Pakistan J. Zool., vol. 53(2), pp 789-792, 2021 DOI: https://dx.doi.org/10.17582/journal.pjz/20191007101045

Short Communication

Association between Myogenin Gene Polymorphism and Slaughter Traits of Meat Quails

Jun Yan Bai*, Kun Peng Shi, Xiao Ning Lu, Xiao Hong Wu, Xue Yan Fu, Heng Cao, Hong Deng Fan, Meng Ke Chen and Yong Kang Ma

Henan University of Science and Technology, College of Animal Science and Technology, Luoyang 471 023, China

Article Information
Received 07 October 2019
Revised 02 December 2019
Accepted 12 December 2019
Available online 26 February 2021

Authors' Contribution
JYB conceived and designed the study and conducted the lab work. KPS, XYF and HDF analyzed the data and wrote the article. XNL, HC and XHW helped in sampling. MKC and YKM helped in analysis of data.

Key words
Meat quail, Myogenin gene, Slaughter performance, Association analysis, SNP

ABSTRACT

To recognize molecular markers of slaughter performance of quail, SNP in control regions of cytogenin gene (*MyoG*) 5' in French giant quail, and Savimit quailwas detected by PCR-SSCP method in this study. Moreover, correlations of control regions of *MyoG* 5' with slaughter performance of quail were analyzed. Results demonstrated that: In meat quail, three genotypes (AA, BB and AB) were detected at locus A in the control region of *MyoG* 5'. For locus A, BB frequency of French giant quail and Savimit quail was the highest (0.531 and 0.750). For locus B, three genotypes (AA, AB and BB) were detected in Savimit quail, but only AA was detected in French giant quail. The BB frequency of Savimit quail was the highest, reaching 0.389. Locus A showed a significant correlation with liver weight of meat quails($P<0.05$), while locus B presented significant correlations with body weight, carcass weight, carcass net weight, liver weight, breast muscle weight and leg muscle weight($P<0.05$). Loci A and B in the control region of *MyoG* 5' can be used as the molecular marker of slaughter performance of meat quails during marker assisted selection.

At present, quail breeding is more and more popular in poultry, and quail is smaller than other poultry, so quail can be used as a good new experimental animal (Zhang *et al*., 2013; Bai *et al*., 2016a, 2016b, 2020). The experimental values of quail in teaching and scientific studies are increasing gradually (Bai *et al*., 2017, 2019; Li *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. As a transcriptional regulatory factor, MyoG triggers synthesis of a series of skeletal muscle specific embryonal receptor and contractile proteins. Therefore, MyoG is the only one irreplaceable MyoGenic regulatory factor (Hasty *et al*.,1993). Subsequently, abundant studies concerning the relationship between mutation of MyoG and human diseases have been reported in the whole world (Knapp *et al*., 2006). Recently, there are extensive studies on MyoG in China. However, most of them concentrate in formation mechanism of muscles as well as genetic expression and regulation of MyoG (Biressi *et al*., 2013). Few studies on correlation analysis between MyoG and slaughter performance of meat quail are available, therefore, correlations of MyoG with slaughter performance of meat quail were discussed in the present study, which provided references for marker assisted selection of meat quail.

Materials and methods

Blood samples (5ml each) were collected from vein in the wings of 50 French giant quail and 50 Savimit quail, which were stored in heparin sodium anticoagulant tubes and kept in a refrigerator at -20℃ for DNA extracting. Quails were slaughtered at the age of 5 weeks and the following slaughter performance indices were recorded: weight, carcass weight, whole net carcass, heart weight, liver weight, breast muscle weight, leg muscle weight, dressing percentage, whole net carcass rate, heart rate, liver rate, breast muscle rate and leg muscle rate.

