Research Article

Identification of Melatonin Receptors Type C (*MTNR1C*) and Neuropeptide Y (*NPY*) Genes Related to Egg Production in Thai Indigenous Chickens

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Abstract | Melatonin receptors type C (*MTNR1C*) and neuropeptide Y (*NPY*) have a significant influence in the chicken egg production characteristic. In our study, we worked with three hundred Thai indigenous chickens. This study employed PCR-RFLP genotyping of the *MTNR1C* and *NPY* genes to investigate their association with egg production traits. The results revealed significant associations between these genes and several egg production parameters on Thai indigenous chicken populations. In the case of the *MTNR1C* gene, significant effects were observed on WE360d, NE360d, and E/M ($P < 0.05$). Specifically, Thai indigenous chickens carrying the AA genotype in the *MTNR1C* gene displayed higher WE360d values compared to GG genotypes. Furthermore, the GG genotypes of *MTNR1C* were associated with higher NE360d and E/M values (195.61 eggs and 16.30 eggs, respectively) compared to the AA genotype (181.09 eggs and 15.09 eggs, respectively). Notably, the *NPY* gene exhibited highly significant associations with NE270d, NE360d, E/M, and E_Mass ($P < 0.01$). Specifically, the BB and Bb genotypes in the *NPY* gene correlated with significantly higher NE270d values (163.25 and 160.10 eggs, respectively) compared to the bb genotype (148.00 eggs). Moreover, the bb genotype exhibited lower NE360d and E/M values (181.64 eggs and 15.13 eggs, respectively), while BB and Bb genotypes displayed the highest NE360d values (203.33 and 194.27 eggs) and E/M values (16.94 and 16.18 eggs). These findings underscore *MTNR1C* and *NPY* gene were the genetic factors influencing egg production in Thai indigenous chickens, offering valuable insights for selective breeding programs aimed at enhancing egg production.

Keywords | Thai indigenous chickens, Marker assisted selection, *MTNR1C*, *NPY,* Association, Egg production

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INTRODUCTION

Thai indigenous chickens are regarded genetic resources due to their adaptability to harder environments, particularly in rural or free-range settings. They adapt well to changes in the environment, especially in nutrition, which leads to an increase in body weight. Due to their resistance to disease and ability to withstand difficult environmental conditions, these chickens are essential sources of high-protein food in rural areas of developing countries. Moreover, they have lower fat and cholesterol levels (Bungsrisawat et al., 2018). Furthermore, it has been

found that native Thai chickens yield carcasses with increased levels of antioxidants and anserine, which improve the quality of the meat (Charoensin et al., 2021). Raising indigenous chickens has a disadvantage versus raising commercial breeds since they produce less egg production at lower rates and grow more slowly (Tenzin et al., 2020). One solution to solve this problem is to improve the genetics of indigenous chickens to increase their capacity for egg production. The performance of indigenous chickens' production must be improved as a result.

The Pradu Hangdum Chiangmai chickens, an indigenous chickens of Thailand breed recognized for their disease tolerant, the meat is low in fat and tastes good. Their phenotype had black feathers, light yellow skin, a red face and a small pea sized comb. Consumers have a great demand for Thai indigenous chickens. Additionally, because it has high-quality meat and less fat, the selling price is higher than that of commercial chicken. For a product to gain consumer acceptance, it must not only exhibit high meat quality but also possess high egg production (Ruangwittayanusorn et al., 2022). One option that can have long-lasting consequences is the genetic selection method of producing eggs via molecular methods. Therefore, it is essential to research the Thai indigenous chickens using a molecular marker technique in order to fast improve the egg production that will help to satisfy the substantial market demand for expanded output. Molecular methods, including molecular technologies and genetic marker approaches, offer promising avenues for genetic enhancement in breeding programs. The ability to use marker assisted selection (MAS) to modify the genetic make-up of native chickens and increase their ability to produce eggs is made possible by the identification of polymorphisms and DNA markers that are associated with egg production characteristics.

