Potential genetic markers and their association with production traits in Thai Pradu Hang Dam and Chee native chickens

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ABSTRACT: Native chickens are increasingly selected for better production and performance as they cannot compete with hybrids and exotic breeds in growth and reproductive traits. Traditionally, phenotypic based selection was used to select animal which took longer to improve the animal and their response to selection was minimal. However, nowadays we can select animals at young age using genetic markers. But we need more genetic markers for a robust selection. Therefore, in this study, we aimed to find the association among MTNR1C, STAT5B, BMP15, and DRD2 with production traits. A sample size of 188 chickens were used with 95 Chee chickens and 93 Pradu Hang Dam chickens. Blood samples were collected from wing vein. The DNA was isolated with modified form of guanidine hydrochloride method. It was genotyped using PCR-RFLP. The GLM procedure was used to compare the least square means between different genotype. Chi-square goodness of fit test was used to test the Hardy-Weinberg equilibrium. All the genes deviated significantly from Hardy-Weinberg equilibrium except DRD2. The MTNR1C was significantly associated with age at first egg (AFE) with a allele giving earlier eggs than G allele in Pradu Hang Dam chickens and subsequent higher egg production. However, we didn't find any association with other genes and productions traits. Moreover, in Chee, we didn't establish any tentative association with all the genes studied and the production traits. We recommend that MTNR1C can be a potential marker for improving egg production traits.
Keywords: Pradu Hang Dam, Chee, Candidate genes, Production traits, Marker-assisted selection

Received December 12, 2018
Accepted June 12, 2019
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Introduction

Pradu Hang Dam (PD) and Chee (CH) chickens are two important indigenous chickens of Thailand (TIC) earmarked as an important contributor to sustainable utilization of native chickens for egg production and meat (Duangjinda, 2015). Moreover, they are adapted harsh tropical environment. In addition, in recent years, consumer's preference for native germplasm has been increasing despite its higher price. They are preferred for its taste, low cholesterol content and firm meaty texture (Jaturasitha et al., 2002). Farmers are however reluctant to rear native chickens unlike commercial breeds as they provide obvious benefit in terms of higher egg production, as well as persistent egg production and higher growth rate. Therefore, breeders and animal scientist need to improve the production performances of native chickens. Traditionally, phenotypic based selection has longer response to selection and higher generation interval. But the modern genetic based molecular approach to selection has removed these barriers. Particularly, candidate gene approach has become significantly important in finding association among genes and their association with production traits (Teneva, 2009). Therefore, in this study, we studied four potential genes (*MTNR1C*, *STAT5B*, *BMP15*, and *DRD2*) as genetic markers for improving egg production.

Melatonin receptor type C (*MTNR1C*), one of the three types of melatonin receptor subtypes which is involved in sexual maturation and ovary development. The tissue expression study found that they are similar to melatonin receptors in neural tissues (Sundaresan et al., 2009). Moreover, Li et al. (2013) found that the melatonin receptors influenced reproductive traits. Signal Activator of Transcription and Transduction (*STAT*s) are shown to be influencing many cytokines and growth hormones (Darnell, 1997). They are found to be related with growth traits as well as reproductive traits (Ou et al., 2009). Bone morphogenetic factors (BMP15) is essential in normal functioning of ovaries in domestic animals (Dube et al., 1998). Also, known as oocyte factor, it serves as growth factor with many functions including bone formation, and follicular growth (Chen et al., 2004). Dopamine influences cognition, emotion and endocrine functions (Cools, 2008) through its receptors like *DRD2*.

The above genes are not studied in Thai PD and CH chickens. Therefore, for a robust selection, many genes are required to arrive at a balanced selection. An objective of this study was to find the association of these genes with production traits in Thai PD and CH chickens.

Materials and methods

Ethical consideration

Institutional Animal Care and Use Committee (IACUC) of Khon Kaen University, Khon Kaen, Thailand, approved the use of animals under this study.

