Research Note: Polymorphisms of gonadotrophin-releasing hormone gene and their association with growth traits in quail (*Coturnix Coturnix*)

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ABSTRACT This study aimed to identify polymorphisms of gonadotropin-releasing hormone (***GnRH***) gene and their association with growth traits in quail by PCR and direct sequencing. Genomic DNA was extracted from quail blood samples of 36 from Savimalt (**SV**) and 49 from French Giant (**FG**). Growth traits were measured and used for candidate gene analysis, as body weight (**BW**), shank length (**SL**), chest width (**CW**), chest depth (**CD**), breastbone length (**BBL**), body length (**BL**), and shank circumference (**SC**). The results showed that a total of 20 SNPs were detected in *GnRH* gene, whereas 8 SNPs were significantly associated with growth traits ($P < 0.05$). The T215C, G279A, C458T, A520G, and C547G were significantly associated with SL at 3 wk of age in the FG strain, whereas A583T was significantly related to BBL and BL, and C591T was significantly related to SL, BBL, and BL, whereas A592G was significantly correlated with SL, CW, CD, BBL, and BL ($P < 0.05$). The 8 SNPs were significantly related to CW, CD, and BBL at 3 wk of age in the SV strain, whereas A583T, C591T, and A592G were significantly associated with BW ($P < 0.05$). The G279A showed significant correlations with SL at 5 wk of age in FG, whereas A583T showed significant associations with SC in FG, and C591T was significantly associated with BW and SC in FG, whereas A592T was significantly related to BW, SL, and CD in FG ($P < 0.05$). The T215C, G279A, C458T, A520G, and C547G were significantly correlated with BW, CW, BBL, and BL at 5 wk of age in SV, whereas A583T, C591T, and A592G were significantly related to BW, SL, CW, BBL, and BL ($P < 0.05$). Haplotypes based on 8 SNPs showed significant correlation with BW, SL, CW, CD, BBL, BL, and SC in FG ($P < 0.05$). In conclusion, the *GnRH* gene could be used as a molecular genetic marker to provide theoretical foundation to improve growth traits in quail.

Key words: *GnRH*, polymorphism, growth trait, association, quail

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INTRODUCTION

Quails are widely raised due to low feeding cost, high nutritional value, high reproductive ability, and economic benefits (Bai et al., 2021). However, improvement growth trait remains a key concern for quail breeding.

Growth traits are influenced not only by environmental factors but also by genetic factors (Gaur et al., 2022). Growth traits such as body weight (**BW**), chest width (**CW**), chest depth (**CD**), shank length (**SL**), average daily gain (**ADG**), and shank circumference (**SC**) have habitually been used in the selection principle of poultry breeding since they are moderate to high heritable and are genetically associated with growth traits. The *GnRH* gene as one of the vital candidate genes was significantly related to major economic traits such as quail growth and carcass (Bai et al., 2021). Identification of single nucleotide polymorphism (**SNP**) in candidate genes and its correlation with economic traits in animals was a powerful method to identify new genetic markers to more accurately select animals to improve growth performance (Bhattacharya et al., 2019; Shafey et al., 2020; Valencia et al., 2022).

GnRH is a key neuroendocrine regulator in the hypothalamic-pituitary-gonadal axis (Şişli et al., 2022). *GnRH-1, GnRH-2*, and *GnRH-3* were 3 isoforms of *GnRH* (Servili et al., 2010). Wang et al. (2020) have proven that SNPs of the *GnRH* gene have a significant correlation with sperm quality traits of Chinese water buffalo. Bai et al. (2021) purported that the SNPs of the *GnRH* gene were significantly associated with growth traits in egg quail. Polymorphisms of the *GnRH* gene are quite different among different species. Different genotypes affect other growth traits, possibly because base

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mutations change the structure and function of amino acids or the transcription efficiency of genes (Huang et al., 2018). These studies have proven a close correlation between *GnRH* and animals' production traits. The study identified the SNPs of the *GnRH* gene and their associated with growth traits in Savimalt meat quail and French Giant meat quail to improve theoretical foundation for 2 quail strains production.

MATERIALS AND METHODS

Ethics Statement

This research is strictly complianced with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). All procedures were passed by the Animal Care and Use Committee of Henan University of Science and Technology (Luoyang, China).

