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Identification of new position of DNA insertion and 24 bp INDEL mutation polymorphism in *Prolactin* gene promoter of Thai native chickens

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Abstract This research identified a 24-bp INDEL (-358) on the promoter region of the *PRL* gene in Thai native chickens. The allele frequencies for allele I and allele D were 0.20 and 0.80, respectively. The genotype frequencies were II (0.028), ID (0.349), and DD (0.622), respectively. The heterozygosity values comprised the *Ho* (0.350), higher than the *He* value (0.323). Moreover, our findings revealed new DNA duplication on the *PRL* promoter region, with three distinct polymorphisms of 30 bp (-358), 24 bp (-358), and 14 bp (-358), respectively.

Keywords: *Prolactin* promoter, INDEL mutation, 24 bp polymorphism, DNA duplication, Chicken

Introduction

Prolactin (PRL) is a protein evolved by lactotroph cells in the anterior pituitary gland. It plays a role in reproductive functions and incubation behavior in many poultry species, such as chickens, turkeys, birds, and ducks (El Halawani *et al*., 1997). While hens laid eggs, the levels of PRL hormone could not induce the hens to hatch the eggs, but the levels of PRL hormone that were induced to be increased caused the hens to stop laying and the induction of the hatching behavior (Pitts *et al*., 1994). PRL hormone is managed by stimulation of the neurotransmitter Vasoactive Intestinal Peptide (VIP) via VIPergic receptors activated sequentially within the VIPergic system. VIP is released from the infundibular nucleus complex (INF) through the hypothalamic-pituitary portal vessel to the mid-pituitary gland, where VIP receptors are located on lactotroph

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cells and inhibit the secretion of Gonadotropin-releasing hormone (GnRH) and Luteinizing hormone (LH) (El Halawani *et al*., 2000; Chaiseha *et al*., 2012).

In birds, the *PRL* gene is located on chromosome 2 chicken PRL is composed of 9.536 bp and separated into 3 parts, including 3 putative promoter1 (330 bp), promoter2 (287 bp) and promoter3 (314 bp) respectively (314 bp) respectively (Au and Leung, 2002; Kansaku *et al*., 2001). The exon part is exon1 (81 bp), exon2 (182 bp), exon4 (180 bp) (Ohkubo *et al*., 2000) exon3 (59 bp) (Miao *et al*., 1999), and exon5 (418 bp) (Cui *et al*., 2004). Four intron parts are intron1 (714 bp), intron2 (406 bp), intron4 (744 bp) (Dhara and Soller, 1999), and intron 3 remains unknown.

The *PRL* gene promoter is most important because it functions as an early activator for the transcription of *PRL* gene expression (Lewin, 1997). Mutations occurring in the promoter region of the *PRL* gene prevent the *PRL* gene expressed to fail brooding behavior, thus increasing egg production (Lumtauw and Mu'in, 2016). Various reports have identified 24-bp polymorphism (INDEL) of the *PRL* gene promoter, which is significantly associated with egg production (Jiang *et al*., 2005; Cui *et al*., 2006; Lumatauw and Mu'in, 2016). Moreover, single nucleotide polymorphisms (SNPs) on *PRL* gene promoters are recorded for egg production traits (Cui *et al*., 2006). This study aimed to detect novel polymorphisms and identify genotype and allele frequencies of 24 bp polymorphisms at position -356 of the *PRL* gene promoter region of Thai native chicken.

Materials and methods

Blood sample

A 22-gauge needle was used to puncture the underside of the wings of 106 female native chickens in Narathiwat Province, and 1 ml of blood was extracted and placed in a microcentrifuge tube with 0.05 mL of 0.5 M EDTA. After being shaken, the sample was used or kept at -20 °C.

Genomic DNA extraction

The genomic DNA extraction method was adapted from Goodwin et al. (2007), with modifications including the incubation of female chicken blood samples at 25 °C for approximately 30 minutes. A volume of 0.05 mL of the blood sample was transferred into a microcentrifuge tube, followed by the addition of 0.5 µL of 0.9% NaCl. The tube was gently inverted and centrifuged for 5 minutes. The supernatant was discarded, and the pellet was retained; this step was repeated twice.

