Research Article

Polymorphism of the Candidate Genes and Their Association with Egg Production Traits in Thai Native Chickens

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Abstract | Polymorphism was detected in the neuropeptide Y (*NPY*), dopamine receptor D2 (*DRD2*) and vasoactive intestinal peptide (*VIP*) genes, and their associations with egg production traits in 300 Thai native chickens were investigated. DNA was extracted from blood samples for genotyping using specific primers and restriction enzymes for each gene, and polymerase chain reaction-restriction fragment length polymorphism was used to identify the genotypes (PCR-RFLP). Three genotypes were found for each gene as BB, Bb and bb for *NPY*; TT, TC and CC for *DRD2* and II, ID and DD for *VIP*. Genotype frequencies of *NPY* (range 0.13-0.58), *DRD2* (range 0.06-0.55) and *VIP* (range 0.14-0.57) were reported. For the *NPY* gene, allele frequency of b (0.72) was greater than allele frequency of B (0.28), while for the *DRD2* gene, allele frequency of T (0.26) was lower than allele frequency of C (0.74). I and D allele frequencies for *VIP* were 0.72 and 0.28, respectively. Statistical analysis results discovered significant associations between the three candidate genes (*NPY*, *DRD2* and *VIP*). Egg production 270EN, 360EN and E_M of BB genotype were higher than bb genotype for the *NPY* gene ($P < 0.01$), while CC and TC genotypes of the *DRD2* gene were associated with high 270EN, 360EN and E_M ($P < 0.01$). The DD genotype had higher 270EN, 360EN and E_M compared to ID and II genotypes, whereas other egg production traits were not influenced by the candidate gene. Results suggested that alleles of *NPY*, *DRD2* and *VIP* genes showed potential as genetic markers for chicken egg production traits in Thai native chicken population selection programs.

Keywords | Candidate gene, Thai native chickens, Egg production, Selection, Animal breeding

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INTRODUCTION

The most significant economic trait in chicken production is egg production traits, which are influenced by a variety of genes. One of the most popular types of meat consumed worldwide is poultry (Hosnedlova et al., 2020). In rural areas of developing countries, native chickens are produced as a high protein. Native chickens can tolerate harsh environmental conditions and are also resistant to diseases. Moreover, the fat and cholesterol levels in native chicken are also lower (Bungsrisawat et al., 2018). Thai native chickens showed high anserine and antioxidant substances in carcasses with good meat quality yields (Charoensin et al., 2021). Native chickens

produce lower eggs and grow more slowly. Thai native chicken, high egg strain Pradu Hangdum Chiangmai chickens have black feathers and a small pea comb with a red face and whitish yellow skin. High meat quality and good appearance characteristics are important for consumer product acceptance (Kammongkun et al., 2015). Higher egg production reduces the cost of fattening the chicks. Recently, genetic improvements have increased egg production and growth performance of native chickens, with selected breeds showing higher growth in open housing (Promket and Ruangwittayanusorn, 2021).

Molecular technologies and genetic marker approaches are now mainstream techniques for genetic improvement in breeding programs. It may be possible to utilize marker assisted selection (MAS) to improve the genetic makeup of native chickens and increase their egg production by identifying polymorphism and DNA markers associated to egg production features. Copious research has focused on genetic markers associated with egg production to improve this trait**.** The outcomes show that selecting chickens with high egg production using genetic markers was successful (Liu et al., 2019; Tenzin et al., 2020; Wang et al., 2022). Therefore, genetic marker that affected the production of eggs in local Thai chicken populations may be utilized to improve the genetics of the chickens to produce more eggs. In chickens, the neuropeptide Y (*NPY*) gene is an important neuromodulator affecting gonadal function and stimulating feeding and insulin secretion. The plasma levels of prolactin, growth hormone, luteinizing hormone, thyrotropin, GnRH, and vasopressin were changed by *NPY* gene injections. According to Dunn et al. (2004), the *NPY* gene was related to age at first egg and may potentially have a benefit on egg production rate due to its control of ovulation regulation. Dopamine is an important neurotransmitter in birds, operating through vasoactive intestinal peptide to stimulate prolactin production via *DRD1* at the hypothalamus level and inhibit secretion of prolactin via *DRD2* at the pituitary level. The negative regulator of avian reproductive activity such as behavior of incubation is prolactin. *DRD2* inhibits prolactin secretion from the pituitary, which increases chicken egg production and reduces incubation activity. Ruangwittayanusorn et al. (2022) showed that egg production in high egg strain Pradu Hangdum Chiangmai hens was regulated by *DRD2* gene polymorphism. specific receptors binding lactotroph cells at anterior pituitary, the vasoactive intestinal peptide (VIP) gene regulates the prolactin hormone, and VIP protein level and gene expression are correlated with circulating prolactin levels during various phases of reproduction (Zhou et al., 2010). In chickens, association studies between VIP gene polymorphism and variables related to egg production have been conducted. Five polymorphisms were found to be related to the total number of chicken eggs (Xu et al., 2011b; Ngu et al., 2015). Nevertheless, there is no information on the *NPY*, *DRD2*, and *VIP* genes as they associated with egg production in Thai native chickens. As a result, this study determined and evaluated the relationship between polymorphisms in three examined genes *NPY* (neuropeptide Y), *DRD2* (dopamine D2 receptor), and *VIP* (vasoactive intestinal peptide) and characteristics related to egg production in Thai native chickens.

