Research Article

Effect of a Polymorphism in Prolactin Gene on Some Reproductive Traits in TB Crossbred Ducks in Southern Vietnam

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Abstract | The prolactin (*PRL*) gene has previously been reported as a potential candidate for egg production in birds. This study identified the prolactin gene polymorphism in the exon 5 region and its association with reproductive traits of TB crossbred ducks. Three hundred and thirty-one crossbred ducks were used in this study, with blood samples collected from the wing vein for DNA extraction to amplify the fragment length with 536 bp by PCR, and then the PCR products were cleaved with the *Pst*I enzyme. The fragment length with 536 bp was successfully amplified in all individual samples, while genetic polymorphism at the PRL/*Pst*I site was identified as C and T alleles, with allele frequencies 0.811 (C) and 0.189 (T). Three genotypes were observed with frequencies 0.647, 0.329 and 0.024 for CC, CT and TT, respectively. The PIC (polymorphic information content) and H_e (expected heterozygosity) were 0.260 and 0.306. The group of ducks with CC/*Pst*I genotype laid the first egg significant earlier (P<0.05) than CT/*Pst*I or *TT/Pst*I genotype ducks (145.3 vs 152.3 days or 153.2 days, respectively), the total of egg number up to 38 weeks of age was higher (P<0.05) in the group of ducks with CC/*Pst*I genotype compared to CT/*Pst*I or *TT/Pst*I genotype (99.3 vs 93.1 or 91.7 eggs). The average egg weight did not significant difference among genotypes (72.3, 72.2 and 70.6g; P>0.05). From these results, the polymorphic site at *PRL/Pst*I was identified, ducks bearing CC genotype possess earlier age at first egg and egg yield up to 38 wks of age and considered as a candidate gene for supporting genetic selection in ducks to improve egg production.

Keywords | Climate change, Crossbred duck, Egg production, Genetic polymorphism, Prolactin gene

Received | Mach 12, 2023; **Accepted** | April 11, 2023; **Published** | May 03, 2023
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Citation | Nguyen NT, Le TL, Vo TKN, Do CH, Hoang TT, Duong NK, Luu QM (2023). Effect of a polymorphism in prolactin gene on some reproductive traits in TB crossbred ducks in Southern Vietnam. Adv. Anim. Vet. Sci. 11(6):886-892.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.6.886.892
ISSN (Online) | 2307-8316

INTRODUCTION

Vietnam ranks second behind China for duck production with a population of around 86.8 million birds (FAOSTAT, 2022). Duck production plays a principal role in meat and egg production to meet market demands. Various local duck breeds are endemic, and these have comparative advantages compared to imported livestock due to high ability to adapt with local environments and good production characteristics as a result of less artificial selection (Subekti et al., 2019; Nova et al., 2020; Rafian et al., 2022). During past decades, climate change has

