# The effect of the multiple genotypes of Myostatin gene on reproductive characteristics of the Iraqi brown local chickens

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## Abstract

The experimental flock consisted of 101 laying hens attributed to the Sire and Dam from the local Iraqi chickens at the age of 74 weeks / the third generation belonging to the fields of the Poultry Research Station in the Agricultural Research Department / Ministry of Agriculture. Hens were selected based on the characteristic of high egg production rate which was more than 60%, the study aims to determine the multiple genetic features of the Myostatin gene (MSTIN) and its contribution to affecting the average body weight in a sample of Iraqi brown local chickens.blood samples were collected and DNAextracted DNA with a concentration of 98% and a purity of 19.8, PCR-Product technique was used to multiply the segment of the Myostatin gene, and sent to the Republic of South Korea, Macrogen Corporation-Korea, and using the Sanger and Coulson method, the genotypes of genetic mutations were,by sequencing the percentages of individuals carrying it contributed to the variance of genetic frequency and chi-square values (24.8, 58.5 and 37.6 for mutations, respectively) and their incompatibility with the Hardy-Weinberg rule. The hybrid genotypes (CG, AB, and TC) showed significant $p<0.05$ in the weekly egg production rate throughout the productive periods,While there were no significant differences in the average weight of the first egg (36.32, 36.37 and 36.32 g for mutations, respectively), as well as no significant difference in the measures of egg quality (white and yolk weight and their percentages) among all individual of the local brown chicken under study. .

**Keywords:** Myosteine. gene, pluripotency technique, whole genome scanning technique, genetic mutations, local chickens.

## Introduction:

Genetic diversity is characteristic of local chicken breeds as a result of genetic mutations and natural selection and for the benefit of current populations that are in equilibrium with environmental conditions as well as the influence of migration and artificial selection programs (19),Genetic regression analyzes studies in domesticated birds indicate that local chickens descended from true ancestors Red Jungle Fowl and the genome of the local chicken happened to be the contribution of cross-breeding between the four species (gray, Ceylon, red and black forest bird) of the genus Gallus (17), Intensive artificial genetic selection caused a significant decrease of 50% or more in genetic diversity in commercial pure breeds compared to their ancestors due to the limited number of primary breeds and inbreeding programs in commercial lines (16),And that the techniques of the global chicken industry had negative effects on the distribution of chicken genetic resources specific to the components of the breeds, as well as that the comprehensive genetic diversity within local breeds enhances the genetic database, especially chickens that have adapted to local environmental conditions (18),The researchers expect that the results of applying artificial selection programs will be more accurate and based on genetic variation in the encoded regions, and it is assumed that regions with effects on quantitative traits in the genome develop differently from the non-coding regions of the genome, and the theory of genetic isolation indicates an increase in genetic variation between genotypes populations of domestic chickens and the effect of increased geographic distance (13and14) ,Egg weight as one of the quantitative traits is greatly affected by the,genetic ability for individual whether these genetic lines are local only or a mixture of genetic structures by cross-breeding with foreign breeds (6), Significant difference in the weight of the first egg from the average production of the population (15) ,The selection of laying hens for the trait of high live body weight has a positive correlation coefficient with egg weight and a negative correlation coefficient with the characteristic of egg production rate (10),The values of the genetic correlation are positive between the age at the time of laying the first egg and the weight of the first egg and the eggs of the beginning of production, as the weight of the egg increases with the increase in age at sexual maturity, and the genetic correlation between the number of eggs and the weight of the egg is often low and negative (12),that the gene expression level of the MSTN gene in broilers is higher than its expression level in laying hens (23), in selected chickens. Genetic mutations of the MSTN gene have been identified that contribute to the effect on muscle mass in broilers and affect egg production in broilers laying hens (22).

The current study aims to determine the multiple genetic features of the myostatin gene (MSTIN) and its contribution to affecting the weight of the first egg, the weekly egg production rate, and the quality of some egg qualitative characteristics in a sample of Iraqi brown local chickens.

