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The Polymorphism of *Prolactin* Gene in Native Chicken Zabol Region

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Abstract: The aim of this study was to determine genetic polymorphism of *prolactin* gene for native chickens Zabol. Avian prolactin hormone located in chromosome No: 2. It is a polypeptide that it molecular weight is 21700-26000 Dalton and effective in increasing broodiness and commonly results in regression of the ovary. In this study, blood samples were collected from 30 native chickens randomly and DNA was extracted. The PCR was used to amplify 439 bp fragments for *prolactin* gene locus. The amplified fragment was digested with Alu I enzyme. Allele frequencies of T and C prolactin hormone gene for native chickens were 0/67, 0/33, respectively. Genotype frequencies TT, CT, CC in herd were 53/33, 26/67 and 20/00%, respectively. The results show that PCR-RFLP method is proper for genotype determination of *prolactin* gene chicken studies.

Key words: *Prolactin* gene, polymorphism, PCR-RFLP, native chicken, genotype, Iran

INTRODUCTION

The native chicken PRL genomic sequence has 6, 163 bp long. The 5 exons are 28, 182, 108, 180 and 192 bp long. The 4 introns are 1,520, 408, 1,348 and 1,909 bp long. (Au and Leany, 2002). Physiologically, it has been well established that prolactin in poultry play an important role in the onset of incubation of hens. Increased plasma prolactin concentration is associated with the occurrence of broodiness. During incubation, prolactin mRNA reaches its highest level which infers that prolactin is important in the maintenance of broodiness. Egg laying pattern in domestic hen is characteristic to the breed of birds. Genetically superior birds' take fewer pauses compared to native breed of birds developed for dual purpose, resistant to diseases and adverse climatic variables as backyard poultry in rural areas (Reddy *et al*., 2006). It has been reported that most of sequence polymorphisms in the chicken *PRL* gene occur in 5 flanking region, 3 flanking region and coding region of the signal peptide (Wong *et al*., 1991; Zhou *et al*., 2001; Cui *et al*., 2006). Therefore, polymorphisms in the promoter region especially those that result in changes of promoter binding sites, most likely influence mRNA expression thus influencing hen incubation behavior and egg production. Polymorphisms of 5 flanking region of chicken *Prolactin* (cPRL) gene were examined in several populations of Chinese native Yuehuang, Taihe Silkie and White Leghorn Layer chickens. About 4 Single Nucleotide Polymorphisms (SNPs) were identified at position -2425 (C/T), -2215 (T/C), -2063 (G/A) and -1967 (A/G). A 24 bp indel (insertion or deletion) and a polyA length polymorphism were also identified. For the 24 bp indel locus, 3 genotypes (AA, AB and BB) were found in Yuehuang chickens while only 2 genotypes were detected in Taihe Silkie (AB and BB) and Leghorn chickens (AA and BB). The genotype frequencies of AA, AB and BB were significantly different among the 3 breeds. For the polyA locus, although 3 genotypes (CC, CD and DD) were found, only one genotype (CC) was detected in White Leghorn chickens while 2 or 3 genotypes were observed in Chinese native chickens. The results show that chickens with genotype AB of 24 bp indel locus which are of the highest incidence of broodiness had the highest cPRL mRNA levels providing the possibility that this polymorphic site might be related to the broodiness in chickens via modulating the transcriptional level of *cPRL* gene (Liang *et al*., 2006). Broodiness is observed in most breeds of domestic fowl with the exception of the White Leghorn which has undergone long-term artificial selection to minimize phenotypic expression of this behavior (Romanov *et al*., 2002). The purpose of this study was to estimate the allelic frequencies at the prolactin gene in native chicken of Zabol region.

