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Characterization of 24bp Insertion Polymorphism of *Prolactin* Gene and its Association with Quantitative Traits in Tellicherry Native Chicken Breed

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ABSTRACT

Background: Over many centuries, the conventional methods of poultry breeding is based on the phenotypic selection of the birds with superior trait in a particular population. *Prolactin* is a physiological candidate gene that has significant effects on production traits in poultry. The objective of this study was to investigate 24 bp insertion (I) polymorphism of *prolactin* gene and its association with quantitative traits in Tellicherry native chicken.

Methods: A total of 200 blood samples were collected from the randomly selected birds of All India Co-ordinated Research Project (AICRP) on Poultry Breeding, Mannuthy. The isolated DNA samples were subjected to polymerase chain reaction (PCR) by using specific set of primers to amplify the 24 bp insertion polymorphism. PCR amplicons were sequenced to study the polymorphism at nucleotide level.

Result: PCR amplification revealed the presence of a specific DNA fragment with 130/154 bp contained 24 bp insertion polymorphism. Based on the polymorphic patterns birds were designated with three genotypes as II, ID and DD. The frequency of I (0.6975) allele was found higher than D (0.3025) allele. Our experimental results revealed that 24 bp insertion polymorphism of *prolactin* did not have a significant association with production traits *viz*, age at sexual maturity, egg weight and egg number in Tellicherry native chicken.

Key words: Allele, Chicken, PCR, Polymorphism, Prolactin.

INTRODUCTION

In birds, prolactin hormone is involves in various physiological functions *viz.* crop sac development, induction and maintenance of broody behavior and regulation of gonadal function (Kulibaba *et al*., 2012). Also, it has a crucial effect on egg production in chicken (Cui *et al*., 2006). It has been observed that most *prolactin* gene polymorphism occurs at the 5' flanking region, 3′ flanking region and coding region of a signal peptide (Li *et al*., 2009). Additionally, the 5′ flanking region of the avian *prolactin* gene has been regarded as a magnificent experimental model for studying both tissue-specific and hormonally regulated activation of gene transcription (Wilkanowska *et al*., 2014). The sequence variation at the 5′ flanking region of *prolactin* gene possibly will consequence in alters the transcription factor binding sites and contribute to the release of prolactin hormone (Rashidi *et al*., 2012). A 24 bp insertion at the promoter region of the chicken *prolactin* gene is associated with suppression of the *prolactin* gene expression causing non-broodiness in hens, it could be used as a genetic marker against broodiness in chicken (Jiang *et al*., 2005). Research studies showed the correlation of different allelic variants of the *prolactin* gene with egg production for the different breeds of chicken (Begli *et al*., 2010). Modern commercial poultry production is mainly aimed at increase in egg production by inhibition of broody behavior (Xu *et al*., 2010). Over the past few years, new DNA technologies have been invented in poultry science which gave rise to marker-assisted and genome selection (Wolc *et al*., 2016). The combination of modern DNA technologies and classic selection approaches paved the way for the creation of highly productive competitive lines and breeds of poultry (Sodhi *et al*., 2013). Based on the studies carried out over the few decades, it has been reported that the *prolactin* gene has a direct and indirect association with various production traits in poultry. Hence, it could be used as a potential marker for the production traits (Wilkanowska *et al*., 2014). The main objective of the present study was identification of 24 bp insertion-deletion polymorphism at the promoter region of the *prolactin* gene and to find out its association with quantitative traits in Tellicherry native chicken of Kerala, India.

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MATERIALS AND METHODS

Experimental birds

A total of 200 birds of Tellicherry native chicken which had undergone five generations of continuous selection were randomly selected from All India Research Co-ordinated Project (AICRP) Farm on Poultry Breeding, Mannuthy, Thrissur, Kerala. The present study was carried out during the period of September 2016 to October 2017 at Avian Biotechnology Laboratory, 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 under aseptic conditions and transferred an EDTA vial. The samples were brought to the laboratory at 4°C in an ice pack.

Isolation of genomic DNA

Genomic DNA was isolated from the whole blood using the ODP304 Origin Genomic DNA isolation kit. The yield and quality of the DNA was checked on Nano drop spectrophotometer. The DNA samples showing the OD260/OD280 value between 1.7 and 1.9 were used for further investigation.

