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Research article

SINGLE NUCLEOTIDE POLYMORPHISMS IN CANDIDATE GENES ASSOCIATED WITH EGG PRODUCTION TRAITS IN NATIVE NOI CHICKEN OF VIETNAM

Chau Thanh Vu and Nguyen Trong Ngu*

College of Agriculture and Applied Biology Can Tho University, Can Tho, Vietnam

ABSTRACT: The aim of this study was to identify single nucleotide polymorphisms (SNPs) in candidate genes known to associate with egg production trait in chicken. In total, 218 hens of native Noi chicken were used for genotyping 14 SNPs of 9 genes namely PRL, GH, VIP, VIPR1, BMPR-IB, MTNR1C, NPY, DRD2 and IGF-I. It was shown that, in Noi chicken population the substitutions of alleles at different loci including C2402T, C2161G, C2983T, G51389822T, A287G, G294A, C31394761T, T5841629C, C1715301T, C1704887T, C338T, D2648 to 2650I and C364T (5'-UTR) were detected with high frequencies of alleles T and G (PRL), A (GH), T and I (VIP), C (VIPR1), G (BMPR-IB and MTNR1C), D and C (NPY), C (DRD2) and B (IGF-I). It is expected that selection based on these polymorphic loci is more effective in increasing reproductive performance of Noi chickens.
Keywords: Noi chicken, Candidate genes, SNPs, PCR-RFLP.

*Corresponding author: Nguyen Trong Ngu., College of Agriculture and Applied Biology, Can Tho University, Can Tho, Vietnam E-mail: ntngu@ctu.edu.vn

INTRODUCTION

Noi chicken is a native breed in Vietnam with dominant characteristics of good meat quality and suitable with consumers' demand, however there exists some deficiencies such as low egg yield (40-50 eggs/hen/year) and low heterogeneity of breed [1]. These characteristics could be improved with conventional breeding methods, but often in a much longer time frame. Recently, approaches of quantitative trait loci mapping and candidate gene analysis have become popular, of which the first technique is to discover genomic regions related to quantitative traits and the other one focuses on detection of mutations in candidate genes and their possible association with economical production traits [2] and thereby exploits information from previous cellular, biochemical or physiological functional studies to target a gene of interest.

In chicken, NPY (Neuropeptide Y) gene controls Gonadotropin-releasing secretion, a hormone that stimulates ovaries activity [3], manipulates food absorption and promotes reproductive activity and the age of sexual maturation [4]. It is thus one of the genes regulating reproductive function in chicken [5]. Besides, the research of Xu *et al*. [6] in Ningdu Sanhuang chicken indicated that PRL (Prolactin), VIP (Vasoactive Intestinal Peptide), VIPR-1 (Vasoactive Intestinal Peptide Receptor 1) and DRD2 (Dopamine Receptor D2) genes were associated with age at first egg and egg number at 300 days [7]. The chicken prolactin hormone, produced in the lactotropes of the pituitary gland, has an important effect on reproduction ability, commonly by regression of the ovary [8] and loss of egg yield [9].

It was additional shown that VIP, a PRL-releasing factor in birds, regulates PRL gene expression [10], stimulates PRL secretion [11] via dopamine D2 receptor [12] and thus affecting egg productivity. Moreover, GH (Growth Hormone) gene was pointed out to impact on physiological functions such as growth and reproduction [13]. In addition, BMPR-IB (Bone Morphogenetic Protein Receptor Type IB) and MTNR1C (Melatonin Receptor 1C) genes were also reported to link with egg reproduction, in which BMPR-IB were involved in ovarian physiology and increased ovulation rate [14] whereas the other (MTNR1C) was found to regulate ovarian physiology and reproduction [15]. Depending on each breed, the presence of alleles in these genes will change and thus it is important to understand how it occurs in the population of interest. The objective of this study was to detect SNPs and investigate their frequencies in native Noi chicken of Vietnam.

