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Association of Prolactin Gene with Egg Production in Khaki Campbell Ducks

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Abstract

Prolactin (*PRL*) is a polypeptide hormone with a variety of physiological functions, and the *PRL* gene in the duck is involved, in reproduction, ovarian follicle development and egg formation process. The objective of this study was to investigate polymorphism of the *PRL* gene and the relationship between *PRL* genotypes with egg production trait in Khaki Campbell ducks (n = 60) by Polymerase Chain Reaction technique (PCR) for 5 sites, Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing. The results showed that only intron1 region was polymorphic. A C/A mutation at the position of the 359 bp in intron1 of the *PRL* gene was found by DNA sequencing, and results in 3 genotypes GG, GT and TT can be detected by *XbaI*. The frequencies of genotype GG and allele G were the highest (0.56 and 0.74 respectively). The association analysis between the polymorphism in intron1 of the *PRL* gene and egg production at 300 days of age (E300D) was carried out. In the Khaki Campbell population, association analysis demonstrated that the C359A polymorphism was significantly associated with E300D ($P < 0.05$), GT genotype was associated with higher egg production than the GG and TT genotype (53.32, 37.50 and 36.67 eggs, respectively).

Keywords: Prolactin gene, Khaki Campbell duck, egg production and polymorphism

Introduction

The prolactin (*PRL*) hormone, an important reproduction hormone, is synthesized primarily by the anterior pituitary gland, is coded from the *PRL* gene and is involved in a broad variety of biological functions in all vertebrates. In mammals, the dominant function of prolactin seems to be lactogenesis. *PRL* may act as a "co-gonadotropin" and fine-tune the process of folliculogenesis by altering the acquisition of granulosa FSH receptors in procine [1]. Prolactin in poultry plays an important role in the onset of incubation and regression of the ovaries in chickens. Prolactin also plays an important role in reducing the number of graafian follicles per unit area of the ovary and thus can be directly involved in loss of egg production and egg quality [2]. The prolactin hormone affects the molting and egg production in Peking and Alabio ducks [3].

The Khaki Campbell (*Anas platyrhynchos*) is a breed of domesticated duck that originated in England and is kept for its high level of egg production. Duck eggs have higher nutrient levels than chicken eggs. The USDA has a complete breakdown of duck egg qualities. Duck eggs have higher levels in 7 of 8 minerals, 7 of 9 vitamins and 11 of 18 amino acids analyzed [4].

The duck *PRL* gene is 10 kb in size and is composed of 5 exons and 4 introns, encoding 229 amino acids, and the duck *PRL* cDNA shares 92.0, 91.7 and 91.4 % sequence homology with chicken, turkey and quail *PRLs*, respectively [5]. The *PRL* gene sequence could provide the basis for investigating the phenotypes of genes affecting the economic traits based on polymorphism trait association. To date,

single nucleotide polymorphisms (SNPs) in poultry have been reported and some SNPs have been revealed to be associated with economic traits. The polymorphisms at the 5'UTR of the *PLR* gene were associated with egg production and egg quality traits in White Leghorn chickens [2]. Therefore an extensive investigation of the duck *PRL* gene was initiated using Khaki Campbell ducks to screen polymorphism for 5 sites of the duck *PRL* gene and determine their association with egg production.

Materials and methods

Animals

This experiment was started on May 3, 2012 to February 28, 2013 with egg production data from December 1, 2012 to February 28, 2013. Sixty Khaki Campbell ducks of the same age and origin were used in this experiment. Each duck was kept in an individual cage from 19 - 43 weeks of age. All ducks were provided with fresh water freely. The laying pellet consisted of 16 % crude protein and 2,700 Kcal of ME/kg. The experiment was reviewed and approved by the Animal Experimentation Ethics Committee of Suratthani Rajabhat University.

Data on egg production including the total egg production were collected daily using wing bands to identify individual ducks. The data for an individual duck were collected for 3 months during the experimental period and recording commenced at 31 weeks of age. Genomic DNA and trait data were obtained on all 60 ducks.

