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Short Communication

Correlation Analysis of Estrogen Receptor Gene Polymorphism and Growth Traits in Meat Quail (*Coturnix coturnix*)

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Authors' Contribution
ZHD conceived and designed the study and conducted the lab work. JYB analyzed the data and wrote the article. YL, XPJ and YBY helped in sampling. GLL and HC helped in analysis of data.

Key words
***Coturnix coturnix*, Estrogen receptor gene, Growth traits, PCR-RFLP, Correlation analysis**

ABSTRACT

In this study, PCR-RFLP was used to analyze the correlation between estrogen receptor (*ESR*) gene polymorphism and growth traits in two populations of quail. The results showed that three genotypes, CC, CT and TT, were detected in exon 1, exon 4 and exon 8 of *ESR* gene. The frequencies of CC genotype in expressed exon 1 of *ESR* gene were the highest in french giant quail and savimit quail (0.531 and 0.778, respectively). The frequencies of TT genotype in expressed exon 4 of *ESR* gene were the highest in french giant quail and savimit quail (0.338 and 0.581, respectively). The frequencies of TT genotype in expressed exon 8 of *ESR* gene were the highest in french giant quail and savimit quail (0.544 and 0.838, respectively). The research in this paper demonstrated the significant associations of exon 1 of *ESR* gene with sternal length and body length ($P<0.05$), exon 8 with body length ($P<0.05$), and exon 4 with body weight, chest width, and tibial circumference of meat quails ($P<0.05$).

The quail (*Coturnix coturnix*) is an ancient bird that is widely distributed in and around around the world. In recent years, the research on molecular marker genetic breeding is being developed, which has became an emerging technology for breeding and improvement of quail varieties Li *et al*. (2019). This study indicated that the black plumage color may be caused by increased production of melanocortin receptors (MCR) and the white plumage color may be caused by increased production of agouti signaling protein (ASIP) in Japanese quail. Azhar *et al*. (2018) studied the effect of different bacteria on the meat production of Japanese quail. Bai *et al*. (2016a, b, c, 2017; 2013) analyzed the polymorphism of quail population by using microsatellite markers and expressed sequence tag-simple sequence repeat (EST-SSR) markers. Zhang *et al*. (2013) shows that the expression of MC1R was higher in black plumage quails than that in maroon plumage quails, whereas the expression of ASIP was higher in maroon plumage quails than in black plumage quails. In recent years, some achievements have been made in the gene polymorphism (Li *et al*., 2019) and production performance (Bai *et al*., 2020) of quails. Many scholars have carried out the research work of *ESR* gene in human, pig, chicken, cattle, sheep, guinea pig, etc. But the correlation analysis between *ESR* gene and growth traits of quail has not been reported. Therefore, in order to provide a reference for marker-assisted selection of quails, the association of *ESR* gene with growth traits was analyzed in two meat quail populations by PCR-RFLP technology.

Materials and methods

In this experiment, blood samples (5ml each) were collected from vein in wings of 50 french giant quail and 50 savimit quail. The blood samples were stored in heparin sodium anticoagulant tubes which were then kept in a refrigerator under -20 °C. DNA was extracted by poultry whole blood DNA kit and kept under -20 °C.

Primers were designed according to the reference of Pu (2016) (Table I). Primers were synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd. The PCR amplification procedure was as follows: pre-denaturation at 94 °C for 4 min, then denaturation at 94 °C for 40 s, annealing at 53-55 °C for 1 min, elongation at 72 °C for 20 seconds, denaturation, annealing and elongation

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Table I. The information of primer sequences.

Name	Primer Sequence (5'-3')	Size (bp)	Annealing temperature (°C)
Exon 1	F: CAAAGCCCTTGGAGTTAC R: AGCAGTTCTCCCTACTCC	370	55.4
Exon 4	F: CGGGCGAATGATGAAACA R: CCCAGTTGATCATGTGCA	301	58
Exon 8	F: CAACAAAGGAATGGAGCA R: CCCTTCTTTTGCTGTTAA	212	53.6

Table II. Correlation between *ESR* gene polymorphism and growth traits (3-5 weeks old) in meat quail.