PCR assay and agarose gel electrophoresis

Polymerase chain reaction was done using specific set of forward (F-5′ TTTAATATTGGTGGGTGAAGAGACA3′) and reverse primer (R-5′ ATGCCACTGATCCTCGAAAACTC3′) to amplify the 130/154 bp fragment of *prolactin* gene containing 24 bp in(s) polymorphism at the promoter region. PCR was done in Bio-Rad thermal cycler and standardization was done for each reaction by mild adjustment of the 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 58°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. The PCR products were loaded along with a molecular weight marker (50 bp) for relative sizing. Electrophoresis was carried out at 5V/cm until the bromophenol blue dye migrated more than two by the third length of the gel and was photographed in a Gel Doc System (Bio-Rad, USA). The amplicons of *prolactin* gene 130/154 bp 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. Based on the polymorphic patterns, birds were categorized into three different genotypes and, the allelic and genotypic frequency was calculated accordingly.

Production traits

The production traits *viz.*, age at sexual maturity (ASM), egg weight (EW) at 28th, 32nd and 40th weeks of age and egg number (EN) up to 40 weeks age were measured in the randomly selected birds of Tellicherry native chicken of AICRP on Breeding, Mannuthy. Birds were assigned various genotypes based on polymorphic patterns and allelic and genotypic frequency were estimated.

Statistical analysis

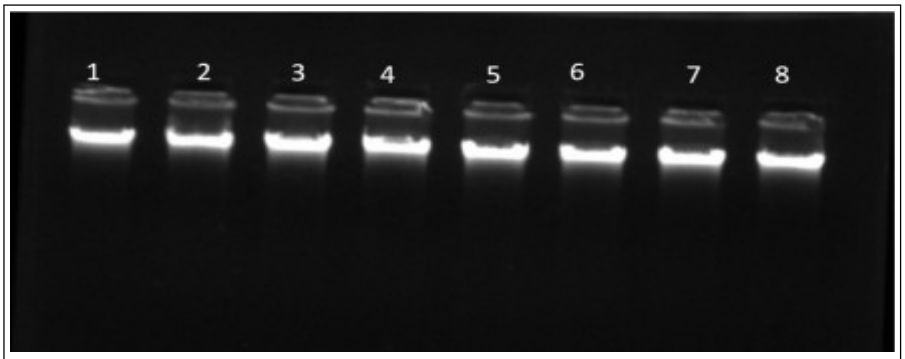
The association of a 24 bp in(s) polymorphism at the promoter region of *prolactin* gene with production traits (ASM, EW and EN) were analyzed by one way ANOVA by using the software SPSS (Version 21.0).

RESULTS AND DISCUSSION

PCR amplification of 130/154 bp fragment (24 bp indel) of the promoter region of *prolactin* gene

On 0.8 per cent agarose gel, a single clear band was obtained without shearing which indicates that the DNA was good in quality and concentration (Fig 1). The size of the amplicons was 130/154 and the polymorphic patterns of promoter region were observed with 24 bp insertion using PCR with 2 per cent agarose gel (Fig 2). Similarly, 24 bp indel polymorphism was identified in different breeds of chicken (Cui *et al*., 2006; Liang *et al*., 2006; Bagheri Sarvestani *et al*., 2010; Rashidi *et al*., 2012).

Based on the polymorphic patterns, all 200 birds were designated with three different genotypes II (insertion-insertion), ID (insertion-deletion) and (deletion-deletion) DD, accordingly. The size of the amplicons were 154, 130/154 and 130 bp representing the II, ID and DD genotypes, respectively. Out of 200 birds of Tellicherry native



Lane 1-8: DNA isolated from blood.

Fig 1: DNA isolated by origin genomic DNA isolation kit (0.8% agarose gel).

chicken, 130 birds were produced II genotype, 19 birds were observed with ID genotype and 51 birds were observed with DD genotype.

Genotype and allele frequency

The genotypic frequency of II, ID and DD of 24 bp indel polymorphism were 0.650, 0.095 and 0.255, respectively. The allelic frequency of I and D was 0.6975 and 0.3025, correspondingly. According to these results, it has been observed the frequency of I allele was found high in Tellicherry native chicken population.

