

Allele Distribution and Genetic Variation of the Leptin Gene in Laying Hens and its Relationship to Egg Production

Ahmed Yassin Taha¹ Esraa Mobasher Tawfeq²

¹Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Iraq

²Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Iraq

* Corresponding author E-mail: ahmed.24agp103@student.uomosul.edu.iq

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ABSTRACT. Egg production is a quantitative trait influenced by the interplay of multiple closely related genetic and physiological factors. Genes contribute to the regulation of hormonal functions and metabolism, playing a pivotal role in controlling egg production rates, continuity, and quality. This study aimed to determine the allelic distribution and genetic diversity of the leptin gene (LEPR) and to investigate its relationship to certain egg production traits in local laying hens. Fifty blood samples were collected from local hens, and DNA was extracted. Tetra-ARMS-PCR was used to determine genotypes and allele distribution. The results of molecular analysis showed the presence of three genotypes: AA, AC, and CC, with frequencies of 58%, 22%, and 20%, respectively. The frequency of the A allele was approximately 69%, compared to 31% for the C allele, indicating the dominance of the A allele in the experimental samples. A significant correlation was observed between genetic variation in the leptin gene, with the AC genotype resulting in the highest percentage of egg production (%HDP), while the AA genotype showed superiority in some traits related to egg weight and internal composition. No significant differences were recorded in most of the studied traits, except for some related to egg weight and shape index. The leptin gene plays a crucial role in regulating the productive performance of laying hens by influencing energy balance and physiological processes associated with reproductive activity. This highlights the potential use of the polymorphism of this gene as an assistive genetic marker in selection and genetic improvement programs, aiming to enhance egg production efficiency and improve the economic performance of local chicken flocks.

Keywords: DNA, Egg production, leptin gene, PCR, genetic.

1. INTRODUCTION

Egg production is one of the most important economic characteristics in the poultry industry. The success of breeding and genetic improvement programs depends heavily on increasing production efficiency and improving traits related to the reproductive performance of laying hens. With the significant advancements in molecular genetics techniques, it has become

possible to identify candidate genes associated with important production traits and utilize them in early genetic selection programs to enhance genetic responsiveness and improve production [1].

The leptin gene is one of the important candidate genes associated with regulating metabolism and energy balance in the bird's body. Leptin acts as a hormone regulating appetite, energy consumption, and nutrient allocation among various vital functions. It also plays a crucial role in regulating reproductive activity through its influence on the hypothalamus-pituitary-ovarian axis, making it one of the candidate genes influencing egg production traits and reproductive performance in laying hens [2, 3]. Recent studies have shown that leptin's role is not limited to regulating feed intake; it also extends to regulating the growth and development of ovarian follicles, as well as affecting ovulation and the continuation of egg production. [4] It was shown that leptin contributes to the activation of primary follicles in the chicken ovary by regulating the insulin-like growth factor system, thus confirming a direct relationship between this gene and the reproductive capacity of birds. Other studies have also indicated that leptin receptors in the ovary play a pivotal role in regulating follicular development and egg production by influencing hormonal pathways associated with ovarian activity. Single nucleotide polymorphisms (SNPs) are among the most important forms of genetic variation used in studying genetic differences between individuals, as these mutations can affect gene expression or the efficiency of the resulting protein, which in turn impacts productive and economic traits. In recent years, there has been increased interest in studying the relationship between polymorphisms in genes regulating energy and metabolism and egg production traits, due to the valuable information these studies provide that can be utilized in genetic marker-assisted selection programs. [5] Several studies have demonstrated a correlation between the genetic variation of certain candidate genes and egg production traits in laying hens. Study [6] showed a significant effect of some gene polymorphisms on productive traits and egg production Eggs.

2. MATERIALS AND METHODS

This study was conducted at the poultry farm of the Department of Animal Production, College of Agriculture and Forestry, University of Mosul, using 50 local laying hens. The birds were raised in individual cages under standardized management and feeding conditions throughout the experiment, with free access to feed and water. Production data were collected weekly and included egg weight, number of eggs laid, average egg production rate (%HDP), and some egg quality characteristics.

2.1 Blood sample collection

Blood samples were drawn from the pterygoid vein using tubes containing the anticoagulant EDTA. DNA was isolated from the blood of all samples included in the study using the modified method by [16].