Name	Varieties	Genotype (Genotype frequency)	Body weight(g)	Tibia length(cm)	Chest width(cm)	Chest depth(cm)	Sternum length(cm)	Body length (cm)	Tibia circumference (cm)
Exon 1	French giant quail	CC(0.531)	122.85±4.90^{a}	3.70±0.02^{a}	2.93±0.05^{a}	3.27±0.03^{a}	3.67±0.09^{b}	8.33±0.16^{a}	1.58±0.01^{a}
		CT(0.306)	127.91±6.01^{a}	3.69±0.03^{a}	3.02±0.05^{a}	3.33±0.04^{a}	3.83±0.11^{ab}	8.51±0.21^{a}	1.59±0.02^{a}
		TT(0.163)	124.31±8.81^{a}	3.66±0.04^{a}	3.02±0.08^{a}	3.28±0.06^{a}	3.85±0.14^{a}	8.37±0.27^{a}	1.58±0.03^{a}
	Savimit quail	CC(0.778)	112.44±5.53^{a}	3.67±0.04^{a}	2.86±0.05^{a}	3.18±0.04^{a}	3.62±0.09^{a}	8.08±0.17^{b}	1.61±0.05^{a}
		CT(0.222)	115.22±9.17^{a}	3.64±0.08^{a}	2.95±0.07^{a}	3.17±0.05^{a}	3.73±0.17^{a}	8.30±0.33^{a}	1.56±0.04^{a}
Exon 4	French giant quail	CC(0.326)	119.70±5.93^{b}	3.65±0.03^{a}	2.91±0.06^{b}	3.24±0.05^{a}	3.72±0.12^{a}	8.23±0.20^{a}	1.56±0.002^{b}
		CT(0.286)	130.93±6.33^{a}	3.73±0.03^{a}	3.10±0.05^{a}	3.36±0.03^{a}	3.83±0.10^{a}	8.60±0.19^{a}	1.62±0.02^{a}
		TT(0.388)	124.29±5.77^{ab}	3.70±0.03^{a}	2.94±0.05^{ab}	3.29±0.04^{a}	3.72±0.11^{a}	8.37±0.19^{a}	1.58±0.02^{ab}
	Savimit quail	CT(0.419)	115.19±7.36^{a}	3.70±0.06^{a}	2.92±0.07^{a}	3.13±0.05^{a}	3.68±0.13^{a}	8.18±0.26^{a}	1.55±0.03^{a}
		TT(0.581)	111.85±8.18^{a}	3.64±0.04^{a}	2.86±0.06^{a}	3.20±0.04^{a}	3.63±0.10^{a}	8.10±0.19^{a}	1.63±0.07^{a}
Exon 8	French giant quail	CC(0.152)	123.43±8.23^{a}	3.69±0.04^{a}	2.95±0.07^{a}	3.32±0.08^{a}	3.81±0.17^{a}	8.44±0.26^{a}	1.58±0.03^{a}
		CT(0.304)	124.05±6.02^{a}	3.68±0.04^{a}	2.99±0.06^{a}	3.29±0.03^{a}	3.73±0.12^{a}	8.40±0.20^{a}	1.59±0.02^{a}
		TT(0.544)	125.45±4.03^{a}	3.70±0.02^{a}	2.97±0.05^{a}	3.28±0.03^{a}	3.74±0.08^{a}	8.38±0.16^{a}	1.58±0.01^{a}
	Savimit quail	CT(0.162)	113.33±10.68^{a}	3.65±0.10^{a}	2.83±0.11^{a}	3.22±0.11^{a}	3.59±0.20^{a}	7.79±0.35^{b}	1.78±0.23^{a}
		TT(0.838)	112.99±5.32^{a}	3.66±0.03^{a}	2.89±0.05^{a}	3.17±0.03^{a}	3.66±0.09^{a}	8.20±0.17^{a}	1.56±0.02^{a}

If the letters in the same column are different, the difference is significant (P<0.05); if the letters in the same column are the same, the difference is not significant (P>0.05).

were carried out for 35 cycles, finally elongation at 72°C for 10 min, the reaction was completed and preserved at 4°C.

The PCR product amplified by exon 1 primers was digested by restriction endonuclease Pvu II. The reaction system was 15 μL: ddH_2O 5 μL, PCR product 8 μL, restriction endonuclease Pvu II (10U / μL) 0.5 μL, 10 × buffer 1.5 μL. The mixture was evenly mixed and digested in a 37 °C water bath for 4 h. The PCR product amplified by exon 8 primers was digested by restriction endonuclease ACC I. The reaction system was 10 μL: ddH_2O 0.5 μL, PCR product 8 μL, restriction endonuclease ACC I (10U / μL) 0.5 μL, 10 × buffer 1.0 μL. The mixture was evenly mixed and incubated at 37°C for 4h. The digested products were detected by 2% agarose gel electrophoresis. Exon 4 was identified by PCR product direct sequencing.

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 and discussion

As can be seen from Figure 1, CC, CT and TT, were detected in expressed exon 1 of *ESR* gene in french giant quail, and only two genotypes, CT and TT, were detected in savimit quail. The frequencies of CC genotype in expressed exon 1 of *ESR* gene were the highest in french giant quail and savimit quail (0.531 and 0.778, respectively). Three genotypes, CC, CT and TT, were detected in expressed exon 4 of *ESR* gene in french giant quail, and only two genotypes, CT and TT, were detected in savimit quail. The frequencies of TT genotype in expressed exon 4 of *ESR* gene were the highest in french giant quail and savimit quail (0.338 and 0.581, respectively). Three genotypes, CC, CT and TT, were detected in expressed exon 8 of *ESR* gene in french giant quail, and only two genotypes, CT and TT, were detected in savimit quail. The frequencies of TT genotype in expressed exon 8 of *ESR* gene were the highest in french giant quail and savimit quail (0.544 and 0.838, respectively).