Association of 24 bp insertion polymorphism of *prolactin* gene with quantitative traits in Tellicherry native chicken

Age at sexual maturity

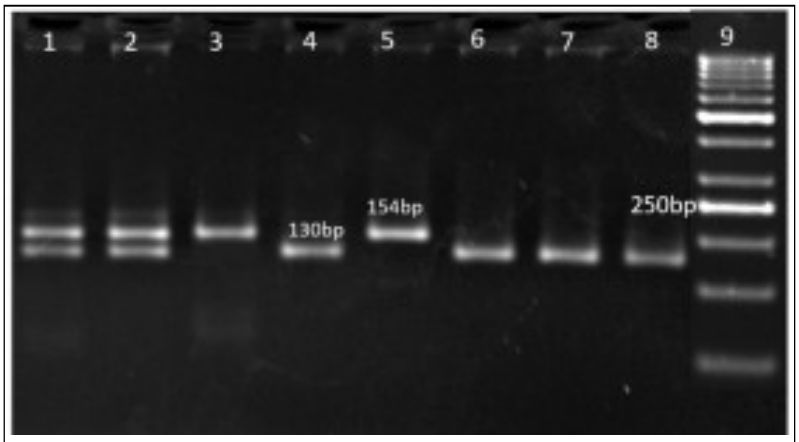
In Tellicherry native chicken, the mean age at sexual maturity was 157.1±8.15, 153.47±14.49 and 154.18±15.61 (days) for the genotypes II, ID and DD, respectively. There was no significant difference (P>0.05) among the genotypes. Though, birds with ID genotype had shown early sexual maturity. Similarly, Begli *et al*. (2010) observed no association between the genotypes of a 24 bp indel site and ASM in native chicken of Yazd province in Iran (Fig 3,4).

Mean egg weight at 28th week

For birds with genotypes II, ID and DD, the mean egg weight at 28 weeks of age was 41.64±5.12, 41.01±3.28 and 41.19 ±3.00 g, respectively. There was no significant difference (P>0.05) among the genotypes.

Mean egg weight at 32nd week

The mean egg weight at 32 weeks of age was 41.30±4.98, 41.18±3.50 and 41.65±3.71 g for the genotypes II, ID and DD, respectively. There was no significant difference (P>0.05) among the genotypes.



Lane 1, 2: 130 and 154 bp fragments of ID genotype.
Lane 3, 5: 154 bp fragments of II genotype.
Lane 4, 6, 7, 8: 130 bp fragments of DD genotype.
Lane 9: 50 bp ladder.

Fig 2: PCR amplification of 130/154 bp fragment (24 bp indel) of promoter region of *prolactin* gene on 2% agarose gel.

ttttaatattggtgggtgaagagacaaggaagacaAgAAgAgACAagaCAaGG
AAGgAagagaaga**cacctgcaggcagggagaataaca**tttacaaacatagagg
ataacaGtctcagaATTgACAactggagttttcgaggatcagtggcatag

Fig 3: Sequence analysis of a 24 bp insertion (I) at the promoter region of *prolactin* gene (154 bp).

ttttaatattggtgggtgaagagacaaggaagacaAgAAgAgACAagaCAaGG
AAGgAagagaaga----------
tttacaaacatagaggataacaGtctcagaATTgACAactggagttttcgagg
atcagtggcatag

Fig 4: Sequence analysis of s 24 bp deletion (D) at the promoter region of *prolactin* gene (130 bp).

Mean egg weight at 40th week

The mean egg weight at 40 weeks of age was 42.02±4.49, 41.70±3.48 and 41.29±3.76 g for the genotypes II, ID and DD, respectively. There was no significant difference (P>0.05) among the genotypes.

In comparison, similar findings were reported in Kadaknath and Aseel birds (Yadav *et al*., 2016; Yadav *et al*., 2018). Additionally, Begli *et al*. (2010) compared different genotypes of 24 bp indel site and found no association with mean egg weight in native fowl of Yazd province. Furthermore, the genotypes of 24 bp indel polymorphism did not show significant association with mean egg weight at 2nd, 4th, 6th weeks of laying period in Japanese quail (Lotfi *et al*., 2013).

