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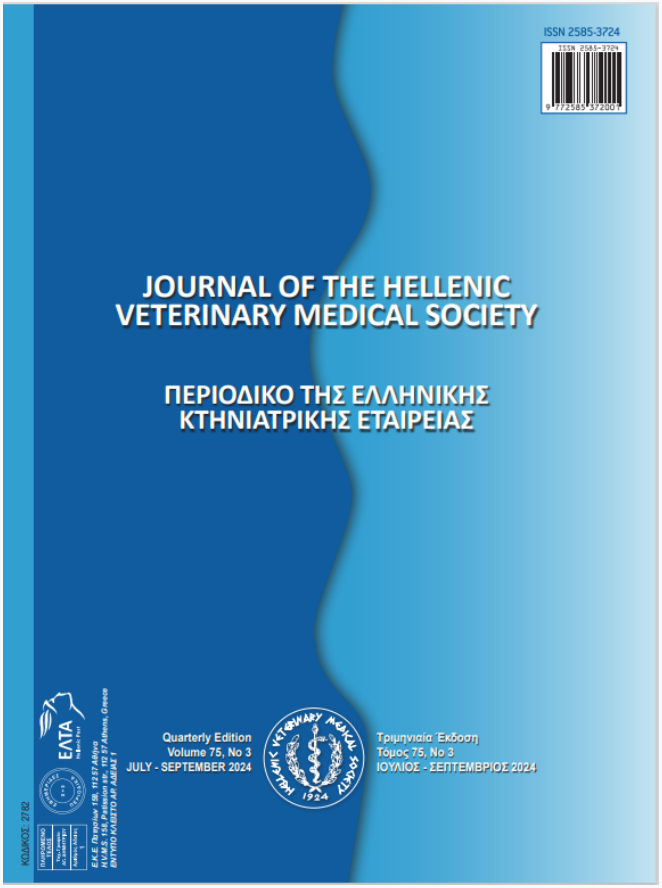

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Research article
Ερευνητικό άρθρο

GH, SEMA3E and TLX gene polymorphism and its association with egg quality traits of quail

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ABSTRACT: This study used the PCR-RFLP technique in Japanese quail to determine the relationship between the GH, Sema3E, and TLX genes and egg quality traits. Three genotypes and two alleles were obtained by cutting the GH gene with the *Msp*I enzyme, the Sema3E gene with the *Hae*III enzyme, and the TLX gene with the *Pst*I enzyme. The allele frequency and the frequency of heterozygous genotypes were higher for all genes. There was no statistically significant relationship between egg quality traits and genotypes. However, it was found that the eggs of the animals with the A allele for the GH gene had a higher shape index. Similarly, eggs from animals with the A allele for the Sema3E gene were found to be heavier. In light of previous studies and these results, it is believed that these genes can be used as selection markers for egg quality traits.

***Key words*:** Quail; PCR-PFLP; GH; Sema3E; TLX

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INTRODUCTION

Quail has become a preferred breeding branch in recent years because it requires less capital and is easy to breed. It is believed that the demand for quail meat will increase daily. After all, it has less fat than chicken and a protein content that promotes brain and body development in children (Priti & Satish, 2014). Also, the fact that quail eggs are richer in essential amino acids and some minerals (Ca, P, and Fe) than chicken eggs (Tolik et al., 2014) indicates that conscious consumers may prefer them. Although quail meat and egg production in the world appears to be low, it is predicted that production will increase due to these characteristics. To make the increased production more economical, quail breeding is gaining importance, and genetic studies are becoming important in this context.

Many gene regions associated with economic yield traits have been studied in poultry such as quail. These important economic yield traits in quail are known as meat yield and egg yield. In the studies, the Growth Hormone (GH) gene region was found to be associated with meat yield traits such as live weight, carcass weight (İlhan, 2021; Lajan & Al-Barzinji, 2022; Nasirifar et al., 2018) and egg (Ahmed & Al-Barzinji, 2020; Johari et al., 2013) production in quail.

