Research Article

GDF9 Gene Polymorphisms and Their Association with Productive Traits in Iraqi Local and Crossbred Chickens

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Abstract | This study was conducted at the Poultry Research Field, Department of Animal Production Research Al-Zaafaraniya, from November 1, 2023, to September 1, 2024, with the aim of analyzing single nucleotide polymorphisms (SNPs) in the GDF9 gene and determining their association with productive traits in Iraqi local and crossbred chickens. The experiment included 50 local hens and 41 crossbred hens, and productive data were recorded across eight consecutive laying periods (19–34 weeks of age). A 711 bp fragment of the GDF9 gene was amplified using polymerase chain reaction PCR, and SNPs were identified using Sanger sequencing.The results in the crossbred chickens revealed that the T>C:340, C>G:100, G>T:282, and A>G:375 polymorphisms had highly significant effects ($p \leq 0.01$) on egg weight across several production periods, where the TC, CG, GT, and AG/GG genotypes outperformed the other genotypes. These polymorphisms also influenced egg number during early production periods and body weight during specific stages, indicating stage-dependent genetic effects related to ovarian function and follicular development.In contrast, the T>C:340 polymorphism in local chickens showed no significant effects on most traits, whereas the A>G:375 polymorphism showed significant differences in mid-production egg weight and in egg number during period 6, as well as significant effects on body weight during later production periods.These findings suggest that certain GDF9 gene polymorphisms may serve as useful molecular markers to support selection programs aimed at improving productive performance. However, the observed associations are correlative rather than causal, and further functional and gene expression studies are needed before practical application in breeding programs.

Keywords | GDF9 gene, Polymorphism, Productive traits, Iraqi local chickens, Crossbred hens, Molecular markers

Received | October 30, 2025; **Accepted** | November 07, 2025; **Published** | December 09, 2025
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Citation | Rasheed SO, Jawad MM, Al-Anbari EH (2025). GDF9 gene polymorphisms and their association with productive traits in iraqi local and crossbred chickens. Adv. Anim. Vet. Sci., 13(12):2648-2658.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.12.2648.2658
ISSN (Online) | 2307-8316

INTRODUCTION

Egg production is one of the fundamental pillars of the poultry industry, not only to meet the growing global demand for food but also for its considerable economic importance. With the world population expected to reach approximately 9.15 billion people by 2050 (United Nations Department of Economic and Social Affairs, 2024; FAO, 2020; El-Sabrout *et al*., 2022), improving egg production and quality has become a pressing necessity to ensure sustainable food security. Egg production serves as a key indicator for evaluating the productive performance of poultry flocks, where parameters such as egg number and egg weight are commonly used as essential performance metrics (Calik and Obrzut, 2023). Egg production and quality traits exhibit remarkable variation among breeds and genetic lines, reflecting the direct influence of genetic factors (Li *et al*., 2025). Iraqi local chickens represent a valuable genetic resource characterized by strong adaptability to harsh environments and distinctive phenotypic and genetic

features, including feather color, comb type, and body conformation (Maharani *et al*.,2021). These local populations serve as vital sources of biodiversity and contribute to supporting small-scale rural production systems. However, their relatively low egg production and small body size remain major constraints that limit their integration into intensive commercial systems. This emphasizes the necessity of improving their productive traits while maintaining their natural adaptive capacity (Balcha *et al*., 2024). To achieve this balance, the utilization of genetic improvement results from high-performance crossbred lines is crucial. Such integration can guide breeding strategies aimed at enhancing the productivity of Iraqi local chickens while preserving their unique genetic characteristics and resilience to Iraq's climatic conditions (Rachuene *et al*., 2025). Reproductive and egg quality traits are strongly influenced by genes regulating follicular development and ovarian function. Among these genes, the Growth Differentiation Factor 9 (GDF9) gene, which belongs to the Transforming Growth Factor-beta (TGF-β) superfamily, plays a critical role in oocyte growth, granulosa cell proliferation, and follicular maturation (Wang *et al*., 2024). So, In chickens, the GDF9 gene is essential for granulosa cell growth and follicular differentiation. It is highly expressed in the granulosa cells of pre-hierarchical follicles, stimulating cell proliferation and early follicular maturation (Hlokoe *et al*., 2023). Genetic variation is the foundation of improvement programs in poultry breeding, and single nucleotide polymorphisms (SNPs) represent the most common and informative form of genetic diversity that can be used to identify desirable alleles associated with superior productive traits (Volkova *et al*., 2025; Guo *et al*., 2025). The integration of molecular markers into breeding programs through marker-assisted selection (MAS) increases selection accuracy and accelerates genetic progress (Yacoub *et al*., 2024). The Iraqi local chicken population remains an important genetic reservoir due to its adaptability and disease resistance but still exhibits lower productivity compared with commercial crossbreeds (Tawfeq and Al-Neemy, 2022). Recent efforts have focused on crossbreeding local hens with high-performing commercial lines, resulting in hybrid vigor (heterosis) expressed as improved egg production and body weight in the first generation (Rachuene *et al*., 2025). Although GDF9 is recognized to play a crucial role in controlling granulosa cell proliferation and early ovarian development in birds, its particular polymorphisms have demonstrated contradictory relationships with productive features in various chicken breeds around the world (Lou *et al*., 2019). Importantly, little is known about the genetic makeup of Iraqi local and crossbred chickens' egg production. Thus, by examining GDF9 SNPs as possible molecular markers for economic features in these particular groups, this work seeks to close this knowledge gap. Therefore, this study was conducted to analyze single nucleotide polymorphisms (SNPs) in the *GDF9* gene and determine their associations with productive traits in Iraqi local and crossbred chickens, aiming to identify alleles that can be utilized as molecular markers to enhance the productivity of Iraqi local chickens while maintaining their strong environmental adaptability. Although GDF9 in mammals predominantly regulates early folliculogenesis, studies in poultry have demonstrated that GDF9 is also expressed in granulosa cells of pre-hierarchical follicles, where it influences follicle recruitment and selection, which ultimately affects the growth of yolk-accumulating follicles and thereby contributes indirectly to variation in egg weight (Habashy and Adomako, 2023).

