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Genetic Polymorphism in Prolactin Gene and its Association with Reproductive Traits in Japanese Quail (*Coturnix japonica*)

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Abstract

The present study was carried out to investigate the polymorphism of intron 3 to exon 3 of prolactin gene containing 24 bpindel at nucleotide position (np) 358 and its association with some reproductive traits in Japanese quail. These traits consisted of weight (WSM) and age at sexual maturity (ASM), mean egg weight at 2nd, 4th, 6th, and 2-6th weeks (MEW), and the number of eggs during the 2nd, 4th, 6th, and 2-6th weeks of laying period (EN). Blood samples of 194 Japanese quail at 13wk of age were collected randomly. DNA was extracted from blood samples and amplified. The association of prolactin genotypes with reproductive traits was analyzed using the general linear model procedure of SAS software. A 24-bp indel[insertion (I) or deletion (D)] at np358 was identified. Based on the results obtained, the frequency of I and D alleles were 0.52 and 0.48, respectively. Frequencies of II, ID and DD genotypes were 0.10, 0.85 and 0.05, respectively. Genotypes II and ID were significantly associated with increased EN ($P<0.01$). The genotypes of the 24-bp indel site were not significantly associated with other traits ($P>0.05$). The results showed that prolactin gene polymorphism could be used to improve egg production in Japanese quail through marker-assisted selection.

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Introduction

Prolactin (PRL) is one of polypeptide hormones secreted by the anterior pituitary gland in vertebrates. It has been proved that PRL plays an important role in the onset of poultry incubation and brooding behavior (Sharp *et al*., 1988; Shimada *et al.,* 1991; Jiang *et al*., 2005). A rise of prolactin level in plasma may induce incubation behavior and then terminate laying (Sockman*et al.,* 2000), resulting in decreased egg production (Reddy *et al.,* 2002). In some of avian species such as ducks and geese, a sharp rise in plasma PRL level occurs during the formation of the final 10 - 20% of the clutch, which females markedly increase their nest-box occupancy (Hall, 1987; 1991; Fang *et al.,* 2005). Elevated levels of PRL decrease the egg sequence lengths (clutch length) by increasing the inter sequence pauses between the sequences of egg laying. This is particularly pronounced in native birds (Reddy *et al*., 2002; 2006).

PRL is also involved in the crop-sac development ofcolumbiforms, induction and maintenance of broody behavior, regulation of gonadal function and immune responses in a variety of species (Kansaku *et al*., 2008). Therefore, PRL exhibits similar changing profiles in the reproductive cycle and exerts biological functions in the similar pattern in different domestic avian species. To elucidate the genetics of PRL, genomic structure of PRL in avian have been investigated extensively (Kansaku *et al*., 2008). An abundance of SNP has been reported in the 5'-flanking region of chicken PRL (Liang *et al*., 2006) and the 24-bp insert-deletion was significantlyassociated with broody behavior and egg production (Jiang *et al*., 2005; Cui *et al*., 2006), indicating its usefulness as a molecular marker for egg production. Cloning of PRL cDNA in avian species was conducted in the chicken (Watahik *et al*., 1989), turkey (Wong *et al*., 1991) and domestic duck (Kansaku *et al*., 2008).

The Japanese quail prolactin gene is 10 KB in size and is composed of 5 exons and 4 introns, encoding 229 amino acids. The Japanese quail PRL has an overall similarity with a comparable region of chicken (96.5%), turkey (93%), duck (93.4%), goose (93.4%), ostrich (91.3%) and budgerigar (92.6%) PRL (Kansaku *et al*,. 2008).

The aim of the present study was to identify prolactin polymorphism and its possible association with reproductive traits in Japanese quail.

Materials and Methods

Birds and traits

This experiment was carried out at the Poultry Research Station of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan,Iran. The experimental Japanese quail population (*Coturnix japonica*) was fed on a diet that consisted of 20% CP and 3000 Kcal/Kg ME. The temperature of the hen house was around 20ºC and the light was given 15 hrs per day (from 6:00 AM to 9:00 PM). Age and weight of each bird when the first egg was laid were considered as age and weight at sexual maturity (ASM and WSM, respectively). Producedegg number (EN) and mean egg weight (MEW) of each bird at 2nd, 4th, 6th, and 2-6th weeks of laying period were recorded.