MATERIALS AND METHODS

DNA extraction

In the present study, 218 Noi laying hens were used for genotyping. Genomic DNA was isolated from feathers by phenol/chloroform extraction. In detail, chicken feathers were taken, chopped into small pieces and mixed with lysis buffer for incubation overnight at 37°C. In the next step, 300 µl phenol: chloroform: isoamyalcohol (25:24:1) were added into the sample, mixed and centrifuged at 10.000 rpm for 5 minutes. 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 minutes. The supernatant was recovered in a new clean tube with 700 µl chloroform, swirled and centrifuged at 10.000 rpm for 5 minutes. 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%). Mixed gently by hand and centrifuged at 10.000 rpm for 5 minutes to collect DNA pellet. DNA was then washed by adding 1000 µl ethanol 75%, air-dried and stored in 1x TE buffer (pH 8.0). The sample was subjected for OD measurement and diluted into 50 ng/µl for further use.

Establishment of PCR-RFLP assay

Based on the primer sequences that have been previously published, primer pairs were prepared to amplify PRL, GH, VIP, VIPR1, BMPR-IB, MTNR1C, NPY, DRD2, IGF-I genes as shown in Table 1. PCR reactions were performed in a final volume of 10 µL containing 25 ng of chicken genomic DNA, 0.25 M each primer, 0.25 M each dNTP, 1x PCR buffer, and 1U *Taq* DNA polymerase. PCR products were digested with restriction enzymes overnight at 37°C for all enzymes with the exception of *Bse*GI (at 55°C) and *Taq*I (at 65°C). The restriction fragments were separated on 3% agarose gel stained with ethidium bromide.

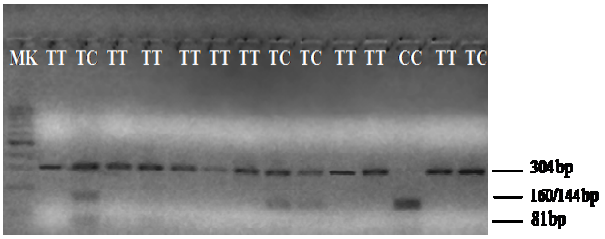
Genotype analysis

Genotype frequencies of the candidate genes were analyzed by excel and the Hardy-Weinberg Equilibrium (HWE) was estimated using the method of Rodriguez *et al*. (2009) [16].

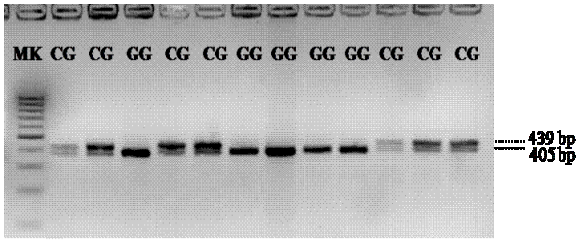
RESULTS

Figure 1 shows the electrophoresis results of PCR products that were digested with restriction enzymes. Bands in gels represented for distinguished genotypes in each SNP observed.

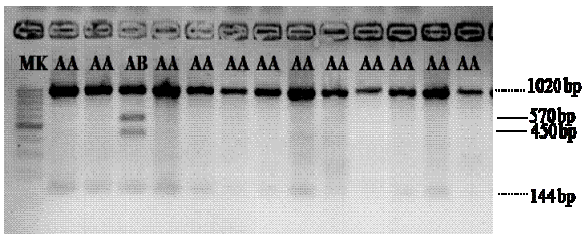
The detection of allelic and genotypic frequency plays an important role in animal breed selection. Based on these frequencies, assumption and their association with phenotype traits would support to create new individuals with desirable genotype and phenotype. The frequencies of PRL, GH, VIP, VIPR1, BMPR-IB, MTNR1C, NPY, DRD2, IGF-I alleles and genotypes are shown in Table 2.



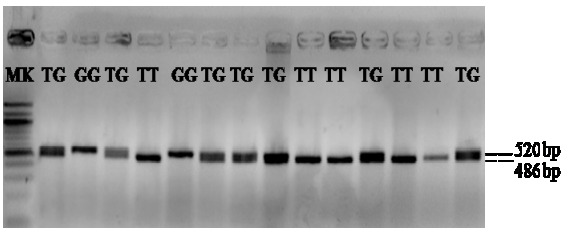
PRL/*Alu*I, C2402T
TT: 304 bp; TC: 304/160/144/81/54 bp
CC: 160/144/81/54 bp