Population and sampling

A total of 188 female chickens were used for this study, of which 93

were Pradu Hang Dam (PD) and 95 were Chee (CH) chickens. A total of 1.5 ml blood sample was collected from wing vein of each individual chicken in a 100 µl of 0.5M ethylenediaminetetraacetic acid (EDTA) tube. The chicken samples were random selected from the PD and CH populations. These chicken populations were maintained at the farm of Research and Development Network Centre for Animal Breeding (NCAB) for Native Chickens at Khon Kaen University, Khon Kaen, Thailand. The production traits for this study were breast circumference of the chicken at 14 days (BC14), body weight at 14 days (BW14), egg number at 240 days (EN240), and age at first egg (AFE).

Genotyping with Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP)

The DNA was isolated using guanidine hydrochloride, a modified method of Goodwin (2011). The quality and quantity of DNA was evaluated using Spectrophotometer ND1000 (Nano-Drop, USA) and density was adjusted to 100 ng/µl with TE buffer and stored at -20 °C for further analysis. The genes were amplified using polymerase chain reaction (PCR). A mixture of 1 µl of genomic DNA, 1 µl of 10X PCR buffer, 1 µl of 2.5 mM of primers (forward and reverse) **(Table 1)**, 1 µl of 1 mM of dNTP, 1 µl of 25 mM $MgCl_2$, 0.1 µl of 5U *Taq* DNA polymerase, and adjust with ddH_2O for 10 µl.

The amplification of DNA by PCR was conducted at 95 °C for initial denaturation for 5 min, and 30 cycles of denaturation at 95 °C for 30 sec, annealing temperature at 60 - 68 °C for 30 sec and extension at 72 °C 5 min and cooled for 1 minute at 25 °C.

Table 1 Primers and enzymes used in the study to find the polymorphisms

Genes	5' - 3' sequence	Tm °C	Enzymes	Sources
MTNR1C	F: GGTGTATCCGTATCCTCTAA R: GACAGTGGGACAATGAAGT	60	*MboI*	Li et al. (2013)
STAT5B	F: TGGAGCTACTGGCATCTCTCA R: TGCTGCAGTTGCTGTGGTCT	68	*HhaI*	Ou et al. (2009)
BMP15	F: TGGAGCTACTGGCATCTCTCA R: TGCTGCAGTTGCTGTGGTCT	68	*Alw21I*	Huang et al. (2015)
DRD2	F: TGCACATAAAAGCCCACTCACTG R: GCCTGAGCTGGTGGGGGG	68	*BseGI/ BtsCI*	Xu et al. (2011)

Genotyping of polymorphism was done using polymerase chain reaction - restriction length polymorphism (PCR-RFLP) and the agarose gel electrophoresis was done with 2 % agarose to detect PCR product using SynGene photography system (SynGene, Madison, WI, USA). Digestion for RFLP was carried out with total of 10 µl solution of which 2 µl was PCR product, 0.1 – 0.3 µl was restriction enzymes, 1 µl of tango buffer

and ddH_2O and incubated overnight at 37 °C for genes except for *DRD2* at 55 °C. The fragments were separated under 2 % agarose gel electrophoresis and stained with GelStar (GelStar Inc, New York, NY, USA) and visualized and photographed under Gel Documentation System standards (SynGene, Madison, WI, USA).

Statistical Analyses

Association of candidate genes and the production traits was performed by using the general linear model (GLM) of SAS 9.2 (SAS Institute Inc, Cary, NC, USA) applying a significance level of $P < 0.05$. The model used is $Y_{ij} = \mu + Gi + H_k + e_{ij}$ where Y_{ij} is production traits, μ is the overall population mean, G_i is the effect of each genotype and H_k is the fixed effect of the hatch and e_{ij} is the error term. The allelic and genotypic was calculated according to (Falconer, 1975). Type III sum of square was used, Tukey HSD was used to test the significance between the LSMeans and a P value of ≤ 0.05 was considered significant.

