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Identification of single nucleotide polymorphism (SNP) of *prolactin* gene in white leghorn and its association with production traits

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Abstract
Marker assisted selection (MAS) is form a genomic selection that can complement the conventional method of selection in poultry breeding. MAS can be done using different molecular markers, one among them is single nucleotide polymorphism (SNP). The main objective of the present study was identification of SNP of *prolactin* gene in White Leghorn and its association with production traits. A total of 200 birds White Leghorn were randomly selected from AICRP on Poultry Improvement, Mannuthy. Blood samples were collected from the randomly selected birds and isolation of genomic DNA was done by using DNA isolation kit. PCR and PCR-RFLP analysis was carried out to find a SNP at C-2402T site of promoter region of *prolactin* gene. The genotypes are designated as CC, CT and TT for C-2402T. All the birds were observed with same genotype (CC) and production performance.

Keywords: Prolactin, SNP, PCR, white leghorn

Introduction
Single nucleotide polymorphisms are the most common type of genetic variation in a particular population, commonly called SNPs (pronounced "snips"). Each SNP represents a variation in a single nucleotide of DNA building block in which nucleotide cytosine (C) replaced with the nucleotide thymine (T) and vice versa in a particular DNA sequence [4]. SNPs occur normally throughout a person's DNA. It occurs almost once in every 1,000 nucleotides on average [11]. In poultry breeding programme, the selection is done mainly based on data on production traits and the extensive breeding records. This conventional method of selection based on phenotypic performance in chicken has successfully increased the quantity of production and reached the optimum level. At this stage, it is necessary to introduce the advanced technologies for the selection and breeding of poultry. SNPs can be used as a molecular marker to choose the superior individual from a particular population for the next generation [10]. The main objective of the present study was identification of single nucleotide polymorphism C-2402T in *prolactin* gene of White Leghorn and its association with production traits.

Materials and Methods
A totally 200 birds of White Leghorn birds which had undergone 28 generations of continuous selection were randomly selected from All India Research Co-ordinated Project (AICRP) farm on poultry improvement, Mannuthy. From each bird, 0.5-1 ml of blood was collected from the wing vein using 2.5 ml disposable syringe in a EDTA vial under aseptic condition. The samples were brought to the laboratory at 4 ºC in ice pack. Isolation of Genomic DNA was done from the whole blood using ODP304 Origin Genomic DNA isolation kit. The yield and quality of the DNA obtained was checked by 0.8% agarose gel electrophoresis as well as by Nano-drop. The DNA samples showing the OD260/OD280 value between 1.7 and 1.9 was used for further investigation. Polymerase chain reaction was carried out using specific set of forward (F-5'AGAGGCAGCCCAGGCATTTTAC3') and reverse primer (R-5'CCTGGGTCTGGTTTGGAAATTG3') to amplify the 439bp fragment of *prolactin* gene containing single nucleotide polymorphism C-2402T in promoter region. Each diluted primer (10 pM/µl) was added to the template DNA [working solutions prepared from stock solution by diluting with sterile distilled water (Millipore) to get a final concentration of 100 ng/µL]

and 2X PCR Smart Mix (origin) in a PCR tube and made upto the final volume of 20 µL using ultra-filtered Millipore water. PCR was done in Bio-Rad thermal cycler and standardization was done for each reaction by mild adjustment of concentration of ingredients and annealing temperature with the following profile: initial denaturation of 5 min at 94 °C; 35 cycles of
94 °C for 30 s, annealing at 67.7 °C for 30 s, and 72 °C for 30 s with a final elongation of 5 min at 72 °C. PCR amplicon was subjected to 2% agarose gel. Five micro litre of the amplified product of promoter region of prolactin gene C-2402T was added with 5 units of *AluI* restriction enzyme and incubated at 37^0C for 1hr. The composition of reaction mixture with the final volume of 12µl contains 5µl PCR product, 1.2µl of 10X buffer, 0.5µl of *AluI* (10U/µl) and 5.3µl of distilled water.
Restriction digestion was carried out for all the PCR amplicons. After restriction digestion, the digested PCR products were separated by electrophoresis in 3% agarose gel in 1X buffer with 50 bp DNA size marker. The restriction pattern was visualized under UV trans-illuminator and documented in gel documentation system. According to the polymorphic pattern birds were categorized in to three different genotypes. Genotypes and allelic frequency was calculated. The production traits viz., age at sexual maturity, egg weight at $28^{th,}32^{nd}$ and 40^{th} weeks of age and egg number up to 40 weeks of ages were measured in the randomly selected birds of White Leghorn and their association with SNP of *prolactin* gene was analyzed by one way ANOVA using the software SPSS (Version 21.0).

