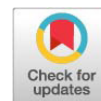


Research Article



Polymorphisms of the OIH Gene and Their Association with Internal Egg Quality Traits in Iraqi Local Chickens

SHAIMA OMRAN RASHEED^{1*}, MUHANNAD MUNTHIR JAWAD², EMAN HASAN AL-ANBARI¹

¹Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq;

²Authority of Scientific Research, Ministry of Higher Education and Scientific Research, Baghdad, Iraq.

Abstract | Internal egg quality traits, including albumen height, Haugh unit, and yolk stability, are essential indicators of egg freshness and market value in laying hens. The ovoinhibitor (OIH) gene may functionally contribute to these traits through protease inhibition. This study aimed to evaluate the association between OIH gene polymorphisms (T>C:77 and T>C:215) and internal egg quality traits in Iraqi local chickens. Fifty hens were monitored across eight production periods. DNA sequencing was used to determine SNP genotypes, and egg quality traits were analyzed using a GLM model including genotype–period interaction. The T>C:77 polymorphism showed significant associations with Haugh unit in Periods 7–8 ($p \leq 0.01$) and with yolk index and shell thickness in Periods 1 and 5 ($p \leq 0.01$). CC hens generally demonstrated higher Haugh unit and yolk index values, indicating improved albumen stability and yolk membrane integrity. In contrast, T>C:215 showed no significant effect on most traits ($p \geq 0.05$). These results suggest that the T>C:77 polymorphism may serve as a molecular marker for enhancing internal egg quality in Iraqi local chickens. This study is among the first to characterize OIH gene polymorphisms in Iraqi indigenous chickens and assess their association with egg quality traits.

Keywords | OIH gene, SNPs, Egg quality, Haugh unit, Yolk index, Iraqi chickens

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***Correspondence** | Shaima Omran Rasheed, Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq;

Email: shaima.o@coagri.uobaghdad.edu.iq

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INTRODUCTION

The ovoinhibitor (OIH) gene encodes a serine protease inhibitor responsible for protecting albumen proteins from enzymatic degradation and maintaining egg white stability (Retnosari and Daryono, 2022). Its expression in the oviduct during egg formation, and its hormonal regulation by estrogen and progesterone, suggests a direct influence on traits related to albumen structure such as Haugh unit, yolk membrane firmness reflected by yolk index, and potentially shell communication with internal content affecting shell thickness (Zhu *et al.*, 2001; Huang *et al.*, 2019).

Previous studies on OIH polymorphisms have focused largely on ducks and commercial strains, demonstrating associations with egg internal and shell traits (Wu *et al.*, 2018; Retnosari and Daryono, 2022). However, these findings cannot be extrapolated to Iraqi local chickens, which represent genetically diverse and environmentally adapted populations.

Therefore, this study investigates whether OIH SNP variants are associated with Haugh Unit, yolk index, and shell thickness in Iraqi local hens, providing foundational evidence for their potential use in marker-assisted selection programs.

BIRDS AND MANAGEMENT

A total of 90 local chicks were obtained at one day of age from the same research field and were reared under controlled housing conditions. The floor was littered with 5 cm wood shavings, and both electric and oil-based heating, as well as ventilation systems, were provided to maintain appropriate environmental conditions. The chicks were fed commercial starter, grower, and layer diets (Al-Shaker Company, Al-Zaafaraniya) twice daily according to their age and production phase. At 6 weeks of age, sexing was performed and all male chicks were excluded from the experiment, leaving 50 confirmed female chicks for further study. At 16 weeks of age, the hens were transferred to individually labeled cages for accurate monitoring of production and egg quality traits.

EGG QUALITY MEASUREMENTS

Egg quality traits were recorded starting at 19 weeks of age and continued for eight production periods, each lasting two weeks (100 days total). Eggshell thickness was measured after drying the eggshell using a digital vernier caliper at the broad and narrow ends, and the mean value was recorded. Haugh unit (HU), an indicator of albumen quality, was calculated using the following equation:

$$HU = 100 \times \log_{10}(H - 1.7 \times W^{0.37} + 7.6)$$

Where H is albumen height (mm) and W is egg weight (g).

