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Original Article

Association Analysis Between Polymorphism of Gonadotrophin Releasing Hormone Genes and Growth Traits of Quail (Coturnix Coturnix)

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ABSTRACT

This study investigated SNP mutation sites of Gonadotrophin releasing hormone (GnRH) gene in China yellow quail, Beijing white quail and Korean quail through PCR amplification and DNA sequencing technologies. Moreover, polymorphism of GnRH gene and its association with growth traits of quail were analyzed, aiming to get molecular markers associated to growth traits of quail, which could provide references for breeding of new quail species. According to research results, a total of 14 SNP mutation sites of GnRH were detected in China yellow quail, Beijing white quail and Korean quail, which were C71T, C108T, C168T, C178T, A184G, C206T, A209C, C215T, A252G, A279T, C281T, C293G, C339T and C458T. Except that only 2 genotypes were detected for A209C and C281T in China yellow quail and Beijing white quail, 3 genotypes were detected for all of the remaining 12 SNP mutation sites in three quail species. Of the 14 SNP sites, C71T, A209C, C215T, C281T, C293G, C339T and C458T were significantly associated with body weight (p<0.05), C71T, C108T, C168T, C178T, A184G, C206T, C215T, A252G, C293G, C339T and C458T were significantly associated with shank length (p<0.05), C71T, C215T, C293G and C458T were significantly associated with breastbone length (p<0.05), A209C and C281T were significantly associated with shank circumference (p<0.05).

INTRODUCTION

Gonadotrophin releasing hormone (GnRH) is a decapeptide firstly gained from hypothalamus of pig by Schally's research team. It mainly includes three types, namely, GnRH-1, GnRH-2 and GnRH-3. Among them, GnRH-1 mainly exists in the hypothalamus and it is vital to gonad development and sexual maturity of animals. Gong *et al*. (2018) reported that the expression and distribution of GnRH were in the preoptic region and in the arcuate nucleus of ewe hypothalamus. He *et al*. (2009) demonstrated that the expression level of GnRH mRNA was significantly higher in the hypothalamus of the egg laying group than in that of the non-egg laying group in Xueshan chicken. Zhang *et al*. (2019) discovered that GnRH mRNA was expressed in corpus leteum tissues of sow during different gestation periods, and the expression level was consistent with corpus leteum functions in the gestation period. Hameed *et al*. (2020) discussed influences of GnRH gene and short-term progesterone combined with electrocardiogram (ECG) on reproduction parameters of anestrous Beetal goat during the non-breeding stage. Wang *et al*. (2020) found a remarkable association between the SNPs of GnRH and sperm quality traits of Chinese water buffalo. Xu *et al*. (2011) found a highly significant association between

G840327C of the GnRH-I gene and age at first egg (AFE) (p<0.01) , but no significant association was detected between G840327C and egg number at 300 days of age (p>0.05) in a Chinese chicken population. In addition, Zhang *et al*. (2016), Han *et al*. (2016), Ding *et al*. (2018) and Liu *et al*. (2017) studied expression laws of GnRH gene at different development stages of Sichuan white goose, Xupu goose, small tailed han sheep and Microtus brandti. These studies all elaborated the close relationship between GnRH and reproduction traits of animals. It can be used as an important candidate gene in the process of animal production.

Quail is a suitable experimental animal in multiple subjects, such as poultry propagation, histology, nutriology, hemadenology, embryology, physiology and pharmacology (Bai *et al*., 2016a, 2016b, 2016c, 2020). Rasul *et al*. (2019) analyzed the effects of different anti stress agents on the growth and meat quality of Japanese quail. Li *et al*. (2019) indicated that the black plumage color might be caused by increased production of MC1R and the white plumage color might be caused by increased production of ASIP in Japanese quail. Therefore, this study analyzed polymorphism of GnRH gene through sequencing technique, and investigated growth traits of quails, aiming to gain molecular markers related to growth traits. Research conclusions can provide references for breeding and marker-assisted selection of new species of egg quail.