Semaphorins are proteins involved in embryonic development of the nervous system (Purohit et al., 2014). The semaphorin 3E gene are one of the members of semaphorin family. Hox genes play an important role in the embryonic development of the skeleton (Pineault et al., 2019). The importance of Hox 11 (TLX) genes, one of the paralogs of Hox genes, in embryonic skeletal development, postnatal growth, and adult fracture repair has been genetically demonstrated (Pineault et al., 2015; Swinehart et al., 2013). There are very few studies on SEMA3E and TLX genes in poultry. In several studies conducted on quails, polymorphisms in the genes mentioned above have been associated to body weight, carcass characteristics, (Ahmed & Al-Barzinji, 2020; Bozkaya et al., 2013) and egg production traits (Lajan & Al-Barzinji, 2022).

In this study, the genotypes of GH, SEMA3E, and TLX genes in quail were determined by PCR-RFLP analysis and an attempt was made to determine the relationship between these genotypes and egg quality traits.

MATERIAL METHOD

Experimental Population and Phenotypic Measurements

The experiment was conducted at the farm of the Faculty of Agriculture, Selcuk University. The experimental material consists of 50 Japanese quails. The quails aged 24 weeks were raised in individual cages for 5 weeks. The birds were fed ad libitum with feed containing 20% protein and 2800 ME from a commercial company. Egg production was determined daily. Egg quality characteristics and feed consumption were determined weekly. During the experiment, the necessary measurements were made on the eggs collected on the last two days of each 7 days to calculate the breaking strength of the eggshell, shell thickness, shell weight, and internal quality characteristics of the eggs. The shape index (%) = [egg width (mm)/ egg length (mm)]x100 is calculated using the formula. The height of the yolk and albumen was determined with a digital height gauge, and the diameter of yolk and the length and diameter of the albumen were determined with a digital calliper. Yolk index (%) = (yellow height/yellow diameter) x 100, albumen index (%) = [white height/(white length + white width)/2] x 100.

PCR-RFLP analysis

After 5 weeks, all raised animals were slaughtered and blood was collected for DNA isolation. The protocol by Miller et al. (1988), based on salt precipitation for the isolation of genomic DNA, was optimized in vitro. In this method, firstly, Erythrocyte Lysis Buffer Solution is added onto the blood in the tubes, and left for 10 minutes. After this period, centrifugation is performed, and the liquid phase remaining on top is removed. This process is repeated three times. The remaining portion in the tube is subjected to centrifugation again after adding Physiological Buffer Solution, and once more the liquid portion is removed. The remaining solid portion is then incubated with Lysis Tris EDTA, Sodium Dodecyl Sulfate, and Proteinase K for one and a half hours at 65 °C. After the incubation, a NaCl solution is added into the tubes, mixed using a vortex mixer, and then centrifuged again. The liquid phase remaining on top is carefully transferred to new tubes, and alcohol is layered on top of the liquid to facilitate DNA precipitation. Following centrifugation, the DNA, now purified with alcohol, precipitates at the bottom of the tube. The upper liquid phase is removed, and the DNA remaining in the lower portion of the tube is prepared for analysis by adding Tris-EDTA buffer solution.

The primers and restriction enzymes used in the study are listed in Table 1.

Table 1: Lengths of amplified fragments, primer sequences and restriction enzymes of studied gene regions

Locus	Primer sequence	Length	Enzyme	References
GH	F5'-ATCCCCAGGCAAACATCCTC-3' R5'-CCTCGACATCCAGCTCACAT -3'	770	*Msp*I	(Johari et al., 2013)
SEMA3E	F5'-ATACTCCAGCTGAGTGGGGA-3' R5'-CAGAAGTATGAGGGAGATCAG-3'	412	*Hae*III	(Ahmed & Al-Barzinji, 2020)
TLX	F5'-ACACTAGGAACATAATGGGCT-3' R5'-TCACTGTGGCGTTTCAGATT-3'	546	*Pst*I	(Ahmed & Al-Barzinji, 2020)

For 10 µl PCR reaction mix, 2 µl DNA (50-100 ng/µl), 0.25 µl (10 pmol/µl) of each primer, 5 µl Taq green PCR Master Mix and 2.5 µl ddH2O were used. The PCR protocol is as follows: after a 5-minute hold at 94 °C, a cycle of 35 cycles was performed; it was held at 94 °C for 30 seconds, at 60 °C for 1 minute, and at 72 °C for 1 minute. Finally, the PCR process was completed by holding at 72 ºC for 10 minutes. The PCR products obtained were then digested overnight at 37 °C by the addition of restriction enzymes, and the bands were analysed on a 2% agarose gel.