The one of genetic marker that is important for egg production, melatonin, an indole hormone primarily synthesized in the pineal gland, plays a crucial role in various physiological processes in chickens, including circadian rhythms and reproduction (Hao et al., 2020; Israa et al., 2021). In chickens, three melatonin receptor subtypes, *MTNR1A*, *MTNR1B*, and *MTNR1C*, belonging to the G protein coupled receptor superfamily. Although *MTNR1A* and *MTNR1B* are two of the high-affinity melatonin receptor types found in mammals, *MTNR1C* is only found in chickens and amphibians (Li et al., 2013). The presence of melatonin binding sites in ovaries of chickens suggests melatonin's involvement in various ovarian activities (Li et al., 2013; Hao et al., 2020). Single nucleotide polymorphisms (SNPs) in melatonin receptors, such as *MTNR1A* and *MTNR1C*, have been linked to age at first egg and are considered potential molecular markers for early maturity traits in ducks (Feng et al., 2018). Furthermore, studies have highlighted the presence of melatonin binding sites in chicken ovaries, and antioxidant therapy has demonstrated positive impacts on chicken reproductive capabilities. Elevated serum estradiol levels and decreased ovarian GnIHR, caused by melatonin, are correlated with an improved egg laying rate, with a likely role played by melatonin receptor activation (Jia et al., 2016). Additionally, domestic chickens exhibit similar pharmacological characteristics in all three melatonin receptor subtypes found in chicken brain tissues, suggesting melatonin's direct influence on female reproductive functions (Li et al., 2013).

Moreover, the neuropeptide Y (*NPY*) gene has a crucial role in gonadal function as well as eating and insulin secretion in chickens. Prolactin, luteinizing hormone, growth hormone, vasopressin, and thyrotropin plasma levels were changed by *NPY* injections. The *NPY* has a variety of physiological roles in chickens, including regulating food intake, sexual development which controls ovulation regulation, was connected to age at first egg, and may possibly improve egg production rate (Sartsoongnoen et al., 2021). Given these findings, *MTNR1C* and *NPY* genes have emerged as promising candidate genes associated with egg production traits. Nevertheless, it remains unclear whether *MTNR1C* and *NPY* nucleotide polymorphisms are linked to factors influencing egg production in Thai indigenous chickens. Thus, the purpose of this research is to find variation in the genes *MTNR1C* and *NPY* and investigate it relates with egg production in Thai indigenous chickens.

MATERIALS AND METHODS

Experimental Chickens And Traits

Our experimental procedures involving animals strictly adhered to the guidance provided by the IACUC (Institutional Animal Care and Use Committee) of Mahasarakham University, Thailand, as outlined in protocol IACUC-MSU-9/2023.

Three hundred High Egg Strain Pradu Hangdum Chiangmai Chickens (Thai indigenous chickens) were provided from the Chiangmai livestock research and breeding center, situated in Chiangmai province, Thailand. The breeding objective of our breeding initiative was to bolster egg production, with a specific target of increasing it by 30% from the initial 147 eggs per year observed in the foundation stock to a goal of 191 eggs per year within the breeding stock. To induce photo responsiveness, the Thai indigenous chickens were maintained under controlled lighting settings, with 16 hours of light and 8 hours of darkness per day. They received a carefully formulated commercial feed, comprising 17% crude protein and 2,900 kilocalories of metabolizable energy per kilogram, tailored for the laying

phase. The Thai indigenous chickens had free access to food and drinking water, in strict accordance with the guidelines stipulated by the National Research Council (NRC, 1994). Each chicken was individually housed in cages measuring 8 x 16 inches in all dimensions, an environmental room temperature, and facilitating precise monitoring and care. Throughout the course of the egg production cycle, we diligently documented a range of egg production traits, including the weight of hens at their first egg (WH_FE), the age at which the first egg was laid (A1E), the weight of the initial egg (WE_1E), egg weights on day 270 (WE270d) and egg weights on day 360 (WE360d), cumulative egg counts at day 270 (NE270d), cumulative egg counts at day 360 (NE360d), as well as the monthly egg production rate (E/M) and the relationship between egg production and egg weight can be described egg mass (E_MASS). This comprehensive data collection served as the foundation for our thorough assessment and analysis of the performance of the Thai indigenous chickens within our research.

The Thai indigenous chickens were classified at cumulative egg counts at day 360 (NE360d) into 2 groups; low egg producing (LEP) and high egg producing (HEP) Thai indigenous chickens. The Thai indigenous chickens from the lowest 10 percentage egg production were defined as low egg producing group and the Thai indigenous chickens from the highest 10 percentage egg production were defined as high egg producing group. To analyze the genotype distribution and egg production between the LEP and HEP populations.

Blood Collection And Dna Extraction

For the purpose of DNA extraction, blood samples of 1 mL each were collected from the wing vein of the subjects and transferred into 1.5 mL microtubes containing 100 L of 0.5 M ethylenediaminetetraacetic acid (EDTA) for prevents blood clotting.