MATERIALS AND METHODS

Genomic DNA extraction of quail

50 female samples of China yellow quail, Beijing white quail and Korean quail were collected, respectively. All samples were cultured in single cages which had been sterilized strictly before the experiment. Spraying sterilization was performed regularly to all cages during the experiment. All cages were piled up completely into four layers. The culture room was provided with 24 hour light/day and the quails were allowed to drink water and eat diet freely throughout the experiment. Feeds were supplemented artificially twice a day (morning and evening). The culture temperature and humidity were determined according to culture management requirements. When the feeding experiment ended at the age of 17 weeks, 5mL blood was collected from the heart of each quail and genomic DNA was extracted by poultry whole blood DNA extraction kit.

Measurement of growth traits

The growth traits such as body weight, chest depth, chest width, breastbone length, body length, shank length and shank circumference were measured from quails at 7-17 weeks. The specific measurement methods were as follows:

Body weight (g): the electronic scale weighs the weight of quail.

Chest depth (cm): use vernier caliper to measure the distance from the first thoracic vertebra to the front of keel.

Chest width (cm): use vernier caliper to measure the body surface distance between the two shoulder joints.

Breastbone length (cm): measure the distance from the front of sternum to the end of sternum with tape measure.

Body length (cm): measure the distance between shoulder joint and ischial node along the body surface with a leather tape.

Shank length (cm): use vernier caliper to measure the linear distance from the joint on the tibia (metatarsal) to the third and fourth toes.

Shank circumference (cm): use cotton thread to circle the tibia and mark on the cotton thread, and then measure the length marked on the cotton thread with a tape measure.

PCR amplification of GnRH gene

In this experiment, GnRH-1 primers were designed according to Pu (2016), which were F-TCTTGGTTGGTGTTCTCCT and R-ATTGCTCAGCCTGGGAT. The expected amplification fragment size was 906bp. The total volume of PCR amplification reaction system was 15 μL, containing 3.5μL deionized water, 1μL upstream primer, 1μL downstream primer, 2μL DNA template and 7.5 μL 2×Taq PCR Mix. The PCR amplification program was as follows: initial denaturation for 5 min at 94 °C, following it was 30 cycles of degeneration for 30s at 94 °C, annealing for 30s at 59 °C and extension for 30s at 72 °C, finally an extension for 7min at 72 °C. The reaction system was stored under 4 °C.

Data analysis

PCR products of GnRH gene were sent to Beijing Qingke biological Co., Ltd. for sequencing. Chromas software was used to determine the genotype of the sequencing results.

The model used for association analysis between GnRH gene and growth traits was as follows:

Analytical model: $y_{ijkl} = \mu + B_i + W_j + M_k + e_{ijkl}$

Y_{ijkl} is the phenotype value of traits, μ is the total mean value, B_i is the effect of the i th variety (i = 1, 2, 3), W_j is the effect of the j th Week age (7,8,9,10,11,12,13,14,15,16,17), M_k is the effect of the k th genotype effect, e_{ijkl} is the residual effect.

SPSS17.0 statistical software was used to analyze the association between different genotypes and growth traits, the final results were represented in the form of mean value + standard error.

RESULTS

Detection SNP sites

PCR amplification products of GnRH gene in China yellow quail, Beijing white quail and Korean quail are shown in Figure 1. Clearly, the amplification fragments of GnRH gene all gave a high-definition bright stripe near 906bp, which conformed to the target size of 906bp. A total of 14 SNP mutation sites of GnRH gene were detected in three egg quail species, which were C71T, C108T, C168T, C178T, A184G, C206T, A209C, C215T, A252G, A279T, C281T, C293G, C339T and C458T (Figure 2). Pu YJ. (2016) studied polymorphism of GnRH-1 in quail, and detected 6 mutation sites including C108T, C168T, C178T, A184G, C206T and C215T, only C108T was silent mutation without causing changes to the corresponding amino acid. In this study, 8 additional mutation sites were detected besides the six ones above, which were C71T, A209C, A252G, A279T, C281T, C293G, C339T and C458T, demonstrating that GnRH-1 gene had rich polymorphism in 3 egg quail species.