Results and Discussion

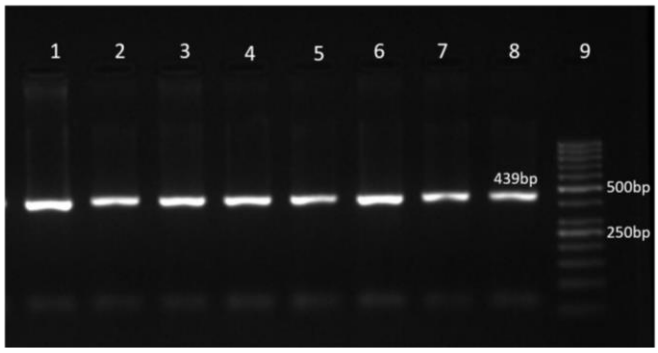

Fig 1: PCR-RFLP analysis of promoter region (C-2402T) of *prolactin* gene on 3% agarose gel (*AluI*)

Lane 1 to 8: 439bp uncut fragment of CC genotype
Lane 9: 50bp Ladder
SNP at C-2402T position of the promoter region of *prolactin* gene was produced an uncut 439bp fragment corresponds to CC genotype (Fig.1). All the 200 birds of White Leghorn were shown to produce the same genotype CC. The genotypic frequency of CC, CT and TT of C-2402T site were1.000, 0.000 and 0.000, respectively and the allelic frequency of C and T were 1.000 and 0.000, respectively in White Leghorn. Our research findings are similar with the studies carried out by [3] they found the genotypic frequency as 1.000, 0.000 and 0.00, respectively and also the allelic frequency as 1.000 and 0.000, accordingly in White Leghorn chicken population for the genotypes of SNP C-2402T. White Leghorn is the best layer breed without broodiness and produces more than 300 eggs per year [3]. The 200 birds which are selected for the present study also observed with same production level (an average of 306 eggs per year). The production traits viz., age at sexual maturity (ASM), egg weight (EW) at 28^{th}, 32^{nd} and 40^{th} weeks of age and egg number up to 40 weeks of age were compared with genotypes CC, CT and TT of SNP C-2402T. No significant difference was found among the genotypes of C-2402T. The obtained results of our studies are accordance with the research conducted by [1] who reported that there was no polymorphism between the *prolactin* gene and production traits (Bodyweight, age at sexual maturity, egg weight and egg number) (P>0.05). Furthermore, they observed that the birds with CC genotype were better than other for production traits. In comparison, similar findings was reported by [3] they observed that there was no correlation between reproductive traits and polymorphism on *prolactin* gene in native chicken and commercial strains. Also, the same investigations were observed on Mazandarani native poultry by [9]. Their studies also showed that there was no significant effect on production traits (body weight at hatch, body weight at sexual maturity, age at sexual maturity, mean egg weight and egg number) of genotype at SNP C-2402T. Studies stated that chickens with CC genotype had significantly greater egg production (egg number) and laying rate than other genotypes (p<0.01) [2]. Similarly in our study, we observed that birds with CC genotype showed higher egg production. Hence, it may be assumed that the *prolactin* gene polymorphism C-2402T has a significant effect on egg production.

Conclusion

This results suggest that all the birds with same genotype and production performance is mainly due to the intense selection of breeding for past 30 years in AICRP on Poultry improvement, Mannuthy. It indicates the efficiency of intensive selection which has been done by AICRP farm. This SNP C-2402T of *prolactin* gene can be used as a molecular marker to determine the efficiency of selection in a particular population. On this SNP C-2402T, further research could be done to identify the association with production traits in other chicken breeds.

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