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Research Note: Association of insulin-like growth factor 1 receptor gene polymorphism with production performance in Savimalt and French Giant meat-type quails

Junyan Bai,[*,1] Xinle Wang [*,1] and Yonggang Zhao[†,2]

[*]*College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, Henan 471023, China; and* [†]*China Animal Health and Epidemiology Center, Qingdao, Shandong 266032, China*

ABSTRACT This study aimed to investigate the association of insulin-like growth factor 1 receptor (***IGF-1R***) gene single nucleotide polymorphisms (**SNPs**) with growth traits and carcass traits of quail by PCR amplification and direct sequencing technology. Genomic DNA was extracted from blood samples collected from 49 female French Giant (**FG**) quails and 36 female Savimalt (**SV**) quails as part of this study. Growth traits and carcass traits were measured and assessed for *IGF-1R* gene analysis in the 2 meat-type quail strains. The results showed that 2 SNPs (A57G and A72T) of the *IGF-1R* gene were detected in the 2 quail strains. The A57G ($P = 0.002$) and A72T ($P = 0.026$) were significantly associated with breastbone length (**BBL**) in FG. Whereas A57G was significantly associated with chest weight (**CW**, $P = 0.004$), BBL ($P = 0.009$), and body length (**BL**, $P = 0.009$) in SV, while A72T was significantly associated with BBL ($P = 0.014$) and BL ($P = 0.028$) in SV. Haplotypes based on these 2 SNPs showed significant effects on BBL in FG strain ($P = 0.000$), and they also had significant effects on CW ($P = 0.007$), BBL ($P = 0.004$), and BL ($P = 0.001$) in SV strain. Additionally, A57G was significantly associated with liver rate (**LR**) in FG strain ($P = 0.017$). A72T showed significant associations with dressed carcass weight (**DCW**, $P = 0.048$) and breast muscle weight (**BMW**, $P = 0.018$) in FG strain. A57G was significantly associated with DCW ($P = 0.048$), whole net carcass weight (**WNCW**, $P = 0.048$), BMW ($P = 0.036$), and liver muscle rate (**LMR**, $P = 0.003$) in SV strain. Haplotypes also displayed significant effects on BMW ($P = 0.029$) and LMR ($P = 0.010$) in FG strain. These findings indicated that the *IGF-1R* gene could serve as a valuable molecular genetic marker for enhancing growth traits and carcass traits in meat-type quails.

Key words: IGF-1R, polymorphism, production performance, quail

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INTRODUCTION

The outbreak of the COVID-19 pandemic has led to an increased awareness of the importance of maintaining good health (Hu et al., 2021). As a result, people are now focusing on improving their immunity by engaging in physical exercise and consuming nutrient-rich foods. Quails offer high nutritional value (minerals, high protein, and beneficial fatty acids), making them a sought-after choice for consumers in recent years (Quaresma et al., 2022). Additionally, quails are small economic poultry that are extensively farmed in China due to their numerous economic advantages (Bai et al., 2023a). They possess several distinctive characteristics, including a short growth cycle, high reproductive ability, and exceptional egg production performance. One of the key benefits of quail farming is its low investment requirement, while still yielding substantial economic returns (Bai et al., 2021, 2023b; Wang et al., 2023). However, it is important to note that there is a limited amount of research conducted on quail breeding domestically and internationally, particularly at the molecular level. This area of study is still in its early stages of development (Priti and Satish, 2014; Bai et al., 2023a). In recent years, marker-assisted selection has emerged as a preferred breeding method over traditional techniques for improving animal economic traits (Abd El-Hack et al., 2018). Several studies have identified candidate genes that are associated with economic traits in livestock. *LEPR, VIPR-1*, and *GnRH* genes are among the most widely accepted candidate genes that have been shown to impact production performance (El-Tarabany et al., 2022; Bai et al., 2023b; Wang et al., 2023).

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[1]These authors contributed equally to this study and share first authorship.