Genomic DNA was isolated from these whole blood samples using the guanidine hydrochloride method, as detailed by Goodwin et al. (2011). The blood samples underwent a process involving protein precipitation and cell lysis buffer. Following this, they were centrifuged at 10,000 rpm and 4°C for a duration of 5 minutes, resulting in the separation of supernatant. The supernatant was carefully transferred to fresh 1.5 mL microtubes, and 100% isopropanol was introduced to facilitate DNA precipitation. Subsequently, the DNA underwent a 5-minute precipitation at 4°C and 10,000 rpm. Two rounds of DNA pellet washing were conducted using 75% ethanol. To assess the concentration and quality of the genomic DNA, a Nanodrop 2000c Spectrophotometer from Thermo Scientific (USA) was employed. The extracted DNA was then stored at -20°C and, prior to use, was diluted to a working solution of 50 ng/L.

Genotyping Of Melatonin Receptors Type C (*Mtnr1c*) And Neuropeptide Y (*Npy*) Genes By Pcr-Rflp

Each genotyping experiment involved a 10 µL total reaction volume for the polymerase chain reaction (PCR). The reaction mixture was composed of the following components: 4.1 µL of nuclease-free water, 0.1 µL of Taq DNA polymerase sourced from Promega (San Diego, CA), 0.8 µL of 50 mM MgCl2, 1 µL of 10X PCR buffer, 1 µL of 1 mM dNTPs, 1 µL of each 5 mM primer (Table 1), and 1 µL of genomic DNA at a concentration of 50 ng/mL.

PCR amplification was carried out using a thermal cycler (iCycler thermal cycler, BioLab, USA; Corbett Research, Australia 2003). The process initiated with a pre-denaturation step at 94 °C, followed by 5 minutes. Subsequently, 35 cycles were performed, each comprising denaturation at 94 °C for 30 seconds, annealing at the specified temperature (as provided in Table 1) for 40 seconds, and extension at 72 °C for 30 seconds, and a final extension phase at 72 °C for 5 minutes. The PCR products were stored at 4°C until further analysis.

The PCR product of the *MTNR1C* and *NPY* gene was assessed by electrophoresis on a 2% agarose gel. After 40 minutes of electrophoresis at 100 V, the gel was stained using GELSTARTM (Gelstar Inc, NY) for 10 minutes. Following this, PCR products underwent overnight digestion with the *Mbo*I for *MTNR1C* gene and *Dra*I for *NPY* gene at a prescribed temperature. Restriction patterns were then visualized through 2.5% agarose gel electrophoresis, staining with GELSTARTM (Gelstar Inc, NY) and documentation using Gel Documentation equipment from Lab Focus, Inc. This method enabled the accurate identification of genotypes.

Statistical Analysis

Proc means by SAS (Statistical Analysis System, Version 9.4, 2019) was used to analyze all of the egg production data in all Thai indigenous chickens (n=300), which was provided as mean and standard deviation (SD).

The chickens were divided into two groups (HEP and LEP) based on the NE360d characteristic, and the means were checked for significance using t-tests to examine the relevance between the LEP and HEP groups. A significant statistical difference was defined as one with a $P < 0.05$. Comparisons of genotype frequencies between the HEP and LEP group were analysed by chi-squared test ($\chi 2$) in SAS software V9.4. Figure 1 illustrates the full process for finding genes that influence traits associated with egg production.

Table 1: Information about the primer used in polymerase chain reaction tests

Gene	Location (bp)	[1/]C.	GeneBank	Primer sequence	[2/] Leng. (bp)	[3/] T ([0]C)	Enzyme
MTNR1C	G294A	4	JQ249896	F: GGTGTATCCGTATCCTCTAA	372	58	MboI
				R: GACAGTGGGACAATGAAGT			
NPY	4bp del 494-499	2	M87298	F: TCTCAGAGCTCCAACGTATGA	240	60	*Dra* I
				R: ATATTTCTGTGCCTGAACAACA			

[1/] C. is chromosome; [2/] Leng. is the length of PCR products; [3/] T is annealing temperature.

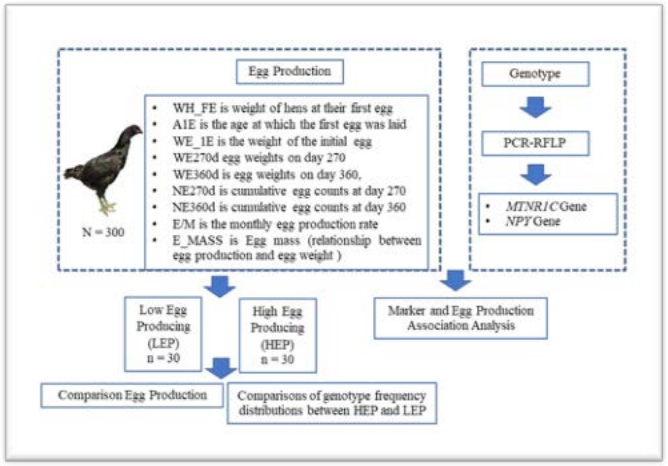

Figure 1: The entire method for identification of *MTNR1C* and *NPY* genes on egg production in Thai indigenous chickens

Marker And Traits Association Analysis

At each locus, genotype and allele frequencies were determined. Chi-square ($\chi 2$) was used to test for Hardy-Weinberg Equilibrium (HWE) and polymorphism information content (PIC) (Falconer and Mackay, 1996).