Primers at loci A and B in the control region of *MyoG* 5' were designed according to Wang *et al*. (2007) and were synthesized by Beijing Dingguo Changsheng Biotechnology Co., Ltd (Table I). The total volume of PCR reactionn mixture was 12 μL, including 8.15 μL of ddH_2O, 1.25μL of 10×buffer, 0.75μL of Mg^{2+} (25 mmol/L), 0.5μL of DNA template, 0.5μL (10 mmol/L) of upstream primers, 0.5μL (10 mmol/L) of downstream primers, 0.25μL of dNTPs, and 0.1μL of taq enzyme. The thermal cycle program was set as follows: pre-denaturation at 94℃

* Corresponding author: junyanbai@163.com
0030-9923/2021/0002-0789 $ 9.00/0

for 4 min, then denaturation at 94℃ for 40 s, annealing at 57-60℃ for 1 min, annealing at 72℃ for 20 seconds, denaturation, annealing and elongation were carried out for 35 cycles, then elongation at 72℃ and finally the reaction was completed and cooled and preserved at 4℃.

After SSCP analysis 5μL denatured buffer (98% formamide, 2% glycerin, 10 m medta, 0.025% bromophenol blue, 0.025% xylene) was taken in 0.2 mL centrifuge tube. 5μL PCR products were added and mixed evenly. After denaturation in water bath at 98℃ for 10min, 10μL of mixed liquid was added into the point sample hole with a pipette gun, another sample hole was added with 5μL of DL2000 marker as reference. The electrophoresis tank was covered with upper cover, connected with the power supply to start electrophoresis. The electrophoresis conditions were 220V electrophoresis for 15min, then 90V electrophoresis for 6h. After electrophoresis, silver nitrate staining was carried out and the results were photographed.

Analytical model: $y_{ijkl} = \mu + B_i + S_j + M_k + e_{ijkl}$, 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), S_j is the effect of the j th sex, M_k is the effect of the k th genotype effect, e_{ijkl} is the residual effect.

Results and discussion

Figure 1 shows the amplified bands of loci A and B in the control region of *MyoG* 5'. Figure 2 shows genotype at loci A and B of the control region of *MyoG* 5' of French giant quail and Savimit quail. For French giant quail and Savimit quail, three genotypes (AA, BB and AB) were discovered at locus A. Besides that, three genotypes (AA, BB and AB) were discovered at locus B.

Tang *et al*. (2013) discovered one mutation site at exon 1 and exon 3 of *MyoG* of Jinghai yellow chicken, which involved 3 genotypes. Zhao *et al*. (2016) discovered 1, 2 and 3 mutation sites in exons 1, 2 and 3 of *MyoG* of three ear duck. Wang *et al*. (2007) discovered one mutation site and 3 genotypes at locus A in the control region of *MyoG*5' of broiler chicken and found 3 SNPs loci and 6 genotypes at loci B. 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 in the control region of *MyoG* 5' of two meat quail groups was tested. Three genotypes were discovered at loci A and B, which were AA, AB and BB. This revealed that *MyoG* had rich polymorphism in meat quail groups, which was similar to polymorphism repored from other poultries.

Gene frequency and genotype frequency at loci A and B in the control region of *MyoG* 5' in French giant quail and Savimit quail are listed in Table II. For locus A, BB frequency of French giant quail and Savimit quail is the highest, reaching 0.531 and 0.750. B allele frequency is the highest, which values 0.582 and 0.833, respectively. Genetic polymorphism of French giant quail is high (He=0.487). For locus B, three genotypes (AA, AB and BB) are detected in Savimit quail, but only AA is detected in French giant quail. For Savimit quail, the highest BB frequency is 0.389 and the highest B allele frequency is 0.569. Genetic polymorphism of Savimit quail is high (He= 0.490).

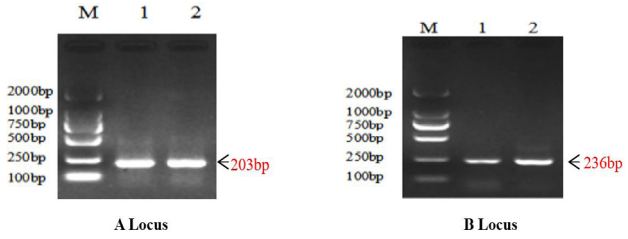

Fig. 1. Agar detection of *MyoG* gene.
Note: M is Marker DL2000. Lane 1 is French giant quail and Lane 2 is Savimit quail.