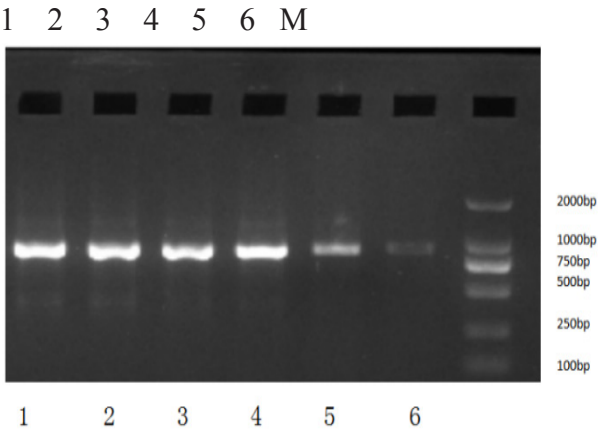

Figure 1 – Agarose electrophoresis results of GnRH-1 gene.

Note: M is marker DL2000, 1,2 represent Chinese yellow quail, 3,4 represent Beijing white quail, 5,6 represent Korean quail.

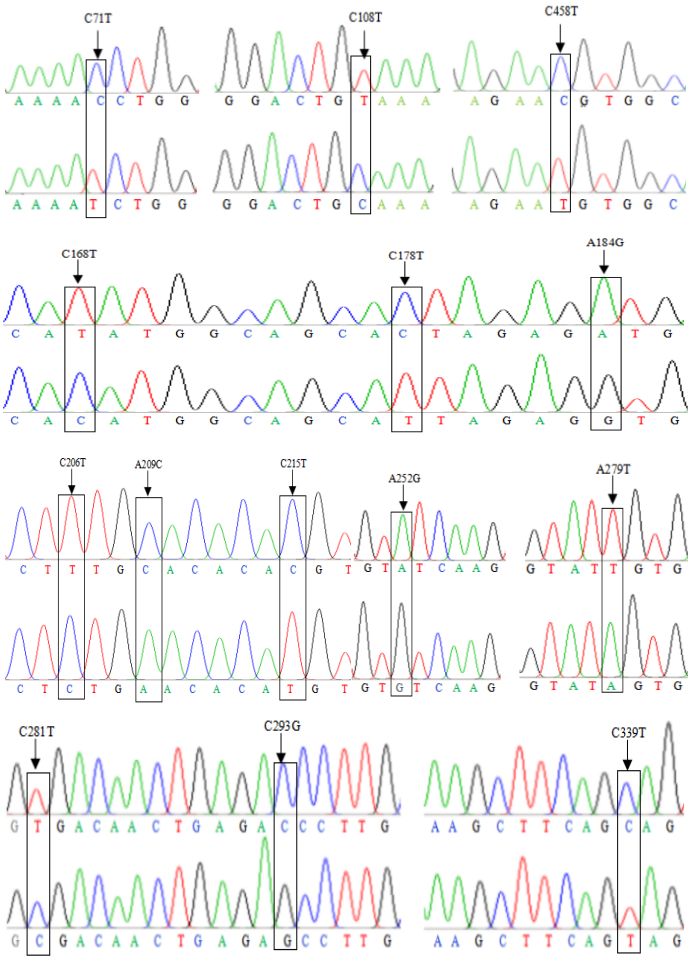

Figure 2 – SNP detection of GnRH-1 gene in quail.

Genotype frequency

Except that only 2 genotypes were detected for A209C and C281T in China yellow quail and Beijing white quail, the remaining 12 SNP mutation sites all detected 3 genotypes in three egg quail species. The frequencies of TT, CC, CC, TT, GG and CC genotypes were the highest for C71T, C108T, A209C, C281T, C293G and C339T respectively, which reached 0.479, 0.522, 0.534 (TT genotype frequency of C71T), 0.458, 0.500, 0.438 (CC genotype frequency of C108T), 0.980, 0.955, 0.624 (CC genotype frequency of A209C), 0.980, 0.955, 0.708 (TT genotype frequency of C281T), 0.458, 0.523, 0.646 (GG genotype frequency of C293G), 0.833, 0.795, 0.688 (CC genotype frequency of C339T) in China yellow quail, Beijing white quail and Korean quail respectively.