Statistical Analysis

Analysis of samples obtained after animal slaughter, allele and genotype frequencies were determined using the POPGEN program. To analyse the obtained data, ANOVA was performed in the statistical program Minitab16 and the Tukey test was used for multiple comparisons. The statistical model used for the analysis is given below.

$$Yij=\mu+Gi+e$$

Yijk: The value of the feature under consideration,

µ: population mean,

Gi: effect of genotype,

eijk: error.

RESULTS AND DISCUSSION

PCR-RFLP

The GH intron 1 gene region was cut with the *Msp*I enzyme, which recognizes the 5'-C/CGG 3' sequence in the 770 base pair PCR product, and the genotypes AA (770 bp), AB (770, 529, 241 bp), and BB (529, 241 bp) were determined (Fig. 1). As a result of digesting the SEMA3E gene with the restriction enzyme *Hae*III, which recognizes the 5'-GG/CC-3' sequence in the PCR product with 412 base pairs, the genotypes AA (412 bp), AB (412, 362, 50 bp) and BB (362, 50 bp) were determined (Fig.2). The TLX gene region was cut with the *Pst*I enzyme recognizing the 5' CTGCA/G-3' sequence in the 546 base pair PCR product, and the genotypes AA (546 bp), AB (546, 404, 142 bp), BB (404, 142 bp) were determined (Fig. 3). The alleles identified are similar to the ones described in previous studies (Ahmed & Al-Barzin-

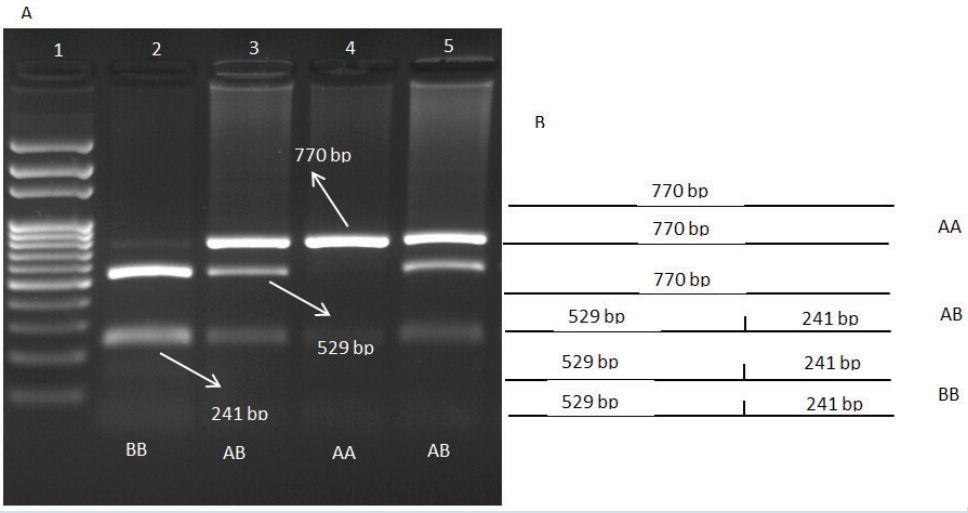

Fig 1. (A) RFLP analysis of PCR products of the GH gene following digestion by MspI restriction enzyme. Lane 1, 100 bp DNA marker. Lanes 2 to 5, RFLP pattern of line 2: BB, 3: AB, 4: AA, 5: AB genotypes. (B) Schematic representation of the MspI enzyme restriction profile of the GH gene region.

ji, 2020; Bozkaya et al., 2013; Lajan & Al-Barzinji, 2022). Allele and genotype frequencies are shown in Table 2. According to the results of the chi-square analysis, the populations used in the study are not in Hardy-Weinberg equilibrium.

Genotyping and Allelic Frequencies

The genotype and allele frequencies derived from the results of PCR-PFLP for the gene regions used in the study are shown in Table 2. The frequency of the A allele was higher than that of the B allele in all three gene regions. However, the frequency of the heterozygous genotypes was found to be higher than the other genotypes. Examination of the chi-square values shows that the population is not in Hardy-Weinberg equilibrium for all three gene regions ($p<0.05$). The results obtained for the GH gene were similar to those obtained by Ahmed and Al-Barzinji (2020).

Association of Genotypes with Egg Quality Traits

ANOVA analysis was performed to determine the relationship between the three genotypes for each of the three genes studied and the egg quality traits, and the results are presented in Tables 3, 4 and 5. It was found that there was no relationship between either of the three genotypes and egg quality traits.