Results and Discussion

Production Performance of Chee (CH) and Pradu Hang Dam (PD) chickens

The descriptive statistics for the study are shown in the **Table 2.** The breast circumference at 14 (BC14) weeks was 22.66 and 24.21 cm for CH and PD, respectively. The BC14 was higher in case of PD than CH. Breast circumference directly correlates with weight of the animals with greater breast circumferences corresponding to more weight of the animals. The average daily gain of weight of native chicken was 10 gm/day which is far lower compared to commercial chickens 30-50 gm/day (Aengwanich, 2007).

Table 2 Mean, standard deviation, Minimum and Maximum of production data

Breed/Traits	Mean	SD	Min	Max	Breed/Traits	Mean	SD	Min	Max
CH	n = 95				PD	n = 93			
BC14	22.66	1.02	20.9	24.9	BC14	24.21	1.31	21.20	26.30
BW14	925.68	82.06	760	1230	BW14	1112.47	96.65	910	1350
AFE	187.96	16.08	154	229	AFE	207.33	17.60	158	260
EN240	36.13	11.95	9	73	EN240	24.80	11.63	1	64

CH = Chee, PD = Pradu Hang Dam, BC14 = Breast circumference at 14 weeks, BW14 = body weight at 14 days, AFE = age at first egg, EN240 = egg number at 240 days

The age at first is an important indicator for producing a greater number of eggs if the chicken produce eggs at earlier age. In this study, the age at first egg (AFE) is 187.96 and 207.33 days in CH and PD, respectively and the subsequent egg number at 240 days is 36.13 and 24.80 eggs, for the former and later. The cumulative egg number was higher in case of CH than PD chickens. The lower egg number produced by the PD chickens is that the

genetic background of the PD is growth and mostly used as broilers. However, the egg number produced by the Thai indigenous chickens are far lower than commercial chickens at 117 eggs per year compared to more than 300 eggs in exotic breeds (Mookprom et al., 2017).

Genotyping with PCR-RFLP

The DNA was isolated, and samples of PD and CH were genotyped using PCR-RFLP. The genotypic fragment length of various genes is shown in the figure 1.

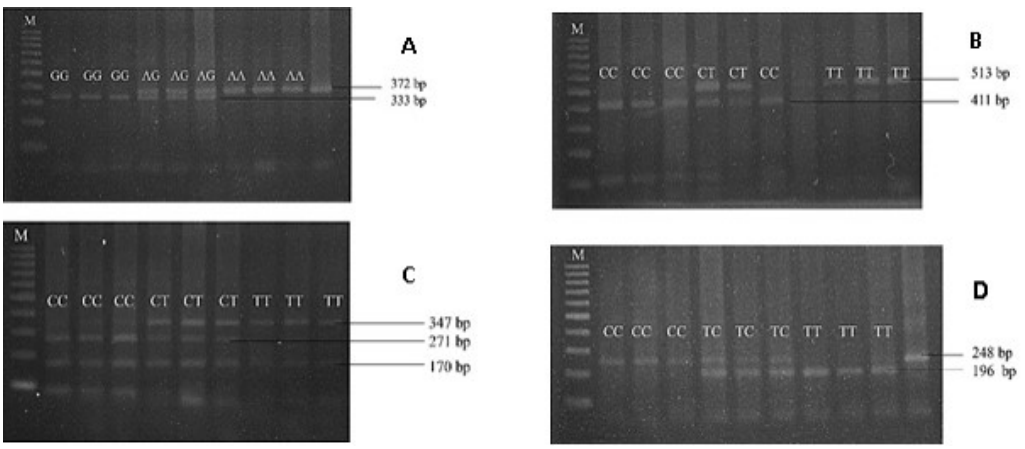

Figure 1 PCR-RFLP and fragment length produced. A is MNTRC (MboI), B is STAT5B (HhaI), C is BMP15 (Alw21I) and D is DRD2 (BSeGI).

