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The Association between POU1F1 Gene Polymorphisms and Growth as well as Carcass Traits of Noi Native Chickens

Nguyen Thi Anh Thu[1], Lam Thai Hung[2], Ly Thi Thu Lan[1*], Nhan Hoai Phong[1], Huynh Tan Loc[3], Luu Huynh Anh[3], Nguyen Trong Ngu[3]

[1]*School of Agriculture and Aquaculture, Tra Vinh University, 126 Nguyen Thien Thanh, Tra Vinh City, Vietnam*
[2]*Science and Technology Department of Tra Vinh Province, 38 Nguyen Thai Hoc, Tra Vinh City, Vietnam*
[3]*College of Agriculture, Can Tho University, Can Tho City, Vietnam*

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ABSTRACT

The study was conducted to detect and analyze the association between single nucleotide polymorphisms (SNP) in the POU1F1 (POU class 1 homeobox 1) gene and growth as well as carcass traits in Noi native chickens. Blood samples were taken at the wings, DNAs were extracted based on the phenol: chloroform technique and genotypes were analyzed by PCR-RFLP method. The frequencies of CC genotypes for three polymorphic sites (POU1F1_*Hha*I SNP, POU1F1_*Eco*RI SNP, POU1F1_*Bsp*HI SNP) were the highest. The corresponding C allele frequencies were higher than those of T alleles. Of three polymorphisms, POU1F1_*Bsp*HI SNP was found to be significantly linked with growth and carcass traits. Chickens bearing TT genotype showed higher body weight at 91 days, carcass weight, breast weight, and thigh weight than those of chickens with CC genotype. This SNP can be a useful marker for the selection of Noi chicken for improvement of growth and carcass traits.

Introduction

Native breeds of chickens are playing a crucial role in the economy in most countries, especially developing and underprivileged countries because of their taste and eggs that are suitable for domestic consumers' demand. Besides, many indigenous poultry breeds have good adaptability to climatic conditions and low nutritional regimes (Tiep, 2011). In the Mekong Delta of Vietnam, Noi chicken is popularly raised and is considered a native breed with dominant characteristics for good meat quality. However, there still exist some deficiencies such as low growth rate and low heterogeneity of breed (Quyen and Son, 2008). Therefore, many solutions including changing husbandry, feeding, and better health protection have been applied in order to improve their performance. Nonetheless, genetic improvement is a method that should be considered due to its long-lasting benefits. Recent achievements of molecular biology techniques such as quantitative trait locus analysis and candidate genes provide better tools for identifying functional genes and breeds to improve economically important traits in animals (Zahra *et al.*, 2011). Therefore, knowledge about functional genes helps the selection of animals with favorable breeding traits and improves growth rates, body weight, and carcass traits (Manjula *et al.*, 2018).

The POU class 1 homeobox 1 is a transcription factor that binds and transactivates promoters of growth hormone, prolactin, and thyroid-stimulating hormone chain encoding genes (Bodner *et al.*, 1988; Ingraham *et al.*, 1988; Steinfelder *et al.*, 1992). Previous studies examined the POU1F1 of pigs and cattle and identified its polymorphisms associated with growth traits (Sun *et al.*, 2002; Xue *et al.*, 2006). Most single nucleotide polymorphisms (SNPs) are reported to be associated with growth-related traits in chickens (Jiang *et al.*, 2004; Nie *et al.*, 2008; Bhattacharya *et al.*, 2012). In the present study, the objective was to detect POU1F1 polymorphisms and evaluate their association with growth and carcass traits of Noi chicken.

Materials and methods

Feed and bird care

A total of 192 Noi chickens of 1-day old were raised with the diet formula (Table 1) according to Hung *et al.* (2020). Feeding diets were a mix of yellow maize, soybean meal, rice bran, synthetic lysine and methionine, dicalcium phosphate,

*Corresponding author: Ly Thi Thu Lan
E-mail address: thulan@tvu.edu.vn

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shell, salt, vitamin premix, and mineral premix. The chemical composition of feedstuffs is presented in Table 2. In the stage of 1-28 days of age, chickens were brooded on rice husks with a density of 50 chickens/m^2 and then were raised on a cage floor with a density of 12 chickens/m^2. Chickens were fed and given fresh water *ad libitum* throughout the experiment. Chickens were vaccinated via eye drops against Newcastle disease on the 5th day and infectious bursa on the 7th and 21st day; via ingestion against Newcastle disease on the 28th day. Chickens were managed separately by numbering their feet.

