * |
| **Week 3** | 158.89 ± 2.08 a | 144.36 ± 4.95 b | 162.28 ± 4.66 a | * |
| **Week 4** | 238.37 ± 2.99 a | 217.73 ± 6.50 b | 243.07 ± 6.53 a | * |
| **Week 5** | 332.77 ± 4.03 a | 306.00 ± 7.77 b | 341.39 ± 8.65 a | * |
| **Week 6** | 441.58 ± 5.13 a | 409.27 ± 9.79 b | 453.11 ± 11.21 a | * |
| **Week 7** | 563.06 ± 6.26 a | 527.54 ± 11.35 b | 578.07 ± 13.47 a | * |
| **Week 8** | 697.78 ± 7.47 ab | 659.72 ± 13.86 b | 715.82 ± 16.03 a | * |

* (P<0.05)Means having with the different letters in same row differed significantly.

**Table 4 :** Relationship of the Genotype of MC4R gene with weekly feed intake.

| Feed intake- | Mean ± SE of FI (gm/bird) | | | Level of sig. |
|---|---|---|---|---|
| **FI : Weeks** | **GG(No. = 79)** | **GC(No. = 11)** | **CC(No. = 28)** | |
| **Week 1** | 39.00 ± 0.14 ab | 38.65 ± 0.37 b | 39.50 ± 0.23 a | * |
| **Week 2** | 75.22 ± 0.39 ab | 74.02 ± 0.80 b | 76.68 ± 0.67 a | * |
| **Week 3** | 125.29 ± 1.09 ab | 121.72 ± 2.21 b | 129.23 ± 1.89 a | * |
| **Week 4** | 181.11 ± 0.77 ab | 178.74 ± 1.66 b | 183.89 ± 1.32 a | * |
| **Week 5** | 230.06 ± 1.64 ab | 225.37 ± 3.71 b | 235.88 ± 2.75 a | * |
| **Week 6** | 277.15 ± 3.12 ab | 267.72 ± 6.73 b | 288.30 ± 5.30 a | * |
| **Week 7** | 326.22 ± 3.22 ab | 315.13 ± 6.24 b | 337.83 ± 5.70 a | * |
| **Week 8** | 419.15 ± 4.39 ab | 404.82 ± 8.89 b | 434.96 ± 7.62 a | * |
| **Total FI** | 1673.23 ±14.74 ab | 1626.20 ±30.45 b | 1726.30 ± 25.44 a | * |

* (P<0.05)Means having with the different letters in same row differed significantly.

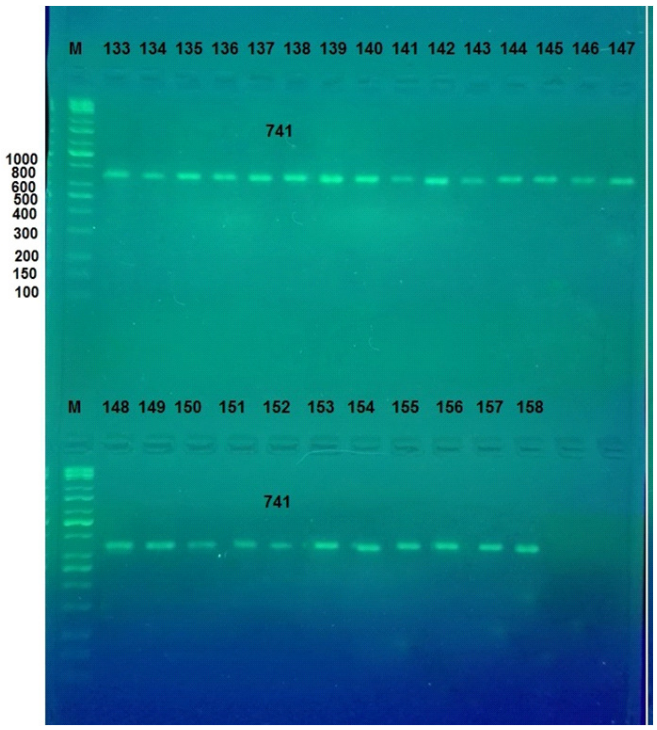


**Figure 1 :** PCR product the band 741bp. The product was electrophoresis on 2% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

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. This finding is agreement with El-Sabrout and Aggag (2017) who found that MC4R plays an area responsible for controlling feed intake behavior, which in turn affect the body weight.

Of the economically significant traits, improvements in body weight can be achieved through mass selection whereas feed conversion is relatively more difficult to improve. Selecting for body weight has been suggested as an effective method of indirectly improving feed conversion ratio. In modern chicken breeding operations, the selection for improved feed conversion ratio is a multi-stage process and is based on predefined traits, whereby individuals are primarily selected for higher growth and improved conformation from which only the best of all individuals are chosen for feed conversion ratio testing. In general, due to impracticability of measurements of feed conversion ratio on the entire population selection procedures are developed which measure feed conversion ratio on a sample of the population (Skinner-Noble and Teeter, 2004).

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