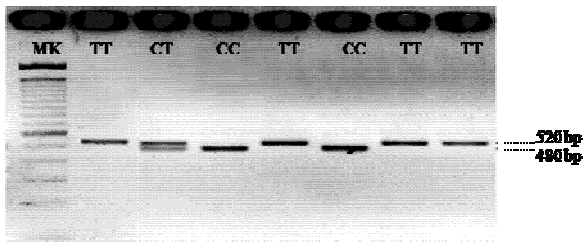
PRL/*Csp*6I, C2161G
CC: 439 bp; CG: 439/405/34 bp; GG: 405/34 bp



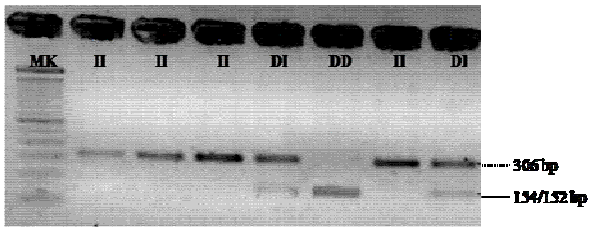
GH/*Sac*I, A2983B
AA: 1020 bp; AB: 1020/570/450/144 bp



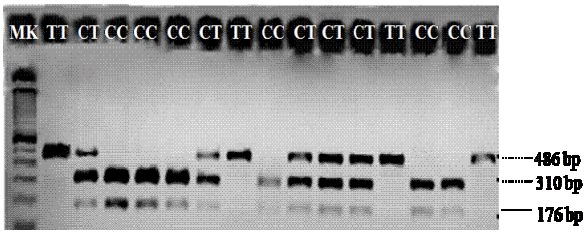
VIP/*Apo*I, G51389822T
GG: 520 bp; TG: 520/486 bp; TT: 486 bp



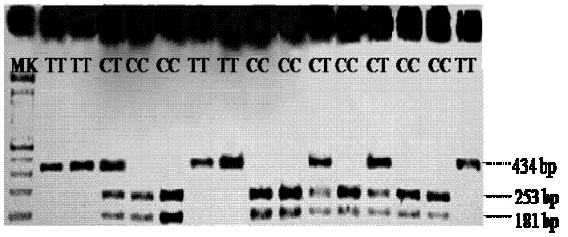
VIP/*Hinf*I, C338T
TT: 520 bp; CT: 520/480 bp; CC: 480 bp



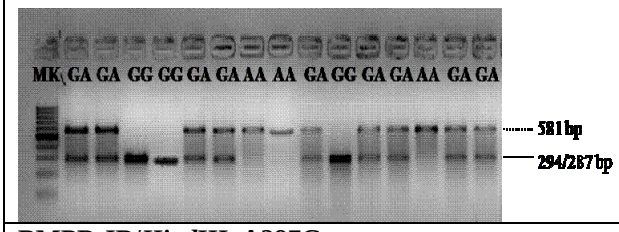
VIP/*Vsp*I, D2648 to 2650I
II: 306 bp; DI: 306/154/152 bp; DD: 154/152 bp



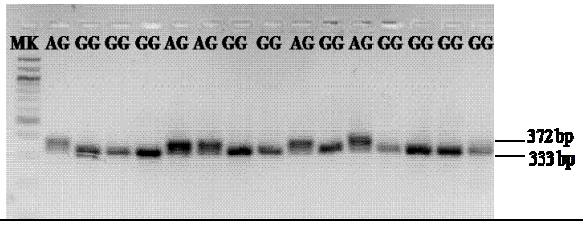
VIPR1/*Taq*I, C1715301T
TT: 486 bp; CT: 486/310/176 bp ; CC: 310/176 bp



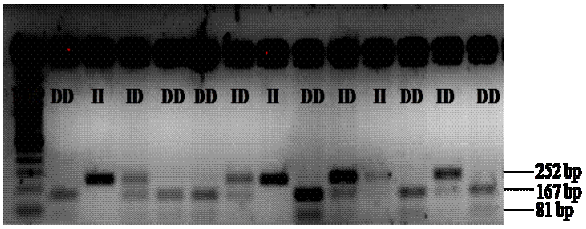
VIPR1/*Hah*I, C1704887T
TT: 434 bp; CT: 434/253/181 bp; CC: 253/181 bp