MATERIALS AND METHODS

Animals and morphology

High egg strain Pradu Hangdum Chiangmai chickens (Thai native chickens) were developed from Pradu Hangdum Chiangmai 1. The Chiang Mai Livestock Research and Breeding Center preserves a flock of Pradu Hangdum chickens. With cooperation from the Agricultural Research Development Agency (Public Organization) and the Department of Livestock Development, the breeding goal was to enhance egg production by 30% from 147 eggs per year of foundation stock to 191 eggs per year of breeding stock. High egg strain Pradu Hangdum Chiangmai chickens have black feathers with a red face and small red pea comb. The shanks, toes and claws are yellow to black (Figure 1). This study was carried out at an experimental open house farm in Chiangmai Livestock Research and Breeding Center, Thailand. A total of 300 sixteen-week-old Thai native chickens were selected at random, and they were fed a commercial feed (17% CP and 2,900 kcal of ME/kg for the laying phase) and given water *ad libitum* (NRC, 1994). The chickens were raised separately in 8 x 16 inch cages, and data were recorded as follows: weight hen at first egg (HW_FE), age at first egg (A_FE), egg weight at first egg (EW_FE), egg weight at day 270 (270 EW), egg weight at day 360 (360EW), cumulative egg number at 270 days (270EN), cumulative egg number at 360 days (360EN) and number of eggs per month (E_M).

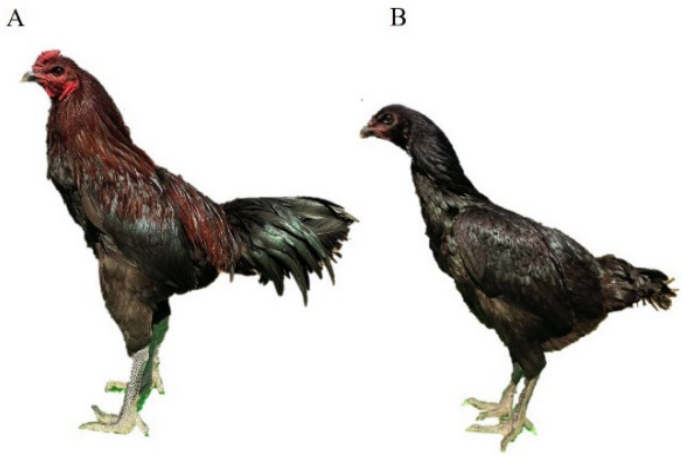

Figure 1: Morphology of male (A) and female (B) high egg strain Pradu Hangdum Chiangmai Thai native chickens.