impacted duck production in the Vietnam, especially in Mekong River Delta region, due to increased salinization. A new line of crossbred ducks was created based on the reciprocal hybridization between Bien strain and TC strain and named BT or TB crossbred ducks. TB ducks are crossbred between TC male and Bien female. Bien is a local duck strain that has a high ability to adapt to increased salinity (Le et al., 2020) and TC is a newly created line with high egg yield from crossing Triet Giang male (imported from China) and Co female (native duck) by Vietnamese scientists (Le et al., 2022). In birds, egg yield has evolved from the interaction of many genes. Recent increased understanding of avian genes and genomes, especially ducks, using different molecular markers has allowed scientists to screen for candidate genes in relation to egg yield productivity traits. Restriction fragment length polymorphism based on polymerase chain reaction (PCR-RFLP) is a regularly used for polymorphism genotyping studies (Hiyama et al., 2012; Roy et al., 2020). Many candidate polymorphism genes in layer ducks have been recognized and correlated with egg production, in which the prolactin (*PRL*) gene has important functions in living things, specifically in egg production in duck (Reddy et al., 2002; Susanti et al., 2012; Irma et al., 2014; Mazurowski et al., 2016; Wang et al., 2011; Chuekwon and Boonlum, 2017; Ghanem et al., 2017; Astuti, 2019; Sabry et al., 2020), goose (Tang et al., 2021) and chicken (Cui et al., 2006; Rashidi et al., 2012; Roy et al., 2020; Manoharan et al., 2021; Rohmah et al., 2022). The prolactin gene was identified in ducks at 10 kb in length and comprising 4 introns and 5 exons (Kansaku et al., 2008). This gene plays a key role in reproductive traits (Wang et al., 2011). Research on *PRL* gene polymorphism in Indonesian local ducks documented exon 2 (Susanti, 2015) and exon 4 (Indriatia et al., 2016) or in chicken (Rohmah et al., 2020). In exon 5, a single nucleotide polymorphism (SNP) showed a positive correlation with egg production in Chinese ducks (Wang et al., 2011), Indonesian local ducks (Susanti et al., 2012; Astuti, 2019; Purwantini et al., 2020; Rafian et al., 2022) and Egyptian duck breeds (Sabry et al., 2020) or in chicken (Rohmah et al., 2022). Current study investigated polymorphism of the prolactin gene in exon 5 to determine the effect of genetic polymorphism on some reproductive traits of BT crossbred ducks.

MATERIALS AND METHODS

Animals

The birds in this study, as 331 TB crossbred ducks (32 males, 299 females, Figure 1), were reared at the VIGOVA Poultry Research and Development Center. Ducks were housed in pens as confined area, and individual ducks were identified by a fitted wing band. The commercial feed was used for ducks during the experiment in according with their age, such as a starter period (0 to 8 weeks) comprising 19-21% crude protein (CP) and 2850-2950 kcal of metabolizable energy (ME) *ad libitum*, a grower period (9 to 17 weeks) consisting of 14-15% CP and 2700-2800 kcal of ME with a restricted feeding regime, and a laying stage (18 to 70 weeks) as 17-18% CP and 2700-2 800 kcal of ME *ad libitum*. Fresh clean water was freely supplied.

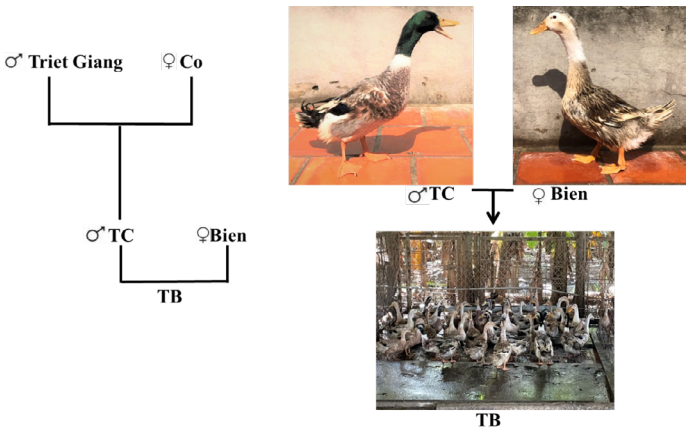

Figure 1: Representative duck mating schemes to produce TB crossbred duck (left) and parents and TB crossbred ducks (right).

The ducks were weighed (BW) in the morning before they were fed at 8 and 18 weeks of age to monitor the feeding regime. Reproductive traits related to egg production were recorded as followed AFE (age at first egg; in days), MEW (mean weight of eggs; in grams) as average daily egg weight collected during weeks 37 and 38 of age, and TNE (total number of eggs) calculating as the number of eggs laid until 38 weeks of age.

Collection of sample and DNA extraction

Blood samples were collected from 331 ducks at 8 weeks of age, with handling in accordance with good practitioner to minimize suffering for animals. The fresh blood (1 mL) was taken from the wing vein by a professional technician, stored in a tube with EDTA, then placed on ice at 4°C and transported to the laboratory within 12 hours.