[2]Corresponding author: byezi@163.com

The insulin-like growth factor (***IGF***) plays a key role in regulating animal growth and development, with the insulin-like growth factor 1 receptor (***IGF-1R***) gene serving as the main effector of *IGF*. Previous research has demonstrated the critical involvement of the *IGF-1R* gene in the regulation of diverse biological processes in animals (Cheng et al., 2016). It not only governs the activity of IGFs but also exerts control over the cellular growth cycle, metabolism, proliferation, differentiation, and immune regulation. Moreover, it exerts regulatory effects on significant processes during adulthood (Cardoso et al., 2021). Multiple studies have investigated the association between the *IGF-1R* gene and the economic traits in different breeds of animals. It has been observed that different mutation sites and genotypes have varying effects on animal growth, carcass, and reproduction traits. Ding et al. (2022) detected 10 SNPs of the *IGF-1* gene in Hulun Buir sheep. Ma et al. (2019) found that the *IGF-1R* CNV was significantly associated with body weight (**BW**) and height in Jinnan cattle, as well as with body height and hucklebone width in Qinchuan cattle. El-Magd et al. (2017) have proven that 2 SNPs (G64A and G280A) of the *IGF-1* gene had a significant impact on growth traits of buffalo. Wu et al. (2017) analyzed the correlation between the *IGF-1* gene and growth traits in Bian chickens, and found a strong correlation with BW ($P < 0.05$). Dao et al. (2014) found that birth weight, weaning weight, and daily gain of calves were related to the *IGF-1R* gene. Szewczuk et al. (2013) reported a significant association between the polymorphism of the *IGF-1R* gene and weight in Angus cattle ($P < 0.05$). Pierzchala et al. (2012) research on the *IGF-1R* gene in pigs showed that the *IGF-1R* gene has a potential impact on the growth and development of pigs after birth and carcass composition. Thus, the *IGF-1R* gene has been widely used to evaluate genetic diversity in many regions with different breeds.

Previous research has demonstrated the significant association between polymorphisms in candidate genes and various important traits such as growth, carcass, egg production, and meat quality traits in several animal species (El-Magd et al., 2017; Wu et al., 2017; Ali et al., 2021). Understanding the specific genetic variations responsible for these traits enables the development of targeted breeding and genetic improvement strategies to enhance productivity and quality in the livestock and aquaculture industries. However, limited studies have been conducted to investigate the association of polymorphisms in the *IGF-1R* gene with economically important traits in poultry species (Li et al., 2008; Pu et al., 2016; Yang et al., 2022). Growth traits (tibial length, tibial circumference, chest width, chest depth, breastbone length, and body length) and carcass traits (BW, dressed carcass weight [**DCW**], whole net carcass weight [**WNCW**], heart weight [**HW**], liver weight [**LW**], breast muscle weight [**BMW**], leg muscle weight [**LMW**]) are important economic traits in poultry breeding. This study aimed to investigate the association between the polymorphism of the *IGF-1R* gene and growth traits as well as carcass traits in French Giant (**FG**) quail and Savimalt (**SV**) quail by PCR sequencing. The findings of this research will contribute valuable reference values for future studies on quail breeding.

MATERIALS AND METHODS

Ethics Statement

All experimental procedures in this study received approval from the Institutional Animal Care and Use Committee of the College of Animal Science at Henan University of Science and Technology (Luoyang, China; Latitude: 34°72′ N; Longitude: 112°45′ E). Animal experimentation in this study was conducted in strict adherence to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China).

Experimental Animals, Management, and Phenotypic Measurements

A total of 49 female FG strain and 36 female SV strain quails were randomly selected from a quail breeding company (Henan University of Science and Technology Quail Breeding Co. Ltd., Luoyang, China). All quail individuals were healthy and fed in individual cages under the same conditions (dry, clean, and good ventilation system) at the experimental farm of Henan University of Science and Technology. The temperature was kept at 37°C from 1 to 3 d of age, at 35°C from 4 to 7 d of age, at 30°C from 8 to 14 d of age, and then it was gradually reduced to room temperature (25°C). The humidity was 20% from 1 to 3 d and then gradually increased to 65% in the room. During the whole investigation, all quails were allowed to feed and drink ad libitum. Supplemental heaters were provided for first 2 wk of growth. The daily lighting schedule was lights on from 5:00 am to 7:00 pm until 35 d. All 2 strains were fed a diet with 2900 kcal/Kg of ME and 24% CP from d 1 to 35. The growth traits of the SV and FG quail strains were recorded at 3 and 5 wk. The growth traits included tibial length (**TL**), chest width (**CW**), chest depth (**CD**), breastbone length (**BBL**), body length (**BL**), and tibial circumference (**TC**). The carcass traits included BW, DCW, WNCW, HW, LW, BMW, LMW, dressing percentage (**DP**), whole net carcass rate (**WNCR**), heart rate (**HR**), liver rate (**LR**), breast muscle rate (**BMR**), and leg muscle rate (**LMR**) were measured at 5 wk of age.