DNA extraction

In 1.5 mL microtubes with 100 µL 0.5 M ethylenediaminetetraacetic acid (EDTA), blood samples (1 mL) were collected from the wing vein. Using the guanidine hydrochloride technique for extracted genomic DNA from whole blood samples (Goodwin et al., 2011). Protein precipitation and cell lysis buffer were given to the blood and centrifuged for 10,000 rpm at 4°C, 5 minutes. Transferred the supernatant to 1.5 mL microtubes and add absolute isopropanol. The DNA was precipitated at 10,000 rpm at 4°C for 5 minutes. The DNA pellet was washed with 75% ethanol (2 times). A Nanodrop 2000c Spectrophotometer (Thermo Scientific, USA) was used to assess the concentration and quality of the genomic DNA. Before use, the DNA diluted to a working solution of 50 ng/µL and kept at -20°C.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis

The polymerase chain reaction (PCR) was carried out for each experiment in a total volume of 10 µL, which contained the following: 0.8 µL of 50 mM MgCl2, 1 µL of 10X PCR buffer, 1 µL of 1 mM dNTPs, 1 µL of 5 µM of each primer, 0.1 µL of *Taq* DNA polymerase (Promega, San Diego, CA), 4.1 µL of nuclease free water and 1 µL of working genomic DNA (50 ng/µL). A PCR thermal cycle (Corbett Research, Australia 2003 and iCycler thermal cycler, BioLab, USA) was used to perform PCR amplification. Table 1 shown the primer sequence sets for the three gene used in PCR experiments. PCR profile included pre-denaturation at 94°C, 5 minutes was followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at temperature (Table 1) for 40 seconds, extension at 72°C for 30 seconds and the final extension was performed at 72°C for 5 minutes. The PCR products were kept at 4°C until needed. Using a 2% agarose gel examine PCR products of the *NPY*, *DRD2*, and *VIP* genes. The gel was stained with GELSTAR™ (Gelstar Inc, NY) for 10 minutes after electrophoresis at 100 V for 35 minutes. The PCR products were digested with one restriction enzyme and incubated overnight (*Dra*I at 37°C for *NPY*, *Bse*GI at 55°C for *DRD2* and *Vsp*I at 37°C for *VIP*). The restriction patterns were shown using 3% agarose gel electrophoresis and staining with GELSTAR™ (Gelstar Inc, NY) then determine the genotypes by Gel Documentation (Lab Focus, Inc.).

Statistical analysis

PROC MEANS was used to evaluate the means of egg production variables (SAS Institute Inc. Cary, NC, 2003). In accordance with Falconer and Mackay (1996), chi-square ($\chi 2$) was used to test for Hardy-Weinberg Equilibrium (HWE), polymorphism information content (PIC), allele frequencies and genotype frequencies. Associations between the three different polymorphisms of *NPY*, *DRD2* and *VIP* genes with egg production variables were studied using the model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where Y_{ij}: egg production variables in chickens, μ: the overall mean, G_i: the fixed effect of the genotype (*NPY*, *DRD2* and *VIP*) and e_{ij}: residual error.

RESULTS AND DISCUSSION

Egg production traits in Thai native chickens

Table 2 shows descriptive statistics data of egg production traits in Thai native chickens. Mean (SD) values of HW_FE and A_FE were 1,927.65 (178.01) g and 154.25 (11.40) days, respectively. The EW_FE was 34.18 g and the 270EW and 360EW were 44.60 g and 45.10 g, respectively. Cumulative egg numbers at 270 days (270EN) and cumulative egg number at 360 days (360EN) were 154.12 and 191.20 eggs, respectively.

Genotypes frequencies of *NPY*, *DRD2* and *VIP* genes in Thai native chickens

Three candidate genes (*NPY*, *DRD2* and *VIP*) were identified from Thai native chickens. PCR products of *NPY*, *DRD2* and *VIP* were 240 bp, 248 bp and 306 bp (Figure 2), respectively. The *NPY*/*Dra*I PCR-RFLP analysis of 300 DNA samples obtained from Thai native chickens showed three genotypes, namely BB (240 bp), Bb (240 bp, 161 bp and 79 bp) and bb (161 bp and 79 bp), as shown in Figure 2A.

Table 1: Details of primer for polymerase chain reaction (PCR) assays.