PCR and DNA sequencing

Blood samples of 194 female birds were randomly collected at 13 wk of age. Blood samples were stored in 2 mL tubes containing EDTA as coagulant agent. DNA was isolated from 200µL blood sample using DNA extraction Kit (ISO Gene, Moscow, Russia) based on Boom *et al.* (1990). Gel monitoring and spectrophotometry were used to determine the quality and quantity of the extracted DNA. The primers used for the amplification of the PRL gene fragments (130 or 154 bp, containing the 24 bpindel at np 358), were those described by Cui *et al*. (2006). The primer sequences were as follows: forward 5´-TTT AAT ATT GGT GGG TGA AGA GAC A-3´ and reverse 5´-ATG CCA CTG ATC CTC GAA AAC TC-3´. The PCR was performed in 25 µL mixture containing 100 ng of genomic DNA, 1X PCR buffer, 0.5 µM of each primer (TakapooZist, Tehran, Iran) and 12.5 µL of master mix (Sina Gene™, Tehran,Iran). 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, 30 Sec at 72°C; and a final extension of 5 min at 72°C. The PCR-products of the 24 bp region was run on agarose gel 3%. Ethidium bromide was used for staining the gels.

Statistical Analysis

Alleles and genotypes frequencies and their accordance to Hardy-Weinberg equilibrium were calculated using POPGENE software (Yeh *et al*., 1997). The association of genotypes with reproductive traits was investigated using the GLM procedure of SAS software (2001). The following model was used:

$Y_{ijk} = \mu + G_i + H_j + e_{ijk}$

Y_{ijk}: observed trait values

μ: overall mean

G_i: genotype effect

H_j: fixed effect of hatch

e_{ijk}: random residual effect

Results

The alignment of the nucleotide sequences of PRL gene is shown in Figure 1. Two alleles, [insertion (I) or deletion (D)] and three genotypes, namely, II, ID and DD, were observed in the population. The observed frequencies of alleles and genotypes for the PRL gene are shown in Table 1. The frequencies of I and D alleles were 0.52 and 0.48, respectively. The observed frequencies of II, ID and DD genotypes were 0.10, 0.85 and 0.05, respectively. The chi-square test revealed that the Japanese quail population is not in Hardy-Weinberg equilibrium for this region of the PRL gene (Table 2). The effect of polymorphism of the PRL gene on reproductive traits was evaluated (Table 3). Insertion-insertion (II) and insertion-deletion (ID) genotypes were significantly associated with increases in EN ($P<0.01$) (Table 3). Nevertheless, genotypes of the 24 bpindel were notsignificantly associated with other traits (EN, MEW, ASM and WASM).

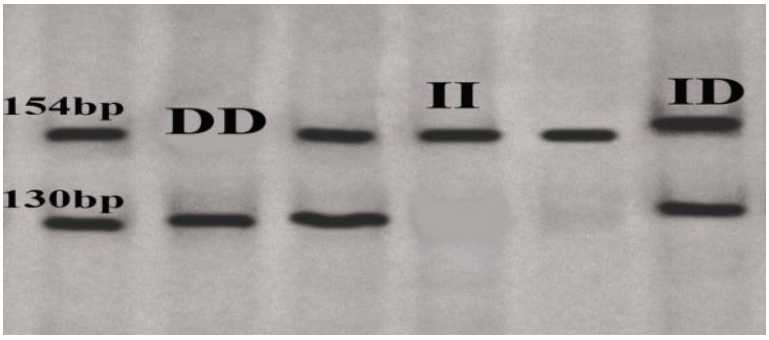

Figure 1.Genotypes of the 24-bp ideal at no 358 by PCR on 6% polyacrylamide.
II: insertion-insertion; DD: deletion-letion; ID: insertion-deletion.