DNA Samples, Primer Designing, PCR Amplification, and DNA Sequencing

Blood samples (5 mL) were taken from the wings of 85 quails (49 FG and 36 SV) at 5 wk of age into a syringe containing 2% EDTA used as an anticoagulant and stored at −80°C for further experiment. Genomic DNA was isolated from venous blood samples using a poultry whole DNA extraction kit (Dingguo Changsheng

Biotechnology Company, Beijing, China). Based on the potential SNPs of the *IGF-1R* gene published in the NCBI database (https://www.ncbi.nlm.nih.gov/), the primer pairs were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA), which were F-AACGCCTGGAGAACTGTACG and R-ATCGCTGAGGCTTTCCAAG. The primer specificity was verified by BLAST at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The expected amplified segment size was 155 bp. PCR was performed in a total volume of 20 μL, which included 10 μL of the 2 × Taq PCR Master Mix, 0.7 μL of upstream and downstream primers, 1 μL DNA sample, and 7.6 μL double-distilled water. The reaction conditions were as follows: initial denaturation at 95°C for 4 min, followed by 35 cycles of 95°C for 40 s, annealing for 56°C for 45 s, extension at 72°C for 35 s, and a final extension at 72°C for 10 min. The reaction system was stored under 4°C (Bai et al., 2023a). Then, the amplified samples of the *IGF-1R* gene were sent to Beijing Tsingke Biological Co., Ltd. for sequencing.

Statistical Analysis

Sequence alignment and SNP identification were conducted via MegAlign program (version 5.0; DNAstar, Madison, WI). Chromas software (version 2.2.2; Technelysium, Queensland, Australia) was used to conduct sequence analyses. Genotypes and alleles were recorded using Excel (version 2016; Microsoft, Redmond, WA). The population's genetic information was statistically analyzed using the Popgene version 1.32 (Yeh and Boyle, 1997). Finally, association analysis of polymorphisms was accomplished with the measured growth traits and carcass traits using Duncan's multiple range test in SPSS (version 26.0; IBM Corp., Armonk, NY) and expressed as means ± standard error (**SE**). Differences were considered highly significant or significant at $P \leq 0.01$ or $P \leq 0.05$, respectively. The association analysis model of growth traits was as follows:

$$Y_{ijk} = \mu + W_i + G_j + e_{ijk}. \quad (1)$$

Y_{ij} is the phenotype value, μ is the total mean value, W_i is the effect of week-age (3, 5), G_j is the effect of genotype, and e_{ijk} is the random error. The association analysis model of carcass traits was as follows:

$$Y_{ij} = \mu + G_i + e_{ij}. \quad (2)$$

Y_{ij} is the phenotype value, μ is the total mean value, G_i is the effect of genotype, and e_{ij} is the random error.

RESULTS AND DISCUSSION

Polymorphisms of IGF-1R Gene

In this study, we have detected polymorphisms of the *IGF-1R* gene in quails. Two SNPs (A57G and A72T) identified in the 2 quail strains of the *IGF-1R* gene were genotyped by sequencing technology (Figure 1). It can be seen from Table 1 that 3 genotypes (AA, AG, and GG) were detected at the A57G site. Three genotypes (AA, AT, and TT) were detected at the A72T site. The frequencies of the AA, AG, and GG genotypes at the A57G site in the FG strain were 34.7, 38.8, and 26.5%, respectively (Figure 2A). The frequencies of the AA, AG, and GG genotypes at the A57G site in SV strain