Association analysis between GnRH gene and growth traits

It can be observed from Table 1 that for C71T mutation site, body weight and shank length of individuals with CT genotype were significantly

Table 1 – Genotype frequency of GnRH gene.

Locus	Genotype	Chinese yellow quail	Beijing white quail	Korean quail	Locus	Genotype	Chinese yellow quail	Beijing white quail	Korean quail
C71T	CC	0.104	0.159	0.042	C215T	CC	0.354	0.659	0.250
	CT	0.417	0.318	0.313		CT	0.438	0.295	0.500
	TT	0.479	0.522	0.534		TT	0.208	0.046	0.250
C108T	CC	0.458	0.500	0.438	A252G	AA	0.104	0.205	0.125
	CT	0.417	0.319	0.417		AG	0.438	0.318	0.458
	TT	0.125	0.181	0.146		GG	0.458	0.477	0.417
C168T	CC	0.125	0.182	0.125	A279T	AA	0.085	0.100	0.109
	CT	0.417	0.341	0.479		AT	0.809	0.800	0.804
	TT	0.458	0.477	0.396		TT	0.106	0.100	0.087
C178T	CC	0.458	0.477	0.396	C281T	CC	0	0	0.063
	CT	0.417	0.341	0.438		CT	0.020	0.045	0.229
	TT	0.125	0.182	0.167		TT	0.980	0.955	0.708
A184G	AA	0.458	0.477	0.375	C293G	CC	0.104	0.159	0.042
	AG	0.417	0.341	0.458		CG	0.438	0.318	0.312
	GG	0.125	0.182	0.167		GG	0.458	0.523	0.646
C206T	CC	0.125	0.182	0.187	C339T	CC	0.833	0.795	0.688
	CT	0.438	0.364	0.542		CT	0.146	0.182	0.186
	TT	0.437	0.454	0.291		TT	0.021	0.021	0.125
A209C	AA	0	0	0.063	C458T	CC	0.563	0.341	0.604
	AC	0.020	0.045	0.313		CT	0.396	0.386	0.313
	CC	0.980	0.955	0.624		TT	0.041	0.273	0.083

higher than those with CC genotype (p<0.05), and the breastbone length of individuals with CC and TT genotypes were far higher than that with CT genotype (p<0.05). For C108T, C168T, C178T and C206T, shank length of individuals with CT genotype was far higher than that with CC and TT genotypes (p<0.05). For A184G and A252G, shank length of individuals with AG genotype was far higher than that with AA and GG genotypes (p<0.05). For A209C, body weight of individuals with AA and AC genotypes was dramatically higher than that with CC genotype (p<0.05), shank circumference of individuals with AA genotype was far higher than that with AC and CC genotypes (p<0.05). C215T played an important role in body weight, shank length, breastbone length and shank circumference, due to the significantly higher body weight, shank length and shank circumference of CC and CT genotypes than TT genotype (p<0.05), and significant higher breastbone length of CC genotype than CT and TT genotypes (p<0.05).

C281T had significant impact on body weight and shank circumference as body weights of CC and CT genotypes were dramatically higher than that of TT genotype (p<0.05), and shank circumference of CC genotype was significantly higher than that of CT and TT genotypes (p<0.05). For C293G, body weight of individuals with CG genotype was significantly higher than that with CC and GG genotypes (p<0.05), breastbone length of individuals with CC and GG genotypes was greatly higher than that with CG genotype (p<0.05). For C339T, body weight and shank length of individuals with TT genotype were far higher than that with CC and CT genotypes (p<0.05). With respect to C458T, body weight of CT and TT genotypes was far higher than that of CC genotype (p<0.05), and shank length of CT genotype was significantly higher than that of CC genotype (p<0.05), breastbone length of TT genotype was far higher than that of CC and CT genotypes (p<0.05). Generally, GnRH gene influenced growth traits of egg quail.