PCR-RFLP analysis

Blood samples were collected from the wing vein of each duck and genomic DNA was extracted by guanidine hydrochloride using the silica gel method. The quality and quantity of DNA was verified by 0.8% agarose gel electrophoresis. All DNA stocks were diluted with TE buffer (Tri-HCl pH = 8.0), 0.5M EDTA (pH = 8.0) to create a standard DNA concentration of 100 µg/µl. Polymerase chain reaction (PCR) was performed to amplify 5' flanking region intron1, inton2, intron4, exon2, exon4, exon5 and 3' flanking region of the duck *PRL* gene. The primers were used to amplify the target region, covering the full length of 5 exons and their flanking region sequence. Primer information is given in **Table 1** [6]. The PCR reaction was performed in 10 µl final volume, containing 1 µl 10X Buffer, 1 µl dNTPs (2.5 mM), 0.8 µl Mg^{2+} (25 mM), 1 µl each primer (25 ρmol/µl), 1.0 µl genomic DNA (100 ng/µl), 0.2 Taq polymerase (5U/µl). Thermal cycling conditions were 5 min preheat at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature, 30 s at 72 °C, a final extension of 10 min at 72 °C and conservation at 4 °C. A total of 60 samples were subjected to genotyping analysis using the PCR-RFLP technique. The PCR reaction was digested using 1 µl *XbaI* enzyme (1U/µl) at 37 °C overnight. The digestion products were characterized in 2 % agarose gel in 1X TAE and 1.0 mM gel star. Individual genotype banding patterns were determined under visible light and samples showing different bands in the gel. The fragments were then cloned with Clone JET^{TM} PCR Cloning Kit (Fermentas) and automated sequencing (Ward Medic, Ltd.).

Genetic variation identification and DNA sequencing

The digestion products from 60 ducks were genotyped using 2 % agarose gel electrophoresis (100 V, 30 min). The digestion products were mixed with loading dye (2:5 µl). Different genotypes of the SNPs were confirmed by sequencing.

Statistical analysis

Data on total egg production to 300 days of age (E300D) was obtained from the records. The frequency of genotypes and alleles were calculated. Genotype distribution was evaluated for the agreement with Hardy-Weinberg equilibrium by the FREQ procedure of SAS system 9.1.3 (SAS Institute, Cary, N.C., USA) using χ^2 test. The effects of *PRL* genotypes on egg production of ducks were analyzed using the general linear model (GLM) procedure base on the following model;

$$Y_{ij} = \mu + G_i + E_{ij} \quad (1)$$

where Y_{ij} is the observed trait values
μ is the overall mean
G_i is the genotype effect (i = 1, 2, 3)
E_{ij} is the random error

Table 1 Primer sequences and annealing temperature of the *PRL* gene in the Khaki Campbell duck.

Primer name	Primer sequence (5' → 3')	Amplification region		Product size (bp)	Tm (°C)
PRL-F1	AAATTCCCTCTCACAGTTACA	23 - 439	5' flanking region and intron1	416	60
PRL-R1	GATGCAGAGACAAGTTTCACC				
PRL-F2	AATCGAATGACTATGCTTGCC	1608 - 2008	exon2	-	-
PRL-R2	TACTGAAGGGATTTTTATATG				
PRL-F3	CTTTTAGTGCTGACCATTGTT	2111 - 2512	intron2	402	55
PRL-R3	CCCTCCGCTCTATCTCACACT				
PRL-F4	AAATAAATTCCTAGATCTCTG	3643 - 4070	exon4 and intron4	428	55
PRL-R4	TAACTGAATCTGAGAACTTTG				
PRL-F5	TGCAAACCATAAAAGAAAAGA	5707 - 6106	exon 5 and 3' flanking region	400	60
PRL-R5	CAATGAAAAGTGGCAAAGCAA				

Results and discussion

In the current study, polymorphism of the duck *PRL* gene was detected by PCR-RFLP and DNA-sequencing methods. Four fragments were amplified by 5 primers, the amplified fragment of PRL-F1 PRL-R1, PRL-F3 PRL-R3, PRL-F4 PRL-R4 and PRL-F5 PRL-R5 were 416, 402, 428 and 400 bp in length respectively. The results showed that, 5SNPs were detected compared with the database in AB158611 (**Table 2**), the A381C site was recognized by *XbaI* PCR-RFLP. As shown in **Figure 1**, PCR-RFLP using *XbaI* was digested into 3 genotypes, GG (416 bp), TT (354 bp/62 bp) and TG (416 bp/354 bp/62 bp). The homozygous individuals of different genotypes were cloned and sequenced. While comparing G and T alleles, nucleotide substitutions were found as C/A mutation at the position of 359 bp in the intron1 region.