Body weight and carcass characteristics

Chickens were weighed individually at 1, 35, 63, and 91 days of age at 6 a.m. before feeding to determine body weight and daily weight gain. At the end of the experiment, all chickens were slaughtered (at 91 days) to determine carcass, breast and thigh weight. The corresponding ratios were calculated based on the description of Faria *et al*. (2010).

Markers and primers

According to the research by Manjula *et al*. (2018), three primers of POU1F1_*Hha*I, POU1F1_*Eco*RI, and POU1F1_*Bsp*HI were used to identify mutations g.6758T>C (Exon 5), g.9432T>C (Intron 5), and g.11041T>C (Exon 6) on the chicken population in the experiment. The detailed information about primers is shown in Table 3.

Extraction of feather DNA

Chicken DNAs were isolated by phenol/chloroform method (Bello *et al*, 2001). Breast muscles were chopped into small pieces and mixed with lysis buffer for incubation overnight at 37°C. In the next step, 300 µL phenol: chloroform: isoamylalcohol (25:24:1) was added into the sample, mixed and centrifuged at 10.000 rpm for 5 min. The supernatant was then transferred into a 2 mL tube and added 700 µL phenol: chloroform, swirled and centrifuged at 10.000 rpm for 5 min. The supernatant was recovered in a new clean tube with 700 µL chloroform, swirled and centrifuged at 10.000 rpm for 5 min. The upper phase was transferred into a new clean tube containing 300 µL 1.2M NaCl; 150 µL 2M sodium acetate and 1000 µL cold ethanol (100%) and mixed gently by the hand and centrifuged at 10.000 rpm for 5 min to collect DNA pellet. A total of 1.000 µL ethanol 75% was used for DNA washing, air-

Table 1. Experimental diets of Noi chickens

Feedstuffs	Diets		
	1-28 day	29-56 day	57-84 day
Yellow maize (%)	56.33	56.13	53.6
Soybean meal (%)	29.28	23.4	16.96
Rice bran (%)	9.53	15.7	25.41
Lysine (%)	0.62	0.54	0.45
Methionine* (%)	0.19	0.18	0.17
DCP (%)	1.6	1.6	1
Shell (kg)	1.8	1.8	1.76
Premix** (%)	0.25	0.25	0.25
Salt (%)	0.4	0.4	0.4
Nutritional value of diets			
ME (kcal/kg of feed)	2,900	2.9	2,900
CP (%)	19	17	15
Lysine (%)	1.1	1	0.9
Methionine (%)	0.79	0.72	0.64
Threonine (%)	0.86	0.81	0.78
Tryptophan (%)	0.35	0.31	0.25
Calcium (%)	1.18	1.18	1.03
Phosphorus (%)	0.84	0.89	0.8

DCP: dicalcium phosphate; *: ingredient source from methionine calculated both methionine and cysteine requirement; **: premix including vitamin and micro-minerals; ME: metabolism energy; CP: crude protein

Table 2. Chemical composition of feedstuffs

Feedstuffs	Chemical composition (% feed)							
	DM	ME, kcal/kg	CP	Lys	Met+cys	Thr	Ca	P
Yellow maize	85.81	3,335	6.94	0.25	0.369	0.234	0.21	0.31
Soybean meal	87.94	2,631	44.87	0.949	1.277	2.124	0.4	0.69
Rice bran	87.86	2,624	12.01	0.61	0.22	1.17	0.375	1.58

DM: dry matter, ME: metabolism energy, CP: crude protein, Lys: lysine, Met+cys: methionine + cysteine; Thr: threonine; Ca: calcium; and P: phosphorus

Table 3. PCR-RFLP primers and restriction enzymes used to identify POU1F1 gene polymorphisms

Markers	Location(1)	Region	Primer (Forward/Reverse) (5'-3')	Restriction enzyme	Amplicon (bp)
POU1F1_*Hha*I	g.6758T>C	Exon 5	AGTATAGCTCTGTGGTGCAC TATGCCCTCAGATGTCCCAG	*Hha*I	626
POU1F1_*Eco*RI	g.9432T>C	Intron 5	GGGGATTTTGCCACTTTAGGG TGGGTAAGGCTCTGGCACTGT	*Eco*RI	442
POU1F1_*Bsp*HI	g.11041T>C	Exon 6	GGGGTACCACTCAACTTCAG TAGGGTACCTGCAATGGGGG	*Bsp*HI	750

dried, and stored in 1x TE buffer solution at pH 8.0.