Egg number up to 40 weeks of age

In Tellicherry native chicken, the mean egg number up to 40 weeks of age were 86.26±18.98, 87.24±20.28 and 80.25±20.31 for the genotypes II, ID and DD, respectively.

Although there were no significant differences among the genotypes of 24 bp indel polymorphism of *prolactin* gene, birds with II and ID genotypes produced more eggs than DD genotype. Hence, it could be assumed that a 24 bp nucleotide sequence insertion at the promoter region of the *prolactin* gene may influences the intensity of egg-laying in birds. In comparison, Kulibaba *et al*. (2012) researched *prolactin* and *growth hormone* gene polymorphisms in chicken lines of Ukrainian selection and reported that increased frequency of I allele corresponds to an egg-laying direction of chicken productivity. Cui *et al*. (2006) reported that the ID genotype of the 24-bp indel (insertion-deletion) site had significantly (p<0.05) higher egg production compared to II and DD contrary to the present results. In addition, Begli *et al*. (2010) found that birds with II and ID genotype had shown a significant association (P<0.01) on egg production than DD in native fowl of Yazd province. Also, Bagheri Sarvestani *et al*. (2013) reported that chicken with II and ID genotypes for the 24 bp insertion/deletion had significantly (P<0.01) greater egg production and laying rate than DD genotype. These findings were contrary to the results of the present study. This may possibly due to small number of sample size and the artificial selection done at AICRP farm. In addition, the frequency I (0.6975) allele was found higher than D (0.3025) allele. This could be due to genetic drift *viz*, bottle neck effect and founder effect.

It has been reported that due to insertion of a 24 bp nucleotide sequence at the promoter region of the *prolactin* gene, a possible ecotropic viral integration site-1 encoded factor (Evi-1) binding site is located in the 52 flanking region of the chicken *prolactin* gene (Cui *et al*., 2005) Evi-1 was shown to be involved as a repressor in the transcription of many genes (Vinatzer *et al*., 2001; Izutsu *et al*., 2002). Additionally, Evi-1 represses the expression of the *prolactin* gene in chickens through binding the Evi-1 binding site which prevents broodiness that can improve egg production to some extent (Jaing *et al*., 2005). In accordance with these results, in the present study frequency of I (insertion) allele, was found to be high in the Tellicherry chicken population based on the obtained results of allelic frequency estimation. In general, annual egg production of indigenous chicken breeds are comparatively less than commercial layers. For example, an average egg production is about 60 to 80 numbers in Tellicherry chicken breed that are reared under backyard system. In the present study, the average egg production of Tellicherry chicken is around 168 numbers up to 72 weeks age which is higher than backyard chicken. This may due to the artificial selection and controlled breeding followed in All India Co-ordinated Research Project Farm on Poultry Breeding, Mannuthy, Thrissur, Kerala. It has been observed that broodiness is less pronounced in native chickens reared under the caged system which may improve the egg production (intensity of laying) to some extent. Also, broodiness and egg production are negatively correlated traits in poultry. Similarly, based on the day-to-day observation and production data maintenance at AICRP farm, we have observed that Tellicherry chicken had shown decreased number of broody days with an increased egg number. Furthermore, it has been found that the presence of a 24 bp insertion (I) at the promoter region of the *prolactin* gene has a positive effect on egg production. Hence, a 24 bp insertion polymorphism of the *prolactin gene* could be used as a molecular marker to improve the egg production in native chickens.

CONCLUSION

In the present study, we have identified a 24 bp insertion polymorphism at the promoter region of *prolactin* gene. The allele frequency for the I (0.6975) allele was found higher than D (0.3025) allele. However, no significant association (p>0.05) were observed among different genotypes (II, ID and DD) with age at sexual maturity, egg weight at 28, 32 and 40 weeks of age and egg number up to 40 weeks of age in Tellicherry chicken. Hence, further studies could be carried out with large number of samples to confirm the association of 24 bp insertion polymorphism of *prolactin* gene with production traits in other breeds of native chicken.

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Conflict of interest

All authors declared that they have no conflict of interest.

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