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Research Article

Turk. J. Vet. Anim. Sci.
2009; 33(3): 193-197
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doi:10.3906/vet-0709-4

Association of polymorphisms in the intron 1 of duck prolactin with egg performance

Hui-Fang LI[1,*], Wen-Qi ZHU[1], Kuan-Wei CHEN[1], Tang-Jie ZHANG[2], Wei-Tao SONG[2]

[1]Institute of Poultry Science, Chinese Academy of Agriculture Science, Yangzhou, Jiangsu Province 225003, P. R. CHINA

[2]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu Province 225009, P. R. CHINA

Received: 03.09.2007

Abstract: Gaoyou duck (Chinese indigenous breed) is famous for double-yolked egg. In the present study, SNP of prolactin (PRL) gene intron 1 of Gaoyou duck was detected by PCR-RFLP and DNA sequencing. The results indicated that there were 2 Dra I recognition sites in intron 1 of the PRL gene, but only 1 was of polymorphism. A T/C mutation at the position of the 1326 bp of the PRL gene was found by DNA sequencing, and resulted in 3 genotypes AA, AB, and BB. The frequencies of genotype BB and allele B were the highest. The association analysis between the polymorphism PRL gene intron 1 and egg performance was carried out. The least square analysis showed that the BB ducks had significant egg weight at the age of 30 weeks than AB ducks ($P < 0.01$), the double-yolk percentage of the AB ducks was much higher than that of BB ducks ($P < 0.05$) in double-yolk ducks, but there was not significant difference in egg number, longest clutch days, and the body weight at first egg among the three genotypes.

Key Words: PRL gene, PCR-RFLP, Gaoyou duck, double-yolked egg

Introduction

Prolactin (PRL) is a polypeptide hormone secreted by the anterior pituitary gland and has been shown to have a diverse spectrum of biological activities and functions in all vertebrates. In mammals, the dominant function of PRL seems to be lactogenesis whereas in fishes PRL is implicated to be involved in osmoregulation. In birds the roles of PRL are not yet understood extensively, but its major function is believed to be manifested during incubation and feeding of nestlings. The chicken prolactin hormone plays a crucial effect in egg production, because the onset of poultry incubation behavior (broodiness) is induced by an increase in prolactin secretion (1-4), which commonly results in regression of the ovary (5) and loss of egg production (2,6). The chicken prolactin gene complete sequence was reported previously (7,8). In recent years, the polymorphism of the cPRL gene was reported (9,10) and the correlation between the gene polymorphism and egg performance had also been analyzed (10). It was reported that 1 SNP of PRL intron 1 had been detected in Wenchang chicken (Chinese indigenous chicken) by PCR-SSCP (11).

* E-mail: lhfxf_002@yahoo.com.cn¬

The duck prolactin gene is 10 kb in size and is composed of 5 exons and 4 introns, encoding 229 amino acids. Duck PRL was found to have 92.0%, 91.7%, and 91.4% sequence identity at the cDNA level compared to PRL of chicken, turkey, and quail, respectively. The mature duck PRL has an overall similarity with a comparable region of chicken (95.5%), turkey (92.5%), and quail (95.5%) PRL (12).

In ducks, various strains and/or lines showing different egg production traits have been established. Gaoyou duck is an excellent dual-purpose (lean type) duck in China. It is famous for double-yolked egg. Generally, double-yolk percent of Gaoyou duck is 1% to 2%. Annual egg production is about 250. Average egg weight is about 85 g. In China, people love double-yolked egg much more than one-yolked egg. They think the double-yolked egg represents happiness and it may bring good luck to them. Therefore, consumption market for double-yolked eggs is reasonably large. It is very indispensable to use MAS method to accelerate the progress of breeding Gaoyou duck.

The single nucleotide polymorphism (SNP) of the PRL gene in China domestic duck has not been reported. The objectives of this study were to search for 1 SNP of PRL gene and evaluate the associations between PRL polymorphism and egg performance.

