Investigation of Prolactin Gene Polymorphism in Japanese Quail

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Abstract

Prolactin (PRL) is a peptide hormone synthesized and secreted by specialized cells in anterior pituitary of vertebrates. The objective of the present study was to investigate the polymorphism of prolactin promoter in Japanese quail. Blood samples were collected and DNA was extracted from the samples according to a commercial kit protocol. This gene was amplificated by polymerase chain reaction (PCR) a 24-bp indel (insertion or deletion) at nucleotide position (np) 358 was identified. Based on our results, we observed two alleles A and B in this locus.

Keywords: Japanese quail, polymorphism, prolactin gene.

1. Introduction

Recently, Japanese quail (Coturnix Japonica) have become an important livestock in Turkey. The advantages of Japanese quail, which have been widely used for biological and genetic studies are because of this bird has a small body size, easily handled, a large number of birds can be kept in a limited space, sexual maturation is rapidly accomplished, turnover of generations is rapid, high egg production and many offspring can be available from certain number of parents [1-3].

Prolactin (PRL) is a peptide hormone synthesized and secreted by specialized cells in anterior pituitary of vertebrates. It has been well established that PRL plays an important role in the onset of incubation and brooding behavior of birds [4-6]. The prolactin gene consists of five exons and four introns [6]. Elevated levels of PRL decrease the egg sequence lengths (clutch length) by increasing the inter sequence pauses between the sequences of egg lay. This is particularly pronounced in native birds [7, 8]. PRL is also involved in crop-sac development of columbiforms, induction and maintenance of broody behavior, regulation of gonadal function and immune responsiveness in a variety of species [9]. Liang *et al*. [10] suggested that these loci might serve as potential genetic markers for chicken breeds, also provide valuable insights into the regulation of chicken PRL gene expression. The flanking regions in chicken and turkey show high similarity [10]. The chicken prolactin gene complete sequence was reported previously [11, 12]. In recent years, the polymorphism of the cPRL gene was reported [13, 14] and the correlation between the gene polymorphism and egg performance had also been analyzed [14]. It was reported that 1 SNP of PRL intron 1 had been detected in Wenchang chicken (Chinese indigenous chicken) by PCR-SSCP [15]. The aim of the present study was to estimate the frequency of PRL gene variants in Japanese quail.

2. Materials and methods

The blood samples were collected randomly from 194 quails reared in Quails Research Station, located in Gorgan University of Agricultural Sciences and Natural Resources, Golestan

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province, Iran. DNA was extracted from 100 µl of blood, using a commercial kit (Diatom DNA Prep100, ISO Gene, Moscow) following the manufacturer's protocol. The quality and quantity of extracted DNA were measured spectrophotometically and gel electrophoresis on 1% agarose.

Polymerase Chain Reaction (PCR) was carried out, using the Personal Cycler™ thermocycler (Biometra, Germany) and the PCR Master Kit (Cinna Gen Inc., Iran). The kit of master mix consisted of 0.04 U/µl of *Taq* DNA polymerase, 10X PCR buffer, 3mM MgCl2 and 0.04 mM dNTPs (each). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (5 pmol/µl) and some deionized water making up a final volume of 25 µl. Amplification for a fragment of PRL gene (130 or 154 bp, containing the 24 bp indel at np 358) was carried out using primers described by Cui *et al*. [14] under following condition:

PRL-F (5′-TTT AAT ATT GGT GGG TGA AGAGAC A-3′) and PRL-R (5′-ATG CCA CTG ATC CTCGAA AAC TC-3′).

The following cycles were applied for the PRL gene amplification: 94°C for 5 min; followed by 35 cycles of 30 sec at 94°C, 30 sec at 54°C, and 30 sec at 72°C; and a final extension of 5 min at 72°C.The PCR-products of the 24 bp and were run on 10% polyacrylamide gel. Silver nitrate was used for staining the gels.

