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Association analysis of ESR gene polymorphism and carcass traits in egg quails (*Coturnix coturnix*)

JUN YAN BAI✉, ZHI HAO DONG, XIAO NING LU, ZHI YANG HUANG, ZI HENG LI, HUI RONG GONG, QI HANG HU, GAO XIANG HUANG and ZHENG YU FAN

College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003 China

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Estrogen receptor (ESR) gene is a kind of nucleic acid receptor in the family of activated transcription factors and could combine with specific hormone response DNA element. Ichikawa (2003) cloned the cDNA of the ESR gene, and since then the cDNAs of ESRα and ESRβ in several species have been cloned. Many scholars have carried out the research work of ESR gene in human, pig, chicken, cattle, sheep, guinea pig, etc. (Zhang *et al.* 2013, Wu *et al.* 2013, Si *et al.* 2016). Quail can be used as a research animal in many subjects such as poultry reproduction, histology, nutrition, endocrinology, embryology, physiology, pharmacology and so on (Zhang *et al.* 2013, Bai *et al.* 2016a, 2016b, 2016c, 2016d, 2017, Azhar *et al.* 2018, Dash *et al.* 2018, Raju *et al.* 2019, Li *et al.* 2019). But the correlation analysis between ESR gene and carcass 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 carcass traits was analyzed by PCR-RFLP technique in egg quails.

In this experiment, egg quails were 80 China quails with yellow feathers, 80 Beijing quails with white feathers and 80 Korean quails. All egg quails were females. Blood samples (5 ml each) were collected at vein in wings of each quail and 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 of ESR gene were designed according to reference of Pu *et al.* (2016). The primer sequences of exon 1 were F: CAAAGCCCTTGGAGTTAC and R: AGCAGTT CTCCCTACTCC with an expected fragment size of 370 bp and annealing temperature of 55.4°C. The primer sequences of exon 8 were F: CAACAAAGGAATG GAGCA and R: CCCTTCTTTTGCTGTTAA with an expected fragment size of 212 bp and annealing temperature of 53.6°C. 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 40s, annealing at 53–55°C for 1 min, elongation at 72°C for 20 seconds, denaturation, annealing and elongation were carried out for 35 cycles, after a final elongation at 72°C for 10 min the reaction was completed and cooled at 4°C.

The restriction endonuclease Pvu a! was used to digest the PCR product amplified by exon 1 primers. The reaction system was 15 μL: dd H_2O 5 μL, PCR product 8 μL, restriction endonuclease Pvu a! (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 restriction endonuclease ACC '! was used to digest the PCR product amplified by exon 8 primers. The reaction system was 10 μL: dd H_2O 0.5 μL, PCR product 8 μ L, restriction endonuclease ACC'! (10U/μL) 0.5 μL, 10 × buffer 1.0 μL. The mixture was evenly mixed and digested in a 37°C water bath for 4 h. The digested products were detected by agarose gel electrophoresis of 2% concentration and then photographed.

SPSS17.0 statistical software was used to analyze the association between different genotypes and carcass traits, and Duncan multiple comparison method was used to make multiple comparison. The final results were represented in the form of mean value ± standard error.

The polymorphism detection results of exon 1 and exon 8 of ESR gene were shown in Fig. 1.

Exon 1 of ESR gene detected three genotypes of CC, CT, TT in Chinese and Korean quails and two genotypes of CC and CT in Beijing white quails. The frequencies of CC genotype were the highest in China yellow quail, Beijing white quail and Korean quail (0.515, 0.614 and 0.723, respectively), while the frequencies of TT genotype were the lowest (0.088, 0 and 0.015, respectively), the frequencies of CT genotype were moderate (0.397, 0.386 and 0.262, respectively). Three genotypes, CC, CT and TT were detected in exon 8 of ESR gene in both China yellow quail and Beijing white quail, and only two genotypes, CT and TT were detected in Korean quail. The highest frequencies of TT genotype in exon 8 of ESR gene were detected in Beijing white quail and Korean quail (0.618, 0.540), while the highest frequency of CT genotype was detected in China