Du QZ (2011) demonstrated that for A883G of GnRH gene, the original sperm activity of individuals with AB genotype was far higher than that with BB genotype in Holstein cattle, based on which, he deduced that A allele might be positively related with characteristics of original sperms. Wang *et al*. (2020) found a remarkable association between the SNPs of GnRH and sperm quality traits of Chinese water buffalo. Xu *et al*. (2011) found a highly significant association between G840327C of the GnRH-I gene and age at first egg (AFE) (p<0.01) in a Chinese chicken population. Li *et al*.(2016) pointed out that for T68G of GnRH, lambing number of GG genotype was far higher than that of the rest two genotypes in Rongjiang little sweet sheep. Yan *et al*. (2011) pointed out that P4 amplification fragment of GnRH gene influenced lambing number of both Sinonsa milk goat and Boer goat significantly. Huang *et al*. (2011) demonstrated that three genotypes

Table 2 – Correlation analysis between GnRH gene and growth traits of quail.

Locus	Genotype	Body weight(g)	Shank length(cm)	Chest width(cm)	Chest depth(cm)	Breastbone length(cm)	Body length(cm)	Shank circumference (cm)
C71T	CC	162.034±2.486^{b}	3.569±0.013^{b}	3.182±0.021^{a}	3.294±0.026^{a}	4.408±0.026^{a}	9.224±0.061^{a}	1.677±0.014^{a}
	CT	165.006±0.975^{a}	3.621±0.008^{a}	3.157±0.011^{a}	3.309±0.013^{a}	4.329±0.011^{b}	9.180±0.031^{a}	1.679±0.006^{a}
	TT	164.014±0.815^{ab}	3.606±0.007^{a}	3.188±0.009^{a}	3.338±0.011^{a}	4.380±0.010^{a}	9.243±0.022^{a}	1.684±0.005^{a}
C108T	CC	163.647±0.865^{a}	3.596±0.007^{b}	3.187±0.010^{a}	3.332±0.012^{a}	4.385±0.011^{a}	9.240±0.025^{a}	1.683±0.005^{a}
	CT	165.264±0.990^{a}	3.632±0.008^{a}	3.162±0.010^{a}	3.321±0.013^{a}	4.342±0.010^{a}	9.190±0.029^{a}	1.677±0.006^{a}
	TT	162.921±1.773^{a}	3.579±0.011^{b}	3.181±0.017^{a}	3.304±0.021^{a}	4.360±0.023^{a}	9.224±0.047^{a}	1.687±0.011^{a}
C168T	CC	162.971±1.856^{a}	3.575±0.011^{b}	3.179±0.017^{a}	3.297±0.021^{a}	4.357±0.024^{a}	9.217±0.049^{a}	1.684±0.011^{a}
	CT	165.173±0.928^{a}	3.630±0.008^{a}	3.166±0.010^{a}	3.325±0.012^{a}	4.348±0.010^{a}	9.201±0.027^{a}	1.680±0.005^{a}
	TT	163.603±0.904^{a}	3.596±0.008^{b}	3.185±0.010^{a}	3.330±0.012^{a}	4.383±0.011^{a}	9.235±0.025^{a}	1.682±0.005^{a}
C178T	CC	163.603±0.904^{a}	3.596±0.008^{b}	3.185±0.010^{a}	3.330±0.012^{a}	4.383±0.011^{a}	9.235±0.025^{a}	1.682±0.005^{a}
	CT	164.832±0.944^{a}	3.628±0.008^{a}	3.162±0.010^{a}	3.321±0.012^{a}	4.345±0.010^{a}	9.202±0.028^{a}	1.679±0.005^{a}