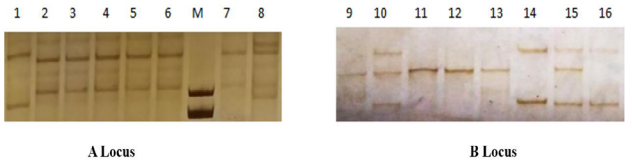

Fig. 2. SSCP results of *MyoG* gene.
Note: M is Marker DL2000; A: Lane1 is AA genotype, Lanes 2, 3, 4, 5, 6, 7 is BB genotype and Lane 8 is AB genotype. B: Lanes 9, 11, 12, 13 are BB genotypes. Lanes 10, 15 are AB genotypes. Lanes14, 16 are AA genotypes.

Correlation analysis between polymorphism of the control region of *MyoG* 5' and slaughter performance of meat quail is shown in Table III. For locus A, liver weight of AA is significantly higher compared with that of AB ($P<0.05$), liver weights of AA and AB are similar with that of BB ($P>0.05$). AA, AB and BB genotypes of A locus in 5'regulatory region of *MyoG* gene here was no significant effect on other slaughter performance ($P>0.05$). For loci B in the control region of *MyoG* 5', weight, carcass weight, carcass net weight, liver weight, breast muscle weight and leg muscle weight of AA and BB are significantly higher than those of AB ($P<0.05$), besides, there's no significant difference between AA and BB in term of weight, carcass weight, carcass net weight, liver weight, breast muscle weight and leg muscle weight ($P>0.05$). Different genotype has no significant influences on other slaughter performances. ($P>0.05$).

In studies on correlation between *MyoG* polymorphism and production performance of poultries,

Table I. Primer sequence of A and B loci of *MyoG*.

Primer	Primer sequence(5'-3')	Annealing temperature (℃)	Fragment size
A locus	F:GGTGGGTGTGGGGAATGTGCT R:CCGGCTTTGCTCTTAACTCT	61.9	203bp
B locus	F:AAACCCACTCCATTGTGC R:CACTACTTGGCTCCTCTAGTT	57.2	236bp

Table II. Polymorphism of myog gene in meat quail.

Polymorphism		A locus		B locus	
		French giant quail	Savimit quail	French giant quail	Savimit quail
Genotype frequency	AA	0.367	0.083	1	0.250
	BB	0.531	0.750	0	0.389
	AB	0.102	0.167	0	0.361
Allele frequency	A	0.418	0.167	1	0.431
	B	0.582	0.833	0	0.569
Heterozygosity	He	0.487	0.278	0	0.490
Number of effective alleles	Na	1.948	1.385	1	1.963
Polymorphism information content	PIC	0.368	0.240	0	0.370

Table III. Association between MyoG gene and slaughter performance of meat quails.