Experimental Populations, Management, and Phenotypic Measurements

A top of 36 Savimalt (**SV**) strain and 49 French Giant (**FG**) strain meat quails were selected from Henan University of Science and Technology Quail Breeding Co. Ltd., Luoyang 434020, Henan, P. R., China. Animals were fed at the experimental farm at Henan University of Science and Technology under the same conditions. All samples were separated into 2 groups as to the population. During the whole investigation, all quails were allowed to feed and drink libitum. Supplemental heaters were provided first 2 wk of growth. The daily lighting schedule was lights on from 5:00 am to 7:00 pm until 35 d. The feeding schedule of these quails was 2,800 kcal of ME/kg and 20% CP for days 1 to 35 in the SV and FG strains. The growth traits of the SV and FG meat quails were recorded at 3 and 5 wk. The growth traits included BW, CD, CW, breastbone length (**BBL**), body length (**BL**), SL, and shank circumference (**SC**).

DNA Extraction, PCR Amplification, and DNA Sequencing

Blood samples were taken from the wings of 85 quails 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). The primer pairs were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA), which were F-TCTTGGTTGGTGTTCTCCT and R-ATTGCTCAGCCTGGGAT. The expected amplified segment size was 906 bp. PCR was performed in a total volume of 15 μL, which included 7.5 μL of the 2 × Taq PCR Master Mix, 1 μL of each primer, 2 μL genomic DNA, and 3.5 μL double-distilled water. The reaction conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing for 59°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The reaction system was stored under 4°C (Bai et al., 2021). The PCR products of the *GnRH* gene were sent to Beijing Qingke biological Co., Ltd. for sequencing.

Statistical Analyses

All SNPs loci were determined by sequencing results, and genotypes and alleles were collected using Chromas (version 2.22; Technelysium, Tewantin, QLD). The population genetic information was statistically analyzed using the POPGENE version 1.32 (Yeh and Boyle, 1997). In addition, haplotype analysis was executed for SNPs with growth traits using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) (Shi and He, 2005). Finally, association analysis of polymorphisms was accomplished with the measured growth 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 model was as follows:

$Y_i = \mu + G_i + e_{ij}$. Y_i is the phenotype value of traits, μ is the total mean value, G_i is the fixed effect of genotype, and e_{ij} is the random error.

RESULTS AND DISSCUSION

Polymorphisms of Meat Quail GnRH Gene

Research on the identification of poultry genes and their association with economic traits is increasing, which has contributed to identifying new genetic markers to more accurately select animals to improve poultry growth performance (Bhattacharya et al., 2019; Asiamah et al., 2022). Gao et al. (2020) reported that 46 SNPs were detected in the *GnRH* gene of 2 Sichuan White goose populations. It is reported that a mutation (g.206G > A) in the 5′-flanking region was correlated with egg-laying performance (Wu et al., 2015). To identify novel DNA markers related to growth traits in quail, *GnRH* gene polymorphisms were identified and assessed their correlations with growth traits. A total of 20 SNPs were detected in the *GnRH* gene, indicating that the *GnRH* gene is rich in polymorphisms in 2 species of meat quail (Figure 1A). The AA, AG, and GG genotype frequencies were 42.9, 40.0, and 17.1% at the A592G locus in SV, respectively (Table 1). Furthermore, allele A (62.9%) was the dominant gene in the population. The TT, CT, and CC genotype frequencies were 24.5, 44.9, and 30.6% at the T215C locus in FG, respectively. Thus the allele C (53.1%) was the dominant gene in this population. The polymorphism information content (**PIC**) analysis results showed that all SNPs were in moderate polymorphism ($0.25 < \text{PIC} < 0.50$) except T215C, G279A, C458T, A520G, and C547G sites of SV and the C108T, T168C, G252A, T281C, and T542 sites of FG that showed a low

