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Identification of Single Nucleotide Polymorphism (SNP) C-2161G in the Promoter Region of *Prolactin* Gene and its Association with Egg Production in Tellicherry Native Chicken

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ABSTRACT

Background: *Prolactin* is a physiological candidate gene which has significant effects on egg production in poultry. Also, it plays a major role on incubation behaviour (broodiness) in birds. The main objective of the present study was identification of single nucleotide polymorphism (SNP) C-2161G in the promoter region of *prolactin* gene and its association with egg production in Tellicherry native chicken population.

Methods: A total of 200 blood samples were collected from the randomly selected birds of Tellicherry native chicken in All India Co-ordinated Research Project on Poultry improvement (AICRP) farm, Mannuthy, Thrissur, Kerala. Isolation of Genomic DNA was done and the isolated samples were subjected to polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis to identify the SNP C-2161G of *prolactin* gene.

Result: On the basis of RFLP patterns, birds were designated with three different genotypes namely CC, CG and GG. The allelic and genotypic frequency was calculated. The observed genotypic frequency at the SNP site C-2161G was CC (0.61), CG (0.160) and GG (0.23) and the frequency of allele was 0.69 for C and 0.31 for G. The egg production was shown to be statistically similar for the genotypes of SNP C-2161G.

Key words: Genotype, PCR, Polymorphism, Prolactin, RFLP, SNP.

INTRODUCTION

The production potential of poultry influenced by various factors which are directly related to their reproductive potential. In poultry, egg production traits are significantly affected by many factors predominantly the environmental factor (Wilkanowska *et al*., 2014). Also, both production and reproduction parameters are dependent on genetic factors in which *prolactin* gene has significant effects on egg production. The avian *prolactin* gene consists of five exons and four introns with a total length of 6.41kbp and it is located on chromosome number 2 (Kulibaba, 2015). It has been documented that most of the *prolactin* gene polymorphism occurs at the 5' flanking region, 3' flanking region and coding region of signal peptide (Li *et al.,* 2009). *Prolactin* gene transcription is regulated by the pituitary specific transcription factor (Pit-1) which is located on the 5´ flanking region of *prolactin* gene. Hence, polymorphisms in the promoter region of *prolactin* gene may results in changes in the transcriptional binding sites which influences broodiness and egg production in poultry (Rashidi *et al*., 2012). In addition, the 5' flanking region of avian *prolactin* gene has been regarded as an excellent exploratory model for studying hormonally synchronized activation of gene transcription (Wilkanowska *et al*., 2014). Chicken *prolactin* gene plays a crucial role on egg production. In general, the prolactin level of plasma reaches its peak during the onset of broodiness and decreased to normal level on second week and remains same till the end of broodiness (Jiang *et al*., 2011). The elevated level of hormone is positively correlated with inter clutch interval and negatively correlated with egg production (Yousefi *et al*., 2012). At present the commercial poultry breeding is mainly aimed to create high performance poultry lines and breeds in order to increase the egg production and inhibit the broodiness (Xu *et al*., 2010).The selection based on these molecular marker will significantly increase the intensity of selection and production potential in poultry (Wilkanowska *et al*., 2014). Therefore, the present study was aimed at identification of SNP C-2161G in the promoter

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region of *prolactin* gene in Tellicherry native chicken population and analysis of its association with egg production.

MATERILAS AND METHODS

Experimental birds

A total of 200 birds of Tellichery native chicken were randomly selected from All India Research Co-ordinated Project (AICRP) farm on poultry improvement, Mannuthy, Thrissur, Kerala. The study was carried out during the period of September 2016 to October 2017 at Avian Biotechnology Laboratory, in the Department of Poultry Science, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala.

Collection of blood samples

From each bird, 0.5-1 ml of blood was collected from the wing vein and transferred to an EDTA vial under aseptic condition. The samples were brought to the laboratory at 4ºC in ice pack.

Isolation of Genomic DNA

Isolation of Genomic DNA was done from the whole blood according to the standard procedure 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 spectrophotometer. The DNA samples showing the OD260/OD280 value between 1.7 and 1.9 was used for further investigation.

PCR assay and agarose gel electrophoresis

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 SNP C-2161G at the promoter region. 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. Electrophoresis was carried out at 5V/cm until the bromophenol blue dye migrated more than two by third length of the gel and was photographed in a Gel Doc System (Bio-Rad, USA).