## Materials and Methods

Iraqi local chicken flock:

A flock of Iraqi brown local chickens, 74 weeks old, the third generation, belonging to the fields of the Bird Research Station, was used in this experiment The poultry house belonging to the Agricultural Research Department / Ministry of Agriculture in Abu Ghraib, selected on the basis of traits of the high egg production rate of more than 60%,This flock consists of 101 laying hens attributed to the father and mother and marked with metal numbers in the wing since the first day of hatchingThey were transferred from the ground breeding halls

DNA extraction

DNA was isolated from blood samples using the Go Taq G2 Green Master Mix Kit, supplied by the American company Promega, according to the Wizard method for purification of genomes (DNA), by following the following steps:

1. 900. Addedmicroliter of Lysis solution (Cell Lysis Buffer) to 300 microliters of a blood sample in the Eppendorf with shaking and stirring

and then incubating for ten minutes at room temperature for the purpose of analyzing blood cells.

2. Then work the samplewas centrifuged in the previous step for a period of twenty seconds at a speed of 1300 rpm, during which the filtrate was separated from the sediment.

3. 100 added microliter of Nuclei Lysis B solution to the precipitate in the previous step and shaken with a shaker overtaxing.

4. 300 added microliter of ethanolsolution to the mixture in the previous step with shaking for twenty seconds and then centrifugationmicroliter of ethanol solution to the mixture in the previous step with shakingfor twenty seconds, then centrifugation for three minutes at speed1300 rpm for protein precipitation.

5. Pregnant deposit has been transferredand the carrier contains the genetic material DNA into a tube containing 300 microliters of isopropanol alcoholwith the mixture with centrifugation for one minute at a speed of 1300 rpm, neglecting the precipitate.

6. 300 added microliters of ethanol alcohol 70 at room temperature to the precipitate in the previous step with the work of centrifugationfor one minute at a speed of 1300 rpm with passing dry air to get rid of ethanol alcohol.

7.100 addedMicroliter of the solution to DNA strands for an hour at temperature65 m for the purpose of returning moisture to the DNA strands.

Studied traits:

1. Weight of the first egg/gm

Record the weight of the first egg for each chicken and then measure itusing a sensitive scale with an accuracy of one gram.

2. Monthly egg production rate.

3.Some qualitative characteristics of the internal eggs absolute white (weight / g, percentage of egg white weight, egg yolk weight / g and %percentage of egg yolk).

Genetic redundancy calculation:

q = 2×the number of individuals carrying the pure gene + Mixed number of people

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2×the total number of clan

Calculation of the percentage of genotypes:

100%
× All Section / = percentage

The chi-square -×² test was used to compare the percentages of the distribution of genetic phenotypes resulting from the studied mutations in the sample.

$$\times^2 = \pounds \frac{(O-E)^2}{E}$$

Repetition of the genotypes of a gene Al-Myostatin

After taking blood samples from the wing vein of local Iraqi brown chickens inglass tubes containing anticoagulant (EDTA), and transported to Ugene Laboratory For Molecular Research / Kufa, to extractthe chicken genome (DNA) and identify the gene segment, and amplify it by PPCR-RFLP and then sent to Korea for DNA sequence analysis and genetic morphogenesis.

Statistical analysis:

The data of the experiment were analyzed using the statistical program(SAS Statistical Analysis System2012) To study the effect of the multiple genotypes of the Myostatin gene on some productive and qualitative traits of eggs The local Iraqi brown chickens, and comparisons between the averages were made using Duncan's polynomial tes (Duncan, 1955),according to the mathematical model.

$$Y_{ij} = \mu + A_i + e_{ij}$$

So that:

Yij = observation value of j due to the polygenic structure of the gene.

μ = general average of the adjective.

Ai = the effect of multiple genetic phenotypes for the mutations under study.

eij = normally distributed random error" with an arithmetic mean equal to zero and a variance of δ2e.

## Results and Discussion:

Determination and distribution of the genotypes of the myostatin gene segment

The genotypes resulting from scanning the sequences of nitrogenous bases are distributed along the Myostatin gene segment using the Sequences technique and according to determining the heterogeneous appearance of the (Single NucleotidPolymorphism)(SNP) in numbers and percentages shown in Table No. 1 and Table No. 1 showed

The number of genotypes AA, AB, and BBthe varied in the presence of the second genetic mutation, which amounted to 68, 24, and 8, with a percentage of 68, 24 and 8%, and with an allelic frequency of 0.80 and 0.20 for each of the A and B alleles, respectively, as well as a significant advantage of $p<0.01$ in favor of The A allelecompared with the B allele in influencing the traits under study.

