University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Impact of genetic polymorphisms of the GnRH gene on productive performance and carcass traits of Japanese Quai

Hazim Razaq , Ali A. Abdulkareem

[1,2]College of Agriculture and Marshes, University of Thi-Qar, Thi-Qar, Iraq.
[1]*E-mail: hazim.razaq.post2024@utq.edu.iq*
[2]*E-mail: ali-ah@utq.edu.iq*

Abstract

This experiment was conducted in the research hall of the College of Agriculture and Marshes at Thi-Qar University, and it included two stages: field work and laboratory work. In the field work stage, 200 quail birds were raised from one day old to sexual maturity (end of the field work). At the end of the fifth week of field work, blood samples were collected from the birds after slaughter, and these samples were taken for genetic analysis in the laboratory of the Marshes Research Station/Thi-Qar University. In the laboratory, the genetic material was extracted, and electrophoresis was performed. The amplification product was then sent to the Korean company Microgen to determine the sequence of nitrogenous bases that occupy part of the gene and determine the genotype (genotype) of the gene. The study investigated the effect of the gene on some productive traits, including internal organs (liver, gizzard, heart) and some carcass cuts (wings, thighs, breast, and eviscerated body weight). The results showed that the studied gene could be amplified, and three genetic mutations were identified. The results also showed... Based on the previous results for the three mutations (67.A>G, 323.C>T, and 277.G>C) and the relationship between the genetic structures of the mutations in the GnRH gene with edible viscera, the results showed significant differences for the 277.G>C mutation at a significance level of P≤0.01 in heart and liver weights. The average heart weight for the genetic makeup (GC, 1.836 ± 0.032 A) was the highest average in heart weight, while the genetic makeup (GG, 1.690 ± 0.042 B) was the lowest significant structure. For liver weight, the genetic makeup (GC, 3.775 ± 0.061 A) was the highest average, while the genetic makeup (GG, 3.551 ± 0.061 B) was the lowest significant structure. There was no significant effect of the two mutations (323.C>T and 67.A>G) on the traits The results also showed significant differences for the 277.G>C mutation at a significance level of P≤0.05 in eviscerated body weight, where the average eviscerated body weight for the genetic makeup (GG) was the highest average .The genetic makeup (GG, 105.767 ± 0.853) had the highest average eviscerated body weight, while the genetic makeup (GC, 103.223 ± 0.728) had the lowest average. There were no significant differences between the genetic makeups for the other traits, including breast weight, thigh weight, and wing weight. Additionally, there was no significant effect of the two mutations (323.C>T and 67.A>G) on the mentioned traits In summary, the results showed that: - The 277.G>C mutation had a significant effect on heart weight, liver weight, and eviscerated body weight. - The GG genetic makeup had the highest average eviscerated body weight. There were no significant differences between the genetic makeups for breast weight, thigh weight, and wing weight. The 323.C>T and 67.A>G mutations did not have a significant effect on the mentioned traits.

I. Introduction

Quail birds are distinguished by their significant importance in reducing meat shortage crises, contributing greatly alongside other white meats to alleviate these crises. They represent a new area of investment and economic opportunity, providing new job prospects. Quail birds have a short capital cycle, allowing for the sale and marketing of good meat breeds within 36-42 days. The variable costs, including feed and others, are much lower compared to other poultry (1). Quail birds are characterized by several desirable traits, including their meat's unique taste, flavor, and high demand among many consumers (2). They also possess encouraging productive traits and low breeding costs compared to chicken, rapid growth, and early sexual maturity (3). Quail birds offer significant opportunities for advancements in genetic and breeding research. Japanese quail is one of the smallest domesticated birds used for egg and meat production (4). The domestication of Japanese quail, derived from wild Japanese quail (Coturnix japonica), occurred in the 11th

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

century as a small songbird for pets. It has become a thriving industry for egg and meat production and is also used as a laboratory animal (5). Quail breeding has expanded widely for egg production in countries such as Japan, Eastern Siberia, Mongolia, North China, and Korea (6). Key differences between wild and hybrid quail include the latter's heavier weight, earlier sexual maturity, higher egg production, and better hatchability. While wild quail lay around 7-14 eggs per year, hybrid quail can lay approximately 280 eggs per year under natural feeding conditions (7). The reason for not breeding wild quail in fields is that Japanese quail and hybrids adapt better to the environment and are more productive (8). Japanese quail are characterized by a short lifespan, with females living around 2.5-3 years and males living around 3-5 years (9). Japanese quail were first used as a research model in 1959 by researchers Padgett and Ivey, and have since been widely used in various studies, including behavioral, physiological, genetic, and biomedical research (10). There is significant interest in quail as an alternative to chicken and turkey in Western markets among consumers (11). Japanese quail can be used as experimental animals to reduce costs and time in poultry research, making them an ideal choice for many research studies (12).