	TT	164.071±1.753^{a}	3.586±0.011^{b}	3.189±0.017^{a}	3.311±0.021^{a}	4.366±0.022^{a}	9.214±0.046^{a}	1.686±0.010^{a}
A184G	AA	163.335±0.912^{a}	3.596±0.008^{b}	3.182±0.010^{a}	3.327±0.012^{a}	4.381±0.011^{a}	9.234±0.026^{a}	1.681±0.005^{a}
	AG	165.094±0.934^{a}	3.628±0.008^{a}	3.166±0.010^{a}	3.324±0.012^{a}	4.347±0.010^{a}	9.203±0.028^{a}	1.679±0.005^{a}
	GG	164.071±1.753^{a}	3.586±0.011^{b}	3.189±0.017^{a}	3.311±0.021^{a}	4.366±0.022^{a}	9.214±0.046^{a}	1.686±0.010^{a}
C206T	CC	164.071±1.753^{a}	3.586±0.011^{b}	3.189±0.017^{a}	3.311±0.021^{a}	4.366±0.022^{a}	9.214±0.046^{a}	1.686±0.010^{a}
	CT	165.610±0.883^{a}	3.630±0.007^{a}	3.174±0.010^{a}	3.330±0.012^{a}	4.349±0.009^{a}	9.214±0.026^{a}	1.681±0.005^{a}
	TT	162.546±0.964^{a}	3.590±0.008^{b}	3.174±0.011^{a}	3.320±0.013^{a}	4.383±0.012^{a}	9.226±0.027^{a}	1.680±0.006^{a}
A209C	AA	169.157±2.391^{a}	3.674±0.027^{a}	3.255±0.035	3.391±0.057^{a}	4.271±0.049^{a}	9.305±0.087^{a}	1.743±0.016^{a}
	AC	171.498±1.852^{a}	3.663±0.015^{a}	3.233±0.016^{a}	3.382±0.021^{a}	4.371±0.022^{a}	9.274±0.046^{a}	1.686±0.011^{b}
	CC	162.970±0.656^{b}	3.598±0.005^{a}	3.166±0.007^{a}	3.313±0.009^{a}	4.366±0.008^{a}	9.208±0.019^{a}	1.679±0.004^{b}
C215T	CC	164.971±0.920^{a}	3.609±0.007^{a}	3.181±0.010^{a}	3.322±0.012^{a}	4.410±0.011^{a}	9.273±0.028^{a}	1.685±0.006^{a}
	CT	166.242±0.943^{a}	3.619±0.008^{a}	3.178±0.010^{a}	3.329±0.012^{a}	4.348±0.011^{b}	9.194±0.027^{a}	1.687±0.005^{a}
	TT	157.095±1.545^{b}	3.577±0.012^{b}	3.161±0.016^{a}	3.312±0.021^{a}	4.294±0.016^{c}	9.141±0.040^{a}	1.657±0.009^{b}
A252G	AA	163.331±1.838^{a}	3.578±0.011^{b}	3.190±0.017^{a}	3.309±0.021^{a}	4.368±0.024^{a}	9.238±0.048^{a}	1.683±0.011^{a}
	AG	164.391±0.937^{a}	3.628±0.008^{a}	3.158±0.010^{a}	3.317±0.012^{a}	4.344±0.010^{a}	9.186±0.028^{a}	1.678±0.006^{a}
	GG	164.236±0.901^{a}	3.598±0.008^{b}	3.189±0.010^{a}	3.334±0.012^{a}	4.383±0.011^{a}	9.242±0.025^{a}	1.683±0.005^{a}
A279T	AA	163.795±1.776^{a}	3.609±0.015^{a}	3.184±0.020^{a}	3.361±0.029^{a}	4.424±0.021^{a}	9.369±0.049^{a}	1.693±0.012^{a}
	AT	165.572±1.611^{a}	3.624±0.015^{a}	3.170±0.029^{a}	3.335±0.023^{a}	4.341±0.020^{a}	9.261±0.056^{a}	1.665±0.011^{a}
	TT	162.034±2.486^{a}	3.569±0.013^{b}	3.182±0.021^{a}	3.294±0.026^{a}	4.408±0.026^{a}	9.224±0.061^{a}	1.677±0.014^{a}
C281T	CC	169.157±2.391^{a}	3.674±0.027^{a}	3.255±0.035^{a}	3.391±0.057^{a}	4.271±0.049^{a}	9.305±0.087^{a}	1.743±0.016^{a}
	CT	167.411±2.143^{a}	3.644±0.018^{a}	3.197±0.017^{a}	3.372±0.024^{a}	4.353±0.027^{a}	9.268±0.053^{a}	1.676±0.012^{b}