Genotyping by PCR-RFLP procedure

Three polymorphisms of chicken POU1F1 gene (POU1F1_*Hha*I, POU1F1_*Eco*RI, POU1F1_*Bsp*HI) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Table 3). Genotyping of SNPs in POU1F1 was conducted by the method of Manjula *et al*. (2018).

The PCR was performed with a volume of 10 µL with 50 ng of genomic DNA, 0.25M each primer, 0.25M each dNTP, 1X PCR buffer, and 1U Taq DNA polymerase. The thermal cycles were as follows: 94°C for 10 min; 35 cycles of 94°C for 30 s, Ta each primer ranged from 60°C to 67°C within 30 s, and 72°C within 30 s; and 72°C within 10 min for the final extension. PCR products were further digested at 37°C overnight with *Hha*I, *Eco*RI, *Bsp*HI restriction enzymes (Table 3). Genotypes of polymorphisms were determined under UV light after electrophoresis on 2.0% agarose gel at 80 V, 400 mA within 30 min.

Statistical analysis

Genotype frequencies of the candidate genes were analyzed by using Microsoft Excel and the method of Rodriguez *et al*. (2009) was applied to estimate the Hardy-Weinberg Equilibrium (HWE). The association between genotypes to production of chicken was analyzed on the GLM program of the Minitab 16 software.

Results

Table 4 showed the genotypes, allele frequencies for three markers (POU1F1_*Hha*I SNP, POU1F1_*Eco*RI SNP, POU1F1U_*Bsp*HI SNP) and Chi-Square tests for Hardy-Weinberg equilibrium. In POU1F1_*Hha*I SNP, the CC genotype was the most frequent (0.89), and the CT genotype was the least frequent (0.11); the TT genotype did not appear. The CC, CT, and TT genotype frequencies of POU1F1_*Eco*RI SNP were 0.51, 0.38, and 0.11, respectively. In POU_*Bsp*HI SNP, the frequency of the TT genotype was 0.17 while the frequency of the CC and CT genotypes were 0.40 and 0.43, respectively. All SNPs followed the Hardy-Weinberg equilibrium (P>0.05).

Association of SNPs with growth traits

Table 5 indicated that genotypes of POU1F1_*Hha*I SNP and POU1F1_*Eco*RI SNP were not importantly associated with any of the growth traits tested in Noi chickens. However, POU1F1_*Bsp*HI SNP was significantly associated with body weight at 91 days old (BW91) and weight gain from 0 to 13 weeks of age (GR1-91) (P<0.05). In POU1F1_*Bsp*HI SNP, the highest BW91 was found in the TT genotype (1,040 g), while the lowest was observed in the CC type (946.0 g). Similarly, for weight gain, GR1-91, the TT animals had a higher weight gain than the CC animals, while the CT type showed moderate weight gain.

Association of SNPs with carcass characteristics

The results in Table 6 showed that POU1F1_*Hha*I SNP and POU1F1_*Eco*RI SNP were not associated with traits of the carcass, breast and thigh weight; and carcass, breast, and thigh ratio. However, POU1F1_*Bsp*HI SNP was clearly associated with the traits of the carcass, breast and thigh weight. In POU1F1_*Bsp*HI SNP, chickens with the TT, CT, and CC genotypes were found to obtain the average carcass weight (706.9 g), (655.0 g) and (635.3 g), respectively; the average breast weight (88.95 g), (84.10 g) and 82.32 g, respectively; the average thigh weight (159.0 g), (147.7 g) and (143.4 g), respectively.

Discussion

The present study was undertaken to identify polymorphisms in the POU1F1 gene that were related to growth, live weight gain, and carcass traits in Noi chicken. The results showed that the CC genotype was more frequently observed than other genotypes in Noi chickens, and the C allele frequency was higher than that of the T allele in all SNPs. Besides, all SNPs were in Hardy-Weinberg equilibrium in the native Noi chicken population. This result was similar to the study of Manjula *et al*. (2018) in a survey on Korean native chickens. The authors also found that the frequency of the CC genotype (ranging from 0.844 to 0.868) as well as the C allele in all three surveyed polymorphisms was higher than that of the TT genotype and T allele in the population. Similarly, the current research results are also supported by Nie *et al*. (2008) that the number of individuals with the CC genotype (335/451 chickens) was higher than that of the TT genotype (11/451 chickens) when surveying of the POU1F1_*Eco*RI SNP polymorphism.

