



Polymorphism analysis of myogenin gene in meat quail (*Coturnix coturnix*)

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Quail can be used as a research animal in many subjects such as poultry reproduction, histology, nutrition, endocrinology, embryology, physiology, pharmacology and so on (Bai *et al.* 2016a, 2016b, 2016c). The research value of quail in teaching and scientific studies is increasing gradually (Zhang *et al.* 2013, Bai *et al.* 2016d, 2017, Li *et al.* 2019, Raju *et al.* 2019). Myogenin gene (MyoG) is a kind of myogenic regulatory factor and it regulates muscle growth together with myogenic determination gene, myogenic regulatory factor 4, myostatin and myogenic factor 5. However, most of the studies about these genes were concentrated in formation mechanism of muscles as well as genetic expression and regulation of MyoG. Few studies on correlation analysis between MyoG and growth performance of meat quail are available. Here correlations of MyoG with slaughter performance of meat quail were discussed, which provided references for marker assisted selection of meat quail.

In this experiment, blood samples (5 ml each) were collected at vein in wings of 50 French giant quails and 50 Savimit quails 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 amplified for loci A and B in the 5' regulatory region of MyoG gene were designed according to Wang *et al.* (2007). Primers were synthesized by Beijing Dongguo Changsheng Biotechnology Co., Ltd. Primers of locus A were F-GGTGGGTGTGGGA-ATGTGCT and R-CCGGCTTTGCTCTTAAGTCT with expected amplification size of 203 bp, primers of locus B were F-AAACCCACTCCATTGTGC and R-CACTAC-TTGG-CTCCTCTAGTT with expected amplification size of 236 bp. The amplification program was as follows: pre-denaturation at 94°C for 4 min, then denaturation at 94°C for 40 sec, annealing at $57\text{--}60^{\circ}\text{C}$ for 1 min, annealing at 72°C for 20 sec, denaturation, annealing and elongation were carried out for 35 cycles, then elongation at 72°C for 10 min, after which the reaction was completed.

The amplified products were first tested by 10% acrylamide gel electrophoresis for 15 min under 220V, then detected by electrophoresis for 6 h under 90V with the same 10% acrylamide gel. Finally, the products were observed by silver staining. Pictures were taken and stored. Analytical model:

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

where 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 j age effect, M_k is the k genotype effect, e_{ijkl} is the residual effect.

For French giant quail and Savimit quail, three genotypes (AA, BB and AB) were discovered at both locus A and locus B (Fig. 1). Wei *et al.* (2014) found 2 mutation sites and 6 genotypes in the third exon of MyoG of Bian chicken. In this study, polymorphism at loci A and B of the MyoG gene in two meat quail groups was tested. Three genotypes

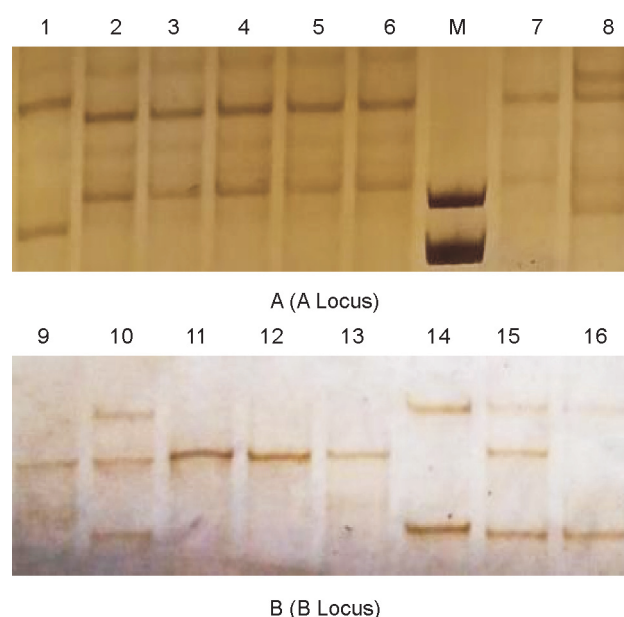


Fig. 1. SSCP results of MyoG gene in meat quail. Note: M is Marker DL2000; A:1 is AA genotype; 2, 3, 4, 5, 6, 7 is BB genotype and 8 is AB genotype; B: 9, 11, 12, 13 are BB genotypes; 10, 15 are AB genotypes; 14, 16 are AA genotypes.

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Table 1. Polymorphism of MyoG gene in meat quail

Loci	Population	Genotype frequency			Allele frequency		Heterozygosity He	Number of effective alleles (Ne)	Polymorphism Information Content (PIC)
		AA	BB	AB	A	B			
A loci	French giant quail	0.367	0.531	0.102	0.418	0.582	0.487	1.948	0.368
	Savimit quail	0.083	0.750	0.167	0.167	0.833	0.278	1.385	0.240
B loci	French giant quail	1.000	0	0	1.000	0	0	1.000	0
	Savimit quail	0.250	0.389	0.361	0.431	0.569	0.490	1.963	0.370

were discovered at loci A and B, which were AA, AB and BB, indicating that MyoG had rich polymorphism in meat quail groups, which was similar with polymorphism research results on other poultries.