2.2 Determining the genetic variation of the Leptin gene using Tetra-ARMS-PCR technology

The presence of the G A mutation at the (rs314805789) site of the Leptin gene was detected by adding 4 microliters (100 ng) of template DNA and 1 microliter (10 pmol) of each mutation-specific primer for the leptin gene, prepared by the Korean company Microgen, to the contents of a premix. Primers designed by the researcher using the primer 3 program were used, as shown in Table (1).

Table (1) Primers used to determine the genetic variation of the leptin gene using T-ARMS-PCR technology

Primer	Sequence	Band size	Melting temperature
F-inner primer (G allele):	TTTGAAAGAAATTGCTGCAATATTTACTG 401	373	63
:R- inner primer (A allele)	AACTGGAAAAAACCTCTCAAATTGCT 401	426	
F- outer primer (5' - 3'):	GAGNACAGACTGGGAGAAGAACTCAC 74	49	
R-outer primer (5' - 3'):	TTTGCTTACAAAANGAAATNACAGTGAA 533	560	
Product size for G allele:		189	
Product size for A allele:		378	
Product size of two outer primers:		512	

Then the reaction tubes were inserted into the Thermocycler to carry out the polymerization reaction using the special program for the reaction as shown in Table (2):

Table (2): The program adopted in T-ARMS-PCR technology to determine the genetic variation of the Leptin gene.

No.	Stage	Temperature	Time	Number of Cycle
1.	Initial denaturation.	95	5 min.	1
2.	Denaturation.	95	45 sec.	35
3.	Annealing.	60	1 min.	
4.	Extension.	72	1 min.	
5.	Final extension.	72	7 min.	1
6.	Stop reaction.	4	5 min	1

2.3 Statistical Analysis

The data were statistically analyzed using a completely randomized design (CRD), and the significance of differences between means was tested using Duncan's multiple range test at a probability level of ($P \leq 0.05$). This was done according to the statistical program [7].

3. RESULTS AND DISCUSSION

The results of the molecular analysis (Figure 1) showed the presence of three leptin gene genotypes in the studied blood samples. The three genotypes were identified as AA, AC, and CC (Table 3). The AA genotype had the highest frequency at 58% of the flock, followed by AC at 22%, and then CC at 20%. The frequency of the A allele was approximately 69%, compared to 31% for the C allele. These results indicate a clear dominance of the A allele in the studied samples, possibly reflecting selection for this allele across generations due to its association with certain desirable productive traits. Furthermore, the high frequency of the AA genotype compared to the other genotypes may indicate that this genotype possesses a greater ability to adapt and produce within the prevailing environmental conditions of local chickens. Since this gene is physiologically linked to these traits, the frequency of this allele increased spontaneously in the flock. The absence of significant differences in the distribution of the genotypes suggests that the flock is in a state of genetic equilibrium.