**Table 1 Number of genotypes, their percentages and allelic frequency at the site of the first mutation in a sample of local brown chickens**

| | | | Second mutation | | |
|---|---|---|---|---|---|
| genotype | No. | % | $\chi^2$ | A llelicrepetition | |
| AA | 68 | 0.68 | | A | B |
| AB | 24 | 0.24 | 58.515** | | |
| BB | 8 | 0.08 | | 0.80 | 0.20 |

****** Level of morality ($p < 0.0\ 1$)

The difference in the frequency of genotypes (genetic alleles) may be attributed to the strength of the association of those genes and their alleles in the genetic loci, or the short crossing distance between genes on the same chromosome (3),In addition, most quantitative traits are affected by several genes that can exist in more than one genetic location and are affected by the type of mixingmating, the genetic composition of the clan and genetic delinquency, and that the extent of the link strength or weakness between genes and their alleles is negative genetic expression. In adjective imprinting (21), and that the different percentages of genotypes may be attributed to the varying impact of the contribution of genes responsible for multiple genetic phenotypes as well as the influence of local environmental conditions (endocrine efficiency, photoperiod length and nutritional conditions), and the emergence of a state of equilibrium with local environmental conditions and a high percentage of Adaptation compared to the genotypes under study, and thus a higher ability to imprint offspring and gene expression (7), Which will prompt more genetic improvement programs in terms of the diverse genetic structures of individuals (which is an important source for the maintenance of genetic resources) , and for several generations to com

The effect of the multiple genotypes of the Myostatin gene on the weight of the first egg in a flock of brown local chickens

The average weight of the first egg in a flock of Dams and a herd of laying hens affects the state of the level of genetic correlation with the characteristic of harmonious body weight, age at sexual maturity and predictive evidence in the rate of productive performance and for any period of the productive season, which lasts up to 75 weeks in most herds of breeding mothers. In employing the frequency coefficient to support the selective program in the direction of genetic improvement and with the least interference from the effects of environmental factors( laying hans in enhancing the average values of this trait, the study of gene properties and expression capabilities is of importance to reveal the contribution of the diversity of genetic

markers in determining the phenotype of the traits that the gene affects itself.

Table No. 2 shows the multiple genetic phenotypes of local brown chickens and their contribution to affecting the weight of the first egg, as the performance of individuals carrying the genotypes CC, CG and GG, with an average weight of the first egg was 36.32 g, while the individuals carrying the genotypes AA, AB and BB The average weight of the first egg was 36.37 g, while the average weight of the first egg was 36.32 g for

**Table 2 Contribution of the multiple genotypes of MSTIN to the characteristic of first egg weight in domestic brown hens (arithmetic mean ± standard error)**

| third genetic mutation | | | second genetic mutation | | | first genetic mutation | | | |
|---|---|---|---|---|---|---|---|---|---|
| M | H | W | M | H | W | M | H | W | Genotype |
| CC | TC | TT | BB | AB | AA | GG | CG | CC | |
| 36.49 ± 2.88 | 37.06 ± 1.09 | 35.40 ± 1.11 | 36.0 ± 1.08 | 36.12 ± 1.01 | 37.00 ± 1.67 | 36.49 ± 2.88 | 37.06 ± 1.09 | 35.40± 1.11 | Weight of the first egg/g |
| | 36.32 | | | 36.37 | | | 36.32 | | Average weight of the first egg/g |
| N.S | N.S | N.S | N.S | N.S | N.S | N.S | N.S | N.S | morale level |

N. S means no significant difference

TT, TC, and CC genotypes, respectively there were no significant differences in the averages of individuals of the same genotype or among all members of the herd under the experimental study, due to the weak collective variance and the internal genetic correlation coefficient due to the lack of influence of the genetic morphology's contribution to the weight of the first egg.