The yolk index, reflecting yolk shape and membrane integrity, was calculated as the ratio of yolk height to yolk diameter. Shell thickness was also measured at the blunt, equatorial, and pointed ends using a digital micrometer, and the mean value was used for analysis.

BLOOD SAMPLING AND DNA EXTRACTION

At 18 weeks of age, blood samples were collected from the brachial vein using 3 mL syringes and transferred into EDTA tubes to prevent coagulation. Samples were stored at -20 °C until molecular analysis. Genomic DNA was extracted at Nabu Laboratory, and DNA quality was verified using agarose gel electrophoresis (1% gel).

DNA EXTRACTION AND QUALITY ASSESSMENT

Genomic DNA was extracted using the GeneJet Genomic

DNA Purification Kit (Thermo Fisher Scientific), following the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific), and the OD260/280 ratio ranged between 1.82 and 1.95, confirming high purity with minimal protein contamination. RNA residues were removed by RNase A treatment during extraction. DNA integrity was additionally confirmed by visualization on 1% agarose gel electrophoresis.

PCR AMPLIFICATION AND SNP GENOTYPING

A fragment of the OIH gene was amplified using conventional PCR in a total reaction volume of 25 µL, which contained 12.5 µL of FirePol Master Mix (2×), 1 µL of forward primer (10 pmol), 1 µL of reverse primer (10 pmol), 2 µL of genomic DNA template (~50 ng/µL), and 8.5 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 57 °C for 30 seconds, and extension at 72 °C for 45 seconds, with a final extension at 72°C for 7 minutes. PCR products were visualized on a 2% agarose gel and subsequently sent to Macrogen, South Korea, for Sanger sequencing to detect single nucleotide polymorphisms (SNPs) in the gene.

PRIMER PREPARATION

Primers specific to the OIH gene were designed at Nabu Laboratory using the NCBI Primer-BLAST program (Table 1). The targeted region was selected within the genomic locus with an amplified fragment length of 403 bp.

DNA SEQUENCING METHOD

Sequencing of the amplified OIH fragment (403 bp) was performed using the Sanger method (Sanger et al., 1977). Genomic DNA from 50 local hens was sent to Macrogen Inc. (South Korea) for sequence analysis to identify SNPs within the target region.

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Data normality was assessed using the Shapiro-Wilk test prior to analysis. The following model was applied under the General Linear Model (GLM):

$$Y_{ijk} = \mu + G_i + P_j + (G_i \times P_j) + e_{ijk}$$

Table 1: Primer sequences of the OIH gene.

Gene name	Reverse primer (5'-3')	Forward primer (5'-3')	Gene Region	Product size (bp)
OIH	CCAACTTCCACACTGTTTGT	CGTGATGAGCTTGTTAACGAG	Chromosome 13	403 bp

Where; Y_{ijk} = observed trait value , μ = overall mean, G_i = fixed effect of genotype, P_j = fixed effect of production period, $(G^i \times P_j)$ = interaction between genotype and period, e_{ijk} = random error term.

Duncan’s multiple range test was used for mean separation at $\alpha = 0.05$ because it provides greater detection sensitivity for biological differences among genotypes and is widely used in poultry egg-quality genetics research. Data are presented as mean $\pm$ standard error (SE).

A total of 1200 egg measurements were obtained for statistical analysis (50 hens $\times$ 3 eggs $\times$ 8 periods). Because only eggs produced naturally under uniform environmental and nutritional management were collected, all measurements were valid and biologically representative, with no missing or excluded data points. This ensured full statistical power and strengthened the reliability of the GLM model and its assumptions.

RESULTS AND DISCUSSION

PCR amplification successfully produced a 403 bp fragment of the OIH gene in all samples, as visualized on a 2% agarose gel (Figure 1). Sequence analysis identified a SNP at nucleotide position 77 within exon 13, resulting in three genotypes: TT, TC, and CC (Figure 2), and another SNP at nucleotide position 215 within exon 13, producing three genotypes: TT, TC, and CC (Figure 3). For the T>C:77 polymorphism, the genotypes TT, TC, and CC were observed with frequencies of 8%, 52%, and 40%, respectively, with a higher C allele frequency (0.66) compared to T (0.34). For the T>C:215 variant, the TT genotype was predominant (74%), followed by TC (22%) and CC (4%), with corresponding allele frequencies of 0.85 (T) and 0.15 (C) (Table 2).