POU1F1 gene is also known as PIT1 that is responsible for regulating a variety of important biological activities, so it is considered a potential candidate gene for the productivity trait. Many studies have shown that the PIT1 gene is related to carcass and growth traits in pigs (Song *et al*., 2002 and Franco *et al*., 2005) and in cows (Zhao *et al*., 2004; Xue *et al*., 2006). In chickens, a total of 23 SNPs and one 57 bp indel were identified on the mysterious 2,400 bp gene of the PIT1 gene, but the genetic effects on the chicken production traits were not clear (Nie *et al*., 2005). It has been proved that a non-synonymous SNP at POU domain (A → T, Asn229Ile) has a significant link to body weight at 8 weeks of age (Jiang *et al*., 2004). In addition, Nie *et al*. (2008) also found 5 polymorphisms (MR1-MR5) on the PIT1 gene that were significantly associated with shank diameters, hatch weight, shank length, body weight, and average daily gain. Research by Jin *et al*. (2018) also found the association between PIT1 gene and body weight, feed intake as well as FCR of chickens, in which TT genotype of rs13687128 polymorphism and AA genotype in rs13905622 polymorphism are potential markers. In the polymorphism POU1F1_*Bsp*HI, Manjula *et al*. (2018) found that the CC genotype is more dominant than CT and TT genotypes in Korean native chickens in terms of body weight and weight gain. In the current study, three mutations of POU1F1_*Hha*I, POU1F1_*Eco*RI, and POU1F1_*Bsp*HI were surveyed on 425 Noi

Table 4. Genotype and gene frequency of POU1F1 SNP polymorphisms in Noi chicken

Polymorphic sites	Genotype frequency			Allele frequency		p-value (Hardy-Weinberg)
	CC	CT	TT	C	T	
POU1F1_*Hha*I SNP	0.89	0.11	0	0.94	0.06	0.509
POU1F1_*Eco*RI SNP	0.51	0.38	0.11	0.7	0.3	0.131
POU1F1_*Bsp*HI SNP	0.4	0.43	0.17	0.61	0.39	0.34

Table 5. Association of POU1F1 polymorphisms and growth traits

Traits[(1)]	Genotype of POU1F1_*Hha*I SNP			p-value
	CC (112)	CT (14)	TT (0)	
BW01	31.9±0.27	31.2±0.77	-	0.346
BW35	182.6±2.47	173.6±6.99	-	0.223
BW63	548.2±8.55	545.1±24.2	-	0.9
BW91	960.7±12.9	1,002±36.4	-	0.276
GR1-35	4.43±0.07	4.19±0.21	-	0.27
GR36-63	13.5±0.28	13.8±0.80	-	0.793
GR64-91	15.3±0.30	16.9±0.85	-	0.064
GR1-91	10.4±0.14	10.9±0.91	-	0.267
	Genotype of POU1F1_*Eco*RI SNP			
	CC (92)	CT (68)	TT (21)	
BW01	31.6±0.30	32.3±0.35	31.6±0.64	0.319
BW35	182.6±2.85	178.1±3.30	182.7±6.08	0.559
BW63	559.4±9.79	551.4±11.3	515.9±20.9	0.173
BW91	972.0±14.3	971.3±16.5	949.2±30.5	0.785
GR1-35	4.44±0.08	4.29±0.10	4.45±0.18	0.471
GR36-63	13.9±0.31	13.8±0.36	12.3±0.67	0.088
GR64-91	15.3 ±0.34	15.6±0.39	16.1±0.72	0.61
GR1-91	10.6±0.16	10.6±0.19	10.3±0.34	0.791
	Genotype of POU1F1_*Bsp*HI SNP			
	CC (47)	CT (51)	TT (20)	
BW35	184.4±3.67	179.1±3.52	189.7±5.62	0.256
BW63	543.8±12.1	552.5±11.6	583.0±18.6	0.21
BW91	946.0±17.8^{b}	968.9±17.1^{ab}	1,040±27.4^{a}	0.018
GR1-35	4.43±0.11	4.31±0.10	4.66±0.16	0.208
GR36-63	13.2±0.38	13.8±0.37	14.4±0.57	0.223
GR64-91	14.7±0.46^{b}	15.4±0.45^{ab}	16.8±0.70^{a}	0.055
GR1-91	10.2±0.20^{b}	10.5±0.19^{ab}	11.2±0.30^{a}	0.015

([1]) BW01, body weight at one-day-old; BW35, body weight at 35 days old; BW63, body weight at 63 days old; BW91, body weight at 91 days old; GR1-35, weight gain from 1 to 35 days old; GR36-63, weight gain from 36 to 63 days old; GR64-91, weight gain from 64 to 91 days old; GR1-91, weight gain from 1 to 91 days old.

a-b Least square mean within a row with different superscript differ significantly (P<0.05).