Primer information

The sequence with access number AB158611.1 (*Anas platyrhynchos*) from GenBank was used to design a primer by primer 3 software. The sequences of primers were 5'- TGCAAAGTCAGATTCCACCA -3' and 5'- GCAAAGCAACAAGAACACCA-3' with 536 bp fragment length as forward and reverse primer used.

DNA amplification, PCR-RFLP assay and electrophoresis

Genomic DNA extraction was performed using a TopPURE® blood DNA exctraction kit (ABT-Vietnam) according the manufacturer's instructions, extracted DNA

were measured OD value using Bio-drop machine (UK) and stored at -80 °C until used (Nguyen et al., 2022). Extracted DNA from 331 individual samples was exposed to genotypic analysis. Polymerase chain reaction (PCR) was applied with a thermal cycler machine (BIOER, China), using 25 µL volume for the reaction following Nguyen et al. (2022), consisting of 2 µL (25 ng/µL) DNA template, 1 µL (10 pM each) primers (Phu Sa, Vietnam), 12.5 µL of My Taq™ Mix 2X (Bioline, UK), with free nucleic ddH_2O added to make up to 25 µL. The PCR procedure was performed with 35 cycles: (1) 95°C for 4', (2) 95°C for 30", (3) 59°C for 30", (4) 72°C for 30", (5) repeated step 2-4 for 35 cycles and (6) 72°C for 5'. The PCR products were observed using 1% agarose gel with GelRed (30 min, 100V) with a 100 bp DNA ladder under UV light (GelDoc It2 - UVP, USA) after electrophoresis applied.

The fragment length with 536 bp of PCR products was digested with 10 units of *Pst*I restriction enzyme at 37°C overnight. The digested products were then electrophoresed at 50V for 1 hr on 2% agarose gel with GelRed. Individual PCR-RFLP fragment sizes in each sample were recorded based on a standard DNA molecular ladder (100 bp) by observing the band pattern under UV light (GelDoc It2-UVP, USA). The expected three genotypes of *Pst*I PCR-RFLP were 536 bp for the CC genotype, 536/406/130 bp for the TC genotype and 406/130 bp for the TT genotype.

Data analysis

Observations of fragments and genotyping were manually performed following the procedure of Darabi et al. (2010). Allele frequency and genotype frequency were calculated according to the formula of Nei and Kumar (2000). Determinations of observed heterozygosity (Ho) and expected heterozygosity (He) were based on the formulae of Weir (2011) and Nei and Kumar (2000), respectively. The polymorphic information content (PIC) was appraised using the formula of Botstein et al. (1980). To test the effect of the prolactin gene polymorphism on reproductive traits, one-way analysis of variance followed by the Tukey's test was applied, with significant difference set at $P<0.05$.

RESULTS AND DISCUSSION

PCR and PCR-RFLP assay

A fragment of 536 bp of the *PRL* gene in exon 5 was successfully amplified in all duck samples and the representative electrophoresis image with the 536 bp fragment was clearly observed in the Figure 2. Similar with current study, many studies have also successfully amplified the target gene on exon 5 of the prolactin gene with various fragment sizes in ducks (Mazurowski et al., 2016; Ghanem et al., 2017; Yurnalis et al., 2019; Purwantini et al., 2020) or in chicken (Rashidi et al., 2012; Mitrofanova et al., 2017; Perdamaian and Daryono, 2020; Rohmah et al., 2022).