Next, 0.3 mL of Solution I (0.03 M Tris-base, 0.20 M sucrose, 0.10 M sodium chloride, and 0.01 M EDTA, pH 8.0) was added and shaken vigorously. Then, 0.6 mL of Solution II (0.05 M Tris-base, 0.05 M EDTA, pH 8.0, and 2.5% SDS) was added and mixed vigorously. The mixture was incubated at 65 °C for 15 minutes. Subsequently, 0.3 mL of Solution III (prepared by mixing 60 mL of 5.0 M sodium acetate and 11.5 mL of glacial acetic acid) and 0.2 mL of Solution IV (a 1:1 mixture of chloroform and saturated phenol) were added, followed by incubation at -20 °C for 10 minutes.

After incubation, the sample was centrifuged at 14,000 rpm and 4 °C for 15 minutes. The supernatant was transferred to a new tube, mixed with 500 µL of isopropanol, and incubated at -20 °C for 1 hour. Finally, the sample was centrifuged at 14,000 rpm and 4 °C for 10 minutes. The supernatant was discarded and 500 µL of 70% ethanol was added. Centrifuge at 14,000 rpm at 4 °C for 5 min. Dry the DNA precipitate and dissolve the DNA with Nuclease free water. The obtained DNA was tested for quantity and purity using a nanodrop and 1% agarose gel.

Amplification of PRL promoter

The amplification of *PRL* gene contained 1 µl (10 pmol) of DNA, 0.005 ml of 10x Taq buffer, 0.005 ml of 25 mM $MgCl_2$, 0.001 ml of 10 mM dNTPs, 0.001 ml of forward primer (PRL_F TTTAATATTGGTGGGTGAAGAGACA) and reverse primer (PRL_R ATGCCACTGATCCTCGAAAACTC) 10 pmol each. 0.002 mL of Taq DNA Polymerase (recombinant) (Thermo Scientific), and adjust the volume with distilled water to 50 µL. The DNA was then amplified using a Biometra Tpersonal Thermocycler with a preheat temperature of 95°C for 3 min, denature temperature of 95°C for 30 s, annealing temperature of 62 °C for 30 s, extension temperature of 72°C for 20 s, for 40 cycles, and final extension temperature of 72°C for 5 min. The obtained PCR products were then examined for genotype on a 3% agarose gel under UV light.

Cloning and sequencing of PRL promoter

The *PRL* fragment was inserted into the pGEM-T easy vector (Promega) and subsequently transformed into E. coli DH5α. Clones containing the PRL insert were screened using the blue/white colony screening method. LA medium containing ampicillin, IPTG, and X-gal was used. The colonies were grown in an LB medium containing 1 µg/ml ampicillin at 37°C for 16 h. The plasmids were then extracted using the FavorPrep Plasmid Extraction Mini kit from Favorgen Biotech Corp. and the plasmids were sent for nucleotide sequencing analysis at Macrogen, Korea.

Genotype and allele frequencies

Genotypic and allele frequencies of *PRL* gene promoter in female native chicken population in Narathiwat Province were analyzed according to the method of Falconer and Mackay (1996).

Results

Nucleotide sequence analysis

The nucleotide sequence of the *PRL* gene promoter was compared between Thai native chicken and 2 haplotypes of gallus gallus *PRL* gene promoter regions (Accession number: MH745024.1 and MH745025.1) from the NCBI database. The polymorphism of *PRL* promoter region was detected 2 different nucleotide fragments on 3% agarose gel consisting of 130 bp and 154 bp due to polymorphic patterns inserted 24 bp (ACAAGAAGAGACAAGACAAGGAAG) at position -385 on promoter region (Figure 1). Three genotypic mutation patterns on promoter region were insertion (II allele), insertion/deletion (ID allele), and deletion (DD allele), respectively (Figure 2).