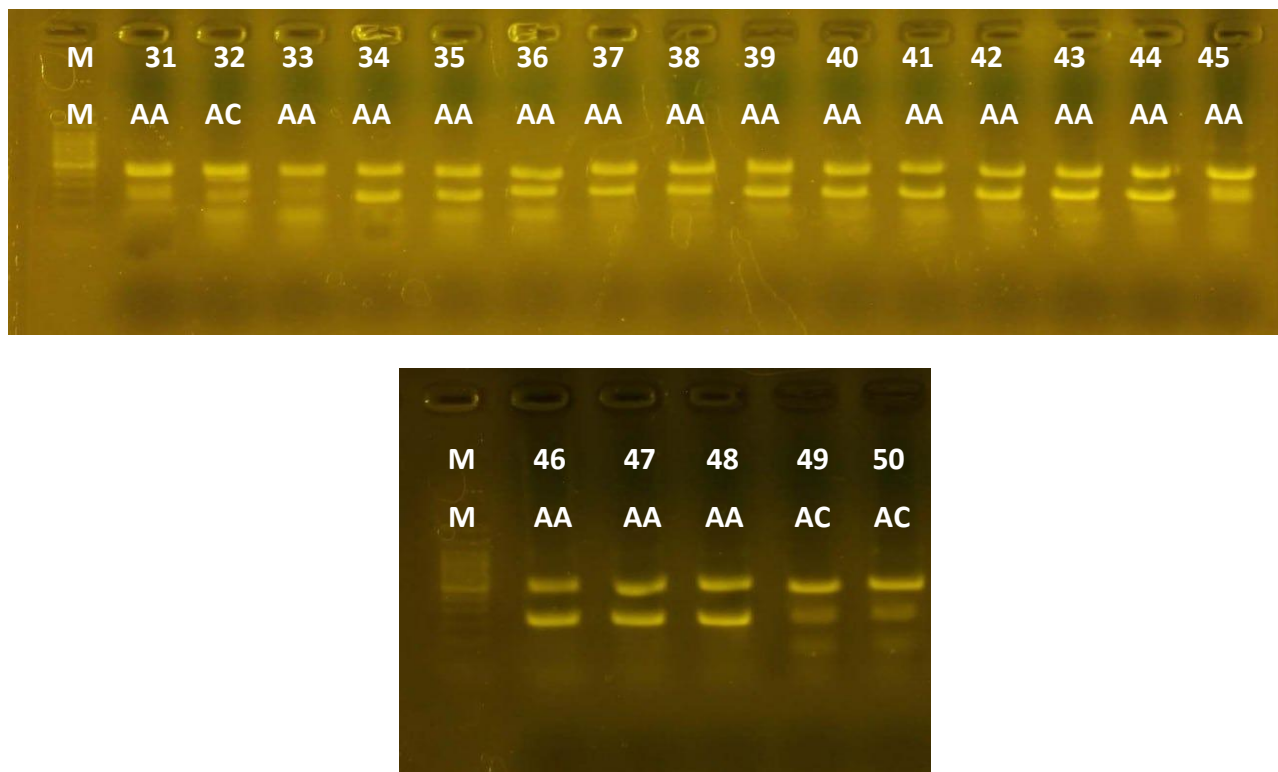


Figure (1) Electrophoresis of the Tetra-ARMS-PCR technology products for detecting genetic variation in the leptin gene, where the 512-base-pair band represents the A allele, the 378-base-pair band represents the G allele, and the 189-base-pair band represents the C allele.

These results are consistent with those of a study [2] conducted on Iraqi local chickens, where the researchers demonstrated clear differences in allele frequencies and genetic makeup of the leptin receptor gene (LEPR), with certain genotypes associated with improved productivity and physiological traits. The study also indicated that genetic variation in energy-regulating genes could be an important tool in genetic selection programs.

Table (3) shows the genetic makeup, percentages and allelic frequency of the leptin gene in chickens.

proportions of genetic composition distribution	percentage (%)	number of chickens	Genetic compositions
0.58	58	29	AA
0.22	22	11	AC
0.2	20	10	CC
1.00	100%	50	Total
Significance Level (N.S.)			

Frequency	Allele
0.69	A
0.31	G

Discussion of production characteristics

Figure (2) illustrates the effect of leptin gene genotypes on the productive performance of local chickens. Significant differences were observed between the different genotypes in the average egg production rate (%HDP). Genotype AC recorded the highest production rate at $77.47 \pm 1.76\%$, followed by genotype AA with an average of $73.40 \pm 0.95\%$, while genotype CC achieved the lowest production rate at $63.07 \pm 0.26\%$. Genotype AC maintained its superiority throughout most of the experiment, indicating a direct effect of leptin gene variance on egg production efficiency. These results are consistent with a study [2] on Iraqi local chickens, which demonstrated a significant effect of genetic polymorphisms in the leptin receptor gene (LEPR) on several productive and physiological traits. This suggests the importance of the leptin and its receptor system in the productive variation among birds. The results were similar to those of [8], who explained that leptin receptor activation affects ovarian follicle development, ovarian function, and egg-laying rate. This indicates that genetic variations associated with the leptin system may be reflected in reproductive efficiency and egg production, which is a complex quantitative trait controlled by a large number of genes in addition to current environmental and physiological factors [9]. However, the results of the current study differ from some studies that did not record a consistent, significant correlation between genetic polymorphisms in the leptin gene or its receptors and certain productive traits. These studies indicated that the main role of this gene is related to regulating feed intake, energy use efficiency, fat deposition, and growth rates, rather than being directly related to egg production. Some research also showed that the effect of leptin receptor genetic polymorphisms varies depending on the studied trait and the breed used showed a clear effect on some productive and physiological traits more than others. This variation may be attributed to differences in the genetic background of the flocks, variations in the studied gene loci, and the influence of environmental, administrative, and nutritional conditions that may interfere with the gene's genetic effect and affect the level of expression of productive traits [2, 10]. Regarding egg weight, the genotypes of the leptin gene did not have a significant effect on the overall average egg weight among the three genotypes. These results indicate that the leptin gene's influence may be more pronounced during the early stages of production, as leptin plays a crucial role in regulating energy consumption, nutrient distribution, and reproductive functions related to egg formation. This finding is consistent with what [2] indicated regarding the influence of certain genotypes associated with the leptin gene on a number of productive and reproductive traits in local chickens, thus confirming the importance of this gene in expressing certain economic traits. It also agrees with what [8] stated that the leptin receptor plays an important role in regulating ovarian follicle development. The study examined ovarian function in chickens, which may influence certain egg production indicators and characteristics throughout different stages of egg production. However, the results of this study differed from some studies that indicated the