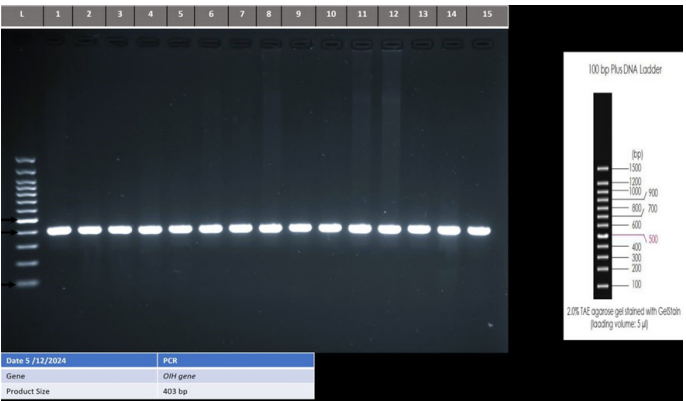


Figure 1: Agarose gel electrophoresis showing the 403 bp PCR amplification of the OIH gene. L = 100 bp DNA ladder.

Table 3 shows that the T>C:77 polymorphism did not influence HU during Periods 1–6, while differences emerged

in Periods 7 and 8, where specific genotypes outperformed TT. Likewise, the T>C:215 polymorphism showed no early effects but became significant from Period 4 onward, with CC consistently maintaining superior albumen quality during later laying Table 3. These outcomes suggest that the OIH variations affect albumen traits primarily in later production stages, rather than exerting constant influence across the laying cycle. During early periods, albumen-secreting tissues likely function at uniformly high efficiency across genotypes, minimizing detectable differences. With age, natural decline in albumen viscosity exposes genetic effects, enabling clearer differentiation among genotypes.

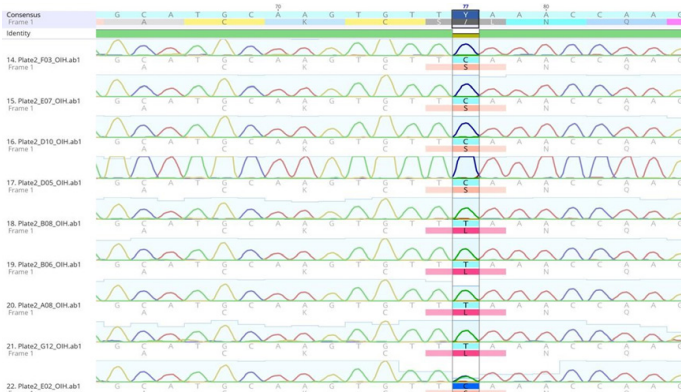


Figure 2: Location of the T>C:77 SNP within exon 13 of the OIH gene.

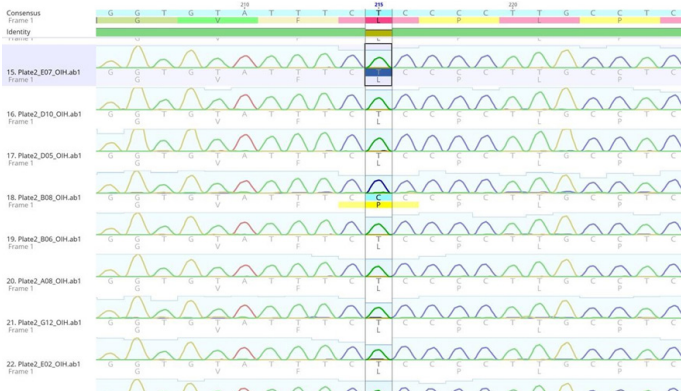


Figure 3: Location of the T>C:215 SNP within exon 13 of the OIH gene.

Table 2: Genotype distribution, percentage frequencies, and allele frequencies of the OIH gene in Iraqi local chickens.

SNP	Geno- type	Num- ber	Percent- age (%)	P value	Allele	Allele frequency
T> C:77	TT	4.00	8.00	0.0001	T	0.34
	TC	26.00	52.00			
	CC	20.00	40.00		C	0.66
T> C:215	TT	37.00	74.00	0.0001	T	0.85
	TC	11.00	22.00			
	CC	2.00	4.00		C	0.15

P-values indicate deviation from Hardy–Weinberg equilibrium.