Materials and Methods

Experimental ducks and traits

On January 23, 2005, this experiment was started to carry out at the Gaoyou duck breeding farm, Gaoyou city, Jiangsu province, P.R.China. Four hundred ducks of the same age and origin were used in this experiment. Each duck was kept in a small separate litter with a small pool. All ducks were raised in the same condition, fed the same diets ad libitum and were provided fresh water access freely. The laying mash consisted of 22.5% CP and 2,815 Kcal of ME/kg. Data on egg production including the total egg production, highest clutch days, egg weight (30 W), and number of double-yolked eggs were collected daily using trap nests to identify individual birds. The data for individual ducks were collected for over 3 months of experimental period and recording commenced at 22-weeks of age. DNA and trait data were obtained from 372 ducks.

PCR-RFLP and DNA sequencing

Blood was sampled from plumage veins and sampled into test tubes containing an anticoagulant solution. Genomic DNA was isolated from it and eluted into 450 μl of TE. Polymerase chain reaction (PCR) was performed to amplify intron 1 of duck PRL gene. The primers designed by myself were used to amplify the target region. The corresponding sequences were 5′-GAATAGAACACTTGACCCTG-3′(forward), and5-′TAGAGGAGGCAAGCATAG-3′ (reverse).

The PCR reaction was carried out on an Eppendorf Mastercycle. The reaction recipe contained 2.5 μl 10 × Buffer, 2.5 μl dNTPs (2.5 mM), 2.5 μl Mg^{2+} (25 mM), 1 μl each primer(25 pmol/μl), 3.0 μl genomic DNA (100 ng/μl), 0.2 Taq polymerase (5 U/μl). The thermal cycling profile for mtDNA was 5 min preheat at 95 °C followed by 35 cycles of 45 s at 94 °C, 45 s at 58°C, 1 min at 72 °C, a final extension of 10 min at 72 °C, and conservation at 4 °C. The PCR product was digested using 1 μL DraI(1 U/μL) enzyme at 37 °C overnight. The digestion products were separated by horizontal electrophoresis (50 volts, 60 min) in 2% agarose gels in 1 × TBE and 1.0 mM ethidium bromide. PCR products were agarose gel-purified and sequenced on an ABI Prism 3730 DNA Analyzer in both directions by primer walking using a BigDye Terminator V. 3.1 Cycle Sequencing Kit (ABI, Foster City, CA).

Statistical Analysis

Data for 100-day egg production (NE 100 d), highest clutch days, egg weight (30 W), and the number of double-yolked (DYE) were obtained from the farm records. Statistical calculations were performed using SPSS procedures. Frequencies of distribution of alleles within the lines were compared with Chi-square test. The effects PRL genotypes on the egg production of chicken were analyzed using GLM procedure with the addition of genotype and genealogy effects.

The following model was used:

$Y_{ijk} = \mu + G_i + I_k + E_{ijk}$

Y_{ijk}: observed trait values

μ: overall mean

G_i: genotype effect

I_k: genealogy effect

E_{ijk}: random error

Results

Sequence variation and PCR-RFLP analysis

For PRL, the 566-bp product from the intron 1 was sequenced for individuals of Gaoyou duck (Figure 1). The following DNA restriction fragments were obtained for PRL-*Dra* I polymorphism: 518bp/47bp for the AA genotype, 518/309bp/209bp/47bp for AB genotype, and 309bp/209bp/47bp for the BB genotype (Figure 2). The homozygous individuals of different genotypes were cloned and sequenced. The results showed that there was a T/C mutation (Figure 3) at the position of 1,326 bp in intron 1 region (Accession NO.AB158611).

The frequency of genotypes was analyzed by X^2 test. The observed distribution of genotypes accorded with the assumption of Hardy-Weinberg equilibrium. Three genotypes, AA, AB, and BB, were found with the preponderant gene B frequency of 0.774 and the gene A frequency of 0.226.