MATERIALS AND METHODS

The experiment was carried out at the Poultry Research Field, Department of Animal Production Research, Al-Zaafaraniya, Ministry of Higher Education and Scientific Research. A total of 160 one-day-old chicks were used, consisting of 80 Iraqi local chicks and 80 crossbred chicks (produced by crossing ISA Brown males with local white females). The chicks were reared in a single poultry house divided into two separate sections using a thick nylon partition, with each section designated for one genotype. The lighting program followed the recommendations of the Lohmann Management Guide and the Department of Animal Production Research guidelines. Light duration and intensity were regulated using automatic timers and 60-watt incandescent bulbs to ensure uniform exposure across both groups. The experiment was conducted during two seasons (winter and summer) using a dual heating system and evaporative cooling units to maintain optimal environmental conditions. Temperature and relative humidity were monitored and recorded daily throughout the experimental period.Commercial ready-made feed from Shukr Company/ Al-Zaafaraniya was provided in three phases:

- Starter diet: 20% crude protein and 2900 kcal metabolizable energy (ME)/kg
- Grower diet: 17% crude protein and 2750–2800 kcal ME/kg
- Layer diet: 17% crude protein and 2800 kcal ME/kg

A standard veterinary vaccination program was applied to all birds. Sex determination was performed at six weeks of age, and any abnormal individuals were excluded. At 16 weeks of age, 50 local hens and 41 crossbred hens were retained and transferred to individual numbered cages for data collection. Productive performance was monitored for 100 consecutive days (from 19 to 34 weeks of age), divided into eight production periods. Body weight was recorded weekly, while egg number and egg weight were recorded daily under uniform management and environmental conditions to evaluate growth and productive performance.

Molecular analyses

At 18 weeks of age, approximately 3 mL of blood was collected from the wing vein of each bird using sterile disposable syringes. Blood samples were placed in EDTA-containing tubes as an anticoagulant and stored at −20°C until further molecular analysis. All laboratory procedures were performed at Nabu Laboratory. Genomic DNA was extracted from blood samples using standard extraction protocols, and the purity and concentration of the DNA were evaluated spectrophotometrically (A260/A280 ratio). The target region of the GDF9 gene was amplified using the Polymerase Chain Reaction (PCR) technique. The amplified fragment size was 711 base pairs (bp). The PCR products were electrophoresed on a 2% agarose gel containing SafeRed™ dye (Biolabs, UK) and visualized under ultraviolet illumination. A 100 bp DNA ladder was used as a molecular size marker to verify the expected fragment size. The electrophoresis results confirmed successful amplification of the target region, indicated by a single distinct band at the expected position, as shown in Figure 1.

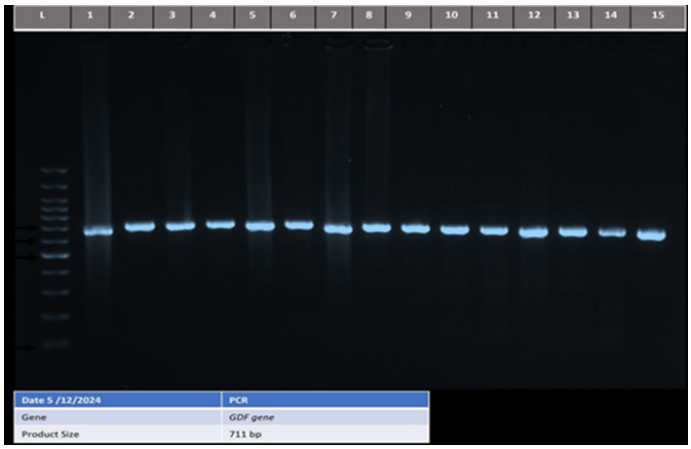

Figure 1: PCR amplification product of the GDF9 gene (711 bp). Electrophoresis was carried out on a 2% agarose gel stained with SafeRed™ dye. A volume of 5 µL from each PCR product was loaded into individual wells, and a 100 bp DNA ladder was used as a molecular size marker.

Primer preparation

The primer sequences used for the amplification of the GDF9 gene were adopted from the study by Liu *et al.* (2018). These primers were utilized in the PCR amplification process, which was carried out by Macrogen Company (Seoul, South Korea). The nucleotide sequences of the primers and their characteristics are presented in Table 1.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure in SAS software (Version 9.4). The following model was used to evaluate the effects of genotype and production period on each productive trait (egg number, egg weight, and shell thickness):

$$Y = \mu + G + P + e$$

Where: Y = observed value of the trait, µ = overall mean, G = fixed effect of genotype, P = fixed effect of production period, and e = random error term.