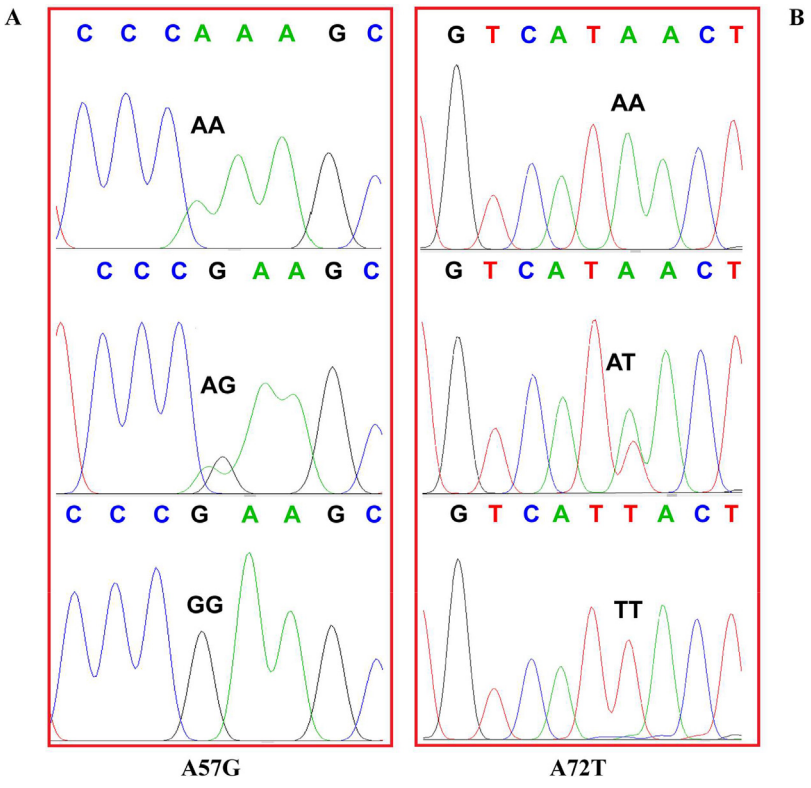

Figure 1. Sequencing results of A57G and A72T sites of *IGF-1R* gene.

Table 1. Genotype frequency, allele frequency, and Hardy-Weinberg's law data of SNPs of *IGF-1R* gene in quail.

SNP[1]	S[2]	Genotypic frequency			Allelic frequency		HWE[3]					
					Major	Minor	χ^2	P	Ho[4]	He[5]	PIC[6]	Ne[7]
A57G	FG(49)	0.347(AA)	0.388(AG)	0.265(GG)	0.541	0.459	2.356	0.125	0.503	0.497	0.373	1.987
	SV(36)	0.028(AA)	0.472(AG)	0.500(GG)	0.736	0.264	1.672	0.196	0.611	0.389	0.313	1.635
A72T	FG(49)	0.163(AA)	0.388(AT)	0.449(TT)	0.643	0.357	1.186	0.276	0.541	0.459	0.354	1.849
	SV(36)	0.028(AA)	0.361(AT)	0.611(TT)	0.792	0.208	0.323	0.570	0.670	0.330	0.275	1.492

Abbreviations: FG, French Giant meat quail; He[5], heterozygosity; Ho[4], homozygosity; HWE[3], Hardy-Weinberg equilibrium test; Ne[7], effective allele numbers; PIC[6], polymorphism information content; SNP[1], single nucleotide polymorphism; S[2], strain; SV, Savimalt meat quail.

were 2.8, 47.2, and 50.0%, respectively (Figure 2B). Furthermore, the dominant gene for the FG strain was allele A (54.1%), while for the SV strain was allele G (73.6%) (Figure 2C). The frequencies of the AA, AT, and TT genotypes at the A72TG site in FG strain were 16.3, 38.8, and 44.9%, respectively (Figure 2D). The