Gene	Location (bp)	Ch. [4/]	Gene ID	Primer sequence (5′-3′)	Length [5/] (bp)	T [6/] (°C)	Enzyme
NPY [1/]	4bp del 494-499	Ch.2	396464	F: TCTCAGAGCTCCAACGTATGA R: ATATTTCTGTGCCTGAACAACA	240	60	*Dra* I
DRD2 [2/]	T5841629C	Ch.24	428252	F: TGCACATAAAAGCCCACTCACTG	248	60	*Bse*GI
				R: GCCTGAGCTGGTGGGGGG			
VIP [3/]	AGG Indel D2648-2650I	Ch.3	396323	F: GAAACCCATCTCAGTCATCCTA R: ACCACCTATTTTTCCTTTTCTAC	306	58	*Vsp*I

Note: [1/]Dunn et al. (2004); [2/] Ngu et al. (2015); Xu et al. (2011b); [3/] Vu and Ngu, (2016); [4/] Ch. is chromosome; [5/] Length is the length of PCR products; [6/] T is annealing temperature.

Table 2: Descriptive statistics of egg production in Thai native chickens.

Trait	Mean	SD	Max	Min	CV (%)
HW_FE (g)	1,927.65	178.01	2,430	1,075	9.23
A_FE (day)	154.25	11.40	198	130	7.39
EW_FE (g)	34.18	5.99	55.00	21.20	17.53
270EW (g)	44.60	3.47	55.00	32.48	7.78
360EW (g)	45.10	3.44	56.00	32.28	7.64
270 EN (egg)	154.12	30.87	221.00	53.00	20.02
360EN (egg)	191.20	30.72	260.00	99.00	16.06
E_M (egg)	15.93	2.56	21.66	8.25	16.06

Note: hen weight at first egg (HW_FE), age at first egg (A_FE), egg weight at first egg (EW_FE), egg weight at day 270 (270 EW), egg weight at day 360 (360EW), cumulative egg number at 270 days (270EN), cumulative egg number at 360 days (360EN) and number of eggs per month (E_M).

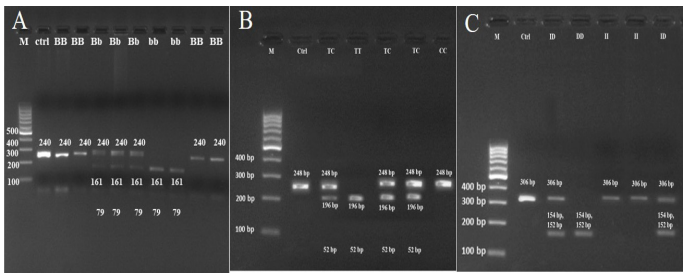

Figure 2: Genotypes of (A) *NPY* gene (BB: 240 bp; Bb: 240 bp + 161 bp + 79 bp; bb: 161 bp and 79 bp), (B) *DRD2* gene (CC: 248 bp; TC: 248 bp +196 bp + 52 bp; TT:196 bp + 52 bp) and (C) *VIP* gene (II: 306 bp; ID: 306 bp + 154 bp + 152 bp; DD: 154 bp + 152 bp), M = marker 100 bp, Ctrl = PCR product.

Distribution of the genotypes and allele frequencies are shown in Table 3. The bb homozygotes (0.58) predominated over BB (0.13) and Bb (0.29) genotype. Frequency of allele b was higher (0.72) than allele B (0.28) in the Thai native chicken population. By contrast, in *DRD2*/ *Bse*GI polymorphism, all three genotypes (TT, TC, and CC) were found. The RFLP patterns of three genotypes on *DRD2* were genotype CC (248 bp), genotype TC (248 bp, 196 bp and 52 bp) and genotype TT (196 bp and 52 bp) (Figure 2B). However, the TT homozygous genotype showed very low frequency (0.06). The most represented genotype was CC with a frequency of 0.55. The frequency of the TC genotype was 0.39 and allele T (0.26), lower frequency than allele C (0.74). Digestion with *Vsp*I of *VIP* was detected in three genotypes: genotype II (306 bp), genotype ID (306 bp, 154 bp and 152 bp) and genotype DD (154 bp and 152 bp) (Figure 2C). The VIP gene found allele frequencies I (0.72) and allele frequencies D were found (0.28). The VIP gene, II pattern had the highest genotype frequency (0.57), followed by ID and DD genotype of 0.29 and 0.14, respectively (Table 3).