Significant differences among means were tested using Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$. All values were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

The results of Table 2 demonstrate a clear and consistent influence of GDF9 gene polymorphisms on egg weight across the production periods. For the T>C:340 polymorphism, highly significant differences ($p \leq 0.01$) were observed among genotypes from the first to the sixth and in the eighth periods, whereas no significant differences appeared in period seven. This lack of significance may be attributed to the natural physiological decline in reproductive efficiency during later laying stages. The TC genotype consistently recorded the highest mean egg weights (59.55–62.24 g) in the periods showing significance, highlighting its favorable role in sustaining egg production performance toward the end of the production cycle. For the C>G:100 polymorphism, highly significant differences ($p \leq 0.01$) were detected from periods one to six, with the presence of the G allele (particularly GG) associated with higher egg weights (57.47–60.24 g) compared to CC. However, no significant differences were found during periods seven and eight, suggesting a reduced genetic influence in late production phases. The G>T:282 polymorphism also demonstrated highly significant differences ($p \leq 0.01$) in most production periods, where the GT genotype achieved the highest egg weight values in periods 1–4, 6, and 8, followed by TT, while GG recorded the lowest values. No significant differences were observed in periods five and seven. Similarly, the A>G:375 polymorphism showed highly significant differences ($p \leq 0.01$) from periods one to six, where AG and GG genotypes outperformed AA, indicating the positive effect of the G allele in enhancing egg weight during early and mid-production stages. The absence of significant differences in periods seven and eight again reflects the impact of advancing laying age. The observed genetic effects are likely indirect, mediated through the role of GDF9 in regulating early follicular growth, granulosa cell proliferation, and yolk deposition efficiency, rather than directly influencing egg component synthesis. These interpretations agree with (Liu, 2018; Hlokoe, 2023; Habashy and Adomako, 2023), who associated elevated GDF9 activity with improved ovarian performance and increased egg component deposition.

Table 1: Nucleotide sequence of primers for the GDF9 gene and PCR product size.

Gene	Accession No.	Primer sequence (5′–3′)	Gene location	Product size (bp)
GDF9	NM_206988	F: GAAGCCGTAAGATGTGAAAG R: GGAAGAAAGCCAGTGAATAG	13th chromosome	711

Table 2: Effect of GDF9 gene polymorphisms on egg weight g in crossbred chickens during eight production periods (mean ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	48.87 ± 0.92b	49.40 ± 1.99b	51.65 ± 0.89b	49.76 ± 1.87b	51.40 ± 0.94b	53.05 ± 0.86b	54.15 ± 0.81b	54.52 ± 0.84ab
	TC	59.55 ± 1.52a	62.08 ± 1.43a	61.02 ± 2.30a	62.24 ± 1.96a	61.35 ± 1.31a	61.19 ± 2.02a	57.98 ± 2.12a	60.58 ± 1.29a
	CC	48.74 ± 2.78b	50.11 ± 1.25b	51.43 ± 2.66b	50.00 ± 1.66b	51.24 ± 2.26b	51.53 ± 1.46b	52.22 ± 2.56b	50.18 ± 2.38b
Significance		**	**	**	**	**	**	NS	**
C>G: 100	CC	47.58 ± 0.69b	47.73 ± 2.19b	50.90 ± 0.98b	48.96 ± 0.87c	51.31 ± 1.05b	52.33 ± 0.85b	53.64 ± 0.91b	54.50 ± 0.93b
	CG	54.96 ± 2.15a	57.51 ± 2.41a	56.33 ± 1.99a	54.64 ± 1.96b	54.81 ± 2.34ab	55.55 ± 1.27b	57.88 ± 1.07a	58.23 ± 1.62a
	GG	57.47 ± 2.64a	59.41 ± 2.93a	58.63 ± 2.71a	59.95 ± 2.05a	58.17 ± 2.57a	60.24 ± 2.92a	55.17 ± 2.79a	55.38 ± 2.86a
Significance		**	**	**	**	**	**	NS	NS
G>T: 282	GG	47.72 ± 0.71b	47.82 ± 2.29b	50.91 ± 1.03b	48.82 ± 0.90b	51.28 ± 1.10a	52.42 ± 0.89b	53.46 ± 0.94b	54.55 ± 0.98ab
	GT	55.19 ± 1.86a	57.37 ± 2.05a	57.57 ± 1.91a	57.38 ± 2.14a	56.21 ± 1.81a	57.10 ± 1.72a	57.81 ± 1.32a	58.24 ± 1.44a
	TT	56.13 ± 2.55a	58.29 ± 2.04a	55.51 ± 2.81ab	55.68 ± 2.74a	56.10 ± 1.16a	58.26 ± 1.28a	52.30 ± 1.63b	50.18 ± 1.38b
Significance		**	**	**	**	NS	**	NS	**
A>G: 375	AA	47.88 ± 0.76b	47.99 ± 2.04b	50.83 ± 0.86b	48.69 ± 0.71b	50.97 ± 1.02b	52.56 ± 0.79b	54.07 ± 0.89b	54.75 ± 0.93b
	AG	57.29 ± 1.52a	59.91 ± 1.80a	57.92 ± 2.28a	58.52 ± 1.13a	58.26 ± 1.03a	56.56 ± 1.50ab	57.85 ± 1.31a	58.17 ± 1.63a
	GG	56.88 ± 2.79a	59.58 ± 2.90a	59.30 ± 2.63a	59.73 ± 2.07a	57.87 ± 2.61a	59.74 ± 3.08a	55.16 ± 2.79a	54.37 ± 3.71a
Significance		**	**	**	**	**	**	NS	NS