Using the least-squares method, the relationships between genotype and characteristics of egg production were investigated (GLM Procedure, Statistical Analysis System, Version 9.4, 2019). The following assumptions were made about the model that was used to analyze the data:

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

where Y_{ij}: the Thai indigenous chicken trait measurements, μ: the population mean values for the traits, G_i: the fixed effects linked to the genotype (*MTNR1C and NPY*) and e_{ij}: residual random error.

RESULTS

Egg Production Performance Of Thai Indigenous Chickens

Table 2 provides a comprehensive overview of the descriptive statistics pertaining to egg production traits in Thai indigenous chickens. These traits encompass a range of parameters, including WH_FE, A1E, WE_1E, WE270d, WE360d, NE270d, NE360d, E/M, and E_MASS. The result showed the dataset of all Thai indigenous chickens reports the mean values, accompanied by their corresponding standard deviations (SD), for these egg production traits: WH_FE: 1,938.81 (187.03) g, A1E: 154.25 (11.40) days, WE_1E: 34.18 (5.99) g, WE270d: 44.60 (3.47) g, WE360d: 45.10 (3.44) g, NE270d: 153.53 (31.90) eggs, NE360d: 188.16 (29.18) eggs, E/M: 15.68 (2.43) egg and E_MASS: 41.36 (7.46) g per hen per day.

Moreover, these characteristics are meticulously compared across two different groups, especially the high egg production (HEP) and low egg production (LEP) groups. The results of this thorough comparative research show that the HEP and LEP groups had highly significant differences in NE270d, NE360d, and E/M ($P < 0.01$). However, there were no statistically significant differences in the variables WH_FE, A1E, WE_1E, WE270d, WE360d, and E_MASS between two comparison groups.

Identified Genotype, Allele And Genotype Frequencies

The study identified two candidate genes, *MTNR1C* and *NPY*, in Thai indigenous chickens, with PCR product sizes of 372 bp and 248 bp, respectively. Subsequently, the PCR products underwent digestion with *Mbo*I for *MTNR1C* and *Dra*I for *NPY*, revealing distinctive restriction fragment length polymorphism (RFLP) patterns. For *MTNR1C*, three genotypes were identified: genotype AA (372 bp), genotype AG (372 bp and 333 bp), and genotype GG (333 bp) (Figures 2A). For *NPY* exhibited three genotypes as well: genotype BB (240 bp), genotype Bb (240 bp, 161 bp, and 79 bp), and genotype bb (161 bp and 79 bp) (Figures 2B).

Table 3 presents the distribution of genotype and allele frequencies. In the case of *MTNR1C*, genotype frequencies ranged from 0.26 to 0.48, with allele A and allele G occurring at equal frequencies (0.50 each). *NPY* gene analysis revealed allele frequencies of 0.28 for allele B and 0.72 for allele b. The most prevalent genotype for *NPY* was bb (0.58), while genotype frequencies for Bb and BB were

Table 2: Characteristics of egg production in Thai indigenous chickens and comparison between HEP and LEP groups

Egg Production	All (n = 300)	SD	Group of egg production		
			HEP (n = 30)	LEP (n = 30)	P-value
WH_FE, g	1,938.81	187.03	1,866.16	1,961.13	ns
A1E, day	154.25	11.40	151.33	153.80	ns
WE_1E, g	34.18	5.99	32.72	36.01	ns
WE270d, g	44.60	3.47	44.22	45.14	ns
WE360d, g	45.10	3.44	45.51	45.41	ns
NE270d, egg	153.53	31.90	191.03	111.60	**
NE360d, egg	188.16	29.18	237.43	133.43	**
E/M, egg	15.68	2.43	19.78	11.11	**
E_Mass, g/hen/day	41.36	7.46	208.66	206.20	ns

WH_FE is weight of hens at their first egg, A1E is the age at which the first egg was laid, WE_1E is the weight of the initial egg, WE270d egg weights on day 270, WE360d is egg weights on day 360, NE270d is cumulative egg counts at day 270, NE360d is cumulative egg counts at day 360, E/M is the monthly egg production rate and E_MASS is Egg mass; ** is extremely significant difference ($P < 0.01$), and ^{ns} is non-significant difference