Present address: College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China. ✉Corresponding author e-mail: junyanbai@163.com

Table 1. Correlation analysis between ESR gene polymorphism and carcass traits of egg quail

Name	Parameter	Carcass traits		Beijing white quail		Chinese yellow quail		Korean quail	
		CC	CT	CC	CT	TT	CC	CT	TT
Exon 1	Body weight	136.700±2.325[a]	138.578±3.479[a]	145.000±2.694[a]	130.692±3.223[b]	134.600±3.100[b]	146.478±3.590[a]	145.627±4.150[a]	158.700±4.214[a]
	Carcass weight	131.138±2.302[a]	132.744±3.285[a]	139.806±2.699[a]	125.692±3.177[b]	128.500±3.122[b]	142.578±3.526[a]	142.073±4.103[a]	155.100±4.102[a]
	Eviscerated weight	83.629±1.516[a]	82.989±2.173[a]	92.712±1.755[a]	85.300±2.442[a]	88.300±2.170[a]	94.617±3.344[a]	90.127±3.119[a]	107.200±3.211[a]
	Heart weight	0.971±0.034[a]	1.111±0.096[a]	1.153±0.048[a]	1.067±0.054[a]	0.800±0.025[a]	1.067±0.034[b]	1.027±0.051[b]	1.600±0.054[a]
	Liver weight	3.871±0.144[a]	4.111±0.184[a]	4.024±0.193[a]	3.283±0.276[a]	3.200±0.245[a]	4.122±0.154[a]	5.773±1.159[a]	7.600±0.741[a]
	Pectoral weight (total)	27.971±0.852[a]	26.878±0.918[a]	29.271±0.744[a]	25.483±1.179[b]	28.600±0.859[a]	30.300±1.219[a]	27.473±1.346[a]	31.700±1.235[a]
	Leg muscle weight (single)	7.181±0.178[a]	7.278±0.237[a]	7.635±0.187[a]	7.400±0.319[a]	6.500±0.415[a]	7.628±0.265[a]	7.591±0.269[a]	7.800±0.281[a]
	Slaughter rate	95.916±0.132[a]	95.799±0.147[a]	96.400±0.206[a]	96.159±0.153[a]	95.468±0.176[a]	97.329±0.149[a]	97.554±0.229[a]	97.732±0.214[a]
	Eviscerating percentage	61.244±0.728[a]	59.911±0.825[a]	64.035±0.919[a]	65.274±1.054[a]	65.602±0.985[a]	64.425±0.995[a]	61.948±1.623[a]	67.549±1.023[a]
	Heart rate	1.165±0.039[a]	1.346±0.120[a]	1.241±0.040[a]	1.261±0.068[a]	0.906±0.074[a]	1.144±0.043[a]	1.147±0.057[a]	1.493±0.046[a]
	Liver rate	4.654±0.177[a]	4.990±0.271[a]	4.344±0.191[a]	3.879±0.329[a]	3.624±0.241[a]	4.446±0.227[a]	6.695±1.602[a]	7.090±0.753[a]
	Pectoral muscle rate (total)	33.356±0.580[a]	32.412±0.847[a]	31.570±0.557[a]	29.833±0.958[a]	32.390±0.845[a]	32.033±0.647[a]	30.448±0.983[a]	29.571±0.874[a]
	Leg muscle ratio (single)	17.174±0.307[a]	17.572±0.495[a]	16.477±0.281[a]	17.306±0.453[a]	14.723±0.413[a]	16.152±0.193[a]	16.870±0.335[a]	14.552±0.314[b]