(**) indicates highly significant differences at the level of p ≤ 0.01within the same column. (NS) indicates no significant differences at the level of p≤0.05among the genotypes of the same polymorphism within each period of the experiment.

The results presented in Table 3 there is a variation in the effect of different genetic polymorphisms of the GDF9 gene on the number of eggs produced during the various production periods. This effect was observed during the early production periods, which are characterized by high ovarian and hormonal activity, which enhances the appearance of genetic differences in reproductive performance. In the T>C:340 polymorphism, the results showed highly significant differences (p ≤ 0.01) among the genotypes during periods one and three, while no significant differences were recorded in the remaining periods. The CC genotype surpassed the others in the first period, recording the highest mean number of eggs produced compared to TC and TT, while the TT genotype surpassed the individuals carrying TC and CC in the third period. This variation indicates the possibility that the C allele may be responsible for early stimulation of GDF9 gene activity at the beginning of the cycle, which increases the ovulation rate, while the effect of the T allele appears in the third period as a result of continued gene expression and hormonal activity balance. These interpretations agree with (Liu *et al*., 2018; Li *et al*., 2019), who explained that

polymorphisms in the GDF9 gene are associated with increased efficiency of follicle growth and responsiveness to reproductive hormones and stimulation of steroid hormone synthesis necessary for follicle growth and oocyte maturation, which may explain the superiority of CC and TT during the early production periods. The results of the C>G:100 polymorphism showed no significant differences among the genotypes CC, CG, and GG in all the studied production periods, and this may indicate that the C>G polymorphism had no effect on determining the number of eggs produced and may be considered a silent polymorphism that does not change the amino acid sequence or the efficiency of the resulting protein. Meanwhile, the G>T:282 polymorphism recorded highly significant differences ($p \leq 0.01$) in the first and third production periods, where the TT genotype surpassed both GT and GG at the beginning of the cycle, recording the highest mean number of eggs produced, while the GT genotype surpassed the other genotypes in the third period. This may suggest that the T allele may contribute to enhancing the gene expression of GDF9 during the early stages of production, leading to stimulation of follicle growth and increased ovulation rate. The A>G:375 polymorphism showed highly significant differences ($p \leq 0.01$) in the fourth production period, where the AG genotype recorded the lowest number of eggs produced compared to AA and GG, indicating reduced efficiency of the AG genotype in the middle of the production cycle. It is likely that this is due to the reduced effectiveness of the G allele during this stage, leading to lower GDF9 gene expression and decreased ovarian activity. This interpretation is supported by (Huang *et al*., 2015), who indicated that some polymorphisms in this gene may limit ovulation efficiency and reduce ovarian responsiveness to reproductive hormones.

Table 4 shows that T>C:340 polymorphism showed highly significant differences ($p \leq 0.01$) during Periods 7 and 8, while no significant differences were recorded from Periods 1 to 6. The CC genotype outperformed both TT and TC

Table 3: Association between GDF9 gene polymorphisms and the number of eggs produced (eggs/hen) in crossbred chickens (mean ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	8.40 ± 0.51b	9.16 ± 0.55b	9.23 ± 0.52a	8.96 ± 0.51a	9.56 ± 0.60a	8.20 ± 0.47a	9.13 ± 0.48a	9.03 ± 0.53a
	TC	10.87 ± 0.74ab	10.37 ± 0.90ab	10.12 ± 1.02a	8.12 ± 0.98a	10.28 ± 1.01a	8.50 ± 0.92a	9.25 ± 0.86a	10.25 ± 0.72a
	CC	12.66 ± 0.33a	7.33 ± 2.18a	5.00 ± 1.73b	7.00 ± 1.73b	8.00 ± 1.00a	9.66 ± 0.88a	10.33 ± 1.20a	7.00 ± 1.00a
Significance		**	NS	**	NS	NS	NS	NS	NS
C>G: 100	CC	8.62 ± 0.56a	9.16 ± 0.59a	8.91 ± 0.61a	8.70 ± 0.56a	10.04 ± 0.63a	8.25 ± 0.55a	9.60 ± 0.51a	9.45 ± 0.58a
	CG	9.50 ± 1.00a	9.62 ± 0.88a	9.50 ± 0.86a	7.87 ± 1.09a	8.87 ± 1.32a	8.75 ± 0.81a	7.62 ± 0.88a	8.62 ± 1.08a
	GG	10.44 ± 1.08a	9.22 ± 1.29a	9.22 ± 1.29a	9.22 ± 0.92a	8.87 ± 0.91a	8.33 ± 0.74a	9.77 ± 0.70a	8.66 ± 0.74a
Significance		NS	NS	NS	NS	NS	NS	NS	NS
G>T: 282	GG	8.56 ± 0.58b	9.34 ± 0.59b	9.08 ± 0.61ab	8.73 ± 0.59a	10.00 ± 0.66a	8.13 ± 0.57a	9.63 ± 0.54a	9.56 ± 0.60a
	GT	9.33 ± 0.68ab	9.66 ± 0.81a	10.00 ± 0.86a	8.16 ± 0.75a	9.41 ± 1.01a	9.08 ± 0.58a	8.41 ± 0.74a	8.66 ± 0.74a
	TT	11.33 ± 1.47a	8.16 ± 1.72a	7.33 ± 1.35b	9.33 ± 1.35a	8.00 ± 0.63a	7.83 ± 1.04a	9.50 ± 0.84a	8.33 ± 1.08a
Significance		**	NS	**	NS	NS	NS	NS	NS
A>G: 375	AA	8.66 ± 0.53a	9.14 ± 0.54a	9.11 ± 0.57a	8.77 ± 0.51a	9.70 ± 0.67a	8.22 ± 0.51a	9.15 ± 0.51a	9.33 ± 0.54a
	AG	10.00 ± 1.04a	9.20 ± 1.01a	9.60 ± 1.43a	5.80 ± 0.58b	10.60 ± 0.92a	9.40 ± 1.02a	9.40 ± 1.46a	10.00 ± 1.09a
	GG	10.33 ± 1.13a	9.66 ± 1.36a	8.77 ± 1.16a	9.88 ± 0.96a	8.50 ± 0.80a	8.22 ± 0.72a	9.44 ± 0.58a	8.00 ± 0.88a
Significance		NS	NS	NS	**	NS	NS	NS	NS