The study aims to investigate and understand the impact of three types of studied mutations that cause genetic variations in the GnRH gene on carcass traits, specifically edible viscera, in Japanese quail.

Keywords: Quail bird, GnRH gene, Quail Weight.

II. Materials and Methods

This study was conducted in the specialized poultry research hall at the Agricultural Research Station, College of Agriculture and Marshes, Thi-Qar University, from October 10, 2024, to March 13, 2025. The study consisted of two stages:

Field Work Stage

Preparation and Rearing of Chicks: 200 Japanese quail chicks were obtained from Mohammed Ali hatcheries in Souk Al-Shuyukh district, Thi-Qar governorate. After hatching, the chicks were transferred to the experimental site in special boxes under suitable thermal and ventilation conditions according to the birds' age. The chicks were numbered with plastic tags on their legs, and their weights were measured weekly. The chicks were raised in an open system with continuous lighting until 35 days of age and were housed in BRC cages made of iron for easy management and control. Sexing of the birds was done at three weeks of age.

Laboratory Work Stage:

Blood Collection Process : Blood samples were collected at the end of the fifth week after slaughtering the birds, following the fieldwork period. 5 ml of blood was collected from each bird into test tubes containing EDTA anticoagulant. The samples were then transported to the laboratory in a cooled box and stored frozen at -20°C until DNA extraction was performed in the lab.

Extraction of Genetic Material

Genetic material was extracted from blood samples taken from birds according to the instructions and guidelines of the diagnostic kit used for molecular laboratory testing of the GnRH gene. The DNA extraction process from blood was carried out according to the instructions of the kit provided by the Korean company Geneaid, using a number of laboratory devices for extraction.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Primer Preparation

The primers specific to the GnRH gene were prepared by the Korean company Macrogene in the form of freeze-dried powder. The primers were separated into two tubes, each containing one primer, with a label indicating the sequence of nitrogenous bases.

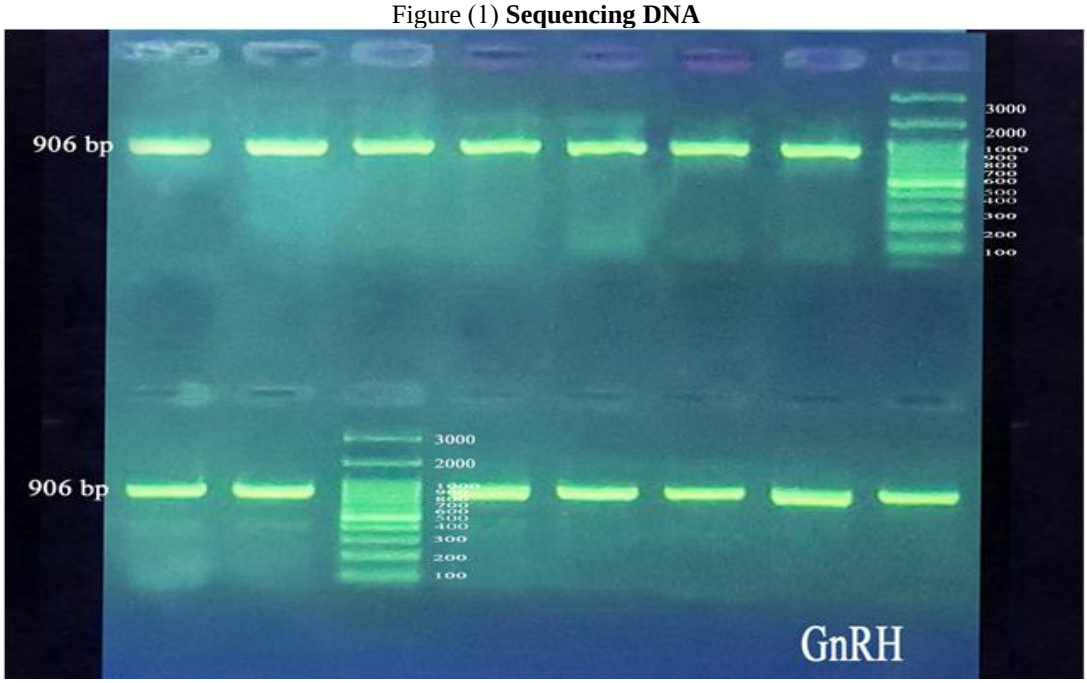
Table (1): Primer sequences for the GnRH gene.