Table 3: Association of OIH gene polymorphisms with Haugh unit in Iraqi local hens (mean $\pm$ SE).

Polymor- phism	Genotype	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8
T>C:77	TT	61.11 $\pm$ 0.46	64.65 $\pm$ 1.22	70.15 $\pm$ 2.28	72.30 $\pm$ 0.28	69.84 $\pm$ 1.55	74.65 $\pm$ 3.76	80.02 $\pm$ 4.85a	70.93 $\pm$ 2.06b
	TC	60.92 $\pm$ 1.59	66.33 $\pm$ 1.14	68.11 $\pm$ 1.15	72.09 $\pm$ 1.20	74.51 $\pm$ 1.07	74.68 $\pm$ 0.69	77.10 $\pm$ 1.09b	85.29 $\pm$ 0.99a
	CC	60.49 $\pm$ 0.70	65.17 $\pm$ 0.89	69.34 $\pm$ 1.14	72.30 $\pm$ 0.95	74.09 $\pm$ 1.19	76.00 $\pm$ 0.99	79.88 $\pm$ 1.28a	83.28 $\pm$ 1.44a
	Significance	NS	NS	NS	NS	NS	NS	**	**
T>C:215	TT	83.32 $\pm$ 1.97	86.61 $\pm$ 2.17	87.32 $\pm$ 1.87	85.21 $\pm$ 1.77b	87.12 $\pm$ 2.02b	90.42 $\pm$ 1.99ab	89.30 $\pm$ 1.52b	88.64 $\pm$ 1.61c
	TC	83.06 $\pm$ 1.92	78.66 $\pm$ 7.45	85.74 $\pm$ 0.87	82.93 $\pm$ 1.88b	86.71 $\pm$ 2.22b	87.41 $\pm$ 1.57b	90.96 $\pm$ 1.89b	93.84 $\pm$ 1.43b
	CC	95.25 $\pm$ 2.02	98.51 $\pm$ 2.29	98.71 $\pm$ 2.84	98.91 $\pm$ 3.04a	97.26 $\pm$ 1.67a	96.99 $\pm$ 2.84a	95.88 $\pm$.48a	97.12 $\pm$ 2.09a
	Significance	NS	NS	NS	**	**	**	**	**

** ($p \leq 0.01$); NS ($p \geq 0.05$)

Table 4: Association of OIH gene polymorphisms with yolk index in Iraqi local hens (mean $\pm$ SE).

Polymor- phism	Genotype	P 1	P 2	P 3	P 4	P 5	P 6	P 7	P 8
T>C:77	TT	0.32 $\pm$ 0.01b	0.34 $\pm$ 0.01	0.34 $\pm$ 0.02	0.32 $\pm$ 0.01	0.28 $\pm$ 0.01b	0.33 $\pm$ 0.01	0.35 $\pm$ 0.01	0.33 $\pm$ 0.01
	TC	0.34 $\pm$ 0.01ab	0.32 $\pm$ 0.01	0.33 $\pm$ 0.01	0.30 $\pm$ 0.01	0.31 $\pm$ 0.01ab	0.34 $\pm$ 0.01	0.33 $\pm$ 0.01	0.34 $\pm$ 0.01
	CC	0.36 $\pm$ 0.01 a	0.33 $\pm$ 0.01	0.34 $\pm$ 0.01	0.29 $\pm$ 0.01	0.31 $\pm$ 0.01 a	0.34 $\pm$ 0.01	0.32 $\pm$ 0.01	0.35 $\pm$ 0.01
	Significance	**	NS	NS	NS	**	NS	NS	NS
T>C:215	TT	0.34 $\pm$ 0.01	0.32 $\pm$ 0.01	0.33 $\pm$ 0.01	0.30 $\pm$ 0.01	0.31 $\pm$ 0.01	0.33 $\pm$ 0.01	0.33 $\pm$ 0.01	0.34 $\pm$ 0.01
	TC	0.35 $\pm$ 0.01	0.34 $\pm$ 0.01	0.34 $\pm$ 0.01	0.29 $\pm$ 0.01	0.30 $\pm$ 0.01	0.36 $\pm$ 0.01	0.33 $\pm$ 0.01	0.35 $\pm$ 0.01
	CC	0.35 $\pm$ 0.01	0.31 $\pm$ 0.01	0.34 $\pm$ 0.01	0.30 $\pm$ 0.02	0.28 $\pm$ 0.01	0.34 $\pm$ 0.02	0.34 $\pm$ 0.02	0.35 $\pm$ 0.01
	Significance	NS	NS	NS	NS	NS	NS	NS	NS