Table 3: Genotype and allele frequencies for polymorphisms

Gene	N	Genotype frequency			Allele frequency		Chi-Square	PIC
		AA	AG	GG	A	G		
MTNR1C	300	0.26 (77)	0.48 (145)	0.26 (78)	0.50	0.50	0.33	0.37
		BB	Bb	bb	B	b		
NPY	300	0.13 (39)	0.29 (88)	0.58 (173)	0.28	0.72	21.11	0.32

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

Table 4: Comparisons of genotype frequency on *MTNR1C* and *NPY* distributions between HEP and LEP groups

Gene	N	Genotype frequency			Allele frequency		Chi-Square
MTNR1C		AA	AG	GG	A	G	ns
HEP	30	0.17(5)	0.47(14)	0.37(11)	0.40	0.60	
LEP	30	0.33(10)	0.47(14)	0.20(6)	0.57	0.43	
NPY		BB	Bb	bb	B	b	*
HEP	30	0.17(5)	0.53(16)	0.30(9)	0.16	0.84	
LEP	30	0.03(1)	0.30(9)	0.67(20)	0.18	0.82	

* represented significant difference between HEP and LEP group ($P<0.05$); ns represented non-significant difference between HEP and LEP group ($P>0.05$)

Table 5: Association between polymorphisms in *MTNR1C* and *NPY* genes and egg production traits in Thai indigenous chicken

Gene	Trait	Genotype			P-value
		AA	AG	GG	
MTNR1C	WH_FE, g	1,941.74	1,946.28	1,922.03	0.60
	A1E, day	155.63	153.65	153.98	0.46
	WE_1E, g	34.19	34.66	33.27	0.30
	WE270d, g	44.68	44.89	44.00	0.18
	WE360d, g	45.83 ^{a}	45.15 ^{ab}	44.29 ^{b}	0.02
	NE270d, egg	152.58	152.24	156.85	0.81
	NE360d, egg	181.09 ^{b}	187.91 ^{ab}	195.61 ^{a}	0.02
	E/M, egg	15.09 ^{b}	15.65 ^{ab}	16.30 ^{a}	0.02

	E_Mass, g/hen/day	40.75	41.25	42.17	0.71
NPY		BB	Bb	bb	
	WH_FE, g	1,899.33	1,969.08	1,932.33	0.11
	A1E, day	153.10	153.35	154.96	0.45
	WE_1E, g	32.49	34.36	34.47	0.19
	WE270d, g	43.83	45.04	44.56	0.18
	WE360d, g	44.57	45.13	45.21	0.75
	NE270d, egg	163.25^{A}	160.10^{A}	148.00^{B}	0.002
	NE360d, egg	203.33^{A}	194.27^{A}	181.64^{B}	0.0001
	E/M, egg	16.94^{A}	16.18^{A}	15.13^{B}	0.0001
	E_Mass, g/hen/day	44.06^{A}	42.44^{AB}	40.20^{B}	0.005

WH_FE is weight of hens at their first egg, A1E is the age at which the first egg was laid, WE_1E is the weight of the initial egg, WE270d is egg weights on day 270, WE360d is egg weights on day 360, NE270d is cumulative egg counts at day 270, NE360d is cumulative egg counts at day 360, E/M is the monthly egg production rate and E_MASS is Egg mass

0.29 and 0.13, respectively. Furthermore, Hardy-Weinberg equilibrium (HWE) testing revealed that the *MTNR1C* alleles were in Hardy-Weinberg equilibrium while the *NPY* genes did not conform to HWE and did not meet the equilibrium assumption. The Polymorphic Information Content (PIC) values calculated for *MTNR1C* and *NPY* genes were similar, at 0.37 and 0.32, respectively. These results indicate that both *MTNR1C* and *NPY* genes exhibit moderate levels of polymorphism in Thai indigenous chickens (Table 3).

Comparisons of genotype frequency distributions of the *MTNR1C* and *NPY* genes between HEP and LEP group in Thai indigenous chickens are shown in Table 4. The result showed *NPY* gene presenting significant difference ($P < 0.05$) between HEP and LEP, while, no significant difference was found at *MTNR1C* ($P > 0.05$). Interestingly, the frequencies of genotype BB at the *NPY* gene in the HEP group have an interesting genetic influence on egg production in Thai indigenous chicken populations. These particular genotypes may be favorable for Thai indigenous chicken to produce greater eggs.