Table 1. Allelic and genotypic frequencies for Hardy-Weinberg equilibrium in prolactin genotypes

Frequency (%)	[1]I	D	II	ID	DD
	52	48	10	85	5

[1]I = insertion allele; D = deletion allele; II = insertion-insertion; ID=insertion-deletion; DD = deletion-deletion.

Table 2. Chi-Square test for Hardy-Weinberg equilibrium in the population

Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	$\chi 2$
II	19	52.47	21.34	93.31***
ID	164	96.85	46.56	
DD	11	44.69	25.40	

*** P≤0.001

Table 3. Prolactin genotype-wise least square means of reproductive traits in Japanese quail

Genotype	ASM (d)	WSM (g)	EN2	EN4	EN6	EN2-6	MEW2	MEW4	MEW6	MEW2-6
II	46.97	233.16	4.90[a]	6.96[a]	6.73[a]	27.4[a]	12.43	12.32	12.29	12.24
ID	48.60	235.75	4.52[a]	6.15[a]	6.58[a]	28.59[a]	11.55	11.96	12.21	11.97
DD	54.63	234.55	2.63[b]	4.84[b]	4.93[b]	22.73[b]	13.42	12.58	12.04	12.68

Values in the same column with different superscripts are significantly different (P< 0.01)
ASM: age at sexual maturity; WSM: weight at sexual maturity; EN2, EN4, EN6, EN2-6: produced egg number during 2nd, 4th, 6th and 2-6th wks of laying period; MEW2, MEW4, MEW6, MEW2-6: mean egg weight during 2nd , 4th, 6th and 2-6th wks of laying period.

Discussion

Molecular characterizations of the PRL gene promoter in Japanese quail are rare, and most of reported data are limited to native or commercial chickens in studies of Cui *et al*. (2006), Emamgholi-Begli *et al*. (2010), Alipanah *et al*. (2011) and Rashidi *et al*. (2012). Prolactin, one of pituitary hormones, regulates important physiological functions, ranging from well-known effects in mammalian reproduction to osmoregulation in fish and its roles are not yet understood extensively in birds, but its major function is believed to be manifested during incubation and feeding of nestlings (Li *et al*., 2009). Several studies showed that pituitary transcription and growth hormone factors (Kurima *et al*., 1995; Frisch *et al*., 2000), estrogen receptors (Maurer and Notides, 1987) and CCAAT enhancer binding protein-α (Day *et al*., 2003; Enwright *et al*., 2003) are essential in regulating the expression of PRL via specific promoter binding sites. In the present study, we found the existence of polymorphism at the 24-bp indel site of prolactin promoter in Japanese quail which is in line with the results obtained by Emamgholi-Begli *et al*. (2010). The allelic and genotypic frequencies obtained at the 24-bp indel site of prolactin gene were different from those reported by Rashidi *et al*. (2012) in Iranian indigenous breeder hens. In their study, allelic frequencies of I and D at 5′-flanking region of prolactin gene were 0.59 and 0.41, respectively and the frequency of the observed genotypes were 0.39, 0.40 and 0.21 for II, ID and DD birds, respectively. At the present study the frequency of I (0.52) and D (0.48) alleles were approximately close to each other but the frequency of heterozygous genotype (ID:0.85) was higher compared to homozygous (II:0.10) and (DD:0.05) genotypes. Insertion of sequences and essential proteins in regulating the expression of PRL via specific promoter binding sites may inhibit pituitary transcription factor 1, vasoactive intestinal polypeptide and other transcriptional factor binding sites for PRL (Jiang *et al*., 2005) or may reduce secretion of stimulatory factors like thyrotropin-releasing hormone, that affect the PRL release, and therefore decrease the expression of PRL. The II and ID genotypes were significantly associated with increased EN which was in agreement with the findings of Emamgholi-Begli *et al*. (2010).

The present results showed that PRL polymorphism could be used as a marker for improving egg production in Japanese quail.

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