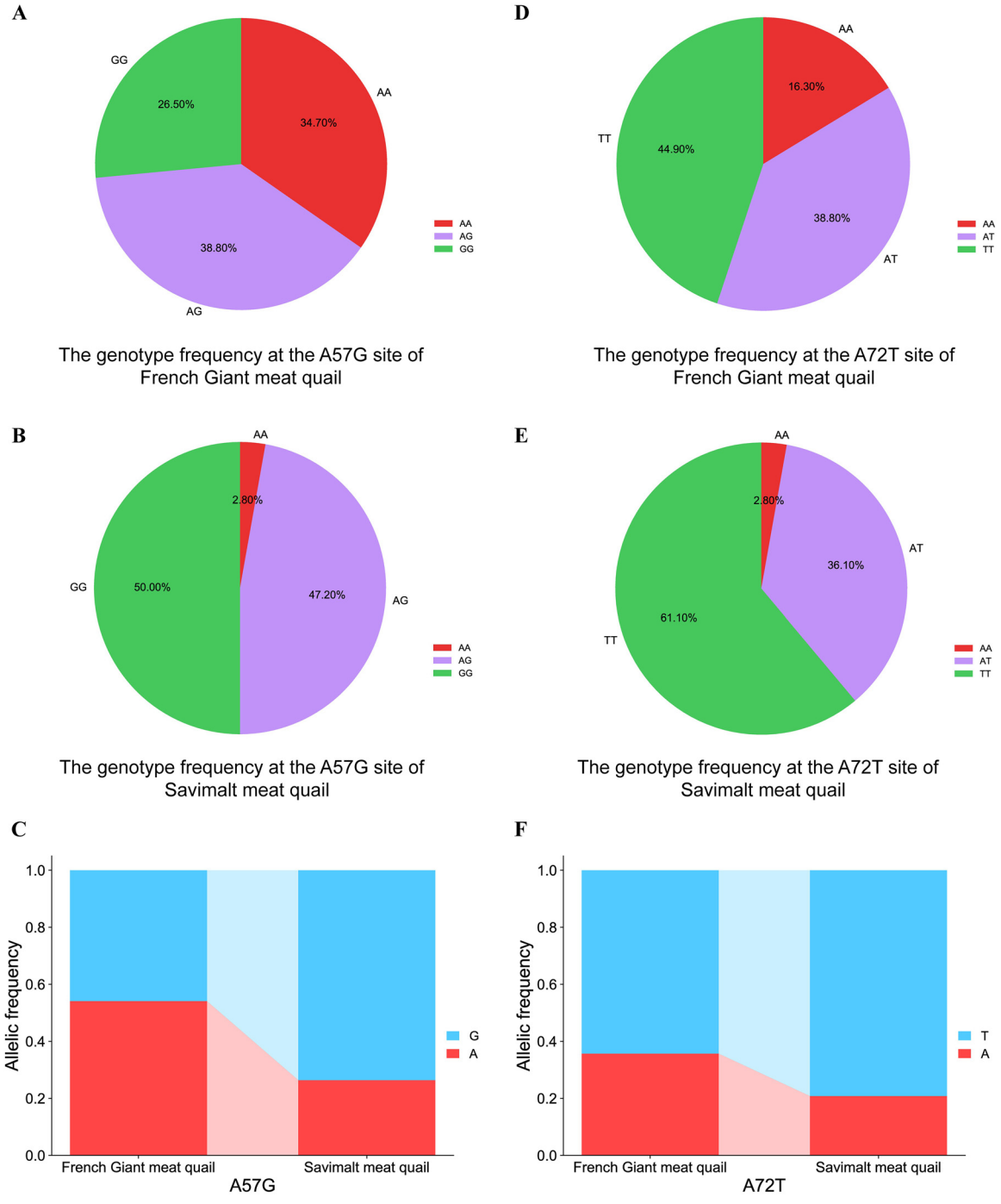

Figure 2. Genotype frequency and allele frequency at the A57G and A72T sites of *IGF-1R* gene.