HWE identified on *DRD2* frequencies ($p < 0.05$) in the total population. The *NPY* and *VIP* genes did not follow HWE and fit the assumption of the equilibrium. Calculated PIC values were similar for *NPY* (0.32), *DRD2* (0.31) and *VIP* (0.32) genes. Results showed that the *NPY*, *DRD2* and *VIP* genes were moderately polymorphic in Thai native chickens.

Association of polymorphism in *NPY*, *DRD2* and *VIP* genes on egg production traits in Thai native chickens

Associations of the polymorphisms in the three candidate genes (*NPY*, *DRD2* and *VIP*) on egg production variable in Thai native chickens were study (Table 4). Significant association was found between the *NPY*, *DRD2* and *VIP* gene polymorphism and egg production traits (270EW, 270EN, 360EN and E_M). A highly significant association ($P < 0.01$) was found between polymorphism of the *NPY* gene and 270EN. The mean 270EN value of chickens with the BB genotype (163.61 eggs) was significantly higher ($P < 0.01$) than chickens with the bb genotype (149.57 eggs). Moreover, the Bb genotype (158.86 eggs) was not significantly associated with the BB genotype. Significant associations ($P < 0.05$) were detected between the *NPY* gene, 360EN and E_M genotypes. The genotype bb was negative for 360EN and E_M at 187.93 eggs and 15.66 eggs, respectively. The BB genotype had the highest number of 360EN and E_M at 198.64 eggs and 16.55 eggs, respectively. Significant effects of *DRD2* polymorphism were detected on 270EN, 360EN and E_M ($P<0.01$). Chickens carrying the CC and TC genotypes had higher values of 270EN, 360EN and E_M than those carrying the TT genotype.

Table 3: Genotype and allele frequencies of polymorphisms.

Gene	Total	Genotype frequency			Allele frequency		$\chi 2$	PIC
NPY	300	BB	Bb	bb	B	b		
		0.13 (39)	0.29 (88)	0.58 (173)	0.28	0.72	21.41	0.32
DRD2	300	TT	TC	CC	T	C		
		0.06 (18)	0.39 (118)	0.55 (164)	0.26	0.74	0.28	0.31
VIP	300	II	ID	DD	I	D		
		0.57(172)	0.29 (87)	0.14 (41)	0.72	0.28	24.09	0.32

Note: PIC is polymorphism information content, $\chi 2$ (2, 0.05) = 5.99

The CC and TC genotypes of *DRD2* had higher 270EN (155.57 and 154.61 eggs, respectively) than the TT genotype (137.77 eggs). Moreover, the CC and TC genotypes gave 360EN of 197.20 and 185.97 eggs, respectively and higher than the TT genotype at 170.83 eggs. The TT homozygotes had the lowest E_M at 14.23 eggs compared to 16.43 eggs for the CC genotype and 15.49 eggs for TC genotype (Table 4). The association between the *VIP* and 270EN, 360EN and E_M were found (P<0.01). The DD genotype had higher 270EN (173.97 eggs) and 360EN (216.53 eggs) compared to the ID (149.41 and 187.49 eggs) and the II genotypes (151.77 and 187.04 eggs). An association of the *VIP* gene was found in E_M. Chickens with the DD genotype (18.04 eggs) showed higher E_M than ID and II genotypes (15.62 and 15.58 eggs, respectively) (P<0.01).

Table 4: Association of polymorphism in *NPY, DRD2* and *VIP* genes and egg production traits in Thai native chickens.