Reference	Annealing TemperatureTa (C)	Amplicon size	Primers	Primer pair cod
Bai, J, Dong (2021)	59	906 bp	F: 5'- TCTTGGTTGGTGTTCTCCT - 3' R: 5'- ATTGCTCAGCCTGGGAT - 3'	GnRH

Sequencing DNA

Agarose gel electrophoresis was used to identify the amplification products, with a 1.5% agarose concentration. This involved dissolving 0.3 grams of agarose in 20 ml of 10x TBE buffer using a microwave. Safe View Dye, an ethidium bromide alternative manufactured by abm Canada, was added at 1 microliter per gel. A DNA ladder of 906 base pairs was loaded into one well, while 4 microliters of PCR product were added to the other wells. Electrophoresis was conducted at 70 volts and 85 milliamps for 35 minutes. Following electrophoresis, the gel was analyzed using a Gel Documentation system to determine the size of the bands.

Figure (1) **Sequencing DNA**



Identification of SNP in the GnRH Gene

University of Thi-Qar Journal of agricultural research

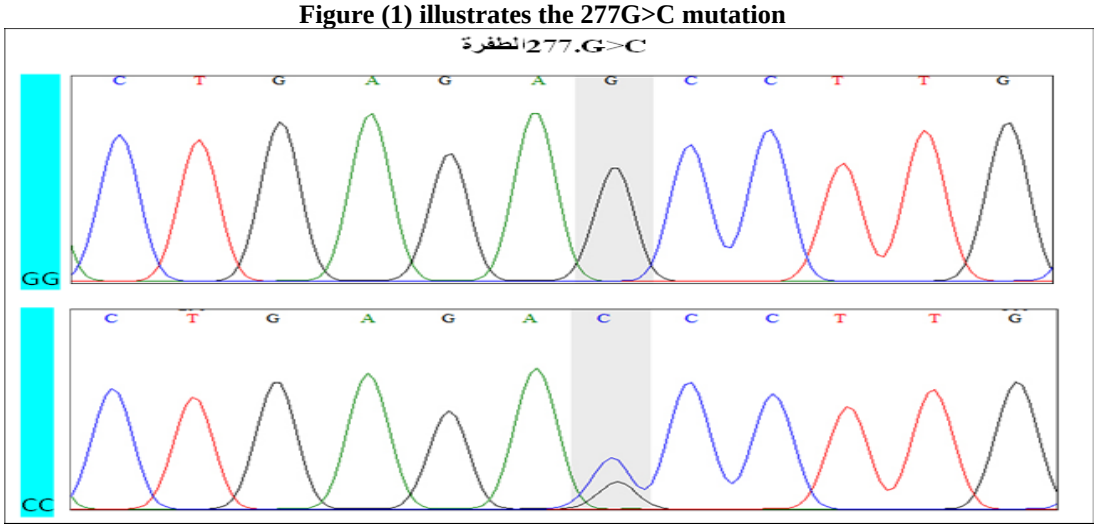
Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