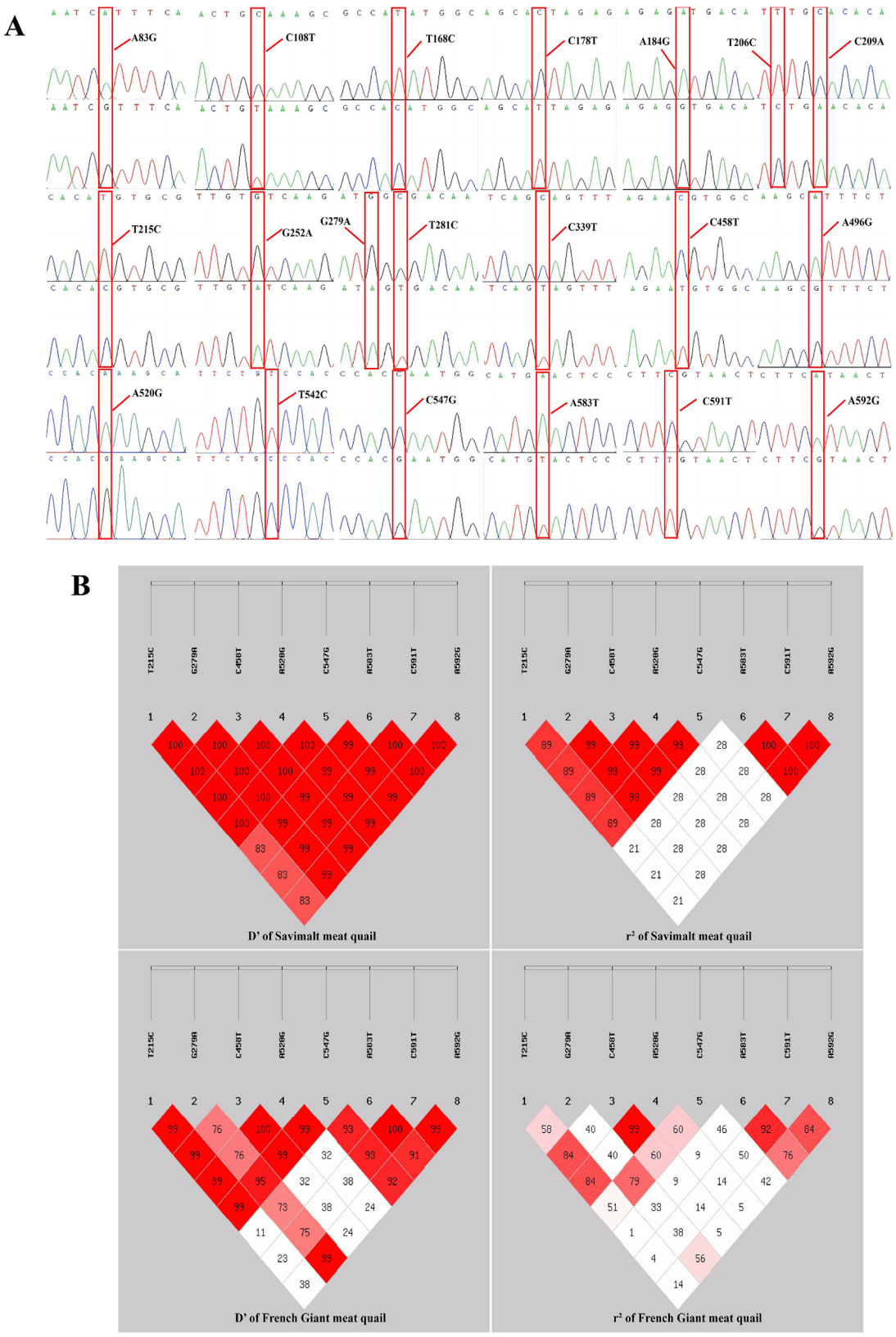

Figure 1. (A) SNPs were identified in quail by direct sequencing. (B) Linkage disequilibrium coefficient between SNPs (D' and r^2) in quail of the Savimalt strain and French Giant strain, the numbers are the D' and r^2 value (%).

polymorphism (PIC < 0.25). All SNPs of SV were in Hardy−Weinberg equilibrium (**HWE**) based on the chi-square test ($P > 0.05$). The T215C, G279A, C458T, A520G, C547G, A583T, C591T, and A592G sites of were in the HWE ($P > 0.05$), and subsequent association analysis could be performed. Other SNPs have significantly deviated from the HWE ($P < 0.01$), which was not statistically significant.

Table 1. Genotype frequency, allele frequency, and Hardy−Weinberg's law data of SNPs of *GnRH* gene in meat quail.