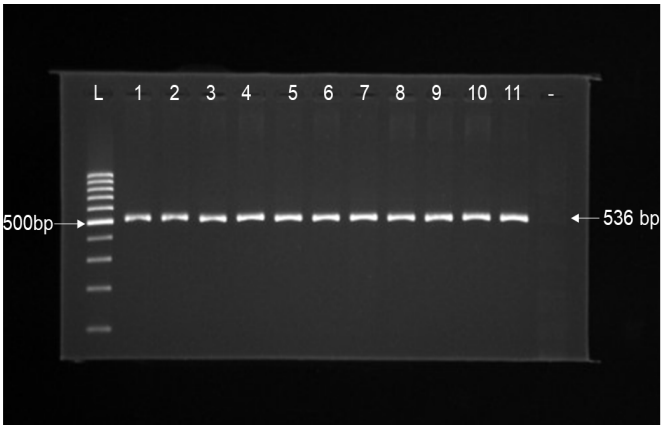

Figure 2: Representative electrophoresis of the PCR products of prolactin in exon 5 on agarose gel. L: DNA ladder (100 bp). No. 1-11: individual samples; (-): Negative control.

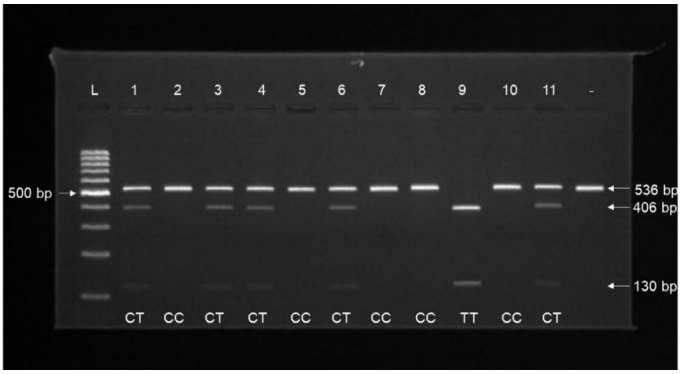

Figure 3: Representative PCR-RFLP pattern of *IGF-1* gene digested with *PstI* and separated on 2% agarose gel, L: DNA ladder (100 bp); lane 2, 5, 8, 10: CC genotype (536 bp); lane 1, 3, 4, 6, 11: CT genotype (536/406/130 bp); lane 9: TT (406/130 bp); (-) negative control (PCR product without enzyme treatment).

As shown in Figure 3, polymorphism at the *PRL*/*Pst*I with two alleles (C and T) and three genotypes (CC: 536 bp; TT: 406/130 bp and TC: 536/406/130 bp) was clearly distinguished. The C and T allele frequencies were 0.811 and 0.189. According to sex, the C and T allele frequencies of in male birds were 0.797 and 0.203, while frequencies of the C and T alleles in female birds were 0.813 and 0.187. The allelic frequency for allele C (0.811) was significantly higher than for allele T (0.189) (Table 1), with the trend of allelic frequency in this study similar to several previous studies in various duck breeds (Chuekwon and Boonlum, 2017; Yurnalis et al., 2019; Sabry et al., 2020), while other research reported balanced allelic frequencies in investigated ducks (Mazurowski et al., 2016; Rafian et al., 2022). The disparities between the study results were caused by different sources of domestic breeds, selected certain traits applied, patterns of genetic mutations and

Table 1: Distribution of genotype and allele frequency in ducks at *PRL/Pst*I polymorphism site.

Generation	Parameter	Genotypic frequency			Allelic frequency		He	PIC
		CC	CT	TT	C	T		
Male	N	19	13	0	0,797	0,203	0,324	0,271
	Observed frequency	0,594	0,406	0				
	Expected frequency	0,625	0,324	0,041				
Female	N	195	96	8	0,813	0,187	0,304	0,258
	Observed frequency	0,652	0,321	0,027				
	Expected frequency	0,661	0,304	0,035				
Total	N	214	109	8	0,811	0,189	0,306	0,260
	Observed frequency	0.647	0,329	0,024				
	Expected frequency	0,658	0,306	0,036				

He: expected heterozygosity; PIC: Polymorphic information content.

levels of material genetic recombination (Rafian et al., 2022). Investigation of *PRL/Pst*I in exon 5 in duck breeds including Shanma, Shaoxing, Jingyun, Jingjiang, Youma and F2 found that some breeds were balanced in allele frequency (Shanma, Shaoxing and Jingyun), while other breeds (Jingjiang, Youma and F2) were dominant in one allele (Wang et al., 2011).

