EFFECTS OF GENETIC POLYMORPHISMS ON EGG PRODUCTION IN INDIGENOUS NOI CHICKEN

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ABSTRACT

The aim of this study was to evaluate the genetic potential of candidate genes involved in egg yield of Noi chicken, a popular backyard chicken breed in the Mekong Delta of Vietnam with limited egg production. A total of 130 hens were individually stalled for daily egg record in 20 weeks. Feather samples were collected for DNA extraction and genotyping was carried out using PCR-RFLP method. Five genes, namely DRD2 (Dopamine receptor 2), IGF-I (Insulin-like growth factor I), NPY (Neuropeptide Y), VIP (Vasoactive intestinal polypeptide) and VIPR-1 (Vasoactive intestinal peptide receptor 1) were used in the present work. It was shown that allele frequencies of DRD2/*Bse*GI, VIP/*Vsp*I and VIPR-1/*Hha*I polymorphisms were of great discrepancy in Noi chicken population and there were significant associations between genotypes and egg numbers ($P < 0.05$). Furthermore, chickens carrying DD (NPY/*Dra*I), CC (VIPR-1/*Taq*I) and CC (VIPR-1/*Hha*I) genotypes had highest egg production with 50.9, 49.8 and 50.4 eggs/hen/20 laying weeks, respectively. These results provide an alternative for breeding selection of Noi chicken towards improving egg production.

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1 Introduction

Indigenous Noi chicken of the Mekong Delta possesses several desired characteristics including the ease of raising and high resistance along with some drawbacks such as low productivity and highly hybridized breeds (Quyen & Son, 2008). Enhancing and stabilizing breeding quality are one of the methods used to improve these indicators. Many candidate genes associated with poultry productivity have been identified (Abdi et al., 2014; Fatemi et al., 2012; Xu et al., 2011a; Xu et al., 2011b), as a result of the unprecedented improvements of genetic technology, especially molecular genetics.

Various researches based on the increasing egg productivity have found that candidate genes such as DRD2 (Dopamine D2 Receptor), IGF-I (Insulin-like Growth Factor I), NPY (Neuropeptide Y), VIP (Vasoactive intestinal peptide) and VIPR-1 (Vasoactive intestinal peptide receptor 1) had significant effects on egg productivity. Chaiseha et al. (2003) cloned cDNA of the DRD2 gene in turkeys and pointed out the associations between DRD2 gene and production and hatching traits. Similarly, Xu et al. (2011a; 2011b) studied the g.5841629T>C polymorphism on DRD2 gene and found a significant association between first laying age and total egg production in chickens at 300 days of age. For IGF-1 gene, Li et al. (2009) suggested that IGF-1 genetic polymorphism at position 5'UTR was related to egg production at 300 and 400 days of age and the number of continuous laying days. In addition, Xu et al. (2011b) also demonstrated the relationship between g.31394761C>T polymorphism of NPY gene and first-laying age in Ningdu Sanhuang chickens, followed by the publication of Fatemi et al. (2012) on the association between NPY gene and production traits as well as growth ability in many different chicken traits. Similarly, for VIP and VIPR-1 receptor genes, El Halawani et al. (2000) found that injecting VIP into 25 week and 54 week-old chickens resulted in an increase in egg production.

It can be therefore remarked that exploiting candidate genes to improve egg production is a potential strategy, yet will need to be examined in each group of chickens. Within the scope of this study, 8 polymorphism positions of the mentioned genes were used to assess their association with egg yield of Noi chicken.

2 Materials and Methods

The experiment was conducted on 130 Noi chickens raised at an experimental farm in Vinh Long province (Vietnam), where all hens were individually kept in cages. The hens in the period of laying eggs (28-47 weeks old) were mated with male Noi chicken at ratio of 1 cock: 8 hens. During the experiment, chickens were fed with diets having metabolizable energy of 2850 kcal/ kg and 17% crude protein and all chickens were vaccinated before and during the experiment. The eggs laid were then collected and data were recorded daily during 20 weeks of laying.

Genotypes of candidate genes were determined by PCR-RFLP technique with some basic steps viz. extraction and purification of DNA from hen's feather (Nozawa et al., 1999); amplification of DNA by using appropriate primers corresponding to each gene; incubation of amplified products with restriction enzymes, and determination of the genotype by electrophoresis band on 2% agarose gel. Details of the primer sequences, annealing temperature, type of restriction enzyme used and size of PCR-RFLP bands are presented in Table 1.