The association between PRL intron 1 polymorphism and egg performance

Association of SNP or haplotypes with egg performance was analyzed using the GLM procedure of SPSS11.0. The results are presented in the Table. The BB ducks had significant egg weight at the age of 30 weeks than that of AB ducks ($P < 0.01$). The

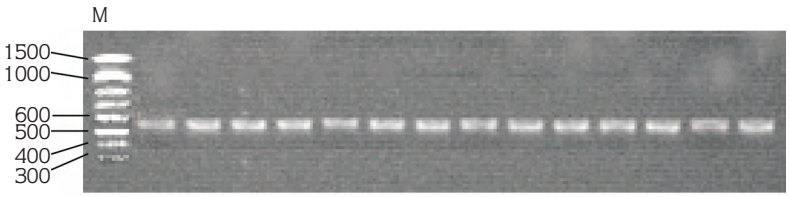
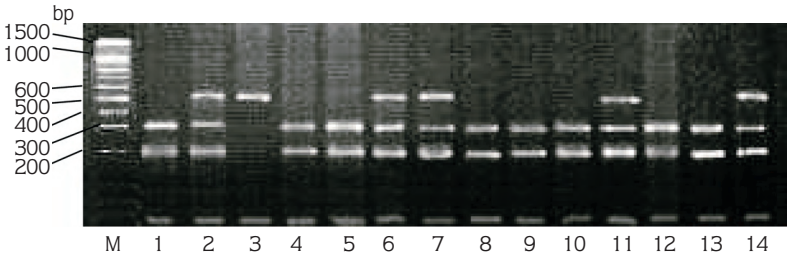


Figure 1. PCR Product of PRL gene M. PCR Marker (100bp+1.5kb DNALadder).



M. PCR Marker (100bp+1.5kb DNA Ladder)
1,4,5,8,9,10,12,13.BB; 2,6,7,11,14.AB; 3.AA

Figure 2. Genotypes of PRL gene intron 1 digested by enzyme *Dra I.*

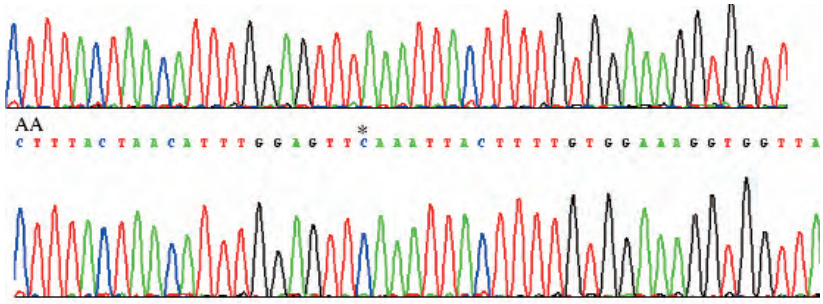


Figure 3. The sequenced results of PRL gene intron 1 BB and AA genotypes.

Table. The interaction of PRL gene intron 1 on egg performance of Gaoyou duck.

Traits	PRL intron 1 genotypes				
	AA(n = 20)	AB(n = 128)	BB(n = 224)	Additive	Dominant
Body Weight at first egg	2.30 ± 0.05	2.26 ± 0.02	2.28 ± 0.02	-0.01	0.0
Highest clutch days	27.2 ± 4.2	28.6 ± 1.6	31.0 ± 1.2	1.9	-0.5
Egg weight	76.80 ± 1.48	74.51 ± 0.58^{A}	76.72 ± 0.44^{B}	-0.04	-2.25
NE (100d)	83.4 ± 1.9	80.7 ± 0.7	81.9 ± 0.6	-0.75	-1.95
Double-yolk duck percent	0	1.88	0.71		
Double-yolk percent	0	2.17^{a}	0.67^{b}		

^{a,b} Means in the same row with different superscripts are significantly different at: small letter- $P < 0.05$, capitals- $P < 0.01$

double-yolk percentage of the AB ducks was much higher than that of BB ducks ($P < 0.05$) in double-yolk ducks. There were no significant association between PRL intron 1 polymorphism and the other traits ($P > 0.05$) in this study.