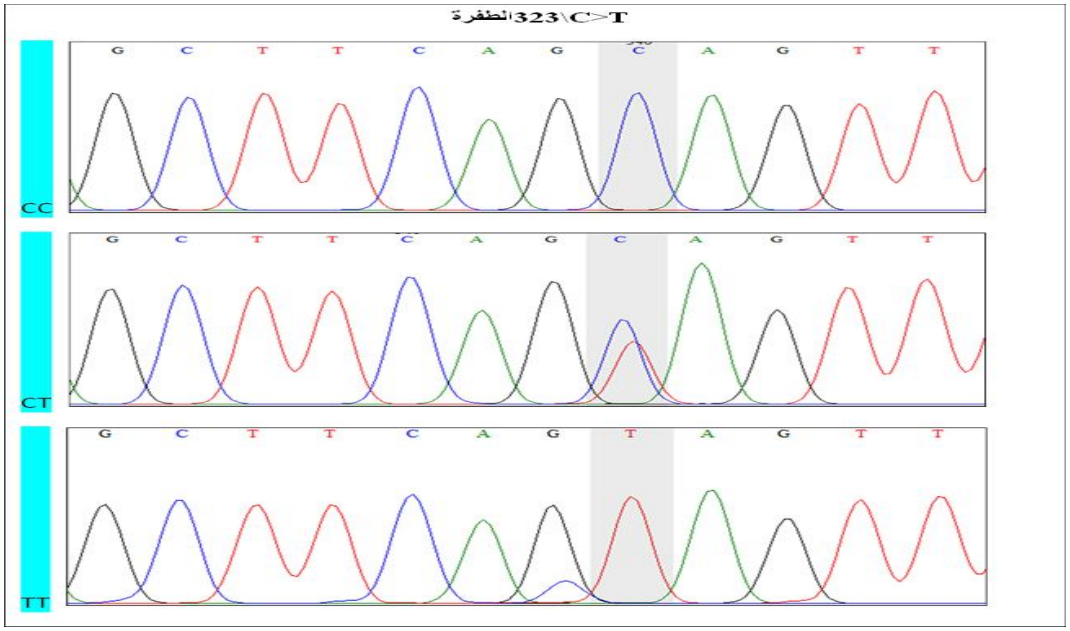
https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

After conducting molecular analysis of the studied region of the GnRH gene, three silent mutations were found: the 277G>C mutation, the 323C>T mutation, and the 67A>G mutation. However, the description of the first mutation's effect seems inconsistent, as it claims the mutation changes serine to thiamine due to both AGC and ACC codons encoding the same amino acid, which doesn't align with standard genetic coding. Typically, AGC codes for serine, and ACC codes for threonine, suggesting a potential change from serine to threonine if AGC mutates to ACC. The diagram illustrates the studied gene region and mutations.

Figure (1) illustrates the 277G>C mutation



. Figure (2) illustrates the 323C>T mutation.



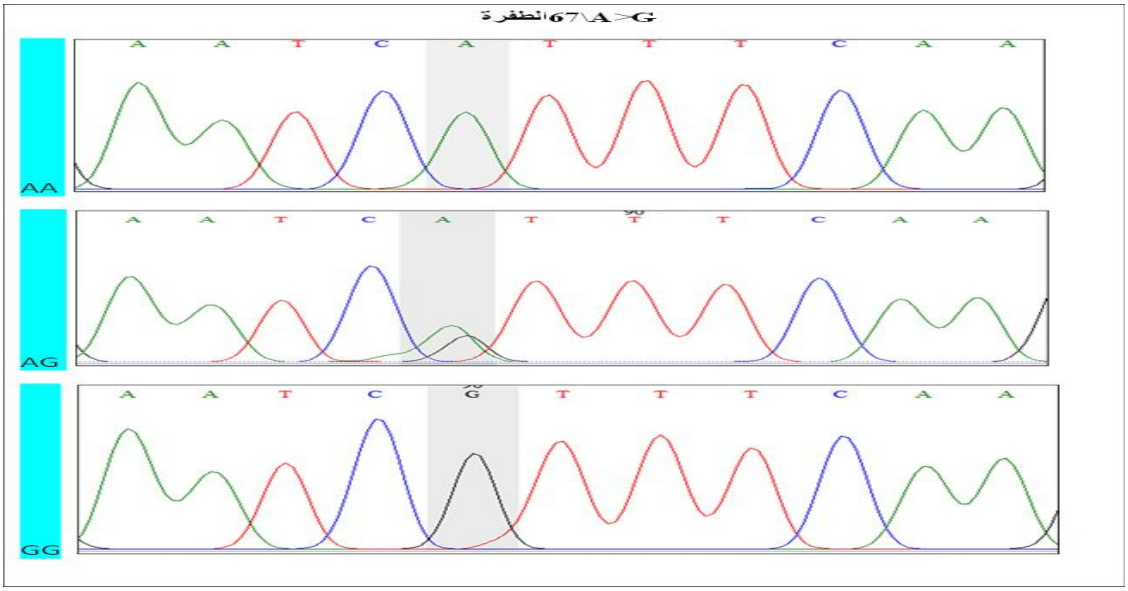
University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Figure (3) illustrates the 67A>G mutation.