Table 6. Association of POU1F1 polymorphisms and carcass characteristics

Traits	Genotype of POU1F1_*Hha*I SNP			p-value
	CC	CT	TT	
Carcass weight, g	646.5±9.54	680.4±26.9	-	0.238
Breast weight, g	83.3±0.91	87.0±2.58	-	0.18
Thigh weight, g	146.6±1.21	149.5±3.41	-	0.423
Carcass ratio, %	67.2±0.24	67.7±0.68	-	0.553
Breast ratio, %	13.0±0.12	13.0±0.33	-	0.99
Thigh ratio, %	22.9±0.20	22.5±0.55	-	0.421
	Genotype of POU_*Eco*RI SNP			
	CC	CT	TT	
Carcass weight, g	657.1±10.7	656.9±12.4	627.9±22.8	0.486
Breast weight, g	84.0±1.06	84.2±1.23	84.2±2.26	0.991
Thigh weight, g	147.7±1.36	148.4±1.57	141.7±2.90	0.121
Carcass ratio, %	67.5±0.27	67.5±0.31	66.2±0.57	0.112
Breast ratio, %	12.9±0.12	12.9±0.14	13.5±0.26	0.136
Thigh ratio, %	22.7±0.21	22.9±0.24	22.7±0.44	0.807
	Genotype of POU_*Bsp*HI SNP			
	CC	CT	TT	
Carcass weight, g	635.3±13.2^{b}	655.0±12.7^{ab}	706.9±0.27^{a}	0.015
Breast weight, g	82.3±1.34^{b}	84.1±1.29^{ab}	88.9±2.06^{a}	0.029
Thigh weight, g	143.4±1.92^{b}	147.7±1.84^{b}	159.0±2.94^{a}	0.001
Carcass ratio, %	67.1±0.31	67.5±0.30	67.9±0.49	0.358
Breast ratio, %	12.9±0.14	12.9± 0.13	12.8±0.21	0.833
Thigh ratio, %	22.8±0.24	22.8±0.23	22.7 ±0.37	0.966

chickens. The results showed that POU1F1_*Bsp*HI polymorphism is significantly associated with BW91, GR1-91, carcass weight, breast weight, and thigh weight, where the TT genotype showed association with the highest value from 0 to 13 weeks of age. In chickens, this gene is located on chromosome 1 and plays an important role in controlling growth performance. Polymorphism in this gene can control the expression of the aforementioned hormone. Therefore, it can affect the metabolic activity and skeletal muscle development of chickens. Based on the results in Tables 5 and 6, it can be seen that the TT genotype is of potential in chicken development concerning the increase in carcass yield and quality.

For the two polymorphisms POU1F1_*Hha*I and POU1F1_*Eco*RI, the current study could not find any association with growth traits. Similarly, at the POU1F1_*Hha*I locus, Manjula *et al*. (2018) also found no association of this polymorphism with chicken productivity traits. However, for POU1F1_*Eco*RI polymorphism, Nie *et al*. (2008) found its association with shank length at 63, 77, 84 days and BW at 84 days, as well as shank diameters at 77 days, where T allele was shown to be potential for chicken growth. Furthermore, in this polymorphism, Manjula *et al*. (2018) found a significant association with body weight and weight gain in Korean native chickens, where the CC genotype was of potential for chicken selection with high productivity.

Conclusion

This study indicated that POU1F1_*Bsp*HI SNP affects the growth of native Noi chickens. The POU1F1_*Bsp*HI SNP is significantly associated with body weight at 91 days old, weight gain from 0 to 13 weeks of age, and carcass characteristics (carcass, breast, and thigh weight). This SNP can be used for improving growth and carcass traits of Noi chickens and is of interest for testing in other native chicken populations.

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Conflict of interest

Authors declare no conflict of interest.

References

Bello, N., Francino, O., Sánchez, A., 2001. Isolation of genomic DNA from feathers. J. Vet. Diagn. Invest. 13, 162-164.

Bhattacharya, T.K., Chatterjee, R.N., Priyanka, M., 2012. Polymorphisms of Pit-1 gene and its association with growth traits in chicken. Poult. Sci. 91, 1057-1064.