	TT	163.695±0.652^{b}	3.602±0.005^{a}	3.172±0.007^{a}	3.316±0.009^{a}	4.368±0.007^{a}	9.211±0.019^{a}	1.680±0.004^{b}
C293G	CC	162.034±2.486^{b}	3.569±0.013^{b}	3.182±0.021^{a}	3.294±0.026^{a}	4.408±0.026^{a}	9.224±0.061^{a}	1.677±0.014^{a}
	CG	165.070±0.961^{a}	3.622±0.008^{a}	3.155±0.011^{a}	3.308±0.013^{a}	4.330±0.011^{b}	9.174±0.030^{a}	1.678±0.005^{a}
	GG	163.957±0.823^{b}	3.605±0.007^{a}	3.190±0.009^{a}	3.339±0.011^{a}	4.380±0.010^{a}	9.247±0.023^{a}	1.684±0.005^{a}
C339T	CC	163.359±0.711^{b}	3.599±0.006^{b}	3.174±0.008^{a}	3.322±0.009^{a}	4.363±0.008^{a}	9.218±0.020^{a}	1.680±0.004^{a}
	CT	164.225±1.366^{b}	3.617±0.012^{b}	3.166±0.015^{a}	3.312±0.019^{a}	4.364±0.020^{a}	9.197±0.044^{a}	1.679±0.008^{a}
	TT	175.214±2.136^{a}	3.695±0.022^{a}	3.243±0.024^{a}	3.369±0.030^{a}	4.395±0.031^{a}	9.288±0.069^{a}	1.711±0.015^{a}
C458T	CC	161.517±0.936^{b}	3.593±0.007^{b}	3.170±0.010^{a}	3.309±0.011^{a}	4.330±0.010^{c}	9.173±0.024^{a}	1.675±0.005^{a}
	CT	167.580±0.890^{a}	3.628±0.008^{a}	3.180±0.011^{a}	3.330±0.013^{a}	4.375±0.012^{b}	9.216±0.030^{a}	1.683±0.006^{a}
	TT	164.919±1.571^{a}	3.608±0.013^{ab}	3.190±0.017^{a}	3.358±0.023^{a}	4.466±0.021^{a}	9.396±0.044^{a}	1.700±0.010^{a}

Note: If the letters in the same column are different, there is a significant difference (p<0.05); if the letters in the same column are the same, there is no significant difference (p>0.05).

of GnRH had evident impacts on egg yield of Liancheng white ducks. Yang T. (2007) proved the significant correlation between genotypes of GnRH and egg yield in Wanxi white goose. Peng L. (2013) detected AA, AB and BB genotypes on GR1 segment of intron 2 of GnRHR gene in muscovy duck, which all showed significant correlations with laying start age, maximum number of continuous laying days, average number of continuous laying days and egg number of 300-day old ducks. Hu *et al*. (2015) pointed out that 3 SNPs of GnRH gene influenced laying start age of Erlangshan chicken significantly and SNP5 played an important role in body weight at the beginning of laying. All these studies show that GnRH gene might regulate the production performance of poultry. Different from the reports above, this study analyzed the relationship between GnRH gene polymorphism and quail growth traits, and detected 14 SNP loci that were significantly related to body weight, shank length, breastbone length and shank circumference of quail. This study

suggested that GnRH gene had certain effect on the growth traits of quail.

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