The GnRH gene sequences were deposited in the NCBI GenBank.

The sequences of the GnRH gene for various genetic constructs were submitted to the NCBI GenBank, and unique accession numbers were assigned. The table below lists the accession numbers and sequence lengths of the deposited sequences in the NCBI GenBank

Table (2) Registration of GnRH Gene Sequence in NCBI GenBank

Sequence Size	Polymorphism	SNP	Accession Numbers
779 bp	AA	67.A>G	LC868287
779 bp	AG		LC868288
779 bp	GG		LC868289
779 bp	GG	277.G>C	LC868290
779 bp	GC		LC868291
779 bp	CC	323.C>T	LC868292
779 bp	CT		LC868293
779 bp	TT		LC868294

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

III. Mathematical Model

YijkL = μ + TI + SJ + CK + EijkL

The Chi-Square test was used to compare the percentages of genotype distributions for the gene, and allele frequencies were calculated using the following equation:

PA = (2 × Number of homozygous individuals + Number of heterozygous individuals) / (2 × Total number of samples)

P + q = 1, then qB = 1 - Pa

Calculation of Chi-Square Value: $\chi^2 = \Sigma [(O - E)^2 / E]$

Frequency of GnRH Gene Genotypes in Japanese Quail Samples The results in Table 2 show highly significant differences in the proportions of genetic structures in the three mutations. For the first mutation (67A>G): The AG genotype had the highest percentage at 27%. Followed by the AA genotype at 18%. The GG genotype had the lowest percentage at 4%.- Allele frequencies were 64% for allele A and 36% for allele G. For the second mutation (277G>C): The GG genotype had the highest percentage at 28%.- Followed by the GC genotype at 21%. Allele frequencies were 77% for allele G and 33% for allele C. For the third mutation (323C>T): The CT genotype had the highest percentage at 24%. Followed by the CC genotype.

Table (2): Distribution of GnRH Gene Genotypes in Japanese Quail Samples

Chi Square	Allele frequency	Allele	Polymorphism frequency	Polymorphism		SNP	GENE
8.510**	36.73	A	18		AA	67.A>G	GnRH
	55.1	G	27		AG		
	8.16		4		GG		
31.408**	57.14	G	28		GG	277.G>C	
	42.85	C	21		GC		
6.918*	38.77	C	19		CC	323.C>T	
	48.97	T	24		CT		
	12.24		6		TT		

Association of Genetic Variants of the 277G>C Mutation in the GnRH Gene with Consumed Organs The results in Table 4 show significant differences (P≤0.01) in heart and liver weights. The GC genotype had the highest mean heart weight (1.836 ± 0.032 A), while the GG genotype had the lowest mean (1.690 ± 0.042 B). For liver weight, the GC genotype had the highest mean (3.775 ± 0.061 A), and the GG genotype had the lowest mean (3.551 ± 0.061 B). However, there were no significant differences in gizzard weight among the genotypes..

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Table 4: Association of GnRH Gene 277G>C Mutation Genotypes with Organ Weights

Mean ± Standard Error			No	Polymorphism
Gizzard	Liver	Heart		
2.738± 0.079	3.551± 0.061 B	1.690 ± 0.042 B	28	GG
2.649± 0.069	3.775 ± 0.061 A	1.836 ± 0.032 A	21	GC
0	0	0	0	CC
NS	**	**	49	Level of significance

Association of GnRH Gene 67A>G Mutation Genotypes with Organ Weights

Table 5 shows that there are no significant differences in heart weight, liver weight, and gizzard weight among the genetic constructs, with no significant variation observed between the means.