Character	Genotype of locus A			Genotype of locus B		
	AA	AB	BB	AA	AB	BB
Weight (g)	145.238±3.925^{a}	138.200±6.459^{a}	140.960±3.010^{a}	145.053±2.296^{a}	122.646±8.229^{b}	145.257±4.002^{a}
Carcass weight (g)	137.638±3.808^{a}	131.018±6.403^{a}	133.575±2.925^{a}	137.700±2.241^{a}	115.338±7.877^{b}	137.507±3.910^{a}
Whole net carcass (g)	99.152±2.840^{a}	95.482±5.018^{a}	97.955±2.448^{a}	100.536±1.829^{a}	81.869±6.139^{b}	102.050±3.180^{a}
Heart weight (g)	1.100±0.068^{a}	1.055±0.097^{a}	1.119±0.044^{a}	1.152±0.039^{a}	0.892±0.108^{a}	1.114±0.069^{a}
Liver weight (g)	3.871±0.113^{a}	3.427±0.166^{b}	3.598±0.080^{ab}	3.733±0.074^{a}	3.169±0.131^{b}	3.714±0.133^{a}
Breast muscle weight (g)	31.514±1.098^{a}	30.127±1.872^{a}	30.940±0.936^{a}	31.995±0.708^{a}	24.692±2.050^{b}	32.593±1.481^{a}
Leg muscle weight (g)	7.300±0.297^{a}	6.582±0.495^{a}	6.845±0.177^{a}	7.191±0.165^{a}	5.631±0.399^{b}	7.014±0.280^{a}
Dressing percentage (%)	94.738±0.179^{a}	94.712±0.272^{a}	94.694±0.150^{a}	94.896±0.120^{a}	93.940±0.223^{a}	94.640±0.319^{a}
Whole net carcass rate (%)	68.216±0.461^{a}	68.916±0.593^{a}	69.165±0.472^{a}	69.177±0.364^{a}	66.275±0.919^{a}	70.182±0.616^{a}
Heart rate (%)	1.114±0.064^{a}	1.100±0.078^{a}	1.151±0.046^{a}	1.152±0.037^{a}	1.105±0.140^{a}	1.092±0.060^{a}
Liver rate (%)	3.942±0.121^{a}	3.639±0.179^{a}	3.786±0.119^{a}	3.775±0.098^{a}	4.074±0.272^{a}	3.682±0.161^{a}
Breast muscle rate (%)	31.688±0.413^{a}	31.492±0.666^{a}	31.390±0.362^{a}	31.735±0.294^{a}	29.976±0.664^{a}	31.802±0.718^{a}
Leg muscle rate (%)	14.720±0.443^{a}	13.721±0.598^{a}	14.077±0.224^{a}	14.345±0.244^{a}	13.974±0.546^{a}	13.746±0.335^{a}

Note: There are significant differences between the lower-case letters in the table ($P < 0.05$) and no significant differences between the same letters ($P > 0.05$).

Bhuiyan *et al*. (2009) discovered that C1111G mutation in *MyoG* gene of castle is significantly correlated with weight of living body ($P<0.05$). Jiusheng *et al*. (2009) reported that polymorphism of *MyoG* was significantly correlated with cross sectional area of psoas and water-holding capacity of Jinhua×Meishan pigs($P<0.05$). Peng *et al*. (2007) studied influences of *MyoG* on some production traits of filial generation of Hubei white pigs, finding two genotypes (AA and AB). These two genotypes had no significant impacts on production traits, such as meat percentage and fat contents in muscles ($P>0.05$).

For correlation between *MyoG* polymorphism and production traits of poultries, Wang *et al*. (2007) discussed correlation of *MyoG* polymorphism with

slaughter traits and meat quality of broiler chicken, finding significantly positive correlation between *MyoG* and muscle fiber growth of chicken ($P < 0.05$). Zhao *et al*. (2016) concluded that two mutations of *MyoG* could influence breast muscle rate, weight and carcass net weight of duck significantly ($P < 0.05$). Wei *et al*. (2014) analyzed correlation between *MyoG* and slaughter performance of Bian chicken, and recognized two same sense mutation sites on *MyoG*, polymorphism of these two mutation sites is correlated with slaughter performance of Bian chicken ($P < 0.05$), expressions of *MyoG* in breast muscle are far higher than that in leg muscle.This study shows that locus A in the control region of *MyoG* 5' has a significant correlation with liver weight of meat quail ($P<0.05$), locus B is significantly correlated with weight, carcass weight, carcass net weight, liver weight, breast muscle weight and leg muscle weight ($P<0.05$). These conclusions are similar to those of Zhao *et al*. (2016) and Wei *et al*. (2014). To sum up, loci A and B in the control region of *MyoG* 5' can be applied as molecular marker of slaughter performance of meat quail during marker assisted selection.