SNP	P	Genotypic frequency			Allelic frequency		HWE		Ho	He	PIC	Ne
					Major	Minor	$\chi2$	P				
A83G	S	0.600(AA)	0.343(AG)	0.057(GG)	0.771	0.229	0.027	0.869	0.647	0.353	0.290	1.545
	F	0.755(AA)	0.122(AG)	0.122(GG)	0.816	0.184	17.153	0.000	0.700	0.300	0.255	1.428
C108T	S	0.571(CC)	0.371(CT)	0.057(TT)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.796(CC)	0.122(CT)	0.082(TT)	0.857	0.143	12.250	0.000	0.755	0.245	0.215	1.324
T168C	S	0.571(TT)	0.371(CT)	0.057(CC)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.796(TT)	0.122(CT)	0.082(CC)	0.857	0.143	12.250	0.000	0.755	0.245	0.215	1.324
C178T	S	0.571(CC)	0.371(CT)	0.057(TT)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.735(CC)	0.122(CT)	0.143(TT)	0.796	0.204	19.023	0.000	0.675	0.325	0.272	1.481
A184G	S	0.571(AA)	0.371(AG)	0.057(GG)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.755(AA)	0.122(AG)	0.122(GG)	0.816	0.184	17.153	0.000	0.700	0.300	0.255	1.428
T206C	S	0.571(TT)	0.371(CT)	0.057(CC)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.755(TT)	0.122(CT)	0.122(CC)	0.816	0.184	17.153	0.000	0.700	0.300	0.255	1.428
C209A	S	0.600(CC)	0.343(AC)	0.057(AA)	0.771	0.229	0.027	0.869	0.647	0.353	0.290	1.545
	F	0.755(CC)	0.122(AC)	0.122(AA)	0.816	0.184	17.153	0.000	0.700	0.300	0.255	1.428
T215C	S	0.743(TT)	0.200(CT)	0.057(CC)	0.843	0.157	2.101	0.147	0.735	0.265	0.230	1.360
	F	0.245(TT)	0.449(CT)	0.306(CC)	0.469	0.531	0.477	0.490	0.502	0.498	0.374	1.993
G252A	S	0.571(GG)	0.371(AG)	0.057(AA)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.796(GG)	0.122(AG)	0.082(AA)	0.857	0.143	12.250	0.000	0.755	0.245	0.215	1.324
G279A	S	0.771(GG)	0.171(AG)	0.057(AA)	0.857	0.143	3.150	0.076	0.755	0.245	0.215	1.324
	F	0.408(GG)	0.388(AG)	0.204(AA)	0.602	0.398	1.784	0.182	0.521	0.479	0.364	1.920
T281C	S	0.600(TT)	0.343(CT)	0.057(CC)	0.771	0.229	0.027	0.869	0.647	0.353	0.290	1.545
	F	0.796(TT)	0.122(CT)	0.082(CC)	0.857	0.143	12.250	0.000	0.755	0.245	0.215	1.324
C339T	S	0.600(CC)	0.343(CT)	0.057(TT)	0.771	0.229	0.027	0.869	0.647	0.353	0.290	1.545
	F	0.653(CC)	0.184(CT)	0.163(TT)	0.745	0.255	13.083	0.000	0.620	0.380	0.308	1.613
C458T	S	0.771(CC)	0.171(CT)	0.057(TT)	0.857	0.143	3.150	0.076	0.755	0.245	0.215	1.324
	F	0.286(CC)	0.449(CT)	0.265(TT)	0.510	0.490	0.506	0.477	0.500	0.500	0.375	1.999
A496G	S	0.600(AA)	0.343(AG)	0.057(GG)	0.771	0.229	0.027	0.869	0.647	0.353	0.290	1.545
	F	0.592(AA)	0.184(AG)	0.224(GG)	0.684	0.316	16.220	0.000	0.567	0.433	0.339	1.762
A520G	S	0.771(AA)	0.171(AG)	0.057(GG)	0.857	0.143	3.150	0.076	0.755	0.245	0.215	1.324
	F	0.286(AA)	0.449(AG)	0.265(GG)	0.510	0.490	0.506	0.477	0.500	0.500	0.375	1.999
T542C	S	0.571(TT)	0.371(CT)	0.057(CC)	0.757	0.243	0.003	0.953	0.632	0.368	0.300	1.582
	F	0.796(TT)	0.122(CT)	0.082(CC)	0.857	0.143	12.250	0.000	0.755	0.245	0.215	1.324
C547G	S	0.771(CC)	0.171(CG)	0.057(GG)	0.857	0.143	3.150	0.076	0.755	0.245	0.215	1.324
	F	0.429(CC)	0.408(CG)	0.163(GG)	0.633	0.367	0.728	0.394	0.535	0.465	0.357	1.868
A583T	S	0.171(AA)	0.400(AT)	0.429(TT)	0.371	0.629	0.719	0.396	0.533	0.467	0.358	1.876
	F	0.327(AA)	0.388(AT)	0.286(TT)	0.520	0.480	2.441	0.118	0.501	0.499	0.375	1.997
C591T	S	0.429(CC)	0.400(CT)	0.171(TT)	0.629	0.371	0.719	0.396	0.533	0.467	0.358	1.876
	F	0.306(CC)	0.388(CT)	0.306(TT)	0.500	0.500	2.469	0.116	0.500	0.500	0.375	2.000
A592G	S	0.429(AA)	0.400(AG)	0.171(GG)	0.629	0.371	0.719	0.396	0.533	0.467	0.358	1.876
	F	0.265(AA)	0.388(AG)	0.347(GG)	0.459	0.541	2.356	0.125	0.503	0.497	0.373	1.987

Abbreviations: F, French Giant meat quail; He, heterozygosity; Ho, homozygosity; HWE, Hardy−Weinberg equilibrium test; Ne, effective allele numbers; P, population; PIC, polymorphism information content; S, Savimalt meat quail; SNP, single nucleotide polymorphism.