Table 5: Association of GnRH Gene 67A>G Mutation Genotypes with Consumed Organ Weights

Mean ± Standard Error			No	Polymorphism
Gizzard	Liver	Heart		
2.673± 0.098	3.572± 0.095	1.724 ± 0.060	18	AA
2.738± 0.071	3.698 ± 0.049	1.771 ± 0.031	27	AG
2.565 ± 0.088	3.646 ± 0.174	1.761± 0.138	4	GG
NS	NS	NS	49	Level of significance

Association of GnRH Gene 323C>T Mutation Genotypes with Consumed Organ Weights

The results in Table 6 indicate no significant differences in heart, liver, and gizzard weights, as there were no significant differences recorded between the means of the genetic constructs for the mentioned traits

Table 6: Association of GnRH Gene 323C>T Mutation Genotypes with Consumed Organ Weights

Mean ± Standard Error			No	Polymorphism
Gizzard	Liver	Heart		
2.702 ± 0.109	3.604± 0.094	1.698± 0.054	19	CC
2.715 ± 0.066	3.697± 0.050	1.786± 0.033	24	CT
2.636± 0.088	3.583± 0.117	1.792± 0.096	6	TT
NS	NS	NS	49	Level of significance

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Relationship between the genetic constructs of the 67A>G mutation in the GnRH gene and the weight of some basic cuts and eviscerated weight

The results in Table 7 indicate no significant differences in breast weight, thigh weight, wing weight, and eviscerated body weight, as there were no significant differences recorded between the means of the genetic constructs for the mentioned traits.

Table 7: Association of GnRH Gene 67A>G Mutation Genotypes with Carcass Traits and Eviscerated Weight

Mean ± Standard Error				No	Polymorphism
Eviscerated	Wings	Thigh	Chest		
104.340± 0.825	10.555 ± 0.417	18.527± 0.447	24.659± 0.119	18	AA
104.671 ± 0.923	10.425 ± 0.288	18.777± 0.340	24.723± 0.098	27	AG
106.228± 1.638	10.125± 0.625	19.750± 1.198	24.953± 0.222	4	GG
NS	NS	NS	NS	49	Level of significance

Association of GnRH Gene 277G>C Mutation Genotypes with Carcass Traits and Eviscerated Weight

The results in Table 8 indicate significant differences at a significance level of P≤0.05 in eviscerated body weight, where the mean eviscerated body weight for the genetic construct (GG, 105.767 ± 0.853) was the highest among the means for this trait. However, for the other traits, including breast weight, thigh weight, and wing weight, no significant differences were recorded between the means of the genetic constructs.

Table 8: Association of GnRH Gene 277G>C Mutation Genotypes with Carcass Traits and Eviscerated Weight

Mean ± Standard Error				No	Polymorphism
Eviscerated	Wings	Thigh	Chest		
105.767 ± 0.853	10.464± 0.286	18.875± 0.325	24.720± 0.091	28	GG
103.223 ± 0.728	10.428± 0.362	18.619± 0.445	24.716± 0.116	21	GC
0	0	0	0	0	CC
*	NS	NS	NS	49	Level of significance

Association of GnRH Gene 323C>T Mutation Genotypes with Carcass Traits and Eviscerated Weight

Table 9 shows that there are no significant differences in breast weight, thigh weight, wing weight, and eviscerated weight among the genetic constructs, with no significant variation observed between the means.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Table 9: Association of GnRH Gene 323C>T Mutation Genotypes with Carcass Traits and Eviscerated Weight

Mean ± Standard Error				No	Polymorphism
Eviscerated	Wings	Thigh	Chest		
103.979± 0.837	10.473± 0.428	18.657± 0.425	24.698± 0.118	19	CC
104.996 ± 0.960	10.395 ± 0.277	18.854± 0.363	24.685± 0.107	24	CT
105.609± 1.685	10.583± 0.624	18.750± 0.989	24.915± 0.143	6	TT
NS	NS	NS	NS	49	Level of significance

IV. Discussion of the Results

I. The results of the three mutations (67A>G, 323C>T, and 277G>C) in the GnRH gene and their association with edible organs showed significant differences (P≤0.01) for the 277G>C mutation in heart and liver weights. The GC genotype had the highest mean heart weight (1.836 ± 0.032) and liver weight (3.775 ± 0.061), while the GG genotype had the lowest means. In contrast, the 323C>T and 67A>G mutations did not show any significant effects on these traits. These findings contradict those of Wali and Abdulkareem (2025), who found no significant differences in relative heart weight among different genotypes. The discrepancy may be attributed to differences in bird body weight, which affects organ weights.