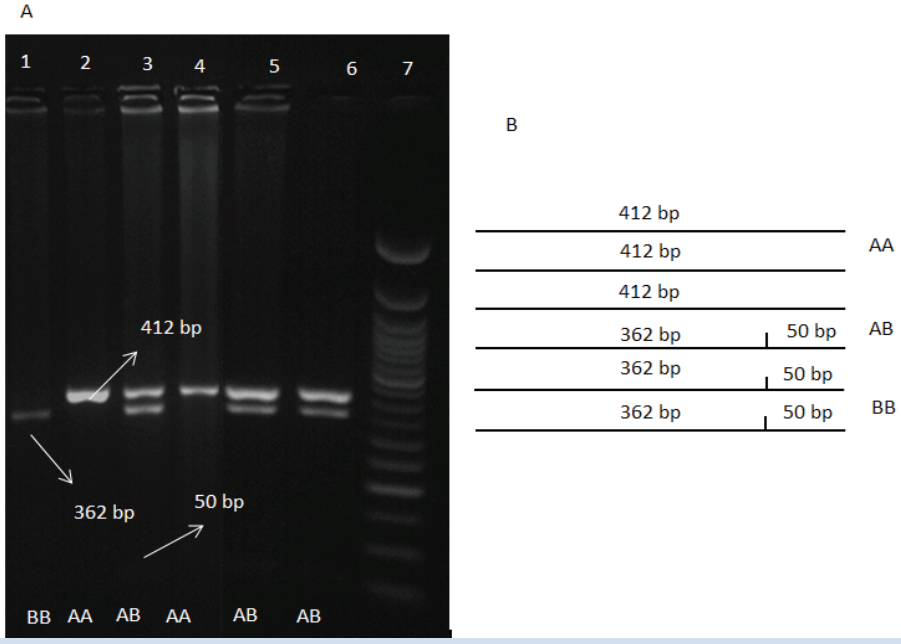

Fig 2. (A) RFLP analysis of PCR products of the Sema3E gene digested by the HaeIII restriction enzyme. Lanes 1 to 6, RFLP pattern of line 1:BB, 2: AA, 3: AB, 4:AA, 5:AB, 6:AB genotypes; Lane 7, 50 bp DNA marker. (B) Schematic representation of the HaeIII enzyme restriction profile of the Sema3E gene region.

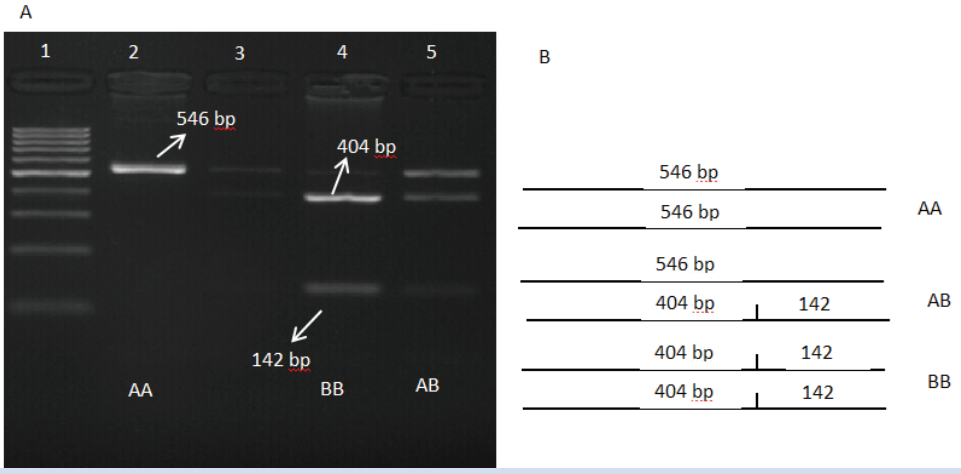

Fig 3. (A) RFLP analysis of PCR products of TLX gene digested by PstI restriction enzyme. Lane 1, 100 bp DNA marker; Lanes 2 to 5, RFLP pattern of line 2: AA, 3:AB, 4:BB, 5:AB genotypes. (B) Schematic representation of the PstI enzyme restriction profile of the TLX gene region.