continued influence of the leptin gene on reproductive performance and egg production for periods longer than the productive lifespan. Furthermore, [9] demonstrated that some genetic loci associated with egg production can continue to exert their effects across multiple stages of the productive lifespan.

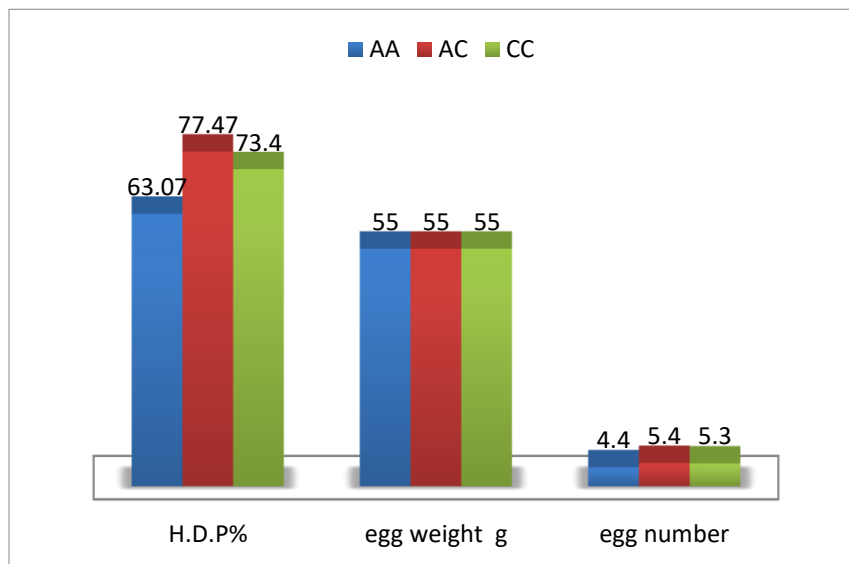


Figure (2) illustrates the productive characteristics of the leptin gene in local chickens

The results showed a significant effect of leptin gene genotypes on the total number of eggs produced over the period. The AC and CC genotypes significantly outperformed the AA genotype ($5.4 \pm 0.15a$, $5.3 \pm 0.16a$, $4.40 \pm 0.25b$, respectively). These results indicate that genetic variation in the leptin gene plays a role in regulating the reproductive efficiency of laying hens, particularly with regard to egg production rate. This can be explained by the vital role leptin plays in regulating energy balance and nutrient utilization, as well as its influence on the hypothalamic-pituitary-ovarian axis, which directly or indirectly affects ovarian activity and egg production rate [8,2]. These results are consistent with the findings of researchers [8] who demonstrated that the leptin receptor plays an important role in regulating ovarian follicle development and ovarian activity in hens, and that changes in leptin signaling affect reproductive performance and egg production rate. The current results are consistent with what [11] stated, namely that leptin receptors are present in ovarian tissue and participate in regulating ovarian follicle growth. However, the results of this study differ from what some researchers have indicated, namely that the effect of leptin is mainly limited to regulating energy consumption and body weight, without showing a clear or consistent correlation with egg production traits. [9] pointed out that egg production traits are subject to the influence of a large number of genes and overlapping genetic loci, and that the effect of a single gene may vary according to breed and production stage. [12] also explained that the response of production traits to the effect of leptin may vary according to the physiological state and energy available to the bird. Furthermore, this study differed from [2] in finding no significant effect of the leptin receptor gene on the number of eggs produced.