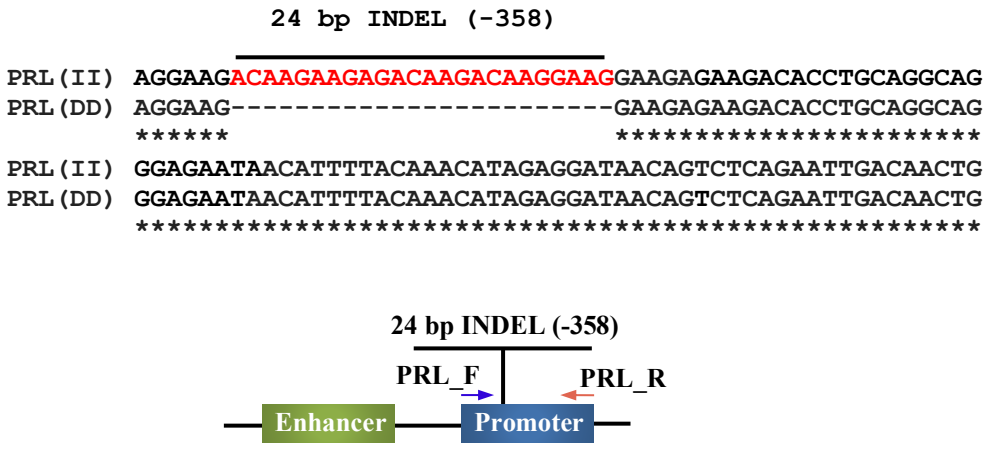

Figure 1. 24 bp INDEL mutation polymorphism at -358 on *PRL* gene promoter of Thai native chicken

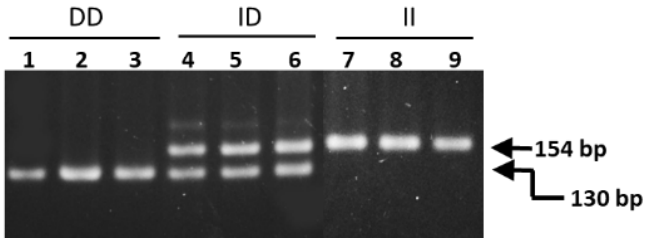

Figure 2. Genotypes of 24 bp Indel at -358 on *PRL* gene promoter of Thai native chicken with 3% agarose gel

Identification of DNA duplication on PRL gene promoter

Based on PCR amplification of *PRL* gene promoter and nucleotide sequence, was found DNA duplication 3 sites at position -333 (30 bp), -329 (24 bp), and -327 (14 bp), respectively (Figure 3).

```
                                                24 bp INDEL (-358)
MH745024.1      TTTAATATTGGTGGGTGAAGAGACAAGGAAGACAAGAAGAGACAAGACAAGGAAGGAAGA  60
MH745025.1      TTTAATATTGGTGGGTGAAGAGACAAGGAAG------------------------GAAGA  36
dah1_PRL        TTTAATATTGGTGGGTGAAGAGACAAGGAAG------------------------GAAGA  36
g1_PRL          TTTAATATTGGTGGGTGAAGAGACAAGGAAG------------------------GAAGA  36
g2_PRL          TTTAATATTGGTGGGTGAAGAGACAAGGAAG------------------------GAAGA  36
                                      ******                            *****

MH745024.1      GAAGACACCTGCAGGCAGGG------------------------------AGAATAACA  89
MH745025.1      GAAGACACCTGCAGGCAGGG------------------------------AGAATAACA  65
dah1_PRL        GAAGACACCTGCAGGCAGGGAGAATAACATTT-ACAAACATAGAGGATAGTAGAATAACA  95  <- 30 bp (-333)
g1_PRL          GAAGACACCTGCAGGCAGGGAGAATAACATTT-ACAAACATAGAGGATA------TAACA  89  <- 24 bp (-329)
g2_PRL          GAAGACACCTGCAGGCAGGGAGAATAACATTTTACAAACA----------------ACA  79  <- 14 bp (-327)
                ********************                                  ***

MH745024.1      TTTTACAAACATAGAGGATAACAGTCTCAGAATTGACAACTGGAGTTTTCGAGGATCAGT  149
MH745025.1      TTTTACAAACATAGAGGATAACAGTCTCAGAATTGACAACTGGAGTTTTCGAGGATCAGT  125
dah1_PRL        TTTTACAAACATAGAGGATAACAGTCTCAGAATTGACAACTGGAGTTTTCGAGGATCAGT  155
g1_PRL          TTTTACAAACATAGAGGATAACAGTCTCAGAATTGACAACTGGAGTTTTCGAGGATCAGT  149
g2_PRL          TTTTACAAACATAGAGGATAACAGTCTCAGAATTGACAACTGGAGTTTTCGAGGATCAGT  139
                ****************************************

MH745024.1      GGCAT 154
MH745025.1      GGCAT 130
dah1_PRL        GGCAT 160
g1_PRL          GGCAT 154
g2_PRL          GGCAT 144
```