Bodner, M., Castrillo, J.L., Theill, L.E., Deerinck, T., Ellisman, M., Karin, M., 1988. The pituitary-specific transcription factor GHF-1 is a homeobox- containing protein. Cell 55, 505-518.

Faria, P.B., Bressan, M.C., Souza, X.R., Rossato, L.V., Botega, L.M.G., Gama, L.T., 2010. Carcass and parts yield of broilers reared under a semi-extensive system. Rev. Bras. Cienc. Avic. 12, 153-159.

Franco, M.M., Antunes, R.C., Silva, H.D., Goulart, L.R., 2005. Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs. J. Appl. Genet. 46, 195-200.

Hung, L.T., Lan, L.T.T., Phong, N.H., Nhan, N.T.H., Ngu, N.T., 2020. Effects of lysine supplementation on growth of Noi broilers. Livest. Res. Rura. Dev. 32.

Ingraham, H.A., Chen, R.P., Mangalam, H.J., Elsholtz, H.P., Flynn, S.E., Lin, C.R., Simmons, D.M., Swanson, L., Rosenfeld, M.G., 1988. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 55, 519-529.

Jiang, R., Li, J., Qu, L., Li, H., Yang, N., 2004. A new single-nucleotide polymorphism in the chicken pituitary-specific transcription factor (POU1F1) gene associated with growth rate. Anim. Genet. 35, 344-346.

Jin, S., He, T., Yang, L., Tong, Y., Chen, X., Geng, Z. 2018. Association of polymorphisms in Pit-1 gene with growth and feed efficiency in meat-type chickens. Asian-Australasian J. Anim. Sci. 31, 1685-1690.

Manjula, P., Choi, N., Seo, D., Lee, J.H., 2018. POU class 1 homeobox 1 gene polymorphisms associated with growth traits in Korean native chicken. Asian-Australasian J. Anim. Sci. 31, 643-649.

Nie, Q.H., Lei, M., Ouyang, J., Zeng, H., Yang, G., Zhang, X., 2005. Identification and characterization of single nucleotide polymorphisms in 12 chicken growth-correlated genes by denaturing high performance liquid chromatography. Genet. Sel. Evol. 37, 339-360.

Nie, Q.H., Fang, M.X., Xie, L., Zhou, M., Liang, Z.M., Luo, Z.P., Wang, G.H., Bi, W.S., Liang, C.J., Zhang, W., Zhang X.Q., 2008. The PIT-1gene polymorphisms were associated with chicken growth traits. BMC Genet. 9, 20-24.

Quyen, N.V., Son, V.V., 2008. Reproductive performance of Noi chickens in the Mekong Delta. Agr. Rural. Dev. J. 3, 46-48.

Rodriguez, S., Gaunt, T.R., Day, I.N.M., 2009. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. American. J. Epide. 169, 505-514.

Song, C., Gao, B., Teng, Y., Wang, X., Wang, Z., Li, Q., Mi, H, Jing, R., Mao, J., 2005. MspI polymorphisms in the 3rd intron of the swine POU1F1 gene and their association with growth performance. J. Appl. Genet. 46, 285-289.

Steinfelder, H.J., Radovick, S., Wondisford, F.E., 1992. Hormonal regulation of the thyrotropin beta-subunit gene by phosphorylation of the pituitary-specific transcription factor Pit-1. Proc. Natl. Acad. Sci. U.S.A. 89, 5942-5945.

Sun, H.S., Anderson, L.L., Yu, T.P., Kima, K.S., Klindt, J., Tugglea, C.K., 2002. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL con centration. Anim. Reprod. Sci. 69, 223-237.

Tiep, N.B., 2011. Anti-disease genes in livestock and potential contributions from local Vietnamese breeds. J. Sci. Dev., Hanoi Uni. Agri. 9, 798-806.

Xue, K., Chen, H., Wang, S., Cai, X., Liu, B., Zhang, C.F., Lei, C.Z., Wang, X.Z., Wang, Y.M., Niu, H., 2006. Effect of genetic variations of the POU1F1 gene on growth traits of Nanyang cattle. Yi Chuan Xue Bao 33, 901-907.

Zahra, R., Masoud, A., Hamid, R.S., Cyrus, A., 2011. Identification of a single nucleotide polymorphism of the pituitary-specific trans criptional factor 1 (Pit 1) gene and its association with body composition trait in Iranian commercial broiler line. African J. Biotech. 60, 12979-12983.

Zhao, Q., Davis, M.E., Hines, H.C., 2004. Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle. J. Anim. Sci. 82, 2229-2233.