(**) indicates highly significant differences at the level of $p \leq 0.01$within the same column. (NS) indicates no significant differences at the level of $p \geq 0.05$among the genotypes of the same polymorphism within each period of the experiment.

Table 4: Association between *GDF9* gene polymorphisms and body weight g in crossbred female chickens (M ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	1340.80 ± 4.33	1387.50 ± 2.03	1432.00 ± 2.12	1463.53 ± 2.66	1495.20 ± 2.55	1539.30 ± 3.90	1614.13 ± 3.96ab	1715.53 ± 6.39b
	TC	1353.88 ± 3.59	1384.00 ± 2.04	1427.00 ± 3.17	1470.25 ± 3.50	1500.75 ± 5.49	1529.63 ± 8.48b	1600.13 ± 3.50b	1716.50 ± 5.22b
	CC	1344.67 ± 4.66	1380.67 ± 1.20	1433.33 ± 2.70	1469.00 ± 3.69	1485.67 ± 6.43	1555.67 ± 8.08a	1634.67 ± 4.37a	1760.67 ± 6.35a
Significance		NS	NS	NS	NS	NS	NS	**	**
C>G: 100	CC	1338.46 ± 5.09b	1387.25 ± 2.21b	1432.25 ± 2.21b	1464.46 ± 3.16b	1496.79 ± 2.95	1539.33 ± 4.41	1614.42 ± 4.36	1713.33 ± 7.56
	CG	1349.38 ± 4.88	1388.22 ± 3.85	1431.88 ± 5.35	1467.38 ± 4.77b	1491.50 ± 4.32	1527.75 ± 7.94	1612.50 ± 7.19	1720.25 ± 10.85
	GG	1352.33 ± 4.10	1383.00 ± 3.25	1427.44 ± 4.11	1465.44 ± 5.04	1496.00 ± 5.40	1546.33 ± 6.95	1609.22 ± 7.40	1733.11 ± 10.37
Significance		NS	NS	NS	NS	NS	NS	NS	NS
G>T: 282	GG	1338.30 ± 5.32	1387.52 ± 2.30	1432.43 ± 2.30	1465.09 ± 3.24	1497.00 ± 3.08	1537.57 ± 4.22	1615.26 ± 4.47b	1711.70 ± 7.71b
	GT	1352.00 ± 3.74	1385.42 ± 3.51	1429.08 ± 3.93	1464.42 ± 4.12	1495.08 ± 3.93	1534.17 ± 7.84	1607.42 ± 5.34	1720.00 ± 8.31ab
	TT	1347.33 ± 4.90	1383.50 ± 4.18	1430.17 ± 5.36	1467.50 ± 6.04	1491.17 ± 6.13	1551.50 ± 5.34	1614.83 ± 8.44	1745.17 ± 8.44a
Significance		NS	NS	NS	NS	NS	NS	NS	**
A>G: 375	AA	1339.22 ± 4.65b	1386.81 ± 2.03ab	1432.07 ± 2.16	1464.59 ± 2.82b	1495.00 ± 2.72	1538.59 ± 4.24	1614.00 ± 4.18	1715.56 ± 7.09
	AG	1353.00 ± 5.54	1393.60 ± 3.34a	1431.60 ± 7.05	1468.20 ± 4.88	1498.80 ± 5.06	1532.20 ± 5.33	1608.00 ± 7.39	1706.20 ± 7.39
	GG	1351.67 ± 3.92b	1380.78 ± 3.07b	1428.00 ± 4.22	1465.56 ± 4.98	1495.56 ± 5.18ab	1542.22 ± 6.86	1612.33 ± 6.81	1736.56 ± 8.64
Significance		NS	**	NS	NS	NS	NS	NS	NS