Figure 3. Nucleotide alignment of *PRL* gene promoter region of 3 samples (dah_PRL, g1_PRL, and g2_PRL) with gallus gallus haplotype 1 and 2 *PRL* gene promoter region (accession number, MH745024.1, and MH745025.1) from NCBI database showing one DNA insertion region and tree DNA duplication regions. The 24 bp (-358) INDEL mutation polymorphism is the red color letter. The DNA duplication at position -333 (30 bp), -329 (24 bp), and -327 (14 bp) are blued color letters

Frequencies of alleles and genotypes for the 24 bp INDEL mutation at location -358

The number of 106 female Thai native chickens found the pattern of genotype type II in 3 samples, ID in 37 samples, and DD in 66 samples. The allele frequency found that allele D was more common (common allele) with a value of 0.80, while allele I was less common with a value of 0.20. The genotype frequencies of forms II, ID, and DD were 0.028, 0.349, and 0.622, respectively (Table 1). The calculated heterozygosity values for Thai native chickens indicate that the observed heterozygosity (0.350) surpassed the expected heterozygosity (0.323) (Table 2).

Table 1. Alleles and genotypes frequencies of *PRL* gene promoter of Thai native chicken

Gene	Allelic frequency		Genotypic frequency		
	I	D	II	ID	DD
PRL promoter	0.20	0.80	0.028	0.349	0.622

Table 2. Estimate of heterozygosity of *PRL* gene promoter of Thai native chicken

Gene	Observed Hetrozygosity (H_o)	Expected Hetrozygosity (H_e)	χ^2 value of the Hardy-Weinberg Law	*P*-value
PRL promoter	0.350	0.323	0.668	0.284

Discussion

Prolactin is a protein secreted by lactotroph cells in the anterior pituitary gland and plays a role in reproductive functions and incubation behavior in many poultry species. Mutations in the *PRL* promoter region, in accord with Lumatauw and Mu'in (2016), prevent the *PRL* gene from being expressed, which enhances egg production by inhibiting the behavior of brooding. The *PRL* promoter is the most important gene because it functions as an early activator for transcription of *PRL* gene expression (Lewin, 1997).

Many research have been reported of 24 bp INDEL mutation at promoter of *PRL* gene in chickens. Results showed that I and D allele frequencies were 1 and 0; 0.05 and 0.95; 0.20 and 0.80; 0.22 and 0.78; 0.17 and 0.83, respectively. Subsequent investigation revealed that the existence of the I allele had a beneficial impact on egg production, and that a polymorphic of 24 bp INDEL was substantially connected with egg production (Cui *et al*., 2005). In this

research, Thai native chickens were present 0.2 and 0.8. The heterozygosity values were presented *Ho* higher than *He*. The heterozygosity is a measure used to evaluate the degree of genetic diversity in a population based on the frequency of alleles at the locus. (Noor, 2010). According to Rohmah *et al.* (2022), a population is diversified with significant heterozygosity, as evidenced by observed heterozygosity values that are higher than expected.

A 24 bp insertion in the *PRL* gene promoter is the main genetic factor responsible for the White Leghorn chicken breed's non-broody disposition. After a 24 bp nucleotide sequence is inserted in the PRL gene's promoter region, a possible binding site for the ecotropic viral integration site-1 encoded factor (Evi-1) is discovered in the 5' flanking region of the PRL gene in chicken. (Cui *et al*., 2005). Numerous *PRL* gene transcription has been demonstrated to be repressed by Evi-1 (Vinatzer *et al*., 2001 and Izutsu *et al*., 2002). According to reports, Evi-1 inhibits the expression of the *PRL* gene in White Leghorn hens by attaching to the Evi-1 binding site and further preventing brooding, which may somewhat increase producing egg (Jiang *et al*., 2005).

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References

Au, W. L. and Leung F. C. C. (2002). Rapid communication: Complete nucleotide sequence of the chicken *prolactin* gene. Journal of Animal Science, 80:1381.