Figure (3) shows the relationship between fertility and hatchability traits. The results indicate a significant effect of leptin gene genotypes on fertility and hatchability traits. The CC genotype achieved the highest fertility rate at 93.9%, significantly surpassing the AA and AC genotypes, which recorded 82.5% and 69.7%, respectively. This same genotype also had a higher hatchability rate of 81.8% compared to 67.4% and 63.6% for the AA and AC genotypes, respectively. Regarding the hatchability rate of fertilized eggs, the CC genotype recorded the highest value at 80.6%, significantly outperforming the other two genotypes, which did not differ significantly from each other. These results are consistent with what was indicated in [8], namely that the leptin system and its receptors play a crucial role in regulating ovarian function, follicle growth, and reproductive development in poultry, which may impact fertility and hatchability efficiency. These findings are consistent with those of researchers [13], who indicated a correlation between leptin receptor polymorphisms and certain reproductive and productive traits, underscoring the importance of this genetic system in influencing avian reproductive performance. These results are similar to those of researchers [14], who suggested that genes regulating hormonal and energy pathways may indirectly affect reproductive efficiency by influencing ovarian activity, germ cell development, and the reproductive capacity of birds. However, these findings differ from those of researchers [15], who indicated that the impact of leptin receptor polymorphisms was more pronounced in growth and feed efficiency than in reproductive traits. Furthermore, researchers [14] explained that the influence of genes associated with reproductive functions may vary depending on the genetic background of the breed and environmental and management conditions, which could explain the discrepancies between the results of different studies.

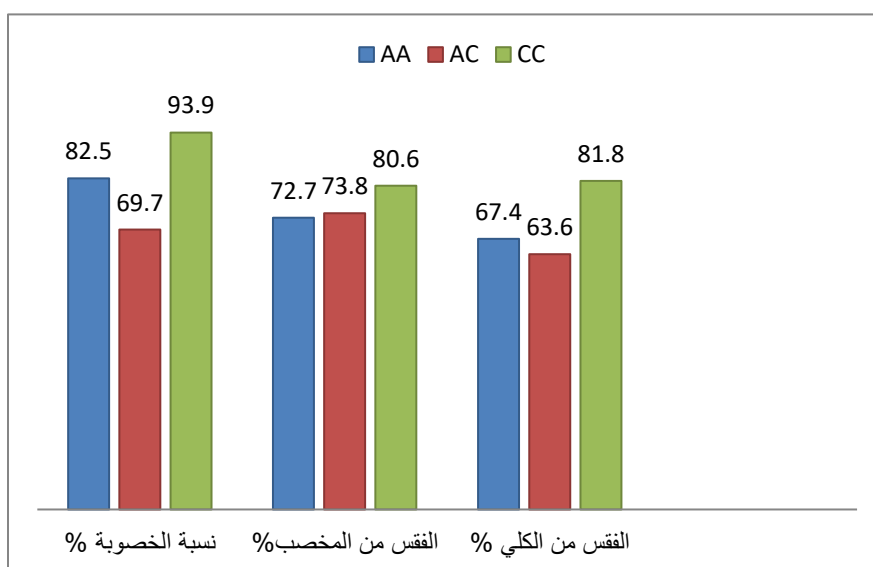


Figure (3) is a graph showing the measurement of fertility and hatchability for the leptin gene in local chickens.

4. CONCLUSION

This study reveals the significant role of leptin gene polymorphisms and the presence of the GA mutation at locus (rs314805789) in enhancing key productive traits. The marked superiority of the AC genotype over AA and CC in most of the studied traits indicates the influence of incomplete dominance on metabolic efficiency. These findings provide a solid molecular basis for improving selection programs using genetic markers, demonstrating that selecting hybrid alleles can significantly increase the production of economic and productive traits. Therefore, future studies of functional genomic characteristics, including gene expression and proteomic analyses, are needed to elucidate the precise molecular pathways through which these productive genotypes enhance their yield.

CONFLICT OF INTEREST

None of the authors will claim a conflict of interest.

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