Restriction fragment length polymorphism (RFLP) analysis

For restriction digestion five micro liters of the amplified PCR product (439bp) of promoter C-2161G was added with 5 units of *Csp6I* enzymes and incubated at 37^{0}C for 1hr. After restriction digestion, the digested PCR products were separated by electrophoresis in 3% agarose gel with 50bp DNA size marker. The restriction pattern was visualized under UV trans-illuminator and documented in gel documentation system.

The amplicons of promoter region of *prolactin* gene 439bp and the digested PCR products were sequenced using respective forward and reverse primers in an automated sequencer using Sanger's dideoxy chain termination method at Agri Genome Labs Pvt. Ltd., Cochin. According to the RFLP patterns, birds were categorized to three different genotypes and, genotype and allele frequency was calculated accordingly. The daily egg production was recorded and the total number of eggs produced by each bird was calculated up to 40 weeks of age.

Statistical analysis

The association between the SNP C-2161G in the promoter region of *prolactin* gene and egg production was analysed by one way ANOVA by using the software SPSS (Version 21.0).

RESULTS AND DISCUSSION

PCR amplification of 439bp fragment of promter region of *prolactin* gene on 2% agarose gel is represented in Fig. 1. PCR-RFLP analysis of promoter region (C-2161G) of *prolactin* gene with *Csp6I* restriction enzyme revealed the presence of different size of fragments on 3% agarose gel (Fig 2). On the basis of RFLP patterns, all the 200 birds of Tellicherry native chicken were classified to three different

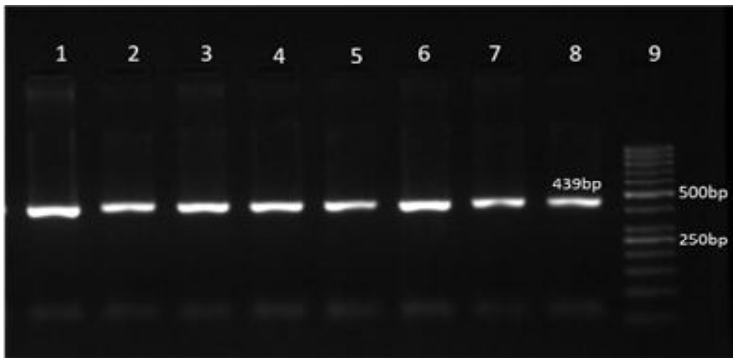

Fig 1: PCR amplification of 439bp fragment of promoter region of *prolactin* gene on 2% agarose gel
Lane 1-8 : 439bp PCR product
Lane 9: 50bp ladder

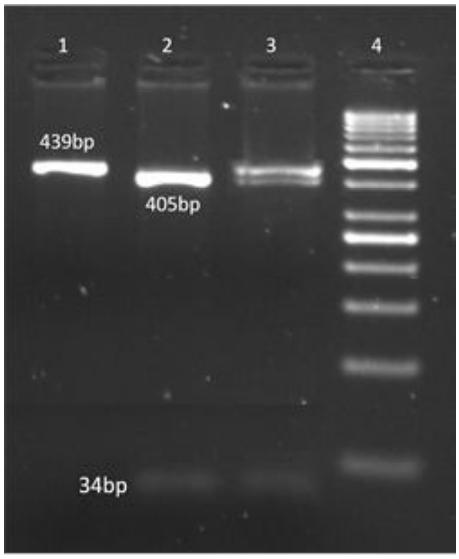

Fig 2: PCR-RFLP analysis of promoter region (C-2161G) of *prolactin* gene on 3% agarose gel (*Csp6I*)
Lane 1: 439bp uncut fragment of CC genotype
Lane 2: 405 and 34bp fragments of GG genotype
Lane 3: 439, 405 and 34bp fragments of CG genotype
Lane 4 : 50bp Ladder

genotypes as CC, CG and GG, respectively. Among the 200 birds of native chicken, 122 birds were observed with CC genotype, 32 birds were produced CG genotype and 46 birds were observed with GG genotype. SNP at C-2161G position in the promoter region of *prolactin* gene was produced an uncut 439bp fragment represents to CC, 439, 405 and 34bp fragments corresponds to CG genotype and 405 and 34bp fragments regarded as GG genotype (Fig 2). Similar findings were reported by Cui *et al*. (2006), Bagheri Sarvestani *et al*. (2013) and Chau *et al*. (2016).