Gene	Trait		Genotype		P value
		BB	Bb	bb	
NPY	HW_FE (g)	1,899.33	1,922.59	1,936.62	0.44
	A_FE (day)	153.10	153.35	154.96	0.34
	EW_FE (g)	32.49	34.36	34.47	0.08
	270EW (g)	43.83	45.04	44.56	0.20
	360EW (g)	44.57	45.13	45.21	0.62
	270EN (egg)	163.61^{A}	158.86^{AB}	149.57^{B}	0.005
	360EN (egg)	198.64^{a}	194.33^{ab}	187.93^{b}	0.02
	E_M (egg)	16.55^{a}	16.19^{ab}	15.66^{b}	0.02
DRD2		TT	TC	CC	P value
	HW_FE (g)	1,977.56	1,914.32	1,931.77	0.34
	A_FE (day)	157.94	154.78	153.45	0.22
	EW_FE (g)	36.33	34.38	33.80	0.20
	270EW (g)	42.39^{B}	44.77^{A}	44.73^{A}	0.01
	360EW (g)	45.01	45.21	45.03	0.90
	270EN (egg)	137.77^{B}	154.61^{A}	155.57^{A}	0.01
	360EN (egg)	170.83^{B}	185.97^{A}	197.20^{A}	0.0001
	E_M (egg)	14.23^{B}	15.49^{A}	16.43^{A}	0.0001
VIP		II	ID	DD	P value
	HW_FE (g)	1,927.49	1,925.63	1,932.63	0.99
	A_FE (day)	153.43	155.03	155.80	0.40
	EW_FE (g)	34.41	33.34	35.03	0.21
	270EW (g)	44.88	44.54	43.60	0.11
	360EW (g)	45.00	45.40	44.87	0.64
	270EN (egg)	151.77^{B}	149.41^{B}	173.97^{A}	0.0001
	360EN (egg)	187.04^{B}	187.49^{B}	216.53^{A}	0.0001
	E_M (egg)	15.58^{B}	15.62^{B}	18.04^{A}	0.0001

Note: ^{ab} means within a row with different superscripts are significantly different (P<0.05). ^{AB} means within a row with different superscripts are significantly different (P<0.01) hen weight at first egg (HW_FE), age at first egg (A_FE), egg weight at first egg (EW_FE), egg weight at day 270 (270 EW), egg weight at day 360 (360EW), cumulative egg number at 270 days (270EN), cumulative egg number at 360 days (360EN) and number of eggs per month (E_M).

Indigenous chickens are very important genetic resources in developing countries to ensure food security (Chomchuen et al., 2022a). Native chicken meat has many advantages, such as low fat and delicious taste. However, genetic barriers in native chickens can result in low growth rate and egg production yield. Native chickens produce low numbers of eggs (Chomchuen et al., 2022b). One way to resolve this problem is by improving the genetic structure of native chickens. Thai native chicken species that is popularly raised by farmers, the mean weight of hen at first egg was 2.05 kg and the weight of first egg was 36.94 g (Tongsiri et al., 2019). Thai native chickens (Pradu Hangdum) produced 117 eggs per year (Mookprom et al., 2017). Egg production of indigenous chickens across different agro-ecologies of Ethiopia was 51.40 eggs/hen/year, with egg weight 48.60 g (Bekele et al., 2022).

Egg production is low to medium heritability and control by polygenic genetic. Traditional breeding strategies for increasing egg production are influenced by environmental factors, which makes genetic improvement challenging. The genetic mechanisms of complex traits can be analyzed using candidate genes. This study investigated the effect of the *NPY*, *DRD2* and *VIP* genes on egg production. Previous research showed that egg production of native chickens was regulated by candidate genes such as *NPY*, *DRD2*, and *VIP* (Ngu et al., 2015; Majid et al., 2019). The size of the DNA fragment of the *NPY* gene as determined by the RFLP method in this investigation has the same size as that reported by Dunn et al. (2004). In this study, allele b for the *NPY* gene, allele C of the *DRD2* gene and allele I of the *VIP* gene showed higher proportions than allele B, allele T and allele D, respectively. The majority of allele frequencies were close to previously observed in Vietnamese chickens (Noi chickens) and Chinese native chickens (Ningdu Sanhuang), with the *DRD2* gene showing a higher frequency of the allele C than the allele T. Additionally, the *VIP* gene indicated a greater proportion of the allele I (Xu et al., 2011a; Ngu et al., 2015). In this population, the PIC values of the *NPY*, *DRD2* and *VIP* genes indicated reasonably informative loci (0.31-0.32). The polymorphism of allele fragments is commonly evaluated using the PIC value. PIC<0.25 indicates slightly informative locus, 0.25<PIC<0.50 shows a reasonably informative locus and PIC>0.50 suggests a highly informative locus, according to Ding et al. (2010). Only *DRD2* genotype frequencies followed the Hardy-Weinberg law because these populations were G_0 flock and selected for egg production traits. They were selection by 2-month cumulative egg production with a mild selection intensity using phenotypic selection. As a result, this gene was in HWE and was not impacted by selection. Frequencies of the *NPY* and *VIP* genes did not follow the Hardy-Weinberg law. The factor of HWE were rate of recombination and mutation, selection, mating system,

genetic linkage, population structure and genetic drift (Kubota et al., 2019).