Association Of Variations In Melatonin Receptors Type C (*Mtnr1c*) And Neuropeptide Y (*Npy*) Genes With Egg Production Traits

The study into the associations between polymorphisms in two candidate genes, *MTNR1C* and *NPY*, and various egg production traits in Thai indigenous chickens, as summarized in Table 5. The findings revealed significant associations between *MTNR1C* and *NPY* gene polymorphisms and key egg production traits, namely WE360d, NE270d, NE360d, E/M, and E_Mass. Particularly noteworthy was the highly significant association ($P < 0.01$) observed between *NPY* gene polymorphisms and NE270d, NE360d, E/M, and E_Mass. Additionally, significant effects of *MTNR1C* polymorphism were detected on WE360d, NE360d, and E/M ($P < 0.05$).

Specifically, Thai indigenous chickens carrying the AA genotype in the *MTNR1C* gene exhibited higher values for WE360d compared to those carrying the GG genotype. However, no statistically significant difference was observed in WE360d between chickens with AA and AG genotypes. Interestingly, the GG genotypes of *MTNR1C* were associated with higher values of NE360d and E/M (195.61 and 16.30 eggs, respectively) in contrast to the AA genotype (181.09 and 15.09 eggs, respectively). Furthermore, the AG genotypes displayed NE360d and E/M values of 187.91 and 15.65 eggs, respectively, with no significant difference from the GG genotype (Table 5).

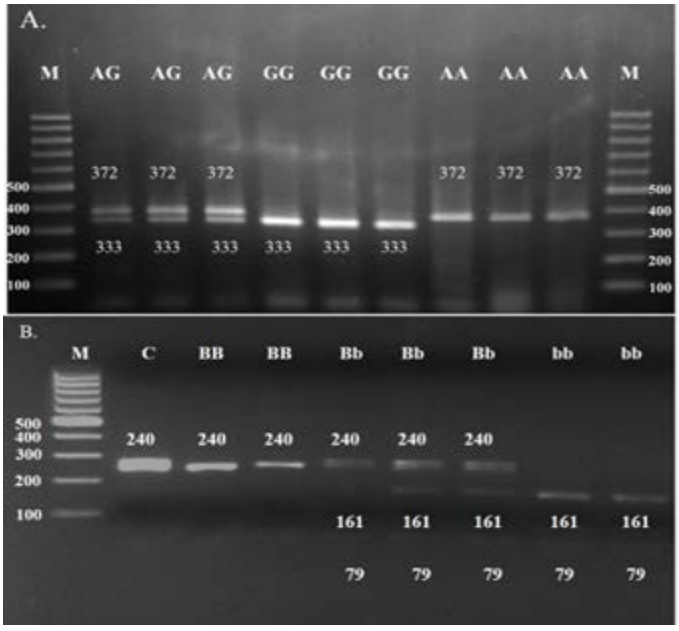

Figure 2: Genotypes of (A) *MTNR1C* gene (M: 100 bp marker; AA: 372 bp; AG: 372 bp + 333 bp and GG: 333 bp) and (B) *NPY* gene (M: 100 bp marker; C: PCR product; BB: 240 bp; Bb: 240 bp +161 bp + 79 bp and bb:161 bp + 79 bp)

Regarding the *NPY* gene, the mean NE270d value for Thai indigenous chickens with the BB genotype (163.25 eggs) and Bb genotype (160.10 eggs) was significantly higher than for Thai indigenous chickens with the bb genotype (148.00 eggs) ($P < 0.01$). The bb genotype exhibited lower values for NE360d and E/M (181.64 eggs and 15.13 eggs, respectively). Conversely, the BB and Bb genotypes demonstrated the highest values for NE360d (203.33 and 194.27 eggs) and E/M (16.94 and 16.18 eggs). Furthermore, the BB genotypes were associated with higher E_Mass values (44.06 g/hen/day) compared to the bb genotype (40.20 g/hen/day) ($P < 0.01$).

DISCUSSION

Indigenous chickens stand as crucial genetic assets in the context of developing nations, serving as a linchpin for ensuring food security (Chomchuen et al., 2022). These indigenous chicken breeds primarily inhabit small-scale poultry production systems, predominantly within rural regions of low and medium-income countries. Consumers are becoming more interested in items from safe animal production such as animal welfare and reduced use of antibiotics. As a result, the indigenous chicken is now the one that is expanding the fastest for these organic foods. One of the key advantages possessed by indigenous chicken, showed the indigenous chicken meat tends to have low fat content and cholesterol, ability to thrive with minimal resources, encompassing basic housing, food, and veterinary services, highlights their suitability for resource-constrained settings (Promket and Ruangwittayanusorn, 2021; Luvanga and Kashoma., 2022; Sari et al., 2023). Moreover, their remarkable resistance to heat stress, their immune responses and maintain metabolic functions of blood cells even in the face of challenging environmental conditions (Pantaya et al., 2021).