The closeness and symmetry in the weight of the first egg and the absence of significant differences between the members of the multiple genotypes in the white herd may be attributed to the fact that these genetic mutations are among the silent mutationsthat code for the same type of amino acid, which does not change the gene expression, so the performance is similar between individuals, or The reason for the decrease in the percentages of mutant and hetrozygote alleles may be due to the fact that the weight of the first egg is a continuous quantitative trait that is affected by a number of Polygenic Effect geneswith cumulative effect.

These results agree with what (11) indicated that the weight of the first egg was significant in individuals that outperformed the hybrid vigor in the

leghornchicken flock, similar results obtained from cross-breeding local Egyptian Fayumi females with Rhode Island Red (RIR) males. varied in weight of the first egg and in favor of members of the RIFI hybrid genotypes (8)These results are in agreement with the results of (5), which indicated that there was no significant superiority in the weight of the first eggs of hybrid individuals (Hetrozygote) due to the effect of the strength of the hybrid that often appears in the first generation of outgrowths, in addition to the fact that the weight of the first egg is affected by age. When the first egg is laid (4).

Whereas, no significant effects were observed for the multiple manifestations of the NPY gene on the characteristic of the weight of the first egg(37.84 g)in the local chickenMazandaran (2).

Contribution of multiple genotypes of MSTIN to the characteristic of weekly egg production (egg/week) in Iraqibrown hens

Egg production is one of the most important productivity indicators in commercial chicken flocks and breeding flockswhich is affected by the length of the productive period (the biological cycle of egg production) and the productive season, as well as the influence of the breed and the multiple genetic manifestations among the members of the herd, especially the diverse environmental conditions and intertwined with genetic factors

Table No. 3 showed the variation in the weekly egg production rate for individuals of various genotypes throughout the productive period from 19` to 74 weeks of age, and this variation was significant, $P<0.05$, in favor of the individuals carrying the CG hybrid genotype, as its weekly production rate was 5.3, 5.3, and 6.0 eggs/week for the first, second and third periods, respectively, while the differences were not significant in the weekly egg production rate of individuals of the various genotypes under study throughout the subsequent periods until the age of 74 w

**Table 3 Contribution of the multiple genotypes of MSTIN to the characteristic of weekly egg production rate (egg/week) in domestic brown chickens (arithmetic mean ± standard error)**

| genetic makeup | first period 26-19 | second period 34-27 | third period 42-35 | Fourth period 50-43 | Fifth period 58-51 | Sixth period 66-59 | Seventh period 74-67 |
|---|---|---|---|---|---|---|---|
| CC | 4.7 ± 0.02 | 4.0 ±0.01 | 4.02 ±0.03 | 4.5 ±0.07 | 4.2 ±0.09 | 3.9 ±0.06 | 3.4 ±0.05 |
| CG | 5.3 ±0.01 | 5.3 ±0.02 | 6.02 ±0.05 | 5.5 ±0.08 | 4.9 ±0.05 | 4.02 ±0.08 | 4.4 ±0.01 |
| GG | 3.5 ±0.01 | 4.6 ±0.02 | 5.02 ±0.07 | 4.5 ±0.08 | 4.1 ±0.03 | 4.0 ±0.07 | 3.1 ±0.04 |
| Morale | * | * | * | N.S | N.S | N.S | N.S |

N.S means there is no significant difference, * means there is a significant difference ( $p < 0.01$)

The superiority of CG individuals in the weekly egg production rate (egg/week) is due to their response to selection programs and genetic improvement, as well as their adaptation to local environmental conditions and their possession of the longest egg chain compared to other genotypeMyostatin has some important roles in chicken development so MSTN is more ubiquitously and more strongly expressed in the ovary (9) ,These results are in agreement with the results of the commercialleghornchicken flocks bred in Iraq, as the weekly production rate for them was 6.7, 7.2, 6.4, 5.5 and 5.0, respectively, (1), and the average weekly egg production was 4.9 and 3.9 eggs/week in commercial Rhode Island red flocks adapted to the local conditions of the Arab Republic of Egypt and Sinai, respectively, at the firstfifteen weeks of production 20.

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