For locus A, B allele frequencies were the highest in French giant quail group (0.582) and Savimit quail group (0.833) respectively. Genetic polymorphism of French giant quail group was high ($He=0.487$). For locus B, three genotypes (AA, AB and BB) were detected in Savimit quail group, but only AA was detected in French giant quail group. For locus B, the BB genotype frequency was the highest (0.389) and the B allele frequency was the highest (0.569) in Savimit quail group. Genetic polymorphism of Savimit quail was high ($He=0.490$, Table 1).

For locus A, weight of individuals with AA genotype was significantly higher than that with AB and BB genotypes ($P<0.05$), and there's no significant difference between AB genotype and BB genotype ($P>0.05$). Body length of individuals with AA genotype was significantly higher than that with AB genotype ($P<0.05$), however, BB genotype had no significant difference with AA and AB genotypes ($P>0.05$). There's no significant difference among three genotypes in term of shank length, breast width, breast depth, breast bone length and shank circumference ($P>0.05$). For locus B, weight, breast bone length and body length of individuals with AA and BB genotypes were significantly higher than those with AB genotype ($P<0.05$), there's no significant difference between AA genotype and AB genotype ($P>0.05$). Moreover, there were no significant differences among three genotypes with respect to shank length, shank circumference, breast width and breast depth ($P>0.05$, Table 2).

Han *et al.* (2016) discussed the correlation between MyoG gene and body traits of Tibetan sheep, they found that individual weight, body height and body length of CC genotype were significantly higher than those of AA genotype ($P<0.05$). Bai *et al.* (2020) showed that MyoG gene had a significant correlation with growth traits such as body length and chest width of sheep ($P<0.05$). Zhao *et al.* (2016) concluded that two mutations of MyoG could influence breast muscle rate, weight and carcass net weight of duck significantly ($P<0.05$). Tang *et al.* (2013) discovered 3 genotypes (AA, AB and BB) on exon 1 of MyoG in Jinghai yellow chicken, the body weight of BB genotype at 2nd, 4th and 6th weeks was far higher than that of AB genotype, and the body weight of BB genotype at 8th week was higher than that of AA genotype ($P<0.05$). Wei *et al.* (2014) found 2 same sense mutation sites on MyoG, polymorphisms of these two mutation sites were correlated with growth performance of Bian chicken ($P<0.05$). These conclusions were similar with research conclusions of Han *et al.* (2016) and Tang *et al.* (2013). In all, loci A and B in MyoG gene can be applied for marker assisted selection of growth traits in meat quail.

SUMMARY

Results demonstrated that in meat quail, three genotypes (AA, BB and AB) were detected at locus A and B in MyoG gene. Locus A is significantly correlated with weight and body length of meat quail ($P<0.05$). Locus B is significantly correlated with weight, breastbone length and body length of meat quail ($P<0.05$). The MyoG gene can be used for marker-assisted selection of growth traits in meat quails.

Table 2. Association analysis between MyoG gene and growth traits of meat quail

Character	Genotype of locus A			Genotype of locus B		
	AA	AB	BB	AA	AB	BB
Weight (g)	125.398±5.194 ^a	116.418±8.142 ^b	118.230±3.737 ^b	123.301±3.269 ^a	105.323±7.204 ^b	118.536±8.221 ^a
Shank length (cm)	3.676±0.038 ^a	3.668±0.064 ^a	3.687±0.024 ^a	3.689±0.022 ^a	3.634±0.056 ^a	3.699±0.056 ^a
Breast width (cm)	2.970±0.053 ^a	2.932±0.077 ^a	2.928±0.038 ^a	2.964±0.034 ^a	2.771±0.079 ^a	2.991±0.072 ^a
Breast depth (cm)	3.285±0.045 ^a	3.271±0.060 ^a	3.225±0.029 ^a	3.259±0.027 ^a	3.130±0.063 ^a	3.300±0.047 ^a
Breast bone length (cm)	3.771±0.102 ^a	3.683±0.145 ^a	3.691±0.066 ^a	3.732±0.061 ^a	3.497±0.139 ^b	3.815±0.127 ^a
Body length (cm)	8.500±0.190 ^a	8.173±0.253 ^b	8.223±0.119 ^{ab}	8.364±0.112 ^a	7.854±0.251 ^b	8.357±0.231 ^a
Shank circumference (cm)	1.667±0.075 ^a	1.582±0.034 ^a	1.570±0.015 ^a	1.610±0.029 ^a	1.538±0.036 ^a	1.586±0.032 ^a

Note: There are significant differences in the lower-case English letters in the shoulder markings of peers ($P<0.05$), but no significant differences in the same letters ($P>0.05$).

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