3. Results and discussion

Allele and genotype frequencies were calculated using Pop-Gene, 1.31 software [16]. A chi-square ($\chi 2$) test was performed to test the goodness of fit to Hardy–Weinberg equilibrium expectations for the distribution of genotypes. The electrophoretic profile of PCR products of PRL gene is shown in Figure 1. The frequencies of alleles and genotypes for this gene are shown in Table 1. Based on present results, the frequencies of A and B alleles were 0.52 and 0.48, respectively. Frequencies of AA, AB and BB genotypes were 0.27, 0.50 and 0.23, respectively. The observed and expected genotypes of PRL were calculated. The analysis of chi-square test showed that this population wasn't in Hardy-Weinberg equilibrium (Table 2). In current study, we detected two alleles (A and B) and three genotypes for this locus. Present results are in agreement with previous reported by Emamgholi Begli et al. [17] that reported two alleles (I=0.76 and D=0.24) in native hens. Alipanah et al. [18] also observed two alleles T and C with frequencies 0.67 and 0.33 in chicken, respectively. Xu et al. [19] observed two alleles (I and D) and three genotypes (II, ID and DD) for this gene in chicken. The frequencies of alleles I and D were studied in native and commercial chickens in a study by Cui *et al*. [14]. In their study, the frequencies of alleles I and D were found to be 1 and 0 in White Leghorn, 0.05 and 0.95 in Yangshan, 0.20 and 0.80 in Taihe Silkies, 0.22 and 0.78 in White Rock and 0.17 and 0.83 in Nongdahe. In addition, Li et al. [20] studied polymorphism of PRL gene in duck and found two alleles (A=0.226 and B=0.774) and three genotypes AA, AB and BB. Jiang et al. [21] studied the P2 locus of PRL gene and found two alleles A and B with frequencies 0.73 and 0.27 in Wan-xi White and 0.59 and 0.41 in Rhine geese, respectively.

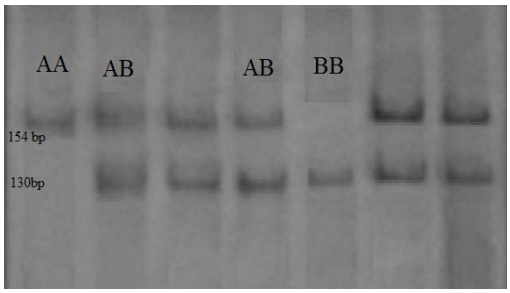

Figure 1. Genotypes of the 24-bp indel at np 358 by PCR on 10% polyacrylamide. AA: insertion-insertion; BB: deletion-deletion; AB: insertion-deletion

In the present study, also there was a 24-bp nucleotide sequence insertion at np 358 of the 5′-flanking region of PRL. Insertion of this sequence in the promoter may inhibit pituitary transcription factor 1 (Pit-1), VIP and other transcriptional factor binding sites for PRL [8] or may reduce secretion of stimulatory factors like thyrotropin-releasing hormone, that affect PRL release and therefore, decrease the expression of PRL. A possible ecotropic viral integration site-1 (Evi-1) binding site was found in the 5′ flanking region of the chicken PRL gene due to the 24-bp insertion [4]. The presence of Evi-1 binding site suggested possible transcriptional regulation of the chicken PRL gene by Evi-1. It is possible that Evi-1 represses the expression of PRL gene in chickens

by binding the Evi-1 binding site, and further prevents broodiness [6], which can improve egg production to some extent.

Table 1: Distribution of PRL alleles and genotypes frequency

Gene	Number	Allele Frequency (%)		Genotype Frequency (%)		
		A	B	AA	AB	BB
PRL	194	0.52	0.48	0.27	0.5	0.23

*A = insertion allele; B = deletion allele; AA = insertion-insertion; AB = insertion-deletion; BB = deletion- deletion.

Table 2: Chi-Square test for Hardy-Weinberg equilibrium in prolactin genotypes

Genotypes	Observed (O)	Expected (E)	$(O-E)^2/E$	$\chi 2$
AA	19	52.45	21.34	92.64
AB	164	97.08	46.12	
BB	11	44.45	25.18	

4. Conclusions

The goal of this study was to determine genetic polymorphism of prolactin (*PRL*) gene in Japanese quail. These results revealed that polymorphism was detected in this population and observed two alleles for this locus. This study also, opens interesting prospects for future selection programs, especially marker assisted selection.

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