There was no statistically significant difference among GH genotypes in egg quality traits except for shape index. However, examination of the data shows that the yolk index and shape index of eggs from animals with the A allele are higher than the ones from animals with the B allele. Shape index has been associated with hatching traits such as hatching, chick survival rate, and embryo mortality (Alasahan & Copur, 2016; Shaker et al., 2017). In this regard, quail egg shape index is particularly important for hatching. Many studies have been conducted to determine the relationship between GH gene region and egg production traits (Kansaku et al., 2008; Kulibaba & Podstreshnyi, 2012; Su et al., 2014). These studies show that the GH gene can be used as a selection marker for both egg production and egg quality.

Table 2. Allele and genotype frequencies of the loci and chi-square results

Gene	Allele Frequencies		Genotypes Frequencies		χ^2
GH	A	0.56	AA	0.21	6.35*
	B	0.44	AB	0.69	
			BB	0.10	
SEMA3E	A	0.58	AA	0.26	3.03*
	B	0.42	AB	0.63	
			BB	0.11	
TLX	A	0.70	AA	0.44	2.60*
	B	0.30	AB	0.52	
			BB	0.04	

*p<0.05

Table 3. Egg quality traits determined according to GH loci.

Traits	AA $X \pm S_x$	AB $X \pm S_x$	BB $X \pm S_x$
Egg weight (g)	11.88±0.805	11.21±0.558	11.81±0.80
Shell break strength (g/cm^2)	1.53±0.33	1.51±0.09	1.64±0.01
Shape index	79.40±3.00	78.65±1.10	74.82±3.08
Albumen index	10.94±0.14	10.34±0.23	11.48±0.12
Yolk index	47.24±3.36	48.69±2.04	42.68±1.63
Shell ratio (%)	8.56±0.60	8.35±0.27	8.41±0.25

Table 4. Egg quality traits determined according to SEMA3E loci.

Traits	AA $X \pm S_x$	AB $X \pm S_x$	BB $X \pm S_x$
Egg weight (g)	12.35±0.18	12.14±0.32	10.82±0.68
Shell break strength (g/cm^2)	1.50±0.35	1.51±0.10	1.77±0.04
Shape index	79.65±2.77	77.32±0.84	75.51±1.23
Albumen index	12.06±0.40	11.48±0.44	10.84±0.62
Yolk index	47.56±4.07	48.03±2.36	49.02±3.18
Shell ratio (%)	8.33±0.28	8.62±0.21	8.95±0.35

Table 5. Egg quality traits determined according to TLX loci.

Traits	AA $X \pm S_x$	AB $X \pm S_x$	BB $X \pm S_x$
Egg weight (g)	12.16±0.35	12.06±0.33	12.56±0.42
Shell break strength (g/cm^2)	1.43±0.24	1.62±0.05	1.35±0.26
Shape index	79.23±1.40	77.56±1.12	78.2±1.35
Albumen index	11.23±0.43	11.85±0.32	11.52±0.34
Yolk index	48.05±2.88	48.36±1.24	48.71±2.67
Shell ratio (%)	8.29±0.22	8.42±0.32	8.36±0.52

There are very few studies on Sema3E and TLX genes in poultry. Ahmed and Al-Barzinji (2020) found that there is a relationship between the genotype of the Sema3E, TLX, and GH genes and age at first laying, the weight of first egg, number of eggs in 150 days, and average egg weight in quail ($p<0.05$). In the present study, when the egg quality characteristics of the Sema3E genotypes were examined, it was found that the eggs of the animals with the A allele were heavier. Considering the previous studies and the fact that Sema3E and TLX are genes related to the embryo, it is believed that these genes can be used as genetic markers, especially in studies related to egg production and hatchability.

CONCLUSION

This study attempted to determine the relationship between the GH, Sema3E, and TLX gene polymorphism and egg quality traits in Japanese quail. No statistically significant relationship was found between egg quality traits and genotypes. However, eggs from animals with an A allele for the GH genotype were found to have a higher shape index and animals with an A allele for the Sema3E genotype laid heavier eggs. In light of these results, it is suspected that these genes are related to egg quality traits, and these relationships may become more evident in studies conducted with larger populations.

DECLATATIONS

Ethics approval: In this study, the animal experiment was conducted according to the guidelines of the local ethics committee of Selcuk University, which were prepared following the “Directive 2010/63/EU is the European Union (EU) legislation”. All procedures in this study complied with the ethical principles of animal welfare.

CONFLICT OF INTEREST

The author declares no competing interests**.**

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