Name	Parameter	Carcass traits		Beijing white quail		Chinese yellow quail		Korean quail	
		CC	CT	TT	CC	CT	TT	CT	TT
Exon 8	Body weight	129.600±7.00[a]	138.208±2.570[a]	137.513±2.933[a]	142.733±4.964[a]	138.670±3.935[a]	138.412±3.438[a]	149.507±3.943[a]	143.640±3.430[a]
	Carcass weight	123.950±6.750 [a]	132.342±2.502[a]	132.038±2.868[a]	137.433±3.954[a]	133.760±4.000[a]	133.153±3.411[a]	145.627±3.871[a]	139.993±3.401[a]
	Eviscerated weight	82.350±4.850[a]	83.958±1.469[a]	83.181±2.010[a]	90.267±1.637[a]	87.460±2.458[a]	90.741±2.256[a]	94.920±3.675[a]	91.860±3.025[a]
	Heart weight	0.950±0.050[a]	1.092±0.050[a]	0.963±0.058[a]	1.133±0.120[a]	1.120±0.057[a]	1.094±0.053[a]	1.047±0.041[a]	1.093±0.052[a]
	Liver weight	4.500±0.700[a]	3.767±0.132[a]	4.006±0.173[a]	3.633±0.088[a]	4.000±0.360[a]	3.535±0.204[a]	4.200±0.173[a]	5.487±0.880[a]
	Pectoral weight (total)	26.200±3.500[a]	28.075±1.070[a]	27.500±0.891[a]	28.867±1.074[a]	26.850±1.228[a]	28.053±1.014[a]	30.787±1.341[a]	27.833±1.137[a]
	Leg muscle weight (single)	7.350±0.450[a]	7.333±0.228[a]	7.100±0.201[a]	7.133±0.233[a]	7.470±0.366[a]	7.588±0.205[a]	7.833±0.300[a]	7.407±0.209[a]
	Slaughter rate	95.638±0.043[a]	95.751±0.173[a]	96.009±0.135[a]	96.326±0.596[a]	96.423±0.214[a]	96.175±0.183[a]	97.398±0.168[a]	97.451±0.184[a]
	Eviscerating percentage	63.525±0.311[a]	60.849±0.981[a]	60.505±0.761[a]	63.335±1.551[a]	63.218±1.522[a]	65.605±0.676[a]	63.347±1.144[a]	63.895±1.333[a]
	Heart rate	1.161±0.129[a]	1.298±0.053[a]	1.167±0.076[a]	1.252±0.114[a]	1.290±0.073[a]	1.205±0.046[a]	1.119±0.051[a]	1.195±0.047[a]
	Liver rate	5.433±0.530[a]	4.503±0.175[a]	4.858±0.230[a]	4.027±0.114[a]	4.586±0.392[a]	3.887±0.181[a]	4.501±0.229[a]	6.215±1.200[a]
	Pectoral muscle rate (total)	31.675±2.385[a]	33.364±0.859[a]	33.029±0.594[a]	31.959±0.675[a]	30.656±1.001[a]	30.861±0.697[a]	32.409±0.636[a]	30.330±0.809[a]
	Leg muscle ratio (single)	17.848±0.042[a]	17.464±0.449[a]	17.096±0.353[a]	15.835±0.819[a]	17.029±0.561[a]	16.747±0.284[a]	16.525±0.216[a]	16.199±0.309[a]

If the small letters of the same row are different, it means the difference is significant ($P<0.05$), if the letters of the same column are the same, it means the difference is not significant ($P>0.05$).