** ($p \leq 0.01$); NS ($p \geq 0.05$)

The period-dependent pattern aligns with the functional role of OIH as a serine protease inhibitor, helping preserve albumen protein stability within the oviduct (Kinoshita *et al.*, 2004; Mukae *et al.*, 2021). Thus, alleles associated with greater proteolytic resistance, such as C, may help maintain albumen integrity when aging-related degradation becomes more pronounced. Because OIH expression is influenced by estrogen and progesterone during the laying cycle (Retnosari and Daryono, 2022), the observed genotype differences may reflect variation in hormonal regulation rather than a direct sole-gene determinant. Similar genetic and age-interaction patterns for albumen quality have been documented in local chicken strains (Bourin *et al.*, 2011; Abdulla *et al.*, 2016; Gao *et al.*, 2017; Xiao *et al.*, 2024). The genotype distribution and allele frequencies of the OIH gene, which provide foundational context for these associations, are presented in Table 2. Collectively, the late-period advantage observed in certain OIH genotypes suggests the potential utility of OIH polymorphisms, particularly T>C:77 and T>C:215, as complementary molecular markers for supporting albumen quality in breeding strategies, while recognizing that the trait remains multifactorial and not exclusively gene-dependent.

Table 4 indicates that the T>C:77 polymorphism influenced yolk index values only during Periods 1 and 5,

with genotypes carrying the C allele showing better yolk firmness compared with TT. During other periods, no genotype effect was detected. The T>C:215 polymorphism showed no association with yolk index across all periods Table 4. These outcomes suggest that the effect of OIH variation on yolk structural traits is limited to specific physiological stages rather than persistent throughout the laying cycle. Since yolk index reflects membrane strength and cohesiveness, the higher values associated with C-carrying genotypes in early and mid-laying may indicate more efficient maintenance of yolk stability during those stages. The functional role of OIH as a serine protease inhibitor provides biological support for this pattern, as it may reduce degradation of proteins associated with yolk membrane cohesion (Mukae *et al.*, 2021). This interpretation is consistent with previous evidence showing that the preservation of yolk and albumen quality is linked to controlled proteolytic activity within the egg (Xiao *et al.*, 2024). The absence of significance for T>C:215 suggests that not all OIH polymorphisms exert functional effects on internal egg traits, and that their impact may depend on the specific genetic site and molecular consequence. Additionally, the overall stability of yolk index across most periods is consistent with reports showing minimal fluctuation in yolk quality in local chickens, with only modest genotype- or age-related variation (Assefa *et al.*, 2023).

Table 5: Association of OIH gene polymorphisms with shell thickness in Iraqi local hens (mean ± SE)

Polymorphism	Genotype	P1	P 2	P3	P 4	P 5	P 6	P 7	P 8
T>C:77	TT	0.32 ± 0.01b	0.34 ± 0.01	0.34 ± 0.02	0.32 ± 0.01	0.28 ± 0.01b	0.33 ± 0.01	0.35 ± 0.01	0.33 ± 0.01
	TC	0.34 ± 0.01ab	0.32 ± 0.01	0.33 ± 0.01	0.30 ± 0.01	0.31 ± 0.01ab	0.34 ± 0.01	0.33 ± 0.01	0.34 ± 0.01
	CC	0.36 ± 0.01a	0.33 ± 0.01	0.34 ± 0.01	0.29 ± 0.01	0.31 ± 0.01 a	0.34 ± 0.01	0.32 ± 0.01	0.35 ± 0.01
	Significance	**	NS	NS	NS	**	NS	NS	NS
T>C:215	TT	0.34 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 ± 0.01
	TC	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.35 ± 0.01
	CC	0.35 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	0.30 ± 0.02	0.28 ± 0.01	0.34 ± 0.02	0.34 ± 0.02	0.35 ± 0.01
	Significance	NS	NS	NS	NS	NS	NS	NS	NS