Chaiseha, Y., Ngernsoungnern, P., Sartsoongnoen, N., Prakobsaeng, N. and El Halawani, M. E. (2012). Presence of prolactin mRNA in extra-pituitary brain areas in the domestic turkey. Acta Histochemistry, 114:116-121.

Cui J. X., Du, H. L., Liang, Y.and Zhang, X. Q. (2004). Polymorphism of chicken prolactin gene. Departement of Animal Genetics, Breeding and Reproduction, College of Animal Science. South China Agricultural University, Wushan Street, Guangzhou, Guangdong, China.

Cui, J. X., Du, H. L. and Zhang, X. Q. (2005). Polymorphisms and bio-informatics analysis of chicken prolactin gene. Yi chuan Hereditas, 27:208-214.

Cui, J. X., Du, H. L., Liang, Y., Deng, X. M, Li, N. and Zhang, X. Q. (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. Poultry Science, 85:26-31.

Dhara, S. K. and Soller, M. (1999). Intragenic haplotypes in chicken prolactin in four diversed breeds. Hebrew Univ. of Jerusalem, Israel.

El Halawani, M. E., Whiting, S. E. Silsby, J. L. Pitts, G. R. and Chaiseha, Y. (2000). Active immunization with vasoactive intestinal peptide in turkey hens. Poultry Science, 79:349-354.

Falconer, D. S. and Mackay, T. F. C. (1996). An introduction to quantitative genetics. 4th ed. Benjamin-Cummings. Longman, Oxford.

Goodwin, W., Linacre, A. and Hadi, S. (2007). An introduction to forensic genetics, 2nd ed. John Wiley & Sons Ltd. West Sussex, UK.

Izutsu, K., Kurokawa, M., Imai, Y., Ichikawa, M., Asai, T., Maki, K. and Hirai, H. (2002). The t (3; 21) fusion product, AML1/Evi-1 blocks AML1-induced transactivation by recruiting CtBP. Oncogene, 21:2695-2703.

Jiang, R. S., Xu, G. Y., Zhang, X. Q. and Yang, N. (2005). Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. Poultry Science, 84:839-845.

Kansaku, N. K., Shimada, T., Ohkubo, N., Saito, T., Suzuki, Y., Matsuda, Y. and Zadworny, D. (2001). Molecular cloning of chicken vasoactive intestinal polypeptide receptor complementary DNA, tissue distribution and chromosomal localization. Biology of Reproduction, 64:1575-1581.

Lewin, B. (1997). Trancription is catalysed by RNA polymerase. In: Genes VI. Oxford University Press, Inc, New York. pp.289-334.

Lumatauw S.and Mu'in, M. A. (2016). A 24-bp Indel (Insertion-Deletion) polymorphism in promoter prolactin gene of Papua local chickens. Animal Production, 18:1-7.

Miao, Y., Burt, D. W. Paton, I. R. Sharp, P. J. and Dunn, I. C. (1999). Mapping of the prolactin gene to chicken chromosome 2. Animal Genetics, 30:473.

Noor, R. R. (2010). Genetika Ternak, Penebar Swadaya, Jakarta, Indonesia, ISBN: 9794893404.

Ohkubo, T., Tanaka, M. and Nakashima, K. (2000). Molecular cloning of the chicken prolactin gene and activation by Pit-1 and cAMP-induced factor in GH3 cells. General and Comparative Endocrinology, 119:208-216.

Pitts, G. R., Youngren, O. M., Silsby, J. L., Rozenboim, I., Chaiseha, Y., Phillips, R. E., Foster, D. N. and El Halawani, M. E. (1994). Role of Vasoactive Intestinal Peptide in the Control of Prolactin-Induced Turkey Incubation Behavior. II. Chronic Infusion of Vasoactive Intestinal Peptide1. Biology of Reproduction, 50:1350-1356.

Rohmah, L., Darwati, S., Ulupi, N., Khaerunnisa, I. and Sumantri, C. (2022). Polymorphism of prolactin (PRL) gene exon 5 and its association with egg production in IPB-D1 chickens. Archives Animal Breeding, 65:449-455.

Vinatzer, U., Taplick, J., Seiser, C., Fonatsch, C. and Wieser, R. (2001). The leukaemia associated transcription factors EVI 1 and MDS1/EVI1 repress transcription and interact with histone deacetylase. British Journal of haematology, 114:566-573.

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