II. Through the previous results of the three mutations (67A>G, 323C>T, and 277G>C) and the relationship of the genetic constructs of the mutations in the GnRH gene with the weight of some main carcass cuts and eviscerated weight, the results showed significant differences for the 277G>C mutation at a significance level of P≤0.05 in eviscerated body weight, where the mean eviscerated body weight for the genetic construct (GG, 105.767 ± 0.853) was the highest among the means for eviscerated body weight, while the genetic construct (GC, 103.223 ± 0.728) had the lowest mean. However, for the other traits, including breast weight, thigh weight, and wing weight, no significant differences were recorded between the means of the genetic constructs. Both mutations (323C>T and 67A>G) did not show any effect on the mentioned traits. These results are consistent with Bai et al. (2021), who found significant differences at a significance level of P≤0.05 in eviscerated body weight for the genetic constructs (CC, CT, TT) with means of (28.860, 25.122, 26.000), respectively.

III. References.

[1] Bakoji, I., Aliyu, M. K., Haruna, U., Jibril, S., Sani, R., & Danwanka, H. (2013). Economic analysis of quails bird (Cortunix cortunix) production in Bauchi local government area, Bauchi state, Nigeria. *Research Journal of Agriculture and Environmental Management*, *2*(12), 420-425.

[2] Kayang, B. B., Vignal, A., Inoue-Murayama, M., Miwa, M., Monvoisin, J. L., Ito, S., & Minvielle, F. (2004). A first-generation microsatellite linkage map of the Japanese quail. *Animal genetics*, *35*(3), 195-200.

[3] Blaszczyk, B., Tarasewicz, Z., Udala, J., Gaczarzewicz, D., Stankiewicz, T., Szczerbiñska, D., ... & Jasieniecka, J. (2006). Changes in the blood plasma testosterone and cholesterol concentrations during sexual maturation of Pharaoh quails. *Animal Science Papers and Reports*, *24*(3), 259-266.

[4] Vali, N., Edriss, M. A., & Rahmani, H. R. (2005). Genetic parameters of body and some carcass traits in two quail strains. *Int. J. Poult. Sci*, *4*(5), 296-300.

[5] Kayang, B. B., Vignal, A., Inoue-Murayama, M., Miwa, M., Monvoisin, J. L., Ito, S., & Minvielle, F. (2004). A first-generation microsatellite linkage map of the Japanese quail. *Animal genetics*, *35*(3), 195-200.

[6] Minvielle, F. (2004). The future of Japanese quail for research and production. *World's Poultry Science Journal*, *60*(4), 500-507.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 2 (2025) PP 17-26

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

[7] NARINC, D., & Aksoy, T. (2012). Effects of mass selection based on phenotype and early feed restriction on the performance and carcass characteristics in Japanese quails. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *18*(3).

[8] Puigcerver, M., Vinyoles, D., & Rodríguez-Teijeiro, J. D. (2007). Does restocking with Japanese quail or hybrids affect native populations of common quail Coturnix coturnix?. *Biological Conservation*, *136*(4), 628-635.

[9] Ottinger, M. A., Abdelnabi, M. A., Henry, P., McGary, S., Thompson, N., & Wu, J. M. (2001). Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail. *Hormones and Behavior*, *40*(2), 234-247.

[10] Huss, D., Poynter, G., & Lansford, R. (2008). Japanese quail (Coturnix japonica) as a laboratory animal model. *Lab animal*, *37*(11), 513-519.

[11] Genchev, A., Mihaylova, G., Ribarski, S., Pavlov, A., & Kabakchiev, M. (2008). Meat quality and composition in Japanese quails. *Trakia Journal of Sciences*, *6*(4), 72-82.

[12] Farag, M., & Abdallah, E. A. (2024). The Effect of Adding Irradiated Mango Peels as a Food Ingredient on the Productive Performance and Biochemical Parameters of Japanese Quail.

[13] Wali, A. H., & Abdulkareem, A. A. (2025, April). Impact of Estrogen Receptor Gene Polymorphisms on Productive Traits and Carcass Quality in Japanese Quail. In IOP Conference Series: Earth and Environmental Science (Vol. 1487, No. 1, p. 012138). IOP Publishing.

[14] Bai,J.Y., Dong,Z.H., Gong,H.R., Lu,X.N.,and LI,H.Z. (2021). Correlation Analysis between Exon 4 of ESR1 Gene and Carcass Traits in Egg Quail , Volume. 53, Page 761-764