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Genetic polymorphism in the promoter of prolactin gene in layer chicken

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ABSTRACT

Genetic polymorphism of promoter (Fragment 1, 2 and 3) of prolactin gene in four strains of White Leghorn chicken layers i.e. IWH, IWI, IWK and layer control was identified. Two alleles (A and B) were found in all the promoter fragments of the gene. In above mentioned layer populations, A allele was predominant. The genotypic and allelic distribution was significantly differed among the populations. Different demographic and divergent parameters were estimated in all the lines. It is concluded that all 3 fragments of promoter of prolactin gene was polymorphic in four strains of White Leghorn layer populations.

Key words: Chicken, Polymorphism, Prolactin, Promoter.

Prolactin hormone is secreted by the anterior pituitary gland in vertebrates. This hormone has a large spectrum of functions, including regulation of broodiness and regression of the ovary and reduction of number of graafian follicles in chickens (Talbot and Sharp, 1994; March *et al.*, 1994 and Crisostomo *et al.*, 1997). The chicken prolactin gene was cloned and characterized (Zhou *et al.*, 2001). For encoding of gene, the promoter played a crucial role, by binding with several factors, e.g. pituitary specific transcription factor-1(Pit-1), v-ets erythroblastosis virus E26 oncogene homolog 1, CCAAT/enhancer binding prolactin, vasoactive intestinal peptide, activin, etc. Therefore, the objective of the current study was to explore polymorphisms in prolactin promoter.

Birds: The study was conducted on 4 strains of White Leghorn chickens, namely IWH, IWI, IWK and layer control, maintained at the Project Directorate on Poultry, Hyderabad, India. The experiment was carried out on 147 birds of IWH line, 132 birds of IWI line, 143 birds of IWK line, and 190 birds of the control layer line. All birds were randomly selected for the present study.

Sample collection and extraction: Blood samples were collected aseptically from the wing veins of the birds with 50 µl of 0.5M EDTA anticoagulant and transported to the laboratory. Genomic DNA was isolated from blood following the protocol of Sambrook and Russel (2001) with slight modifications. The quality and quantity of DNA was verified by 0.8% agarose gel electrophoresis. All DNA stocks were diluted with sterile double distilled water to create a standard DNA concentration of 100 µg/µL.

PCR-SSCP: The whole promoter of prolactin gene was divided into 3 fragments for SSCP genotyping as the SSCP method has limitation that the fragment size should be restricted to 300 bp for efficient typing. A total of 3 fragments namely fragment 1, fragment 2 and fragment 3 of prolactin promoter were grouped in the present study. Primers were designed with DNASTAR software (Lasergene Inc) using the prolactin gene sequence available at NCBI (NCBI Accession number-AB011438). The primer sequences have been stated in Table 1. The PCR reaction included 50 µg of DNA template,10 ng of each primer, 1.5 mM of $MgCl_2$, 100 µM of each dNTP, 1X assay buffer and 0.25 U of Taq DNA polymerase (MBI Fermentas). The PCR programme was initial denaturation at 94^0C for 5 minutes, 30 cycles of denaturation at 94^0C for 30 sec, annealing at 62^0C (For fragment 1)/ 62^0C (For fragment 2)/ 58^0C (For fragment 3) and extension at 72^0C for 30 sec with a final extension at 72^0C for 10 minutes.

A 12% native PAGE (50:1, acrylamide and bis-acrylamide) with 5% glycerol was prepared for SSCP study. The PCR product was electrophoresed at 200 V for 6 h and stained with silver nitrate to visualize banding patterns of the fragment (Vohra *et al.*, 2006).

Statistical analysis: Gene and genotype frequency was calculated by gene counting method (Falconer and Mackay, 1996). The genotypic and allelic distribution between populations was compared by Student's t test. Chi-square and likelihood ratio test was performed to test the Hardy-Weinberg equilibrium in the populations. Demographic

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TABLE 1: Primer details for amplification of prolactin promoter

Primer Name	Primer sequence (5'-3')	Fragments	Location in the Accession number- AB011438
PRL1F	CAT ACT CAG CAT CCC ACA GC	Fragment 1 (278)	-213 to 64
PRL1R	TGT TGC TCA TGG TAG GGA TTC		
PRL2F	GGT GGG TGA AGA GAC AAG G	Fragment 2 (201)	-403 to -203
PRL2R	TGC TGA GTA TGG CTG GAT GT		
PRL3F	GCC AGA AGC CTC CAT TTA C	Fragment 3 (118)	-502 to -385
PRL3R	CCT TGT CTT TCA CCC ACC A		

Values within parenthesis is fragment size (bp)

parameters like Shanon's information index, Nei's expected heterozygosity and Wright's fixation index were estimated following POPGENE32 software.