Discussion

It has been reported that the avian PRL gene is highly conserved most of sequence polymorphisms in the chicken PRL gene occur in 5′ flanking region, 3′ flanking region, and the coding region of the signal peptide (3,9,13). Cui et al (10) reported that 3 SNPs had been detected in cPRL exon 2 and exon 5, and 1 SNP had been detected in cPRL intron 2. In this study, for the first time 1 SNP was detected in duck PRL intron 1 and this polymorphism locus can be identified by restricted enzyme *Dra* I. The frequency of allele A was 0.226, the frequency allele B was 0.774, so the allele B was a preponderant gene in Gaoyou duck population. The sequenced results showed that there was a T/C mutation (Figure 3) at the position of 1326 bp in intron 1 region (Accession NO.AB158611). The frequency of genotypes was analyzed by X^2 test. The observed distribution of genotypes accorded with the assumption of Hardy-Weinberg equilibrium.

The heritability of egg weight is high. Besides the genetic factor, egg weight was mainly influenced by the age of the laying hen, and also related with body size, nutrition and environment. In this study, we chose the same age ducks with the same genetic background, kept them in the same feeding condition and body size, the day of laying first egg were nearly the same. So, we could analyze the association between polymorphism of PRL gene and the egg performance more exactly. PRL gene as a candidate gene had been studied in laying hen (10). In this study, the least square analysis showed that the BB ducks had significant egg weight at the age of 30 weeks than AB ducks ($P < 0.01$). So, we presumed that the PRL gene influenced the egg weight by regulating the activity of reproduction. Although intron did not participate in protein synthesis, the variance of the intron 1 may affect 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. The T/C mutation at the position of the 1326 bp may influence the PRL gene expression. The regulatory mechanism for PRL gene expression has been well documented in mammals. In mammals, Pit-1 plays an important role in regulating the PRL gene (14,15). The involvement of Pit-1 in controlling PRL gene expression is very complex in birds. On the other hand, details of the control mechanism in other orders are not understood. Further studies are required to know the exact regulatory mechanism of PRL gene expression.

The double-yolk percentage of the AB ducks was much higher than that of BB ducks ($P < 0.05$) in double-yolk ducks. It was reported that 1 SNP of PRL intron 1 in Wenchang chicken (Chinese indigenous

chicken) had been detected by PCR-SSCP. The result showed that there was no significant correlation between PRL polymorphism and the number of double-yolked egg (DYE), but PRL polymorphism has additive effect on the number of double-yolked eggs, the E_1E_2 genotype had greater DYE at 0.37 compared to 0.23 and 0.20 for E_1E_1 and E_2E_2 genotype, respectively (11). Although we had not found the dominant effect of PRL intron 1 on the trait of double-yolked egg, the hybrid vigor might exist. So, to identify the existence of hybrid vigor, further researches will be carried out. Generally, there were 2 mechanisms of forming double-yolked egg, they were physiological and genetic mechanism. In physiological mechanism, the young female duck with hearty reproductive function excreted more hormone of PRL, estrogen and other reproductive hormone, thus lead to superovulation. In the genetic mechanism, the heritability of double-yolked egg is very low. The trait may affect by minor gene and environment together. Firstly, we studied the trait of double-yolked egg in Gaoyou duck by molecule biological method, but study on the reproductive traits of Gaoyou duck required analysis of more genes in further studies. The genetic mechanism refers that many genes are very complex. So, it will need more hard work in a long time to know the genetic mechanism of forming double-yolked egg.

Acknowledgement

This research was supported by the Programs of the National Technological Supporting Project of P.R. China (No. 2008BDAB2B08) and the High Technological Project of Jaingsu Province of P.R. China (No. BG2007323).

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