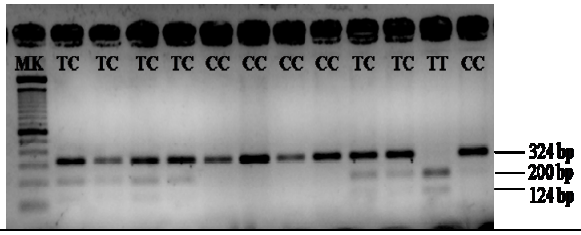
BMPR-IB/*Hind*III, A287G
GG: 581 bp; GA: 581/294/287 bp; AA: 294/287 bp



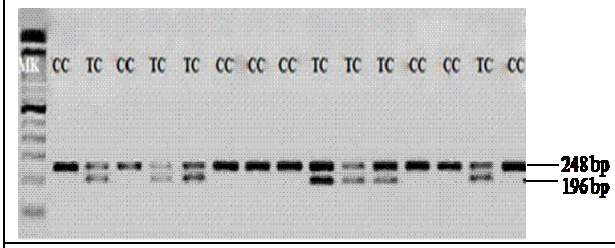
MTNR1C/*Mbo*I, G294A
AA: 372 bp; AG: 372/333 bp; GG: 333 bp



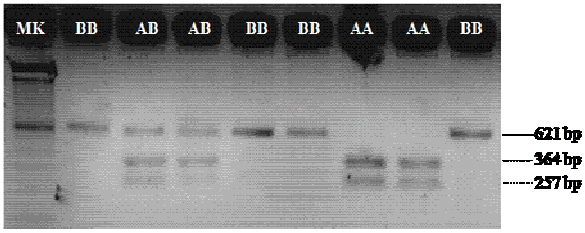
NPY/*Dra*I, I31391359D
II: 252 bp; ID: 252/167/81 bp; DD: 167/81 bp



NPY/*Kpn*I, C31394761T
CC: 324 bp; TC: 324/200/124 bp ; TT: 200/124 bp



DRD2/*Bse*GI, T5841629C
CC: 248 bp; TC: 248/196 bp; TT: 196 bp



IGF-I/*Pst*I, C364T (5'-UTR)
BB: 621 bp; AB: 621/364/257 bp; AA: 364/257 bp

MK: 100-bp DNA ladder, Fermentas

Figure 1: Agarose gel electrophoresis of PCR-RFLP profiles of candidate genes.

Table 1: Primers used for the SNP identification in Noi chicken.

Gene	Sequence (5'-3')	GenBank	Length (bp)	T(a)* (°C)	Restriction enzyme	Reference
PRL	F: AGAGGCAGCCCAGGCATTTTAC R: CCTGGGTCTGGTTTGGAAATTG	AB011438.2	439	58	*Alu*I	[18]
	F:AGAGGCAGCCCAGGCATTTTAC R:CCTGGGTCTGGTTTGGAAATTG	AB011438.2	439	58	*Csp6*I	[18]
GH	F: CTAAAGGACCTGGAAGAAGGG R: AACTTGTCGTAGGTGGGTCTG	NC_006114.3	1164	62	*Sac*I	[19]
VIP	F: GCTTGGACTGATGCGTACTT R: GTATCACTGCAAATGCTCTGC	NC_006090.3	520	58	*Apo*I	[6]
	F:GCTTGGACTGATGCGTACTT R:GTATCACTGCAAATGCTCTG	NC_006090.3	520	55	*Hinf*I	[19]
	F:GAAACCCATCTCAGTCATCCTA R:ACCACCTATTTTTCCTTTTCTAC	NC_006090.3	306	58	*Vsp*I	[19]
VIPR1	F:CTCCTCAGGCAGACCATCATG R:CTTGCACGTATCCTTGGGTAGC	NC_006089	486	61	*Taq*I	[6,7]
	F:CCCCGTTAAACTCAGCAGAC R:CCCAAAGTCCCACAAGGTAA	NC_006089	434	61	*Hha*I	[6,7]
BMPR-IB	F: CCATAGCAAAACAGATTCAG R: TCAGGA CAGTTTGGTAGATT	EF530593	581	52	*Hind* III	[14]
MTRN1C	F: GGTGTATCCGTATCCTCTAA R: GACAGTGGGACAATGAAGT	JQ249896	372	49	*Mbo*I	[15]
NPY	F: TCTCAGAGCTCCAACGTATGA R:ATATTTCTGTGCCTGAACAACA	M87298	252	56	*Dra*I	[6]
	F:CGTGGCTGCTTTGCTTCCTTTC R:GGGGTACGAGGCAAGGACATG	GI:357973829	324	58	*Kpn*I	[6]
DRD2	F:TGCACATAAAAGCCCACTCACTG R:GCCTGAGCTGGTGGGGGG	NC_006111	248	60	*Bse*GI	[6,7]
IGF-I	F: GACTATACAGAAAGAACCCAC R :TATCACTCAAGTGGCTCAAGT		621	56	*Pst*I	[27]

F: Forward primer; R: Reverse primer; T (a): Annealing temperature

Table 2: Allele and genotype frequencies of genes in Noi chicken.