Sequence analysis of depicted samples for representative genotypes was showed in the Figure 4. The results indicated that the transition mutation T>C was found (site 403 in the Figure 4) and that resulted in three genotypes were observed as illustrated in Figures 3 and 4. The similar patterns are also reported in several reports in duck (Cui et al., 2006; Kansaku et al., 2008; Purwantini et al., 2020) or chicken (Rohmah et al., 2020).

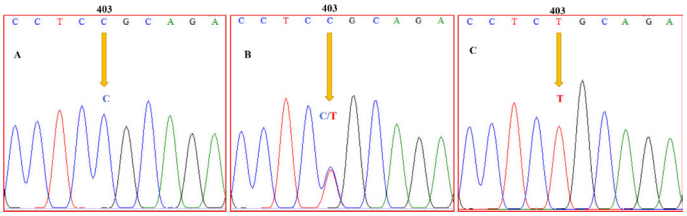

Figure 4: Partial sequencing of exon 5 prolactin gene showing mutation sites; A: CC genotype; B: CT genotype; C: TT genotype; 403: indication of mutation site based on sequences used.

As for genotype frequencies, the CC, CT and TT genotypes were 0.647, 0.329 and 0.024 for total populations or 0.652, 0.321 and 0.027 for the female group (Table 1). However, in the male group, only two genotypes were identified as CC and CT with observed frequencies 0.594 and 0.406, respectively while the TT genotype was not detected. Rafian et al. (2022) reported that monomorphism in the *PRL/Pst*I locus was found in local Joti ducks from Indonesia, contrasting with other research (Wang et al., 2011; Ghanem et al., 2017; Yurnalis et al., 2019) as well as our present study. Abdel-Kafy et al. (2015) mentioned balanced allelic frequency in the population due to higher frequency of heterozygoty (TC) than homozygoty (CC and TT) in the *PRL/Pst*I locus in ducks. Differences among reports might due to long term genetic selection that resulted in high homogeneity and frequencies of unfavorable alleles accumulated under artificial selection (Perdamaian and Daryono, 2020) or the directed selection for increased egg production has supported the favourable allele (Roy et al., 2020).

The observed heterozygosity value was 0.306 and equally to the expected heterozygosity (Table 1), it indicates that the population is relatively diverse. According to Wang et al. (2011), heterozygosity is an index to use for estimating the genetic diversity levels in the population, and the heterozygosity value more than 0.5 is considered high genetic diversity (Karabag et al., 2016). The PIC value as showed in the Table 1 was 0.260 that indicated the polymorphic at *PRL/Pst*I locus had moderate level because of the PIC ranking in 0.25<PIC<0.5 according to Botstein et al. (1980).

Table 2: Effect of *PRL/Pst*I genotype on some reproductive traits in TB crossbred duck.

Geno-type	N	Age first egg (days)	Egg yield up to 38 weeks of age (egg)	Egg weight (g)
CC	188	145.3^{a}±1.8	99.3^{a}±1.5	72.3±0,3
CT	89	152.3^{b}±1.7	93.2^{b}±1.6	71.2±0.6
TT	6	153.2^{b}±3.8	91.7^{b}±2.2	70.6±0.7

The relationship between *PRL/Pst*I polymorphism and some reproductive traits are presented in Table 2. The data from Table 2 elucidated that the group of ducks with the CC genotype achieved sexual maturity earlier than the CT or TT genotypes (145.3 vs 152.3 and 153.2 days, respectively). Contrary, no association between the polymorphism at *PRL/Pst*I site and age at the first egg was found in native Chinese ducks (Wang et al., 2011)

or Indonesian local duck (Purwantini et al., 2020). It was documented that high body weight at hatch (BWH) had earlier sexual maturity in the association with PRL gen polymorphism (Rashidi et al., 2012). In fact, the association of polymorphism at *PRL/Pst*I locus in exon 5 on sex maturity is scare and still unknown the mechanism and it needs more study for clearly explanation.