Genotype and allele frequency

The observed genotypic frequencies of CC, CG and GG for the SNP site C-2161 G were 0.610, 0.160 and 0.230, respectively in Tellicherry native Chicken. In contrast, Bagheri Sarvestani *et al*. (2013) was reported the genotype frequencies as CC (0.437), CG (0.435) and GG (0.101) in Fars native chicken of Iran. Also, Chau *et al*. (2016) was found the genotype frequencies as CC (0.10), CG (0.45) and GG (0.45) in Noi native chicken of Vietnam. These contradictory findings in genotypic frequency may be due to the variation in the chicken breeds. The allelic frequency of C and G were 0.690 and 0.310, respectively for the SNP site C-2161G in Tellicherry native chicken. In comparison, Bagheri Sarvestani *et al*. (2013) also reported the same findings in Fars native chicken of Iran as 0.68 for the allele C and 0.32 for the allele G. Similarly, Chau *et al*. (2016) carried out the research on SNP in candidate genes associated with egg production in native Noi chicken of Vietnam and found two alleles for the SNP site C-2161G with frequency of 0.67 for C and 0.33 for G. As stated by these research findings, it has been observed that frequency of C allele was found to be higher in all chicken breeds. In addition, it has been noted that although there were differences in genotype frequency among chicken breeds, the allelic frequencies were similar in all the chicken breeds with dominant of C allele. These results were contrary to the findings of Cui *et al*. (2006) who conducted the study in six different chicken population (White Leghorn, Yangshan, Taihe Silkies, White Rock, Nongdahe and Teihe Silkies). Furthermore, in these six chicken population, it has been observed that the frequency of G allele was dominant than C allele.

Association of SNP at C-2161G in the promoter of *prolactin* gene with egg production in Tellicherry native chicken

A total number of eggs produced by each bird was recorded up to 40 weeks of age. Also, the daily egg production was recorded individually. On the basis of obtained statistical results, the mean egg production was 85.69 ±21.50, 85.36 ±17.27, 84.09 ±20.04 for the genotypes CC, CG and GG, respectively which were statistically similar. There was no significant association among the genotypes of SNP site C-2161G in the promoter of *prolactin* gene. In comparison, Cui *et al*. (2006) performed a research on association of polymorphisms in the promoter region of chicken *prolactin* with egg production and revealed six SNPs (C-2402T, C-2161G, T-2101G, C-2062G, T-2054A and G-2040A) and one 24-bp insertion-deletion (indel) at the site of “358 of *prolactin* gene. However, only a 24bp indel polymorphism had shown the significant association with egg production. Although, some researchers have reported the significant association of egg production for the SNP site C-2161G of *prolactin* gene. Bagheri Sarvestani *et al*. (2013) found that Fars native chicken with CC genotype had significantly greater egg production (number) and laying rate than GG genotype of SNP site C-2161G. In addition, Chau *et al*. (2016) also reported the association of SNP at C-2161G of *prolactin* gene with egg production in Noi native chicken of Vietnam. Therefore, it may be assumed that SNP at C-2161G site of *prolactin* gene has significant effects on egg production in chicken. Additionally, it has been reported that among the candidate genes of poultry, *prolactin* gene shows a predominant effect on egg production. Hence, SNP C-2161G could be used as molecular genetic marker in poultry breeding to improve the intensity of selection and production potential in chicken breeds.

CONCLUSION

In the present study, PCR-RFLP analysis of promoter region (C-2161G) of *prolactin* gene with *Csp6I* restriction enzyme revealed the presence of different size of fragments. According to the RFLP patterns, a total of 200 birds of Tellicherry native chicken were designated with different genotypes as CC, CG and GG. The frequency of C allele was found to be high and the association of SNP site C-

2161G of *prolactin* gene with egg production was statistically similar. In general, *prolactin* gene is considered as a good phyiological candidate gene and its genetic polymorphisms (SNPs) has great impact on economic traits in poultry. Hence, these SNPs could be applied in marker assisted selection in poultry breeding to improve the selection intensity and production potential of chicken breeds. Further studies could be performed in other indigenous chicken breeds in order to confim the association of SNP C-2161G of *prolactin* gene with production traits.

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