Table 2 The mutations in intron1 of the *PRL* gene in Khaki Campbell.

Location (bp)	Mutation	Restriction enzyme site
55	G ⟶ A	No
191	G ⟶ T	No
245	G ⟶ A	No
287	T ⟶ G	No
359	C ⟶ A	*XbaI*

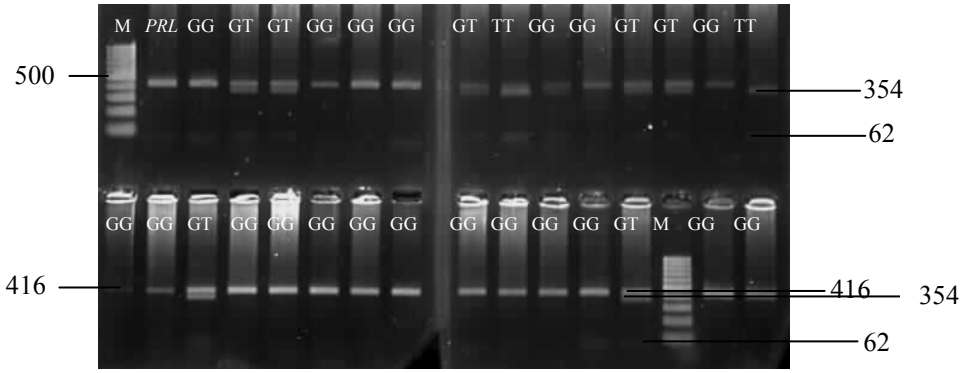

Figure 1 Representative digestive PRL-F1 PRLR1 products of 60 ducks of the experiment population. M: Ladder 100 bp *PRL*: PCR products of a duck. GG genotype shows one fragment (416 bp) GT genotype shows 3 fragments (416, 354 and 62 bp) and TT genotype shows 2 fragments (354 and 62 bp).

Results of the chi-square goodness of fit test revealed that the Khaki Campbell population was in Hardy-Weinberg equilibrium ($P > 0.05$). Moreover, these genotyping results indicated that the GG genotype was preponderant in tested Khaki Campbell ducks (frequency of 0.56 compared to 0.37 for GT and 0.07 for TT genotypes, respectively) with higher frequency of the G allele (0.74) (**Table 3**). The results of the association analysis showed significant association ($P < 0.05$) between *PRL* intron1 gene polymorphism and egg production in Khaki Campbell ducks (**Table 4**). The genotype GT had higher positive effect on E300D than GG and TT ducks. Although introns do not participate in protein synthesis, the polymorphism of the intron1 may affect the translation process by some unknown factors. Some introns have translatable nucleotide sequences that in the absence of splicing can result in production of novel peptides fused to the peptide encoded by the N-terminal exons [7]. The A/C mutation located in the non-coding region has an effect on gene expression by affecting regulatory elements and some intronic SNPs activate cryptic splice sites, leading to alternative splicing [8].

Table 3 Genotypic and allelic frequency in Khaki Campbell breed.

Locus	Allele frequency		Genotype frequency			χ^2
	G	T	GG	GT	TT	
PRL1	0.74	0.26	0.56	0.37	0.07	2.05

Table 4 Statistical analysis of *XbaI* genotypes and egg production.

Trait	LSMeans of genotypes			SEM	P-value
	GG	GT	TT		
Egg production (E300D)	37.50^{b}	53.32^{a}	36.67^{b}	2.98	0.030

^{a,b} Within each column, there was a difference among genotypes ($P < 0.05$).

Conclusions

The results of this study showed that intron1 of the *PRL* gene was polymorphic, and has significant associations with egg production in Khaki Campbell ducks. These findings indicate that the polymorphism at the intron1 of the PRL gene can be utilized in selection programs to improve egg production in Khaki Campbell ducks by culling for the TT genotype.

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