For egg yield, individuals with the CT and TT genotypes had significantly lower egg numbers up to 38 weeks of age compared to CC (92.4 and 91.7 vs 99.3 eggs/bird, $P<0.05$). No significant differences in average egg weight were found among the three genotypes (CC, CT and TT: 72.3, 71.2 and 70.6 g/egg, respectively; $P>0.05$). Investigated from five native duck breeds (Shanma, Shaoxing, Jinyun, Jingjiang and Youxian), Wang et al. (2011) reported that polymorphism at *PRL/Pst*I in exon 5 was detected, and ducks bearing CC genotype possessed higher egg production as well as egg weight than those of the CT genotype. Similar results of polymorphism at the *PRL/Pst*I locus in different duck breeds and its relationship with reproductive traits such as egg production ability was also reported in other studies in ducks (Ghanem et al., 2017; Bai et al., 2019; Rohmah et al., 2022) or in chicken (Cui et al., 2006). In chicken, *PRL/Pst*I $^{+/+}$ genotype was associated with higher egg production at the age of 40 wks (Nagaraja et al., 2000) or at 48 and 57 weeks of age (Li et al., 2008) than *PRL/Pst*I $^{-/-}$ genotype have reported similar trend of results on egg production in this current study.

The prolactin gene is considered as candidate marker gene for egg production in birds because it plays an important role in broodiness behavior that specifically controls the egg yield variability due to reducing egg biosynthesis during the period of broody (Chen et al., 2007; Perdamaian and Daryono, 2020). When the onset of broodiness is activated, the elevation of plasma prolactin, which can be variation in tropical chicken breeds, causes follicle regression on the ovary and then ceases egg production (Li et al., 2013; Banu et al., 2017) of birds in general, especially in ducks. Several factors affect broodiness, including intrinsic factors such as genetic diversity due to mutation and other mechanism related to epigenetic condition, for example: DNA methyl- or demethylation, histone acetyl- or deacetylation) or extrinsic factors such as ambient temperature, humidity, availability of feed compositions and sources and photoperiodicity which alter organism behavior toward broodiness associated with egg production ability (Geng et al., 2014; Perdamaian and Daryono, 2020).

CONCLUSIONS AND RECOMMENDATIONS

This document is the first report of genetic polymorphism of the *PRL/Pst*I locus in exon 5 of TB crossbred duck in Vietnam. Two kinds of alleles with three genotypes were identified for *PRL/Pst*I polymorphism. Ducks with CC genotype possess a lower age at first egg and higher total egg numbers up to 38 wks of age than the group of ducks with CT and TT genotypes. The polymorphic site at PRL/*Pst*I can be considered a candidate gene for supporting genetic selection in ducks to improve egg production.

ACKNOWLEDGMENT

Our sincere appreciation goes to all the staff at VIGOVA for carefully recording the phenotype data of the ducks during this experimental study.

NOVELTY STATEMENT

To the best of our knowledge, this is the first study in Vietnam reporting on the polymorphism of the prolactin gene on exon 5 in TB crossbred ducks as an alternative crossbred duck with high ability to adapt to salinity conditions in response to global warming due to climate change.

AUTHOR'S CONTRIBUTION

All authors generally discussed about the experimental design, read and approved the manuscript in each step. NGUYEN, N.T covered all the research, wrote and revised the manuscript. LE, T.L., VO TKN and DO CH contributed equally to the work on DNA extraction, design primer and amplification of target gene and sequence analysis. HOANG, T.T and LUU, QM donated to sample collection and statistical analysis, respectively. DUONG, N.K. was responsibility for evaluating the manuscript and checking the plagiarism.

Conflict of interest

The authors have declared no conflict of interest.

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