(**) indicates highly significant differences at the level of p ≤ 0.01within the same column. (NS) indicates no significant differences at the level of p ≥ 0.05among the genotypes of the same polymorphism within each period of the experiment.

in the final two periods. This is consistent with findings that the genetic effects of SNP loci on growth traits may become more pronounced in later stages of development due to hormonal and physiological changes associated with maturity, during which the efficiency of energy utilization and mineral deposition increases as muscle and skeletal tissues continue to develop (Richter et al., 2024). This may explain the significant differences observed in the advanced production stages in the present study. In addition, hybrid vigor has been reported to contribute significantly to improving growth traits and body weight in chickens during late production phases, which further supports the superiority of the CC genotype at these stages (Teshome *et al*., 2025). The C>G:100 polymorphism did not show any significant differences among genotypes. Some genetic polymorphisms may not exhibit noticeable effects on productive traits if they occur in non-functional regions of the gene, preventing their influence from being expressed phenotypically (Abu-Rekaiba *et al*., 2021).For the G>T:282 polymorphism, no significant differences were observed from Periods 1 to 7, whereas highly significant differences (p ≤ 0.01) were recorded in Period 8, where the TT genotype exhibited the highest mean body weight, followed by GT, while GG showed the lowest mean. Regarding the A>G:375 polymorphism, highly significant differences (p ≤ 0.01) were recorded in Period 2, where the AG genotype outperformed both GG and AA. The genetic architecture of body weight in chickens varies across production periods, as the effects of SNPs may change with age due to interactions between gene expression and metabolic and hormonal activity associated with sexual maturity and aging, which may explain why certain genetic loci exert their influence only at specific stages of growth (Zhong et al., 2024).

Table 5 shows There was a non-significant variation among the genotypes of the T>C:340 polymorphism across all production periods, as the values did not show any significant differences at the level of (p ≥ 0.05). This indicates that this polymorphism did not have a clear effect

Table 5: Association between GDF9 gene polymorphisms and average egg weight (g) in local female chickens (M ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	33.01 ± 0.97	36.54 ± 0.74	39.17 ± 0.81	40.14 ± 0.74	42.36 ± 1.28	42.92 ± 1.09	47.09 ± 1.17	48.55 ± 2.03
	TC	33.60 ± 1.04	36.74 ± 0.90	38.45 ± 0.88	41.69 ± 0.85	42.69 ± 0.69	43.48 ± 0.33	44.61 ± 0.76	51.19 ± 0.30
	CC	32.57 ± 0.54	36.48 ± 0.67	39.05 ± 1.10	41.27 ± 0.96	42.11 ± 1.13	43.69 ± 0.90	46.43 ± 1.30	48.06 ± 0.05
Significance		NS	NS	NS	NS	NS	NS	NS	NS
A>G: 375	AA	33.43 ± 0.75	36.70 ± 0.66	38.64 ± 0.64ab	41.18 ± 0.66ab	42.64 ± 0.65ab	43.07 ± 0.46b	45.30 ± 0.71	50.47 ± 0.64
	AG	31.93 ± 0.64	35.50 ± 0.64	38.66 ± 1.45b	40.02 ± 0.80b	40.07 ± 0.95b	42.55 ± 1.37b	45.04 ± 0.97	48.06 ± 0.05
	GG	33.94 ± 0.79	37.86 ± 1.11	40.10 ± 0.97	43.82 ± 1.56a	44.30 ± 1.24a	45.72 ± 0.62a	47.24 ± 1.64	41.85 ± 1.13
Significance		NS	NS	NS	**	**	**	NS	NS

(**) indicates highly significant differences at the level of p ≤ 0.01within the same column. (NS) indicates no significant differences at the level of p ≥ 0.05among the genotypes of the same polymorphism within each period of the experiment.

on gene expression or protein activity during the different stages of production, and this may be due to the mutation occurring in a non-functional region of the gene (Qin *et al*., 2015; Liu *et al*., 2018) reported that some mutations in the *GDF9* gene exhibit a variable effect associated with differences in the level of gene expression across production periods, where the activity of the gene increases at certain stages of follicular development and decreases in others, leading to variation in egg weight among periods. As for the A>G:375 polymorphism, the results showed highly significant effects (p ≤ 0.01) in the fourth, fifth, and sixth periods, while no significant differences were recorded in the remaining periods. It is noted that individuals carrying the GG genotype achieved higher mean egg weight during these periods compared to the AA and AG genotypes, while the AG genotype showed a decrease during these stages, indicating that the G allele may contribute to enhancing gene expression or increasing physiological efficiency during the mid-production phase. This result is consistent with Lou *et al*. (2019), who stated that differences in the nature of genotypes lead to variation in genetic efficiency, and therefore differences in productive traits such as egg weight, which explains the reduced performance of the AG genotype compared to both GG and AA (Abdulla *et al*., 2016; Hassan et al., 2024) clarified that egg weight increases gradually with the progression of production periods as a result of the interaction between genetic and physiological factors, which agrees with the upward trend observed in this study from the first to the eighth period, where the average egg weight increased gradually for all genotypes (Abu-Rekaiba *et al*., 2021; Abdel-Karim, 2023) also confirmed that some genetic polymorphisms do not show their effects continuously, but rather their influence appears in specific periods of the production cycle, which explains why the effect of the A>G:375 polymorphism was limited to the mid-production periods and did not extend to the early or late stages of production.