Association Analysis Between GnRH Gene SNPs and Growth Traits in Meat Quail

In this study, association results showed that the T215C, G279A, C458T, A520G, and C547G sites were significantly related to SL at 3 wk of age in the FG stain, and individuals with the wild genotype of this 5 SNPs had significantly higher SL in FG ($P < 0.05$; Table 2). A583T site was significantly associated with BBL and BL, and individuals with the wild genotype of A583T site had significantly lower BBL and BL ($P < 0.05$). C591T site was significantly related to SL, BBL, and BL, and individuals with the homozygous mutation genotype of this site had significantly lower SL, BBL, and BL ($P < 0.05$). Furthermore, A592G site was significantly correlated with SL, CW, CD, BBL, and BL in FG, and individuals with the homozygous mutation genotype of this site had significantly lower SL, CW, CD, BBL, and BL in FG ($P < 0.05$). The T215C, G279A, C458T, A520G, and C547G sites were significantly associated with CW, CD, and BBL at 3 wk of age in SV, and individuals with the homozygous mutation genotype of this 5 SNPs had significantly lower CW, CD, and BBL ($P < 0.05$). While A583T, C591T, and A592G sites showed significant association with BW, CW, CD, and BBL, and individuals with the wild genotype of this 3 SNPs had significantly lower BW, CW, CD, and BBL ($P < 0.05$).

In addition, the G279A site was significantly associated with SL at 5 wk of age in FG, and individuals with the GG genotype of this site had significantly higher SL ($P < 0.05$). A583T site showed a significant correlation with SC, and individuals with the AT genotype of this site had significantly higher SC ($P < 0.05$). C591T site was significantly associated with BW and SC, and individuals with the mutation genotype of this site had significantly lower BW and SC ($P < 0.05$). While A592T site was significantly associated with BW, SL, and CD ($P < 0.05$), and individuals with the mutation genotype of this site had significantly lower BW, SL, and CD ($P < 0.05$). T215C, G279A, C458T, A520G, and C547G sites were significantly related to BW, CW, BBL, and BL ($P < 0.05$) at 5 wk of age in SV, and individuals with the homozygous mutation genotype of 5 sites had significantly lower BW, CW, BBL, and BL ($P < 0.05$). A583T, C591T, and A592G sites were

Table 2. Association analysis of SNP in *GnRH* gene with growth traits of meat quails.