** (p ≤ 0.01); NS (p ≥ 0.05).

The genotype distribution and allele frequencies of the OIH gene, which provide context for these associations, are presented in Table 2. Collectively, these findings indicate that the T>C:77 variant rather than T>C:215 may have relevance as a supportive genetic indicator of yolk stability and firmness during specific laying intervals, while recognizing that yolk quality remains governed by multiple genetic and physiological factors.

Table 5 shows that the T>C:77 polymorphism influenced shell thickness only during Periods 1 and 5, where C-carrying genotypes exhibited thicker shells than TT. During other periods, no genotype-dependent effects were detected. The T>C:215 polymorphism showed no association with shell thickness across all periods Table 5. These findings indicate that the effect of OIH variation on shell characteristics is limited to specific intervals of the laying cycle rather than being constant. Since OIH functions primarily as a serine protease inhibitor that supports internal egg stability (Mukae *et al.*, 2021), its effect on shell thickness is likely indirect. Enhanced protection of albumen and yolk proteins may contribute to improved egg stability, which supports shell integrity during particular physiological phases (Yacoub *et al.*, 2024). The period-specific pattern may reflect fluctuations in reproductive hormonal regulation of secretory activity within the oviduct (Retnosari and Daryono, 2022), rather than a continuous effect of genotype. In contrast, the absence of significance for T>C:215 suggests that this site does not alter OIH functional capacity in a way that influences shell structure. Previous reports have noted that shell thickness is governed by a combination of multiple genes and shell-gland-dependent physiological processes rather than single loci (Benavides-Reyes *et al.*, 2021; Tawfeq and Al-Neemy, 2022). Accordingly, OIH variation may contribute secondarily via internal egg stability, while shell formation itself remains primarily determined by shell gland activity. The genotype distribution and allele frequencies of the OIH gene, which provide context for these associations, are presented in Table 2. Overall, the

T>C:77 polymorphism may serve as a supportive rather than primary genetic indicator for shell stability during specific production periods, while recognizing that shell thickness remains a multi-factorial trait.

CONCLUSION

The findings of this study suggest that specific OIH polymorphisms may be associated with variability in internal egg quality traits in Iraqi local hens, particularly during certain phases of the laying cycle. The T>C:77 variant showed period-dependent trends for albumen quality, yolk index, and shell thickness, while T>C:215 exhibited more limited associations. These results indicate that OIH genetic variation could serve as a supplementary marker for egg quality assessment, although egg characteristics are influenced by multiple genetic and physiological factors. Further functional and molecular analyses are recommended to clarify the biological implications of these SNPs at the protein and gene-expression levels.

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NOVELTY STATEMENT

This study is among the first to characterize OIH gene polymorphisms in Iraqi local chickens and to evaluate their association with internal egg quality traits across multiple production periods. The findings highlight the potential of the T>C:77 polymorphism as a genetic marker for improving albumen and yolk stability in breeding programs targeting indigenous chicken populations.

SOR: Conducted the experimental work, laboratory analysis, data interpretation, and manuscript preparation. MMJ: Provided scientific supervision, guidance in molecular genetics, and manuscript review. EHA-A: Oversaw research design, supervised statistical analysis, and contributed to final manuscript editing. All authors reviewed and approved the final manuscript.

GENERATIVE AI OR AI-ASSISTED TECHNOLOGY STATEMENT

Only language-editing assistance was used. No generative AI tools were used for data analysis, interpretation, or scientific conclusion development.

ETHICAL APPROVAL

All experimental procedures adhered to institutional animal welfare standards. The protocol and reporting were reviewed and approved by the Animal Ethics Committee, College of Agricultural Engineering Sciences, University of Baghdad (Approval No.: AEC-COAES-UB/AN.PROD/2025/21; 09/01/2025). The experimental work was conducted from November 1, 2023 to September 1, 2024, with all efforts made to minimize stress and ensure animal welfare.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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