frequencies of the AA, AT, and TT genotypes at the A72TG site in SV strain were 2.8, 36.1, and 61.1%, respectively (Figure 2E). Furthermore, allele T was the dominant gene in both the FG (64.3%) strain and the SV (79.2%) strain (Figure 2F). The PIC analysis results showed that all SNPs were in moderate polymorphism ($0.25 < \text{PIC} < 0.50$). The A57G and A72TG of FG and SV strains were in Hardy-Weinberg equilibrium (**HWE**) based on the chi-square test ($P > 0.05$) (Table 1). Several studies have reported the presence of different polymorphisms in the *IGF-1R* gene in animals. SNPs within the gene can lead to substitutions of amino acids, thereby altering the protein's binding affinity to growth factors and disrupting growth regulatory pathways. It is reported that 10 SNPs of the *IGF-1* gene were detected in Hulun Buir sheep. SNP8, haplotype combinations H5H5 and H5H6 of the *IGF-1R* gene showed superior growth traits during the early stage (Ding et al., 2022).

Association Analysis of IGF-1R Gene and Haplotype Combinations With Growth Traits in Quail

The present study was conducted to correlate the growth traits in quail (Table S1 and Figure 3). The results showed that there was a significant association between the A57G site of the *IGF-1R* gene with BBL in FG strain ($P = 0.002$). The A57G site was significantly associated with the CW ($P = 0.004$), BBL ($P = 0.009$), and BL ($P = 0.009$) in SV strain, and individuals with the GG genotype had significantly higher CW, BBL, and BL (Figure 3A). For the A72TG site, there was a significant association with BBL in FG strain ($P = 0.026$), and individuals with the AT genotype had significantly higher BBL. In addition, the A72TG site was significantly associated with the BBL ($P = 0.014$) and BL ($P = 0.028$) in SV strain, and individuals with the AA genotype had better performance on BBL and BL (Figure 3B). The growth traits and carcass traits are important economic traits in poultry breeding, which are controlled by genetic, environmental, and nutritional factors. By identifying and selecting quail individuals with favorable *IGF-1R* genotypes, breeders can potentially improve growth rates and overall productivity in quail populations. In conclusion, polymorphisms in the *IGF-1R* gene have been shown to influence growth traits in quail.

In the linkage between 2 SNPs (A57G and A72T), there were 9 and 6 haplotype combinations (combinations with the number of individuals higher than or equal to 3) in FG and SV quail strains, respectively (Table S2). The result showed that haplotype combination AAAT and GGAA had significantly higher BBL ($P = 0.000$) than AGAA in FG strain (Figure 3C). AGAA, AGAT, GGAT, and GGTT combinations had significantly higher CW ($P = 0.007$) than AATT in SV strain (Figure 3D). AGAA combination had significantly higher BBL ($P = 0.004$) and BL ($P = 0.001$) than AATT in SV strain (Figure 3E and F). Similar to this study, a previous study in Japanese quail showed that a SNP (c.2293G>A) of the *IGF-1R* gene was significantly associated with growth traits (Moe et al., 2007). Jin et al. (2014) showed that a haplotype comprising of 3-SNP (rs14011783, rs14011780, and rs14011776) of *IGF-1R*