MATERIALS AND METHODS

A total of 100 samples were genotyped for *Prolactin* gene. The birds used for this study was a Iranian native breed in Zabol region. The chicken produces almost <100 egg year^{-1} and exhibits broodiness. Genomic DNA Extraction Venous blood was collected from the wing vein of each individual with EDTA as an anticoagulant. Blood samples were stored at -30°C till the time for DNA extraction. Genomic DNA was extracted from the whole blood by the conventional phenol/chloroform extraction method or with a DNA extraction kit (Genefanavaran Iran). The prolactin genotypes were analysed using the PCR-RFLP method. PCR products were amplified using primers as previously described (Cui *et al*., 2006):

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5FA: Forward 5'-AGA GGC AGC CCA GGC ATT TTA-C-3' Reverse 5' -CCT GGG TCT GGT TTG GAA ATT G-3'

Cycles applied were denaturation -94°C 5 min^{-1}, followed by 35 cycles 94°C 30 sec^{-1}, annealing 60°C 30 sec^{-1}, extension 72°C 30 sec^{-1} and final extension 72°C 5 min^{-1}. PCR conditions: 2.5 μL 10×PCR buffer (15 mM $MgCl_2$) 1.5 μL dNTP-mix (2 Mm each), 1.5 μL of primer (100 pmol μL^{-1} each), 0.5 μ Taq DNA polymerase (Genefanavaran, Iran) Amplified DNA was digested with Alu I enzyme. Digestion products were separated electrophoretically in 3% (w/v) agarose gel. Frequencies of distribution of alleles within the herds were compared with χ^2-test.

RESULTS AND DISCUSSION

The electrophoresis profiles fragment of *prolactin* gene obtained from primer pair 5FA are shown in Fig. 1.

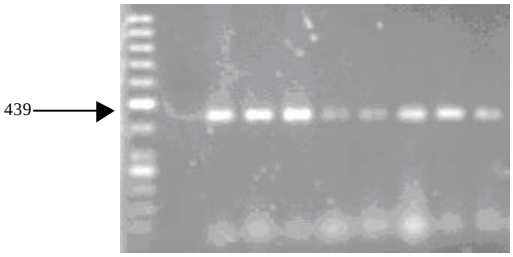

Fig. 1: Gel picture of prolactin fragment amplified by primer pair 5FA before digestion

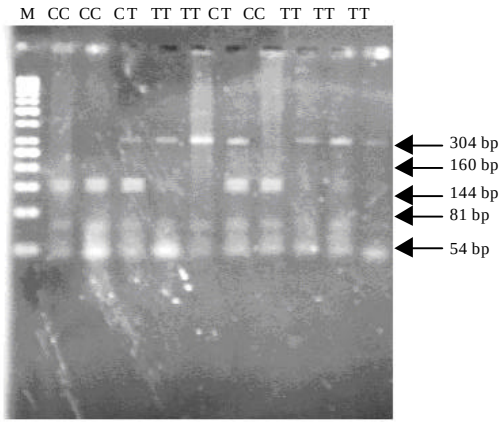

Fig. 2: Restriction analysis of cPRL 439 bp PCR products digested with Alu I by 3% w/v agarose gel electrophoresis stained with ethidium bromide. CC genotype = 160, 144, 81 and 54 bp; CT genotype = restriction fragments of 304, 160, 144, 81 and 54 bp; TT genotype = restriction fragment of 304, 81 and 54 bp

For the polymorphism, 3 genotypes and 2 alleles were distinguishable according to their restriction fragment lengths: 160, 144, 81 and 54 bp (C allele) and 304, 81 and 54 bp (T allele). Genotypes and alleles of the *prolactin* gene are shown in Fig. 2. In this breed the frequencies of alleles were as follows; T = 0.67, C = 0.33. The frequencies of TT, TC and CC genotypes were 0.53, 0.27 and 0.20, respectively.

Frequency of prolactin allele A obtained in this study are similar to those reported by Jiang *et al.* (2005) for white rock (0.65 and 0.35, respectively) no the results was differ for whit leghorn and Yangshan (1.00, 0.00, 0.05 and 0.95, respectively).

Prolactin one the pituitary hormones, regulates important physiological functions in animals also have effect on nesting behavior (broodiness) in bird (Elkins *et al*., 2000).

CONCLUSION

Comparison among chicken breeds show that selected breeds for egg production as leghorn is seldom broodiness however, native chicken breeds have current broodiness in order to maintain normal reproduction. Therefore, if we decided to select native chicken for egg production, we can use of polymorphism *prolactin* gene for reduction broodiness.

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