Data on the frequency and chi-squared value were calculated using SNPStats program (Solé et al., 2006) (<http://bioinfo.iconcologia.net/index.php>). The association between genotype and egg production was analyzed based on General Linear Model of Minitab software version 16.0: $Y_{ij} = \mu + G_i + \xi_{ij}$ (where Y_{ij} : traits observed; μ : general mean, G_i : Influence of genotype; ξ_{ij} : random error).

Table 1 Information regarding the polymorphisms studied

Gene	SNP	Sequencing primer (5'-3')	Ta (°C)	Enzyme	PCR-RFLP size (bp)	References
DRD2	T5841629C	F: tgcacataaaagcccactcactg R: gcctgagctggtggggggg	60	<i>BseGI</i>	248 196/52	Xu et al., 2011a
IGF-I	C364T	F: actatacagaagaaccac R: tatcactcaagtggctcaagt	60	<i>PstI</i>	621 364/257	Nagaraja et al., 2000
NPY	AATA Indel (I31391359D)	F: tctcagagctccaacgtatga R: atattctgtgcctgaacaaca	56	<i>DraI</i>	248(252) 167/81	Xu et al., 2011b
NPY	C31394761T	F: cgtggctgcttgcctccttc R: gggtacgaggcaaggacatg	58	<i>KpnI</i>	324 200/124	Xu et al., 2011b
VIP	C+338T	F: gcttgactgatgcgtactt R: gtatcactgcaaatgctctg	55	<i>HinI</i>	520 480/40	Zhou et al., 2010
VIP	AGG Indel (D2648-2650I)	F: gaaacccatctcagtcaccta R: accacctattttcctttctac	58	<i>VspI</i>	306 154/152	Zhou et al., 2010
VIPR-1	C1715301T	F: ctctcagcgagaccatcatg R: ctgacgtatccttgggtagc	61	<i>TaqI</i>	486 310/176	Xu et al., 2011a
VIPR-1	C1704887T	F: ccccgtaaaactcagcagac R: cccaaagcccacaaggtaa	61	<i>HhaI</i>	434 253/181	Xu et al., 2011a

F: Forward primer; R: Reverse primer; Ta: Annealing temperature

M : Standard DNA 100 bp scale (Fermentas); Ctrl: Non-incubated PCR products with restriction enzymes

Figure 1 Genotyping of different SNPs by agarose gel electrophoresis

3 Results and Discussion

In the current study, 6 Single Nucleotide Polymorphisms (SNPs) and 2 INDELs (Insertion/Deletion) for five candidate genes were identified from Noi chicken breed. The above SNPs were in transition mutations (T>C or C>T), whereas 2 INDEL variations were based on the addition or removal of 4 AATA nucleotides (NPY/*Dra*I) or 3 AGG nucleotides

(VIP/*Vsp*I). The number of bands and PCR-RFLP band size, genotype and allele frequencies of these polymorphism are shown in Figure 1 and Table 2. For the DRD2/*Bse*GI locus, the number of Noi chickens that had T allele accounted for a very low proportion of the population (0.12). Similar trend was found in other loci such as C allele (0.21) (IGF-1/*Pst*I) or T (0.22) (VIPR-1/*Hha*I) and the frequency of these mutations followed Hardy-Weinberg's law ($P > 0.05$).

Table 2 Genotype and allele frequencies of polymorphisms.

SNP	n	Genotype frequency			Allele frequency		χ^2
DRD2/ <i>Bse</i> GI	123	0.01 (TT)	0.23 (TC)	0.76 (CC)	0.12 (T)	0.88 (C)	0.49 ^{ns}
IGF-1/ <i>Pst</i> I	93	0.06 (CC)	0.29 (CT)	0.65 (TT)	0.21 (C)	0.79 (T)	1.43 ^{ns}
NPY/ <i>Dra</i> I	130	0.22 (II)	0.42 (ID)	0.36 (DD)	0.43 (I)	0.57 (D)	2.38 ^{ns}
NPY/ <i>Kpn</i> I	123	0.20 (TT)	0.51 (TC)	0.29 (CC)	0.46 (T)	0.54 (C)	0.03 ^{ns}
VIP/ <i>Hinf</i> I	107	0.12 (CC)	0.31 (CT)	0.57 (TT)	0.28 (C)	0.72 (T)	5.55 [*]
VIP/ <i>Vsp</i> I	118	0.58 (II)	0.4 (ID)	0.02 (DD)	0.78 (I)	0.22 (D)	3.64 ^{ns}
VIPR-1/ <i>Taq</i> I	111	0.48 (CC)	0.33 (CT)	0.19 (TT)	0.64 (C)	0.36 (T)	8.27 ^{**}
VIPR-1/ <i>Hha</i> I	125	0.64 (CC)	0.29 (CT)	0.07 (TT)	0.78 (C)	0.22 (T)	2.80 ^{ns}