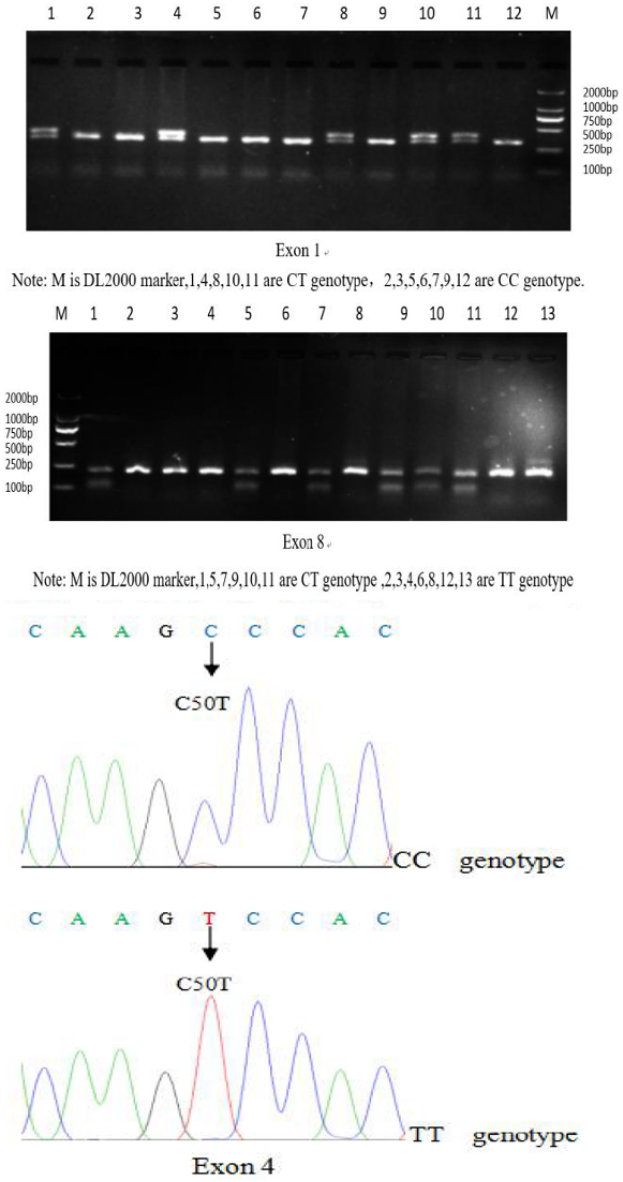

Fig. 1. Detection results of *ESR* gene polymorphism.

Table II showed the results of association analysis between *ESR* gene polymorphism and growth traits of meat quails. As with exon 1 of *ESR* gene in french giant quails, sternal length by TT genotype was significantly greater than that by CC genotype ($P<0.05$), while those by CT genotype had no difference with that by CC or TT genotypes ($P>0.05$), accompanied by hardly any obvious effects on any other growth traits ($P>0.05$). In savimit quails, the body length by CT genotype was larger than that by CC genotype ($P<0.05$), without obvious influences imposed on the rest of their growth traits ($P>0.05$). It therefore suggested a remarkable correlation of exon 1 of *ESR* gene with the sternal length and body length of meat quails($P<0.05$).

Exon 4 of *ESR* gene had no significant effects on the growth traits of savimit quails ($P<0.05$). In french giant quails, the body weight, chest width and tibial circumference of CT genotype were greater than those of CC genotype ($P<0.05$), such indicators by TT genotype were not notably different with that of CT or CC genotype ($P>0.05$), the rest of the traits were not affected. These results told that exon 4 of *ESR* gene had been correlated with the body weight, chest width, and tibial circumference of meat quails ($P<0.05$). Not any growth traits of french giant quails were notably affected by exon 8 of *ESR* gene ($P>0.05$), but among savimit quails, the body length of CT genotype was significantly higher than that of TT genotype ($P<0.05$), indicating the exon 8 and the body length of meat quails in somehow relevance ($P<0.05$).

By now the studies about association between *ESR* gene and growth performance in livestock and poultry were few. Wang *et al*. (2009) and others indicated that individuals with AA genotype of *ESR* gene produced higher body length, and more significant body height, chest circumference and body weight than that with BB genotype in Su Jiang pigs. Liu (2007) found that *ESR* gene had obvious influences on the daily gain of Jiangxi and Anhui Large White pigs, but showed little effect on their body height, body length and chest circumference. Li (2005) suggested that there was no significant difference in early growth traits among Suhuai pigs with various genotypes of *ESR* gene. The results of this research were similar to those findings by Wang *et al*. (2009) but inconsistent with results by Liu (2007) and Li (2005), the research in this paper demonstrated the significant associations of exon 1 of *ESR* gene with sternal length and body length ($P<0.05$), exon 8 with body length ($P<0.05$), and exon 4 with body weight, chest width, and tibial circumference of meat quails ($P<0.05$). In the selection of meat quails therefore the sternal length, body length, the body weight, chest width, and tibial circumference *et al*. shall be considered comprehensively.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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