Acknowledgements

Sincere gratitude goes to the sponsor of National Natural Science Foundation (31201777) and Industry-University-Research Cooperation Project in Henan Province (152107000095.0).

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Bai, J.Y., Pang, Y.Z., Qi, Y.X., Zhang, X.H. and Yun, Y.X., 2017. *Indian J. Anim. Res.*, **51**: 851-855.

Bai, J.Y., Pang, Y.Z., Zhang, X.H., Yun, Y.X. and Qi, Y.X., 2016a. *Brazilian J. Poult. Sci.,* 18: 519-524. https://doi.org/10.1590/1806-9061-2015-0101

Bai, J.Y., Pang, Y.Z., Qi, Y.X., Zhang, X.H. and Yun, X.Y., 2016b. *Brazilian J. Poult. Sci.,* **18**: 027-032. https://doi.org/10.1590/1806-9061-2015-0124

Bai, J.Y., Yang, S., Pang, Y.Z., Wu, X.H. and Li, G.L., 2019. *Pakistan J. Zool*., 51: 1663-1666. https://doi.org/10.17582/journal.pjz/2019.51.5.1663.1666

Bai, J.Y., Cao, H., Yang, S., Pang, Y.Z., Jiang, M.J., Fan, H.D., Fu, X.Y., Zhang, J.Y. and Shi, H.J., 2020. *Indian J. Anim. Res.,* **54**: 392-395.

Bhuiyan, M.S.A., Kim, N.K., Cho, Y.M., Yoon, D., Kim, K.D., Jeon, J.T. and Lee, J.H., 2009. *Livest. Prod. Sci.*, **126**: 292-297. https://doi.org/10.1016/j.livsci.2009.05.019

Biressi, S., Bjornson, C.R.R., Carlig, P.M.M., Nishijo, K., Keller, C. and Rando, T.A., 2013. *Devel. Biol.*, **379**: 195-207. https://doi.org/10.1016/j.ydbio.2013.04.021

Hasty, P., Bradley, A. and Morris, J., 1993. *Nature*, **364**: 501-506. https://doi.org/10.1038/364501a0

Jiusheng, W., Yuehuan, L. and Ningying, X., 2009. *Meat Sci.*, **81**: 108-115. https://doi.org/10.1016/j.meatsci.2008.07.010

Knapp, J., Davie, J., Myer, A., Meadows, E., Olson, E.N. and Klein, W.H., 2006. *Development*, **133**: 601- 610. https://doi.org/10.1242/dev.02249

Li, Y.X., Zhang, X.H., Pang, Y.Z., Qi, Y.X. and Zhao, S.J., 2019. *J. Poult. Sci.*, **56**: 84-90. https://doi.org/10.2141/jpsa.0180058

Peng, X.W., Mei, S.Q., Sun, H., Li, L.H., Song, Z.X., Cheng, N., Huan, S., Guo, W.Z., 2007. *J. Huazhong agric. Univ.*, **26**: 809-812.

Tang, Y., Wang, J.Y., Zhang, G.X., Shi, H.Q. and Zhang, T., 2013. *Chinese J. Anim. Sci.*, **49**: 5-8.

Wang, Q., Liu, Y.P., Jiang, X.S., Yang, C.W., Du, H.R., Qiu, M.H. and Zhu, Q., 2007. *Hereditas.*, **29**: 1089-1096.

Wei, Y., Zhang, G.X., Wang, J.Y., Zhang, T., Tang, Y., Fan, Q.C., Xue, Q., Wang, Y.N., Ding, F.X., 2014. *Chinese J. Anim. Sci.*, **50**: 1-5.

Zhang, X.H., Pang, Y.Z., Zhao, S.J., Xu, H.W., Li, Y.L., Xu, Y., Guo, Z. and Wang, D.D., 2013. *Br. Poult. Sci.,***54**: 306-311.

Zhao, Z.h., Li, H., Yi, H.J., Yang, S.L., Peng, B.X. and Bu, X.Y., 2016. *Scient. Agric. Sin.*, **49**: 3649-3661.