*: $P < 0.05$; **: $P < 0.01$; ^{ns}: $P > 0.05$

In the 2 INDEL variants, genotypic frequencies of VIP/*Vsp*I mutation did not follow the Hardy-Weinberg law ($P < 0.05$) and comparable results were also found in other chicken populations. For example, on Ningdu Shanghuang breed, the frequency of C allele was higher than T allele in DRD2/*Bse*GI mutation point (Xu et al., 2010; Xu et al., 2011a; Xu et al., 2011b). In addition, Kim et al. (2004) showed that C allele frequency (0.31) was lower than T allele (0.69) at IGF-1/*Pst*I polymorphic position in Ogot chicken, but on the same Madandaran chicken, the distribution of 2 alleles was relatively equal (0.51 and 0.49) (Abbasi & Kazemi, 2011). In addition, a comparison of allele frequency on VIPR-1/*Taq*I polymorphism also pointed out that most of the experimental chickens in the population had a higher proportion of C allele.

The effects of polymorphic positions on egg production indicators are displayed in Table 3, in accordance to which the SNPs on NPY and VIPR-1 genes showed a significant association with the egg productivity of Noi chicken in 20 weeks of laying ($P < 0.05$). The highest egg yield was reported in chickens with 4 nucleotides lost (AATA INDEL) at NPY/*Dra*I locus or chickens with CC genotype at VIPR-1/*Taq*I or VIPR-1/*Hha*I position (49.8 to 50.9 eggs). It should be noted that the frequency of this genotype was also in relatively high proportion (0.36 to 0.64), which suggested that the process of natural selection had contributed to the improvement of laying productivity, thereby increased the proportion of beneficial alleles in populations. For the remaining polymorphisms of the current study, the effect of genotype on egg production was not significant ($P > 0.05$).

An important neural regulator that affects reproductive function in mammals and birds is neuropeptide Y (Hilal et al., 1996). In chickens, experiments have shown that when injected into the brain, NPY stimulated intake, increased insulin secretion process and altered levels of certain hormones in the blood such as prolactin, thyrotropin and GnRH (reproduction hormone) (Willoughby & Blessing, 1987; Kuenzel & McMurtry, 1988). Although there are evidences that NPY may play a role in the regulation of hormone secretion mechanism mentioned above, the association between polymorphisms in this gene and egg production is less evident and depends heavily on each different population. Earlier, in a population of commercial laying hens, Dunn et al. (2004) discovered INDEL

(4 bp) mutation at 700 bp position before the start of NPY gene transcription, but no relationship with the total number of eggs was found. Similar findings were also described by Fatemi et al. (2012) on the Iranian Mazandaran local breed; Xu et al. (2011b) on Ningdu Shanghuang breed and Abdi et al. (2014) on West-Azarbaijan native chickens. In contrast, the report of Li et al. (2009) suggested that NPY/*Dra*I polymorphism affect the total egg count of 300 day-old Wengchang chickens, which is confirmed by the current study and this can be explained as NPY gene variations have different effects to chicken ovulation in different breeds (Dunn et al., 2004).

VIP gene regulates GnRH secretion in both humans and poultry (Christian & Moenter, 2008; Li et al., 2009). The effects of VIP on the body depend upon VIPR-1 and VIPR2 receptors, in which VIPR-1 gene is considered selective support indicator to reduce hatchability and improve egg quality (Zhou et al., 2008a). Results from VIPR-1/*Hha*I polymorphism research on Noi chickens showed that chicken with CC genotype had the highest egg production yield. Research by Xu et al. (2011b) also found the influence of this polymorphism on total egg production of Ningdu Sanhuang laying chicken of 300 days, in which C allele benefited more in the selection process. Besides, Zhou et al. (2008b) previously reported a similar result in many different chicken populations; and a number of other research results as well showed that VIPR-1/*Taq*I polymorphism was closely associated with incubation time and the first laying age and individuals carrying CC genotype had a longer incubation period and earlier first laying age (Zhou et al., 2008a; Zhou et al., 2008b). Recently, Xu et al. (2011b) reported that chickens with CC genotype had lower total egg production after 300 days as compared with chickens carrying TT genotype. This also implied that egg production had a negative correlation with hatching time and therefore the two polymorphisms on VIPR-1 gene could be potential molecular markers for the improvement of egg production in Noi chickens.