With the recent advances in molecular genetics and the availability of candidate gene polymorphism markers, many studies have been performed to explain the genetic makeup that controls egg production traits in chickens. *NPY* is one interesting candidate gene, which is control the release of gonadotrophin releasing hormone (GnRH) and is regulate feed intake in chickens. It may also be able to coordinate with reproductive activity and the timing of puberty (Dunn et al., 2004). In broilers, decreased GnRH reduced ovarian activity. Xu et al. (2011a) reported that injection of the *NPY* gene induced precocious puberty in chickens. Therefore, the *NPY* gene may provide genetic markers for control of ovulation and have impact on egg production rate. The association of SNP on the *NPY* gene with age at the first egg in chickens. Additionally, associations found in heterozygous hens indicated that the *NPY* genes may controlling age of hen at first egg traits. Age at first egg was reduced by 8.6 days for *NPY* gene heterozygotes (+/-) compared to homozygotes (-/-), and by 4.2 days for *NPY* gene homozygotes (+/+) (Dunn et al., 2004).

Various studies have shown association of the *DRD2* and *VIP* genes on egg production in chickens. Our findings of significant differences between genotypes of the *DRD2* gene with 270EN, 360EN and E_M correspond with results of association genes performed by Ngu et al. (2015). Xu et al. (2011b) found association between the polymorphism of the *DRD2* and *VIP* genes and eggs production. Additionally, chickens with the TT genotype produced more eggs than chickens with the CC and TC genotypes. According to Tenzin et al. (2020), *DRD2* was associated to egg weight at first egg in Pradu Hangdam chickens, with the TC and TT genotypes having greater breeding values than the CC genotype. In the *VIP* gene, we found association of the *VIP* genotype on 270EN, 360EN and E_M similar to results reported by Zhou et al. (2010). They demonstrated that the VIP gene locate 5' region was related to egg production. While Xu et al. (2011b) demonstrated that the *VIP* gene was not associated with the number of eggs at 300 days. The regulation of the avian reproductive system is significantly influenced by the dopaminergic system. In chickens, vasoactive intestinal peptide releases prolactin. This promotes and controls prolactin hormone secretion, which is important for the behavior of chickens during incubation (Zhou et al., 2010). Results of this study indicate that polymorphism of the *NPY*, *DRD2* and *VIP* genes is associated with egg number. Thus, BB, CC and DD genotypes of the *NPY*, *DRD2* and *VIP* genes are important for high egg numbers in chickens.

CONCLUSIONS AND RECOMMENDATIONS

Polymorphism of the *NPY*, *DRD2* and *VIP* genes and their effect on egg production was analyzed using the PCR-RFLP technique. Significant association was confirmed between the candidate *NPY*, *DRD2* and *VIP* genes and egg production traits 270EN, 360EN and E_M. Thai native chickens with BB, CC and DD genotypes produced higher egg numbers. These genes could be used as genetic markers to increase egg production in Thai native chicken breeding programs.

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NOVELTY STATEMENT

This study focuses on the polymorphism of candidate genes (NPY, DRD2 and VIP genes) in Thai Native chickens (high egg strain Pradu Hangdum Chiangmai population) could therefore allow for the development of breeding program on Thai native chickens.

AUTHOR'S CONTRIBUTION

Doungnapa Promket: DNA and genotype extraction, data analysis and interpretation, development of a draft article, and approval of the final manuscript version.
Khanitta Pengmeesri: Conception and design of the research, analysis data and approval of the manuscript.
Jennarong Kammongkun: Collecting data on egg production, taking blood samples and finalizing the final draft of manuscript.
Thassawan Somchan: Approving the final version of the manuscript.

Ethical considerations

This research was reviewed and approved for the Institutional Animal Care and Use Committee (IACUC) of Mahasarakham University, Mahasarakham, Thailand (IACUC-MSU-9/2023).

Conflict of interest

The authors have declared no conflict of interest.

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