W	S	SNP	GT	Traits (Mean ± SE)		
3	FG	T215C	SL	3.710 ± 0.054^{a} TT	3.551 ± 0.042^{b} CT	3.516 ± 0.038^{b} CC
		G279A	SL	3.645 ± 0.046^{a} GG	3.562 ± 0.045^{ab} AG	3.481 ± 0.032^{b} AA
		C458T/A520G	SL	3.679 ± 0.051^{a} (CC/AA)	3.551 ± 0.042^{b} (CT/AG)	3.519 ± 0.044^{b} (TT/GG)
		C547G	SL	3.649 ± 0.041^{a} CC	3.546 ± 0.045^{ab} CG	3.477 ± 0.040^{b} GG
		A583T	BBL	3.063 ± 0.091^{b} AA	3.297 ± 0.072^{a} AT	3.236 ± 0.089^{ab} TT
			BL	7.131 ± 0.155^{b} AA	7.516 ± 0.120^{ab} AT	7.564 ± 0.160^{a} TT
		C591T	SL	3.596 ± 0.053^{ab} CC	3.629 ± 0.033^{a} CT	3.499 ± 0.056^{b} TT
			BBL	3.227 ± 0.083^{ab} CC	3.297 ± 0.072^{a} CT	3.061 ± 0.097^{b} TT
			BL	7.587 ± 0.151^{a} CC	7.516 ± 0.120^{a} CT	7.080 ± 0.156^{b} TT
		A592G	SL	3.612 ± 0.061^{ab} AA	3.629 ± 0.033^{a} AG	3.499 ± 0.049^{b} GG
			CW	2.796 ± 0.080^{a} AA	2.741 ± 0.055^{ab} AG	2.590 ± 0.052^{b} GG
			CD	3.196 ± 0.075^{ab} AA	3.239 ± 0.052^{a} AG	3.054 ± 0.060^{b} GG
			BBL	3.246 ± 0.095^{ab} AA	3.297 ± 0.072^{a} AG	3.065 ± 0.086^{b} GG
			BL	7.638 ± 0.165^{a} AA	7.516 ± 0.120^{a} AG	7.100 ± 0.141^{b} GG
3	SV	T215C	CW	2.636 ± 0.046^{a} TT	2.539 ± 0.100^{ab} CT	2.212 ± 0.095^{b} CC
			CD	3.099 ± 0.068^{a} TT	3.062 ± 0.140^{a} CT	2.533 ± 0.249^{b} CC
			BBL	3.188 ± 0.089^{a} TT	3.029 ± 0.171^{a} CT	2.362 ± 0.202^{b} CC
		G279A/	CW	2.632 ± 0.045^{a} (GG/	2.541 ± 0.118^{ab} (AG/	2.212 ± 0.095^{b} (AA/
		C458T/	CD	3.101 ± 0.065^{a} CC/	3.047 ± 0.165^{a} CT/	2.533 ± 0.249^{b} TT/
		A520G/	BBL	3.181 ± 0.086^{a} AA/	3.034 ± 0.203^{a} AG/	2.362 ± 0.202^{b} GG/
		C547G		CC)	CG)	GG)
		A583T/	BW	72.033 ± 6.968^{b} AA/	87.521 ± 3.259^{a} AT/	80.973 ± 3.756^{ab} TT/
		C591T/	CW	2.395 ± 0.103^{b} TT/	2.662 ± 0.050^{a} CT/	2.606 ± 0.073^{ab} CC/
		A592G	CD	2.781 ± 0.176^{b} GG	3.210 ± 0.050^{a} AG	3.029 ± 0.105^{ab} AA
			BBL	2.702 ± 0.185^{b}	3.287 ± 0.122^{a}	3.105 ± 0.111^{ab}
5	FG	G279A	SL	3.856 ± 0.026^{a} GG	3.801 ± 0.034^{a} AG	3.738 ± 0.039^{b} AA
		A583T	SC	1.638 ± 0.022^{b} AA	1.716 ± 0.021^{a} AT	1.686 ± 0.027^{ab} TT
		C591T	BW	159.220 ± 4.431^{a} CC	158.768 ± 3.715^{a} CT	146.840 ± 4.109^{b} TT
			SC	1.673 ± 0.028^{ab} CC	1.716 ± 0.021^{a} CT	1.647 ± 0.022^{b} TT
		A592G	BW	161.900 ± 4.685^{a} AA	158.768 ± 3.715^{a} AG	146.247 ± 3.633^{b} GG
			SL	3.857 ± 0.034^{a} AA	3.837 ± 0.028^{ab} AG	3.746 ± 0.035^{b} GG
			CD	3.486 ± 0.065^{a} AA	3.451 ± 0.040^{ab} AG	3.348 ± 0.037^{b} GG
5	SV	T215C	BW	150.327 ± 4.525^{a} TT	147.886 ± 9.275^{a} CT	109.750 ± 19.550^{b} CC
			CW	3.248 ± 0.045^{a} TT	3.212 ± 0.068^{a} CT	2.747 ± 0.247^{b} CC
			BBL	4.277 ± 0.070^{a} TT	4.229 ± 0.146^{a} CT	3.550 ± 0.250^{b} CC
			BL	9.381 ± 0.144^{a} TT	9.029 ± 0.283^{a} CT	7.800 ± 0.900^{b} CC
		G279A/	BW	150.015 ± 4.365^{a} (GG/	148.883 ± 10.911^{a} (AG/	109.750 ± 19.550^{b} (AA/
		C458T/	CW	3.247 ± 0.043^{a} CC/	3.212 ± 0.081^{a} CT/	2.747 ± 0.247^{b} TT/
		A520G/	BBL	4.285 ± 0.068^{a} AA/	4.183 ± 0.164^{a} AG/	3.550 ± 0.250^{b} GG/
		C547G	BL	9.367 ± 0.139^{a} CC)	9.033 ± 0.335^{a} CG)	7.800 ± 0.900^{b} GG)
		A583T/	BW	124.317 ± 10.424^{b} (AA/	155.193 ± 5.272^{a} (AT/	149.640 ± 6.289^{a} (TT/
		C591T/	SL	4.014 ± 0.116^{a} TT/	3.815 ± 0.041^{b} CT/	3.867 ± 0.047^{ab} CC/
		A592G	CW	3.007 ± 0.112^{b} GG)	3.297 ± 0.052^{a} AG)	3.215 ± 0.064^{ab} AA)
			BBL	3.867 ± 0.198^{b}	4.314 ± 0.056^{a}	4.287 ± 0.109^{a}
			BL	8.667 ± 0.468^{b}	9.507 ± 0.174^{a}	9.173 ± 0.199^{ab}

Abbreviations: BBL, breastbone length; BL, body length; BW, body weight; CD, chest depth; CW, chest width; FG, French Giant meat quail; GT, growth traits; S, strain; SC, shank circumference; SL, shank length; SNP, single nucleotide polymorphism; SV, Savimalt meat quail; W, week.