Gene/locus	Observed population			Allele		Expected population			HWE
	Genotype					Genotype			
PRL/*Alu*I	**TT**	**CT**	**CC**	**T**	**C**	**TT**	**CT**	**CC**	
(n=218)	0.69	0.28	0.03	0.83	0.17	0.69	0.28	0.03	NS
PRL/*Csp*6I	**CC**	**CG**	**GG**	**G**	**C**	**CC**	**CG**	**GG**	
(n=211)	0.10	0.45	0.45	0.67	0.33	0.11	0.44	0.45	NS
GH/*Sac*I	**AA**	**AB**	**BB**	**A**	**B**	**AA**	**AB**	**BB**	
(n=214)	0.98	0.02	0.00	0.99	0.01	0.98	0.02	0.00	NS
VIP/*Apo*I	**TT**	**GT**	**GG**	**T**	**G**	**TT**	**GT**	**GG**	
(n=204)	0.50	0.38	0.11	0.56	0.44	0.49	0.19	0.32	<0.001
VIP/*Hinf*I	**CC**	**CT**	**TT**	**C**	**T**	**CC**	**CT**	**TT**	
(n=107)	0.12	0.31	0.57	0.28	0.72	0.08	0.40	0.52	<0.05
VIP/*Vsp*I	**II**	**DI**	**DD**	**I**	**D**	**II**	**DI**	**DD**	
(n=116)	0.59	0.41	0.00	0.8	0.2	0.64	0.32	0.04	<0.01
VIPR1/*Taq*I	**TT**	**CT**	**CC**	**T**	**C**	**TT**	**CT**	**CC**	
(n=111)	0.19	0.33	0.48	0.36	0.64	0.13	0.46	0.41	<0.001
VIPR1/*Hha*I	**TT**	**CT**	**CC**	**T**	**C**	**TT**	**CT**	**CC**	
(n=125)	0.07	0.29	0.64	0.22	0.78	0.05	0.34	0.61	NS
BMPR-IB/*Hind*III	**AA**	**GA**	**GG**	**A**	**G**	**AA**	**GA**	**GG**	
(n=205)	0.18	0.47	0.36	0.41	0.59	0.17	0.48	0.35	NS
MTNR1C/*Mbo*I	**AA**	**AG**	**GG**	**A**	**G**	**AA**	**AG**	**GG**	
(n=207)	0.06	0.40	0.54	0.26	0.74	0.07	0.39	0.54	NS
NPY/*Dra*I	**DD**	**ID**	**II**	**D**	**I**	**DD**	**ID**	**II**	
(n=130)	0.36	0.42	0.22	0.57	0.43	0.33	0.48	0.19	NS
NPY/*Kpn*I	**CC**	**TC**	**TT**	**C**	**T**	**CC**	**TC**	**TT**	
(n=123)	0.29	0.51	0.20	0.54	0.46	0.29	0.50	0.21	NS
DRD2/*Bse*GI	**CC**	**TC**	**TT**	**C**	**T**	**CC**	**TC**	**TT**	
(n=123)	0.76	0.23	0.01	0.88	0.12	0.77	0.21	0.02	NS
IGF-I/*Pst*I	**AA**	**AB**	**BB**	**A**	**B**	**AA**	**AB**	**BB**	
(n=93)	0.06	0.29	0.65	0.21	0.79	0.04	0.33	0.62	NS

HWE: Hardy–Weinberg Equilibrium; NS: non-significant

At the PRL/*Alu*I and VIP/*Hinf*I polymorphic loci, T allele showed higher frequency than C allele and accounted for more than 50% in the population (0.69 in PRL/*Alu*I and 0.57 in VIP/*Hinf*I, respectively). In contrast, number of C alleles were higher than those of T at VIPR1/*Taq*I, VIPR1/*Hha*I, NPY/*Kpn*I and DRD2/*Bse*GI polymorphisms. Moreover, in the DRD2/*Bse*GI SNP, the appearance of C allele was higher than T resulting in highest percentage of CC genotype in Noi chickens (76%).