Important economic characteristics for chickens include egg production, which is complex and controlled by the genes, environment, or interactions between both of them (Lien et al., 2020; Dodamani et al., 2023). Other research asserted the benefit of selecting for features that increase egg production, considering the higher genetic diversity and predictable selective response. As previously reported in other avian species, the heritability estimates for the egg production traits ranged from 0.18 for egg number at 66 weeks to 0.26 for egg number at 43 weeks (Yang et al., 2023). Moreover, the monthly egg production of native chickens was shown to have a heritability of 0.15, indicating that a significant portion of the variation in egg production is caused by genetic variables (Loengbudnark et al., 2023). According to the previously research reported, the heritability of egg production trait is low to medium. The usual breeding approach was ineffectual because egg production was regulated by polygenes with low to moderate estimated heritability ranging from 0.15 to 0.26. As a result, molecular assisted selection emerges as a potent tool for enhancing the economic advantages and features associated with egg production.

One of the main techniques for figuring out whether specific genetic markers are associated to economically significant features in chickens is the research of candidate genes. Candidate genes can be used to examine the genetic bases of complex traits. The polymorphism analysis of egg production from different breeds of chickens identified functional genes, which is closely related to egg production. These findings suggest that by understanding the molecular regulatory mechanisms underlying egg production, it is possible to genetically improve egg production traits in indigenous chicken breeds. Marker and trait association analysis has also shown significant differences between genotypes for egg production, indicating the potential use of molecular markers in chicken breeding programs. This study investigated the effects of the genes *MTNR1C* and *NPY* on egg production and showed a relationship between genes and the phenotype. According with the previous studies shown that candidate genes such *MTNR1C* and *NPY* controlled the production of eggs in native chickens (Majid et al., 2019; Tenzin et al., 2020; Israa et al., 2021).

The RFLP method used in this study determined that the size of the DNA fragment from the *MTNR1C* (372 bp) and *NPY* (240 bp) genes was the same as that reported by Israa et al. (2021) and Bora et al. (2023). Moreover, there are three genotypes of the *MTNR1C* gene in chickens: AA, AG, and GG, with AG having the greatest genotype frequency. Furthermore, genotype frequency of bb was higher than BB and Bb of *NPY* gene. Allele b of the *NPY* gene shown larger proportions than allele B, while alleles A and G for the *MTNR1C* gene are equivalent in this study. According to Israa et al. (2021), in local Iraqi chicken, heterozygous genotypes of *MTNR1C* gene are more common than homozygous genotypes. Bora et al. (2023) found two genotypes (AG and GG) for the *MTNR1C* gene in the Zo-ar chicken population, with the G allele being predominantly present. The genotype frequencies for the bb variant of the *NPY* gene in this study were found to be significantly different from those previously observed in native chickens from Mazandaran and local brown chickens from Iraq. In contrast to findings reported by Majid et al. (2019) and Fatemi et al. (2012), the *NPY* gene showed a higher prevalence of the BB genotype than the Bb and bb genotypes, the explanation is that difference chicken populations.

The population was found to conform to the Hardy-Weinberg Equilibrium (HWE) at the *MTNR1C* locus. The

MTNRC gene reached medium polymorphism in our study and retained the HWE because of the G_0 flock populations and selection for traits linked to egg production. Over the course of a 2-month cumulative egg production period, they received phenotypic selection with a low level of selection intensity. Selection had no impact on this gene because it was present in HWE. The PIC value is frequently employed to evaluate the polymorphism of allele fragments. In this study, the PIC values for the *MTNR1C* and *NPY* genes indicated that the loci were moderately informative (0.32-0.37). Strong, medium, or low locus polymorphism, respectively, are indicated by PIC > 0.5, PIC > 0.25-0.50, or PIC < 0.25 (Qi et al., 2022). The *NPY* genes frequencies did not conform to the HWE. According to Kubota et al. (2019), the migration, mutation, mating system, genetic linkage, selection, genetic drift, and population structure all have an impact on HWE.