[ab]The difference between genotypes with different lowercase letters was significant ($P < 0.05$).

significantly associated with BW, SL, CW, BBL, and BL at 5 wk of age in SV, and individuals with the heterozygous mutation genotype of 3 sites had significantly higher BW, CW, BBL, and BL, and individuals with the wild genotype of 3 sites had higher SL ($P < 0.05$). Similar to this study, a previous study in 3 strains of egg quail showed that 13 SNPs of the *GnRH* gene was significantly associated with growth traits (Bai et al., 2021). This finding indicates that 8 SNPs of the *GnRH* gene may affect growth traits and could be used as novel molecular marker to in SV and FG strains in quail.

Linkage Disequilibrium Analysis and Haplotype Analysis of SNPs of GnRH Gene in Meat Quail Population

The linkage disequilibrium (**LD**) analysis of 8 SNPs genotyped of the Hardy-Weinberg equilibrium law showed that T215C, G279A, C458T, A520G, and C547G loci were completely linked (D' = 1) in SV, whereas A583T, C591T, and A592G loci were completely linked to each other (D' = 1). There was no significant LD (D' > 0.8 and $r^2 > 0.33$) at the remaining SNP loci, indicating that they tend to be genetically independent of each other (Figure 1B). There was significant LD between T215C and 4 SNPs (G279A, C458T, A520G, and C547G), whereas G279A had a significant LD with C547G and A592G, yet the C458T, A520G, and C547G sites were significant LD with each other (D' > 0.8 and $r^2 > 0.33$). Furthermore, C547G, A583T, C591T, and A592G loci strong (D' > 0.8 and $r^2 > 0.33$) LD with each other (Figure 1B). Haplotypes based on the 8 SNPs showed that a total of 3 (S1, S2, S3) and 5 (F1, F2, F3, F4, F5) haplotypes (frequencies greater than 3%) were identified in SV and FG strains.

Table 3. Association analysis of *GnRH* haplotype combinations with growth traits.