Table 3 Genotype and allele frequencies of *STAT5B*, *DRD2*, *BMP15*, and *MTNR1C* in Pradu Hang Dam (PD) and Chee (CH) chickens

Genes	Genotype	Frequency (N)			χ^2			
		PD	CH	Alleles	PD	CH	PD	CH
	AA	0.06 (6)	0.07 (7)	A	0.27	0.2	45.52*	91.65*
MTNR1C	AG	0.40 (37)	0.25 (24)	G	0.73	0.8	-	-
	GG	0.54 (50)	0.67 (64)	-	-	-	-	-
	CC	0.83 (77)	0.36 (34)	C	0.89	0.57	165.71*	6.07*
STAT5B	CT	0.11 (10)	0.41 (39)	T	0.11	0.43	-	-
	TT	0.06 (6)	0.23 (22)	-	-	-	-	-
	CC	0.03 (3)	0.98 (93)	C	0.31	0.99	29.97*	269.23*
BMP15	CT	0.54 (50)	0.01 (1)	T	0.69	0.01	-	-
	TT	0.43 (40)	0.01 (1)	-	-	-	-	-
	CC	0.24 (22)	0.72 (68)	C	0.5	0.85	0.1	113.97*
DRD2	TC	0.51 (47)	0.27 (26)	T	0.5	0.15	-	-
	TT	0.26 (24)	0.01 (1)	-	-	-	-	-

PD = Pradu Hang Dam, CH = Chee, * There is significant deviation of the gene and genotype frequency from Hardy-Weinberg Equilibrium at $P < 0.05$

Allele and genotypic frequencies

The allele and genotypic frequencies are shown in **Table 3**. Except *DRD2,* other genes significantly deviated from Hardy-Weinberg equilibrium. This deviation might be due to the selection that was carried out over 9 generations for the chickens studied for this study. For CH chickens, the allele frequency was less than 2 % and the gene was excluded from further analysis as didn't reach the population threshold of greater than or equal to 2 % (Falconer, 1975).

Table 4 Association of *STAT5B*, *DRD2*, *BMP15*, and *MTNR1C* with egg production and growth traits in Pradu Hang Dam chickens

Genes/Traits	Genotype	AFE (days)	BW14 (g)	BC14 (cm)	EN240 (count)
	AA	203.97 ± 7.55[a]	1144.17 ± 39.54	23.97 ± 0.54	29.27 ± 2.36
MTNR1C	AG	201.78 ± 5.24[a]	1139.78 ± 29.85	24.59 ± 0.41	25.06 ± 4.39
	GG	210.19 ± 5.70[ab]	1118.66 ± 32.99	24.40 ± 0.45	24.77 ± 5.51
	CC	205.76 ± 4.07	110.95 ± 20.39	24.26 ± 0.28	27.09 ± 3.89
STAT5B	CT	202.71 ± 6.49	1106.67 ± 37.92	24.40 ± 0.52	27.42 ± 2.91
	TT	207.48 ± 8.44	1184.99 ± 99	24.30 ± 0.65	24.59 ± 3.80
	CC	202.03 ± 11.03	1133.95 ± 58.06	24.72 ± 0.79	32.39 ± 6.72
BMP15	CT	207.19 ± 4.29	1139.70 ± 25.11	24.07 ± 0.34	23.77 ± 2.90
	TT	206.72 ± 3.92	1128.95 ± 22.44	24.17 ± 0.31	22.94 ± 2.60
	CC	204.06 ± 6.01	1132.18 ± 33.62	24.37 ± 0.46	29.33 ± 4.57
DRD2	TC	204.31 ± 4.71	1140.73 ± 25.19	24.55 ± 0.34	27.20 ± 3.45
	TT	207.57 ± 5.91	1129.70 ± 32.83	24.03 ± 0.45	22.57 ± 3.82

AFE = age at first egg, BW14 = body weight at 14 days, BC14 = breast circumference at 14 days, EN240 = egg number at 240 days; Genotype with superscript is significant at $P \leq 0.05$