In animals, dopamine (DA) plays an important role in regulating the physiological effects of the reproductive system such as creating excitement in mating. Besides, DA inhibits prolactin secretion through DRD2 in the pituitary gland. DRD2 gene functions to regulate and control the release of DA and

Table 3 Association between polymorphic positions and Noi chicken egg production (egg/hen/20 laying weeks).

SNP	Genotype	n	Egg production	P
DRD2/ <i>Bse</i> GI	TT	1	26.0 [*]	0.233
	TC	27	50.7 ± 3.9	
	CC	91	45.3 ± 2.3	
IGF-I/ <i>Pst</i> I	CC	6	47.0 ± 9.3	0.930
	CT	27	43.9 ± 4.4	
	TT	60	45.5 ± 2.6	
NPY/ <i>Dra</i> I	II	28	38.9 ± 3.8 ^b	0.040
	ID	55	48.7 ± 2.9 ^{ab}	
	DD	47	50.9 ± 3.0 ^a	
NPY/ <i>Kpn</i> I	TT	25	43.4 ± 4.2	0.710
	TC	62	47.8 ± 3.0	
	CC	36	46.6 ± 3.6	
VIP/ <i>Hinf</i> I	CC	13	45.9 ± 6.3	0.130
	CT	33	40.6 ± 3.7	
	TT	61	49.9 ± 2.8	
VIP/ <i>Vsp</i> I	II	69	46.6 ± 2.6	0.863
	ID	47	47.3 ± 3.1	
	DD	2	58.5 ± 16.3 [*]	
VIPR-1/ <i>Taq</i> I	CC	53	49.8 ± 2.7 ^a	0.045
	CT	35	44.0 ± 3.2 ^{ab}	
	TT	21	37.7 ± 4.5 ^b	
VIPR-1/ <i>Hha</i> I	CC	78	50.4 ± 2.2 ^a	0.047
	CT	34	40.7 ± 3.3 ^b	
	TT	9	42.9 ± 6.7 ^{ab}	

^{*}Data were not subjected for statistical analysis; Values without common letters differ statistically at $P < 0.05$.

thus mutations in the gene DRD2 may affect the reproductive performance of domestic chickens. The research by Xu et al. (2011b) revealed that DRD2/*Bse*GI polymorphism affiliated with egg production of 300 days old, but this result was not supported by the present report.

Similarly, IGF-I gene had no effect on egg production of domestic chickens, despite its great contribution in stimulating growth, protein synthesis, cell proliferation and differentiation, egg development (McMurtry et al., 1997; Yun et al., 2005; Li et al., 2006;) and IGF-I/*Pst*I has been proven to have correlation with egg production and days of continuous egg-laying (Kim et al., 2004; Li et al., 2009).

VIP gene is also believed to be related to egg production of poultry due to its involvement in the synthesis and secretion of prolactin hormone by the pituitary gland. The mRNA concentration of VIP varies with hens' physiological status (Mauro et al., 1989; Chaiseha et al., 1998) and VIP enhances the stability of mRNA prolactin *in vivo* (Tong et al., 1998) as well. The activities that affect the VIP concentration in the body were suggested to influence the reproductive performance and hatching behavior (Caldwell et al., 1999). Zhou et al. (2010) found 69 polymorphisms when they studied 7 chicken breeds in China, among which AGG INDEL had significant effects on the total egg production during the period

of 90-300 days old when chicken with D allele (with three nucleotides AGG inserted) yielded higher productivity. However, the percentage of DD genotype present in the surveyed population was very low (1.2%). Results of the present study are in agreement with the finding of Zhou et al. (2010) that DD genotype proportion in Noi chickens was very low (1.7%) and egg production averaged at 58.5 eggs/20 laying weeks/hen (the highest in all the genotypes studied). However, it was the average of only two individuals and would be just only for reference and no specific conclusions about the effect of this genotype in the population could yet be made.

Conclusions

In Noi chicken population, at each studied polymorphic locus two alleles existed with three different genotypes, including DRD2/*Bse*GI, VIP/*Vsp*I and VIPR-1/*Hha*I with significant differences in allele distribution. Besides, genotypes at the NPY/*Dra*I, VIPR-1/*Taq*I and VIPR-1/*Hha*I mutation points showed association with total egg production of laying Noi chickens of 20 laying weeks. These results provide an additional solution in the selection process towards improving egg production of Noi chicken.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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