Genotypes: In IWH, IWI, IWK and layer control lines, 2 genotypes namely AA and AB were observed for fragment 1 in which AA was the most frequent genotype in IWH, IWI and IWK lines. In layer control, equal genotypic frequency (0.50) was observed in layer control line. Consequently, A allele was the most predominant one with a magnitude ranging from 0.75 to 0.94. Our results were in agreement with that of Kulibaba and Podstreshnvi (2012) who reported the presence of 2 alleles, C and T in prolactin promoter in both in egg type and meat type chicken. Cui et al (2006) observed 6 single nucleotide polymorphisms in the prolactin promoter region of White Leghorn, Yangshan, Taihe Silkies, White Rock, and Nongdahe breeds of chicken. The frequency distribution of genotypes and alleles were found to be significantly ($P<0.05$) different between IWH with IWI, IWK and layer control and between layer control with IWI and IWK. The distribution of genotypes and alleles between IWI and IWK did not differ significantly. In fragment 2 locus, 2 genotypes (AA and AB) were found of which AA had the highest frequency in IWH, IWK and layer control lines. In IWI line, AA and AB genotypes had similar frequency (0.50). In all the populations, A allele was the most predominant one having frequency in the range of 0.60 to 0.80. The frequency distribution of genotypes and alleles between layer control with IWH, IWI and IWK were found to be significant ($P<0.05$) while among IWH, IWI and IWK lines, distributions were not significantly different. In fragment 3 locus, 3 genotypes such as AA, AB and BB were found in all the lines in which AB genotype was most frequent one and BB was the least frequent one. Consequently, two alleles were observed of which A allele had the highest frequency ranging from 0.47 to 0.64. The frequency distribution of genotypes and alleles were not found to be significantly ($P<0.05$) different from each other.

Demographic parameter: For fragment 1 locus, chi-square test revealed that IWH, IWI, IWK and layer control lines follow Hardy-Weinberg equilibrium. The likelihood ratio test also indicated similar pattern of allelic distribution in all the population. In case of fragment 2 locus, IWH and IWK populations followed Hardy-Weinberg equilibrium. For fragment 3 locus, IWK and layer control populations followed Hardy-Weinberg equilibrium. Bhattacharya et al (2011) also revealed the test of Hardy-Weinberg equilibrium based on allelic distribution of candidate gene in broiler chicken lines. However, in all the lines, the number of polymorphic alleles observed was 2. In IWH line, the effective number of polymorphic alleles were 1.1 (fragment 1), 1.4 (fragment 2) and 1.9 (fragment 3). The Shanon's information index varied from 0.2 to 0.6 across the fragments. In IWI line, the effective number of alleles varied from 1.2 (fragment 1) to 1.9 (fragment 3) and the Shanon's information index varied from 0.35 (fragment 2) to 0.67 (fragment 3). In IWK line, the effective number of alleles varied from 1.2 in fragment 1 to 1.9 in fragment 3. The Shanon's information indices were highest (0.65) in fragment 3 and lowest (0.34) in fragment 1. In layer control, the effective number of allele was higher (1.9) in fragment 2 and fragment 3, and lower (1.6) in fragment 1. The Shanon's information index was highest (0.68) in fragment 3 and lowest (0.56) in fragment1.

Genetic diversity: The observed heterozygosity in IWH population varied from 0.12 for fragment 1 to 0.69 for fragment 3 and the similar trend was observed in Nei's heterozygosity where the magnitude was 0.11, 0.31 and 0.49 in fragment 1, fragment 2 and fragment 3, respectively. The heterozygosities observed in IWI population for fragment 1, fragment 2 and fragment 3, respectively were 0.22, 0.50 and 0.65 in while Nei's heterozygosity were 0.20, 0.37 and 0.48. In IWK population, fragment 3 revealed highest (0.42) heterozygosity and fragment 1 had the lowest (0.22) heterozygosity. The Nei's heterozygosity in fragment 1, 2 and 3 were 0.19, 0.23 and 0.46, respectively in this population. In layer control line, the highest and lowest heterozygosity were 0.50 for fragment 1 and 0.80 for fragment 2. The Nei's heterozygosity in the population was 0.56, 0.67 and 0.68 for fragment 1, 2 and 3, respectively. The Wright's fixation index, which is a measure of

heterozygote deficiency or excess were estimated in the populations. In IWH line, heterozygote deficiency was found for the alleles ranging from -0.06 in fragment 1 to -0.39 in fragment 3. In IWI line, heterozygote deficiency observed for all loci in which the magnitude varied from -0.12 (fragment 1) to -0.35 (fragment 3). In IWK line, heterozygote deficiency was found in fragment 1 and fragment 2 having magnitude of -0.12 and -0.15, respectively while in fragment 3 hehetozygote excess (0.08) was observed. In layer control, heterozygote deficiency was found in all the fragments and the magnitude varied from -0.26 in fragment 3 to -0.67 in fragment 2.

The Ewens-Watterson test for neutrality revealed significant contribution of natural selection in the populations. The birds were selected randomly for this study. Nature decides which birds are the fittest for its survival. The Ewens-Watterson neutrality test corroborated the participation of natural selection in the birds selected for the present study depending on the allelic diversity. In IWH, IWI and IWK lines, the Ewens-Watterson statistics for natural selection was around 0.81±0.02 while in layer control the magnitude was 0.77±0.02 in fragment 1 or 0.78±0.02 in fragment 2 and 3. In all the population, the statistics was significant. The Nei's genetic distance estimated from all the loci revealed that IWH and layer control related most distantly (0.044) while IWI and IWK related most closely (0.006). Thus, all the population parameters indicated the existence of genetic diversity in all the chicken populations. In conclusion, it may be stated that promoter of prolactin gene was polymorphic revealing different allelic distributions in the various layer chicken populations.

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