The analysis of GH/*Sac*I and IGF-I/*Pst*I loci additionally indicated that homologous AA genotype was dominant (98%) in the population. Moreover, at the VIP/*Vsp*I and NPY/*Dra*I loci, two indels (insertion/deletion/) were observed; however, with mutant at VIP/*Vsp*I locus, DD genotype did not appear in Noi chicken population, while the II genotype contributed up to 59%. At the NPY/*Dra*I locus, ID genotype frequency was the highest (0.42) followed by DD (0.36) and II (0.22) genotypes.

DISCUSSION

The findings of genes responsible for genetic variation in the traits of interest in animal species are of importance in genomic analysis [17]. In this study, some candidate genes previously known to affect egg production in various chicken breeds were applied in Noi native chickens for later use in breeding.

Previously, it was indicated that alleles of T and C (PRL/*Alu*I) or C and G (PRL/*Csp*6I) were presented in several breeds such as Yangshan, Taihe Silkies 1, White Rock, Nongdahe and Taihe Silkies 2 [18], in which T and G alleles constituted more than 50%. Those data were similar to the present study in Noi chickens; however, in the commercial White Leghorn chicken population, T and G alleles were not available [18]. In addition, with the GH/*Sac*I polymorphism, this study showed that BB genotype was absent and AB genotype was only at 2%; therefore, the frequency of A allele made up to 99% while B allele only existed at 1%. This was in agreement with the finding of Makhsous *et al*. (2013) [19], who observed higher frequency of A allele in indigenous chicken of Iran but all three genotypes (AA, AB and BB) were existent in the population.

The VIP gene was known to influence reproduction yield and hatching habit of chicken [20]. Zhou *et al*. (2010) [21] examined 644 Ningdu Sanghuang chickens and inferred that VIP/*Vsp*I SNP linked with total number and qualified eggs from 90 to 300 day of age and VIP/*Hinf*I associated with broodiness trait. Xu *et al*. (2011a) [6] also detected some polymorphisms in VIP gene linked to egg number at 300 days of age such as VIP/*Apo*I, VIPR1/*Taq*I and VIPR1/*Hha*I.

The association of BMPR-IB gene with egg reproduction traits in chicken was also observed by Zhang *et al*. (2008) [14]. The authors found higher frequency of A allele in Luqin broiler line, commercial egg-laying line, Jining Bairi and Wenchang chickens. However, in Zang chicken, G allele was of higher frequency than A allele, which was similar to Noi chickens. It was further noted that although there were differences in allele frequency among chicken breeds, genotype frequencies were similar in most of them with dominant heterogeneous GA genotype observed.

At NPY/*Dra*I locus, the frequency D allele was slighly higher than I allele in Noi chickens and it was in agreement with those reported by Li *et al*. (2009) [22] in Wengchang and Xu *et al*. (2011a) [6] in Ningdu Sanhuang chickens. In contrast, Fatemi *et al*. (2012) [23] found the predominance of I allele in Iran's Mazandaran chicken and the II homologous genotype frequency contributed up to 55% in the observed population. For the NPY/*Knp*I SNP, alleles C and T were observed in Noi chickens with similar frequency, which supported the results of Xu *et al*. (2011a,b) [6,7] in Ningdu Shanghuang chickens. Finally, the IGF-I gene was found to be significantly associated with egg yield at 300 and 400 days of age in Wenchang chicken [24] and egg yield in Ogol chicken [25]. Two alleles (A and B) of IGF-I gene were detected in above breeds, where B allele was more dominant than the other one. Those findings were in line with the present work; however, in another investigation Abbasi and Kazemi (2013) [26] found a balance of both alleles in Madandaran chicken population.

CONCLUSION

In conclusion, SNPs and desired alleles of candidate genes (PRL, GH, VIP, VIPR1, BMPR-IB, MTNR1C, NPY, DRD2 and IGF-I) known to associate with egg reproduction were detected in Noi chickens. The present results indicated great potential of genetic resources for egg producing traits and provided evidences for selection to enhance reproductive performance under breeding programs.

ACKNOWLEDGMENTS

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