The genetic makeup that controls egg production traits in chickens has been the focus of many studies utilizing candidate gene polymorphism markers. The effect of the melatonin receptor 1C (*MTNR1C*) gene on egg production in chickens has been studied in multiple papers. The present study findings suggest that the *MTNR1C* in the Thai indigenous chicken population were an important role in the regulation of egg weights and cumulative egg counts at day 360 and monthly egg production rate. Recent experiments have been conducted to investigate genetic variation within the *MTNR1C* gene on egg production traits in the India native chicken (Zo-ar chicken) and explore the possibility of genetic improvement in egg production (Bora et al., 2023). The single nucleotide polymorphisms (SNPs) has been conducted to understand the molecular mechanisms underlying the differences in egg number of Thai native chickens, resulting in the identification of SNPs and *MTNR1C* genes related to egg number at 300 day and reproductive traits (Tenzin et al., 2020). In sample of local Iraqi chickens, identified a specific polymorphism (JQ249896: g.294G>A) within the melatonin receptor *MTNR1C* gene. Notably, we observed statistically significant associations between this *MTNR1C* and several reproductive traits. These included egg number at 100 days, as well as body weight at the first egg. These findings underscore the potential utility of the *MTNR1C* gene as a valuable marker for enhancing genetic breeding programs in local Iraqi chicken populations through marker-assisted selection. The hormone melatonin, regularly referred to as N-acetyl-5-methoxytryptamine, is produced in the pineal gland and plays an essential function in a variety of physiological and reproductive activities in addition to controlling the circadian rhythm of the body. *MTNR1C*, the third melatonin receptor, is not found in mammals but only in birds and amphibians. The last two are typical to animals. *MTNR1C* is a melatonin-binding G-protein coupled receptor. Addition, eating habits, thermoregulation, circadian rhythm, and neuroendocrine activities are all regulated by the *MTNR1C* in chickens (Bora et al., 2023). Chickens' ovaries, ovarian follicular fluid, and granulose layer of cells were likewise found to contain the *MTNR1C* gene, indicating a potential role for melatonin and its.

The *NPY* gene has been identified as a potential candidate gene associated with egg production traits in Thai indigenous chicken. Within the *NPY* gene, we observed three distinct genotypes, namely BB, Bb, and bb. Our comprehensive statistical analysis revealed noteworthy findings. Specifically, we uncovered significant associations between these *NPY* gene genotypes and key egg production traits. *NPY* genes affect the neuropeptide that helps control a chicken's feed intake and reproductive processes. It can boost appetite and increase food consumption since it is expressed in the skeletal muscles of birds. As a neuromodulator in the central nervous system, *NPY* affects pathways involved in food intake and stress response (Cattaneo et al., 2021). *NPY* contributes to the growth of adipose tissue and the buildup of lipids in avian species, demonstrating its function in fat storage. Therefore, *NPY* might play a coordinating role in chicken reproduction and puberty timing. The association between the *NPY* gene and egg production has been observed in different chicken breeds, including the Iraqi local brown chicken flock and Mazandaran native chickens (Fatimi et al., 2012; Majid et al., 2019; Majid, 2021). A previous study from Promket et al. (2023) reported that variations in the *NPY* gene have been identified as potential markers for breeding to increase egg number in Thai native chickens. These findings suggest that chickens carrying the BB genotype exhibited markedly higher levels of egg production when compared to individuals with the bb genotype. Additionally, the *NPY* gene greatly altered body weight at sexual maturity and is linked to both growth performance and egg production, suggesting its potential as a genetic marker for controlling ovulation and influencing egg production rates (Fatimi et al., 2012). In summary, the study revealed significant associations between genetic polymorphisms in the *MTNR1C* and *NPY* genes and various egg production traits in Thai indigenous chicken. These findings underscore the potential influence of genetic variations in these candidate genes on egg production performance, providing valuable insights for breeding programs aimed at enhancing egg production in this Thai indigenous chicken population.

CONCLUSIONS

In conclusion, our research provides compelling evidence affirming that genetic variations within the *MTNR1C* and *NPY* genes play a significant role in the variability on egg production traits among Thai indigenous chickens. This

comprehensive study has established associations between genetic polymorphisms within the *MTNR1C* gene and WE360d, NE360d and E/M traits. For *NPY* genes had significant effected on NE270d, NE360d, E/M, and E_Mass. These findings remarkable influence wielded by specific gene variants over the egg production performance of Thai indigenous chickens. The implications of these findings for poultry breeding programs are of profound significance. Implementing selective breeding strategies based on *MTNR1C* and *NPY* gene variants holds the potential to substantially enhance the egg production capabilities of Thai indigenous chicken populations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

NOVELTY STATEMENT

The construction of a breeding program using Thai native chickens may be made possible by this study, which focuses on the genotype of *MTNR1C* and *NPY* in Thai Indigenous Chickens.

AUTHOR CONTRIBUTIONS

Doungnapa Promket: DNA and genotyping gene, analysis and interpretation of data, article writing, and final manuscript version approval are all included in this process.
Khanitta Pengmeesri: Approval of the article, analysis of data and study conception.
Jennarong Kammongkun: Collecting information on egg traits, collecting sample, proofreading the manuscript's last draft.
Thassawan Somchan: Approving the manuscript's final draft.

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