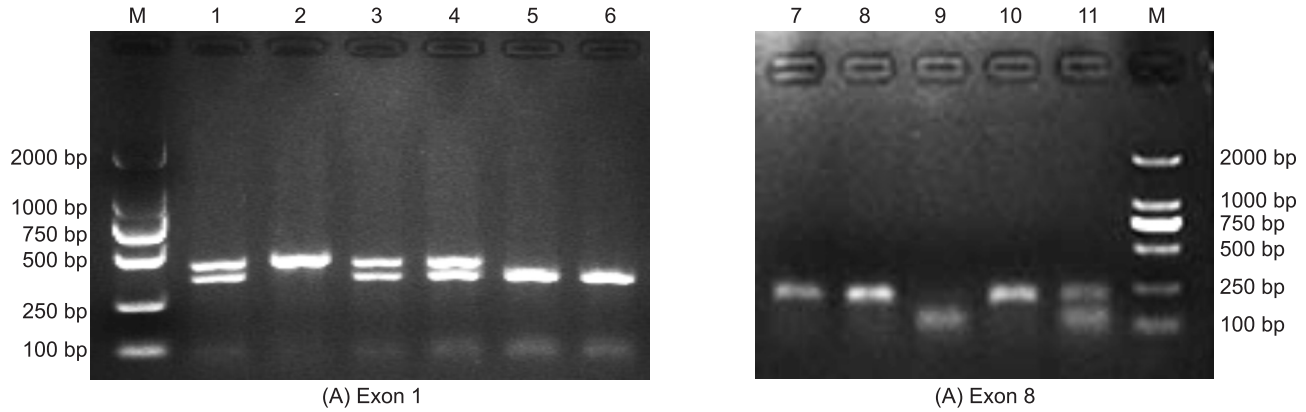


Fig. 1. Polymorphism detection of ESR gene in quail.

Note: M is marker DL2000; 1, 3, 4 are CT genotype; 2 is TT genotype; 5,6 are CC genotype; 7, 8, 10 are TT genotype; 9 is CC genotype; 11 is CT genotype.

yellow quail (0.476). In this study, TT, CT and CC genotypes were detected in exon 1 and exon 8 of egg quail, which is coincident with the results provided by Pu *et al.* (2016).

The correlation analysis results between polymorphism of ESR gene and slaughter traits of egg quails were shown in table 1, which indicated that the exon 1 of ESR gene had no significant effects on the carcass traits of Beijing white quail (P>0.05). The body weight and carcass weight of CC genotype were significantly higher than that of CT and TT genotypes (P<0.05) and the pectoral weight of CC and TT genotypes was significantly higher than that of CT genotype (P<0.05) in China yellow quail. The heart weight of TT genotype was significantly higher than that of CT and CC genotypes (P<0.05) and the leg muscle rates of CC and CT genotypes were significantly higher than that of TT genotype (P<0.05) in Korean quail. It was found that exon 1 of ESR gene was significantly associated with body weight, carcass weight, pectoral weight, heart weight and leg muscle rate of egg quail (P<0.05). Exon 8 of ESR gene had no significant effect on the slaughter traits of China yellow quail, Beijing white quail and Korean quail (P>0.05).

Wang *et al.* (2009) and others indicated that individuals with AA genotype of *ESR* gene produced greater body length, and more significant body height, chest circumference and body weight than that with BB genotype in Su Jiang pigs. Liu *et al.* (2007) found that ESR gene had obvious influences on the daily gain of Jiangxi and Anhui Large White pigs, but showed little effects on their body height, body length and chest circumference. Liu *et al.* (2016) suggested that there were notable divergence in the total litter size, number of living piglets and birth weight between GG genotype and GA genotype of ESR gene in Jinhua sows. Wu *et al.* (2013) detected some polymorphisms at the 5′ lateral region of ESR gene in Female Line of Shaobo Chicken, and found the egg yields of individuals with EE and EF genotypes were both higher than that of individuals with FF genotype. As demonstrated in this study, there were significant correlations between exon 1 of ESR gene and body weight, carcass weight, breast muscle weight, heart weight and percentage of leg muscles of egg quails (P<0.05).

SUMMARY

The polymorphism of estrogen receptor (ESR) gene was detected by PCR-RFLP in China yellow, Korean and Beijing white quails and the association between ESR gene polymorphism and carcass traits of egg quails was analyzed. The results showed that there were three genotypes including CC, CT and TT detected in exon 1 and exon 8 of ESR gene in egg quails. The frequencies of CC genotype in exon 1 of ESR gene were the highest in China yellow, Beijing white and Korean quail (0.515, 0.614, 0.723). The highest frequencies of TT genotype in exon 8 of ESR gene were detected in Beijing white and Korean quails (0.618 and 0.540), while the highest frequency of CT genotype was detected in China yellow quail (0.476). As demonstrated in this study, there were significant correlations between exon 1 of ESR gene and body weight, carcass weight, breast muscle weight, heart weight and percentage of leg muscles in egg quails (*P*<0.05).

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