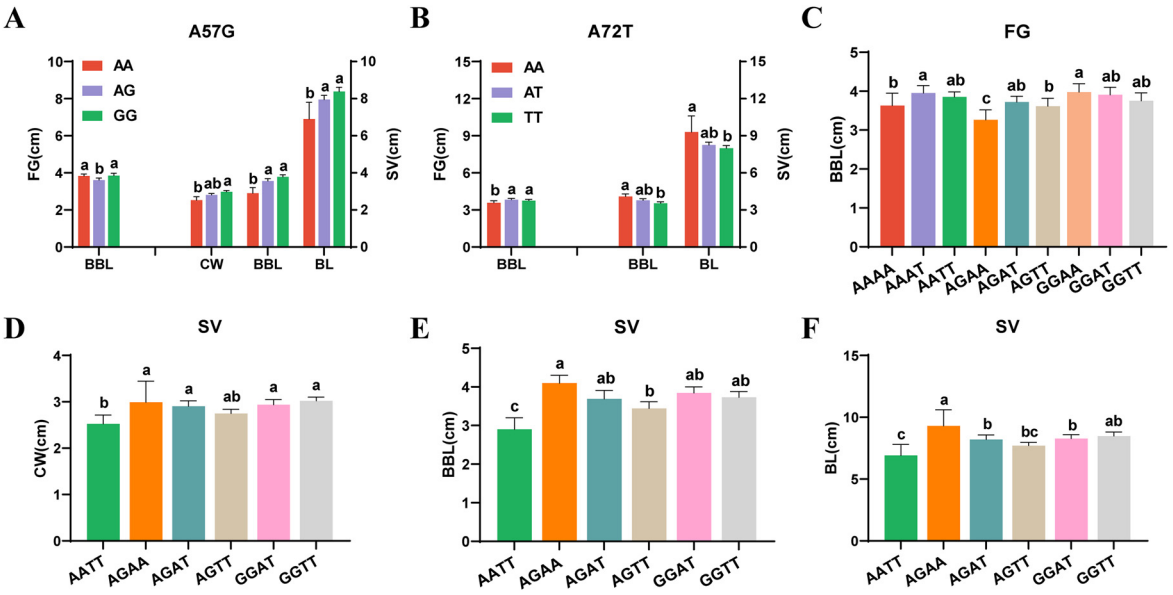

Figure 3. Association analysis of the A57G and A72T sites and haplotype combinations of *IGF-1R* gene with growth traits in quail; FG, French Giant meat quail; SV, Savimalt meat quail. (A) Association analysis of the A57G site of *IGF-1R* gene with growth trait in quail; BBL (first), breastbone length in FG; CW, chest width in SV; BBL (second), breastbone length in SV; BL, body length in SV. (B) Association analysis of the A72T site of *IGF-1R* gene with growth trait in quail; BBL (first), breastbone length in FG; BBL (second), breastbone length in SV; BL, body length in SV. (C) Association analysis of the haplotype combinations of *IGF-1R* gene with growth trait in French Giant meat quail; BBL, breastbone length. (D–F) Association analysis of the haplotype combinations of *IGF-1R* gene with growth trait in Savimalt meat quail; CW, chest width; BBL, breastbone length; BL, body length. [ab]The difference between genotypes or haplotype combinations with different lowercase letters was significant ($P < 0.05$).

gene was significantly associated with BW49, BW70, and FCR ($P < 0.05$), which was similar to this study. It can be seen that there was a significant correlation between the *IGF-1R* gene and economic traits of animals.

Association Analysis of IGF-1R Gene and Haplotype Combinations With Carcass Traits in Quail

The present study was conducted to correlate the carcass traits of quail at 5 wk of age (Table S3 and Figure 4). The result showed that A57G site was significantly related to LR in FG quail strain, and individuals with the AG genotype had significantly higher LR than those of AA and GG genotypes ($P = 0.017$). The A57G site was significantly associated with the DCW ($P = 0.048$), WNCW ($P = 0.048$), BMW ($P = 0.036$), and LMR ($P = 0.003$) in SV quail strain, and individuals with the GG genotype had significantly higher DCW, WNCW, and BMW, while individuals with the AA genotype had significantly higher LMR (Figure 4A). The A72T site was significantly associated with the DCW ($P = 0.048$) and BMW ($P = 0.018$) in FG quail strain, and individuals with the AT genotype had significantly higher DCW and BMW than those of AA and TT genotypes (Figure 4B).

In the linkage between 2 SNPs (A57G and A72T), 9 haplotype combinations (combinations with the number of individuals higher than or equal to 3) were formed in FG strain (Table S4). The result showed that haplotype combinations had no significant association with carcass traits in FG strain ($P > 0.05$). In the linkage between 2 SNPs (A57G and A72T), 6 haplotype combinations (combinations with the number of individuals higher than or equal to 3) were formed in SV strain (Table S5). Haplotype combination AGAA had a significantly higher BMW ($P = 0.029$) than of AATT combination in SV strain (Figure 4C). Haplotype combination AATT had significantly higher LMR ($P = 0.010$) than of AGAT combination in SV strain (Figure 4D). Similar to this study, a previous study in chicken showed that A17299834G SNP of the *IGF-1R* gene was significantly associated with carcass traits, and a haplotype-based on 2 SNPs (A17299834G and C17293932T) showed a significant correlation with most of the early growth traits and carcass traits (Lei et al., 2008). The *IGF-1R* gene may be used as the major gene affecting quail carcass traits. These studies suggest that the associations of the SNP or haplotype with economic traits in the present study were reliable.