Association of the candidate genes with production traits in Chee (CH) and Pradu Hang Dam (PD) chickens

The association of the genes with production traits for PD is shown in Table 4 and for CH in Table 5. There was significant association of between *MTNR1C* with age at first egg (AFE). The chickens with A allele had earlier eggs than the chickens with G allele. Subsequently the number of eggs produced in 240 days was higher in the chickens with A allele. A similar study by Li et al. (2013) found significant association with melatonin receptors with reproductive traits including AFE

and egg number at 300 days. Moreover, it was shown to be expressed in ovarian tissues and involved in ovarian development and sexual maturation (Sundaresan et al., 2009). In mammals, it was also found to be associated with normal reproduction and physiology (Malpaux et al., 2001). Alsiddig et al., (2017) found that melatonin receptor type A (*MTNR1A*), another receptor subtype, was associated with egg production traits in Yangzhou geese. *MTNR1C* is located on chromosome 4 and the quantitative trait loci (QTL) was mapped on the in earlier studies (Tuiskula-Haavisto et al., 2002).

However, in PD as well as Chee, we didn't find any association with other genes and traits. In CH, we didn't find any association among genes and production traits. However, earlier we discussed that the egg production in CH is higher than PD. To find the association among genes and production traits, a greater number of samples can be considered as the effect couldn't be established with small number of samples.

Several studies found association between reproductive and growth traits (Cui et al., 2011; Sadeghi et al., 2012; Ye et al., 2017). *STAT5B* was found to be associated with reproductive and growth traits (Niknafs et al., 2014). *STAT5B* was shown to be influencing sexual maturation and ovary development. *BMP15* is involved in bone formation, ovary development and sexual maturation (Juengel et al., 2004) and the association with egg production trait was reported (Huang et al., 2015). Dopamine with its action through its receptors influences normal physiology including reproduction (Beaulieu and Gainetdinov, 2011). It was found to be associated with early egg production traits Leishu Chickens (Zhu et al., 2015).

Table 5 Association of *STAT5B* and *MTNR1C* with egg production and growth traits in Chee (CH) chickens

Genes/Traits	Genotype	AFE (days)	BW14 (g)	BC14 (cm)	EN240 (Count)
	CC	190.34 ± 3.32	901.23 ± 16 53	23.01 ± 0.32	33.51 ± 2.46
STAT5B	CT	187.15 ± 3.26	938.72 ± 16.23	22.45 ± 0.28	35.87 ± 2.41
	TT	190.36 ± 3.83	929.37 ± 19.08	22.58 ± 0.31	36.66 ± 2.83
	AA	191.70 ± 6.18	927.68 ± 30.78	22.98 ± 0.51	34.10 ± 4.57
MTNR1C	AG	188.39 ± 3.45	910.55 ± 17.17	22.32 ± 0.29	34.93 ± 2.55
	GG	187.76 ± 2.08	931.10 ± 10.36	22.82 ± 0.19	37.00 ± 1.54

AFE = age at first egg, BW14 = body weight at 14 days, BC14 = breast circumference at 14 days, EN240 = egg number at 240 days; Genotype with superscript is significant at $P \leq 0.05$

However, in this study, we couldn't establish the tentative association of *STAT5B, BMP15* and *DRD2* genes with production traits.

Conclusions

We aimed to find the preliminary association among *MTNR1C*, *STAT5B*, *BMP15*, *DRD2* genes and production traits. We found that the *MTNR1C* was associated with AFE in the PD chickens with AA genotype giving earlier eggs than the GG genotype. However, we couldn't establish any association with other genes and production traits. Moreover, in CH chickens we couldn't find any tentative association between the genes and production traits despite higher egg production when compared to PD. The *MTNR1C* can be considered as gene marker to produced earlier eggs in Pradu Hang Dam chickens. Therefore, we recommend *MTNR1C* as a potential marker for improving egg production in Pradu Hang Dam chickens.

Acknowledgements

This research was granted by Thailand International Cooperation Agency (TICA).

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