Table 6 shows the effect of GDF9 gene genotypes on egg production in local chickens across eight production periods. For the T>C:340 polymorphism, no significant differences (p ≥ 0.05) were detected among the TT, TC, and CC genotypes during all production periods, as the mean egg number remained relatively similar across the laying cycle. This indicates that this polymorphism does not contribute to variation in egg production in local chickens, and the minor fluctuations observed are likely associated with normal physiological changes during the laying period rather than genetic influence. In contrast, the A>G:375 polymorphism showed a significant association (p ≤ 0.01) only during Period 6, while no significant differences were observed in the remaining periods. During this mid-production stage, the GG genotype recorded the highest egg production, whereas the AG genotype showed a noticeable decline, suggesting a possible reduction in functional efficiency associated with the AG genotype at this stage. However, this effect was not sustained before or after Period 6, indicating a stage-dependent genetic influence that becomes detectable only when reproductive and metabolic demands increase during the mid-laying phase. The performance of the AA and GG genotypes remained relatively stable across the production cycle, suggesting more efficient regulatory control over ovarian follicle recruitment and development. However, the explanation that the AG genotype may reduce gene expression or mRNA stability remains hypothetical, as direct molecular analysis was beyond the scope of the present study. These findings are consistent with (Lou *et al*., 2019; Abdulla *et al*., 2016; Karim and Noori, 2023), who reported that productive traits in local chicken populations

Table 6: Association between GDF9 gene polymorphisms and egg number in local female chickens across eight production periods (mean ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	4.08a±0.43	4.58a±0.41	4.10a±0.48	3.54a±0.47	4.50a±0.56	3.75a±0.62	4.00a±0.47	4.00a±0.60
	TC	3.77a±0.31	3.95a±0.40	4.50a±0.40	4.19a±0.41	3.78a±0.37	4.21a±0.30	4.86a±0.46	5.91a±1.75
	CC	3.78a±0.40	4.38a±0.40	3.85a±0.36	4.36a±0.63	4.15a±0.43	4.71a±0.59	4.08a±0.60	4.28a±0.41
Significance		NS	NS	NS	NS	NS	NS	NS	NS
A>G: 375	AA	3.84a±0.25	4.18a±0.31	4.35a±0.32	3.83a±0.32	4.06a±0.32	4.15±0.29b	4.53a±0.34	5.24a±1.25
	AG	3.80a±0.46	3.77a±0.49	4.00a±0.47	5.25a±0.52	4.33a±0.55	3.60b±0.58b	4.50a±1.00	4.60a±0.45
	GG	4.00a±0.73	5.16a±0.30	3.80a±0.48	3.60a±1.12	3.50a±0.50	5.83a±0.90a	3.83a±0.60	4.16a±0.70
Significance		NS	NS	NS	NS	NS	**	NS	NS

Table 7: Association between GDF9 gene polymorphisms and body weight (g) in local female chickens (M ± SE).

Polymorphisms	Genotypes	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
T>C: 340	TT	1289.92 ± 5.30	1340.17 ± 3.85	1380.75 ± 3.64	1415.83 ± 2.63	1438.67 ± 1.09	1452.50 ± 1.07	1474.08 ± 2.40	1518.50 ± 5.64
	TC	1287.35 ± 4.01	1341.74 ± 2.57	1378.17 ± 2.46	1418.39 ± 1.52	1439.35 ± 0.90	1454.87 ± 0.77	1473.91 ± 1.26	1514.04 ± 3.40
	CC	1279.64 ± 5.42	1337.71 ± 3.18	1380.64 ± 3.00	1415.50 ± 2.47	1437.57 ± 1.42	1453.14 ± 0.98	1473.71 ± 2.88	1517.57 ± 4.36
Significance		NS	NS	NS	NS	NS	NS	NS	NS
A>G: 375	AA	1288.79 ± 3.28	1341.55 ± 2.20	1379.12 ± 2.11	1417.42 ± 1.38	1439.12 ± 0.73a	1454.15 ± 0.64	1473.73 ± 1.20	1517.00 ± 2.92
	AG	1279.20 ± 5.41	1340.80 ± 3.82	1383.10 ± 2.75	1415.00 ± 3.01	1435.30 ± 1.53b	1453.70 ± 1.20	1473.30 ± 3.47	1516.30 ± 5.59
	GG	1280.17 ± 10.08	1331.83 ± 3.40	1375.67 ± 5.45	1417.50 ± 3.62	1441.83 ± 0.60a	1452.00 ± 1.78	1475.83 ± 3.88	1511.17 ± 7.51
Significance		NS	NS	NS	NS	**	NS	NS	NS

(**) indicates highly significant differences at the level of p ≤ 0.01within the same column. (NS) indicates no significant differences at the level of p ≥ 0.05among the genotypes of the same polymorphism within each period of the experiment.

exhibit variable genetic influence across production periods and that the heritability of egg production is affected by the stage of the laying cycle, and that some reproductive-related polymorphisms only express their genetic effects during specific physiological phases, which aligns with the restricted effect of the A>G:375 polymorphism observed in Period 6 in the present study.