Certain variations in the *IGF-1R* gene are associated with economic traits in animals, such as body weight, carcass traits, and muscle development. Quail individuals with specific genotypes of the *IGF-1R* gene may exhibit improved growth performance and meat production characteristics. Haplotype analysis based on SNP loci can help breeders understand the genetic characteristics of animals and determine the combined effects of these loci on traits. These analyses can aid in identifying superior genotypes and facilitating selective breeding and reproduction. However, it is important to consider other unanalyzed genetic markers and use comprehensive approaches like whole-genome sequencing or haplotype-based analyses to capture a broader range of genetic variations and fully understand the genetic complexity underlying these traits.

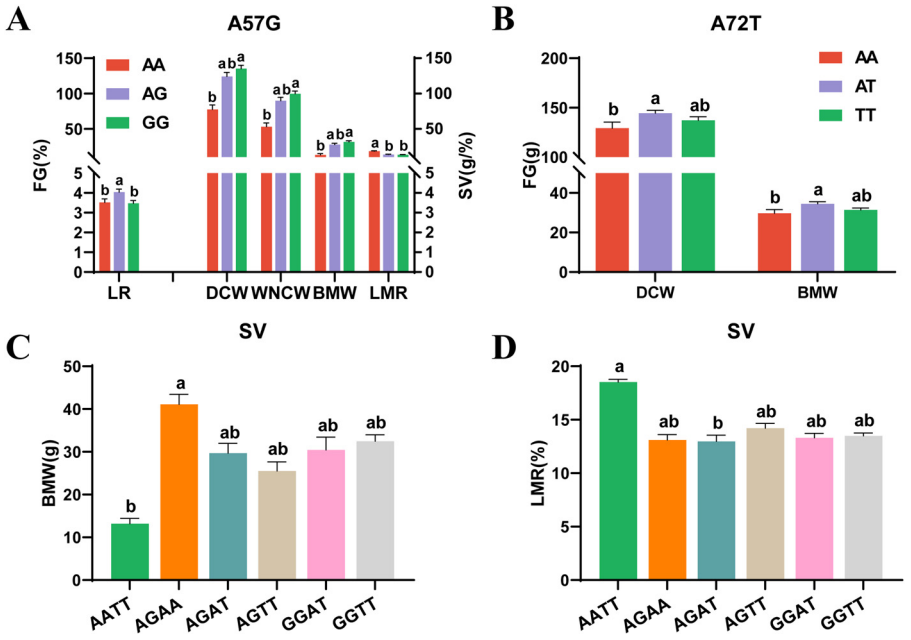

Figure 4. Association analysis of the A57G and A72T sites and haplotype combinations of *IGF-1R* gene with carcass traits in quail; FG, French Giant meat quail; SV, Savimalt meat quail. (A) Association analysis of the A57G site of *IGF-1R* gene with carcass traits in quail; LR, liver rate in FG; DCW, dressed carcass weight in SV; WNCW, whole net carcass weight in SV; BMW, breast muscle weight in SV; LMR, leg muscle rate in SV. (B) Association analysis of the A72T site of *IGF-1R* gene with carcass traits in quail; DCW, dressed carcass weight in FG; BMW, breast muscle weight in FG. (C–D) Association analysis of the haplotype combinations of *IGF-1R* gene with carcass traits in Savimalt meat quail; BMW, breast muscle weight; LMR, leg muscle rate. [ab]The difference between genotypes or haplotype combinations with different lowercase letters was significant ($P < 0.05$).

In conclusion, 2 SNPs (A57G and A72T) or haplotype combinations of the *IGF-1R* gene were significantly correlated to growth traits and carcass traits in Savimalt and FG meat-type quails. Therefore, the *IGF-1R* gene could be a molecular genetic marker to improve economic traits with Savimalt and FG meat-type quails.

ACKNOWLEDGMENTS

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I declare that research on live animals is in line with the guidelines approved by the institutional animal care and use Committee (IACUC) through the use of appropriate management and laboratory techniques to avoid unnecessary discomfort of animals.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103074.

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