W	D	Traits (Mean ± SE)						
		BW (g)	SL (cm)	CW (cm)	CD (cm)	BBL (cm)	BL (cm)	SC (cm)
3	S1S3	84.100 ± 6.043	3.487 ± 0.096	2.630 ± 0.125	3.098 ± 0.130	3.130 ± 0.242	7.300 ± 0.238	1.425 ± 0.025
	S2S3	88.890 ± 3.985	3.531 ± 0.070	2.675 ± 0.054	3.255 ± 0.046	3.350 ± 0.145	7.460 ± 0.200	1.450 ± 0.027
	S3S3	81.364 ± 4.013	3.444 ± 0.096	2.611 ± 0.078	3.020 ± 0.113	3.113 ± 0.119	7.029 ± 0.234	1.421 ± 0.026
5	S1S3	159.875 ± 9.290	3.831 ± 0.108	3.274 ± 0.095	3.436 ± 0.149	4.275 ± 0.131	9.275 ± 0.320	1.800 ± 0.071
	S2S3	153.320 ± 6.583	3.808 ± 0.043	3.306 ± 0.066	3.336 ± 0.046	4.330 ± 0.063	9.600 ± 0.210	1.760 ± 0.040
	S3S3	150.193 ± 6.729	3.881 ± 0.048	3.215 ± 0.069	3.261 ± 0.066	4.271 ± 0.116	9.186 ± 0.214	1.907 ± 0.209
3	F2F2	94.050 ± 4.403^{a}	3.477 ± 0.040^{ab}	2.637 ± 0.064^{ab}	3.151 ± 0.060^{ab}	3.200 ± 0.100^{a}	7.313 ± 0.146^{a}	1.475 ± 0.025^{ab}
	F3F3	92.900 ± 8.979^{a}	3.580 ± 0.122^{a}	2.656 ± 0.155^{ab}	3.097 ± 0.229^{ab}	3.180 ± 0.152^{a}	7.300 ± 0.339^{a}	1.475 ± 0.075^{ab}
	F2F4	73.867 ± 7.565^{b}	3.276 ± 0.150^{b}	2.389 ± 0.129^{b}	2.865 ± 0.086^{b}	2.567 ± 0.203^{b}	6.233 ± 0.328^{b}	1.433 ± 0.033^{b}
	F2F5	95.027 ± 3.316^{a}	3.616 ± 0.035^{a}	2.746 ± 0.057^{a}	3.243 ± 0.057^{a}	3.327 ± 0.071^{a}	7.553 ± 0.142^{a}	1.527 ± 0.023^{ab}
	F4F4	99.850 ± 4.961^{a}	3.711 ± 0.088^{a}	2.692 ± 0.129^{ab}	3.179 ± 0.123^{ab}	3.153 ± 0.147^{a}	7.250 ± 0.222^{a}	1.575 ± 0.025^{a}
	F5F5	97.740 ± 2.679^{a}	3.629 ± 0.087^{a}	2.747 ± 0.139^{a}	3.188 ± 0.083^{ab}	3.196 ± 0.171^{a}	7.560 ± 0.279^{a}	1.440 ± 0.040^{b}
5	F2F2	150.350 ± 2.853^{ab}	3.765 ± 0.044^{ab}	3.263 ± 0.039^{a}	3.402 ± 0.042^{a}	4.413 ± 0.074^{a}	9.525 ± 0.098^{a}	1.675 ± 0.016^{a}
	F3F3	159.400 ± 4.175^{ab}	3.881 ± 0.035^{a}	3.192 ± 0.024^{a}	3.610 ± 0.147^{a}	4.400 ± 0.082^{ab}	9.550 ± 0.210^{a}	1.650 ± 0.050^{a}
	F2F4	136.667 ± 15.828^{b}	3.635 ± 0.075^{b}	2.951 ± 0.123^{b}	3.139 ± 0.099^{b}	4.000 ± 0.252^{b}	8.633 ± 0.664^{b}	1.600 ± 0.058^{a}
	F2F5	157.353 ± 4.002^{ab}	3.816 ± 0.031^{a}	3.285 ± 0.033^{a}	3.456 ± 0.049^{a}	4.387 ± 0.040^{ab}	9.420 ± 0.078^{a}	1.713 ± 0.024^{a}
	F4F4	147.450 ± 9.901^{ab}	3.850 ± 0.084^{a}	3.211 ± 0.032^{a}	3.417 ± 0.052^{a}	4.140 ± 0.295^{ab}	9.500 ± 0.280^{a}	1.625 ± 0.063^{a}
	F5F5	162.140 ± 9.934^{a}	3.888 ± 0.066^{a}	3.330 ± 0.145^{a}	3.422 ± 0.062^{a}	4.140 ± 0.154^{ab}	9.300 ± 0.300^{a}	1.720 ± 0.058^{a}

Abbreviations: D, diplotype; W, week.
^{ab}The difference between genotypes with different lowercase letters was significant ($P < 0.05$).

Association Analysis of Haplotype Combinations With Growth Traits

In the linkage between 8 SNPs, 3 and 6 research prominent combinations (combinations with the number of individuals higher than or equal to 3) were formed in SV and FG, respectively (Table 3). The result showed that 3 haplotype combinations were not significantly related to growth traits in SV ($P > 0.05$). Haplotype combination F2F4 had significantly lower BW, BBL, and BL than the other 5 haplotype combinations at 3 wk of age in FG ($P < 0.05$). F3F3, F2F5, F4F4 and F5F5 combinations had significantly higher SL than F2F4 in FG ($P < 0.05$). F2F5 and F5F5 combinations had significantly higher CW than F2F4 in FG ($P < 0.05$). F2F5 combination had significantly higher CD than F2F4 in FG ($P < 0.05$). F4F4 combination had significantly higher SC than F2F4 and F5F5 in FG ($P < 0.05$). F5F5 had significantly higher BW than F2F4 at 5 wk of age in FG, yet F3F3, F2F5, F4F4 and F5F5 combinations had significantly higher SL than F2F4 in FG ($P < 0.05$). Also, F2F2, F3F3, F2F5, F4F4, and F5F5 combinations had significantly higher CW, CD, and BL than those of F2F4 ($P < 0.05$), whereas F2F2 combination had significantly higher BBL than F2F4 ($P < 0.05$). This haplotype association analysis was consistent with the significant effect detected by the SNP association analysis, which was similarly reported in goose (Gao et al., 2020).

The identified molecular markers which were significantly correlated to growth traits could be used by quail breeders to improve growth traits. However, due to limitation of the number of quail population, further studies in large populations with different quail strains are required to further assess the associations of the polymorphisms of *GnRH* gene with growth traits. Moreover, more studies on the polymorphism of *GnRH* gene are required to improve quail carcass traits.

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DISCLOSURES

The authors declare no conflict of interest. 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.

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