Table 7 shows the T>C:340 polymorphism did not exhibit any significant differences among the TT, TC, and CC genotypes across all eight production periods. This indicates that this polymorphism had no discernible effect on body weight in local hens, suggesting that the nucleotide substitution may occur in a non-functional region of the gene or that its influence on gene expression is too subtle to produce a phenotypic response in this trait. In contrast, the A>G:375 polymorphism demonstrated a highly significant effect (p ≤ 0.01) in the fifth production period, where the AA and GG genotypes were significantly superior to the AG genotype in body weight, while no significant differences were observed during the remaining periods. Although the GG genotype recorded the highest numerical mean, the absence of statistical significance between AA and GG indicates comparable functional impact of these genotypes at this stage. This suggests that the genetic influence of GDF9 on body weight is phase-specific, potentially corresponding to physiological transitions in the ovarian or endocrine activity that occur during mid-laying stages. Body weight in local chickens is a complex quantitative trait regulated by multiple loci that interact both additively and epistatically, alongside substantial environmental modulation (Wang *et al.*, 2024). This multi-factorial architecture contributes to variation in growth patterns across different production stages and may explain why the impact of the A>G:375 polymorphism was observed only during a single period. Therefore, the absence of significant differences in the T>C:340 polymorphism and the limited, stage-specific effect of the A>G:375 polymorphism may reflect the dynamic nature of gene expression regulation throughout the growth and

reproductive phases in local chickens.

We recognize that this study's findings are statistical correlations rather than necessarily causal relationships. Alternative explanations must be taken into account. The *GDF9* gene's polymorphisms could simply represent markers in close linkage disequilibrium with another causative gene situated in the nearby genomic region, according to one such theory. For example, the actual causal factor may be a neighboring regulatory element that influences the expression of GDF9 or another related gene.

Furthermore, population structure may have an impact on the relationship results even though the indigenous and crossbred chicken populations under study are native to the area. We did not conduct formal testing for population structure or linkage disequilibrium due to methodological limitations. To ascertain if these SNPs are just employed as linkage disequilibrium markers or if they have a direct impact on gene expression or protein function, a thorough analysis would be an essential next step (Wang *et al*., 2019). To verify the underlying causal mechanism, we suggest that future research include direct measures of gene expression (e.g., via qPCR) in hens with the superior genotypes.

Also, we recognize that performing multiple statistical tests (96 comparisons) increases the risk of Type I errors (false positives). While our study did not apply a formal correction for multiple testing (such as Bonferroni or FDR, we believe the results showing significance are biologically plausible. However, we acknowledge that the lack of consistency across periods requires cautious interpretation. Therefore, the significant associations found, particularly the stage-specific effects, should be viewed as preliminary candidates for molecular markers, requiring further independent validation before incorporation into breeding programs. This is a crucial area for future research.

CONCLUSION

In conclusion, this study found a number of single nucleotide polymorphisms (SNPs) in the GDF9 gene in Iraqi local and crossbred chicken populations. These SNPs were found to be significantly associated with productive traits, such as egg weight and egg number, especially during later production periods. In both populations, the A>G:375 polymorphism was found to be a significant potential marker that had stage-specific effects on performance (e.g., Period 6). In order to increase the productivity of these native Iraqi strains, we confirm that GDF9 SNPs are useful initial candidates for use as genetic markers in Marker-Assisted Selection (MAS) procedures. Importantly, the findings establish correlation, not causation. Future research must concentrate on functional studies, such as gene expression analysis qPCR and evaluation of Linkage Disequilibrium LD in the genomic region surrounding the *GDF9* gene, in order to confirm the useful application of these SNPs and to definitively comprehend the underlying biological mechanism.

ACKNOWLEDGEMENTS

The authors would like to express their sincere appreciation to the Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, and to the Poultry Research Field at Al-Zaafaraniya, Ministry of Higher Education and Scientific Research, for providing the facilities and technical support necessary to complete this study. Deep gratitude is also extended to Nabu Laboratory for their assistance in conducting the molecular analyses.

NOVELTY STATEMENT

This study is among the first to investigate the relationship between *GDF9* gene polymorphisms and productive traits in both Iraqi local and crossbred chickens using molecular genetic techniques. The identification of specific nucleotide substitutions associated with egg production, egg weight, and body weight provides novel molecular insights that can be utilized for marker-assisted selection to enhance poultry productivity under local environmental conditions.

AUTHOR'S CONTRIBUTION

SOR conducted the experimental work, collected and analyzed the data, and drafted the manuscript. MMJ supervised the experiment, guided the methodology, and reviewed the manuscript. EHA-A designed the research framework, provided overall scientific supervision, revised and finalized the manuscript, and served as the corresponding author. All authors read and approved the final manuscript.

Ethical statement

All experimental procedures were performed in accordance with the ethical standards of the College of Agricultural Engineering Sciences, University of Baghdad. The study protocol was reviewed and approved by the Animal Care and Use Committee under approval number (Ethical Approval No. 1083/2024). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Animal Care and Use Committee, Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, and approved by the Ministry of Higher Education and Scientific Research, Iraq.

Generative AI or AI-assisted technology statement

No generative AI or AI-assisted technology was used in the preparation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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