ASSOCIATION OF *PROLACTIN* GENE POLYMORPHISM WITH PRODUCTION TRAITS IN WHITE LEGHORN

M. Azhaguraja, S. Sankaralingam, P. Anitha, Binoj Chacko and T.V. Aravindakshan

College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala - 680 651

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ABSTRACT

The study was aimed at the identification of polymorphism in promoter region of *prolactin* gene using polymerase chain reaction (PCR) and find out their association with production traits in White Leghorn (IWN strain). A total of 200 birds of White Leghorn (IWN strain) which had under gone 28 generations of continuous selection were randomly selected from All India Co-ordinated Research Project (AICRP) on Poultry for eggs, Mannuthy. Blood samples were collected from all the birds and isolation of genomic DNA was done using DNA isolation kit. PCR was done to reveal the 24-bp insertion-deletion (indel) at the site of -358 in promoter region of *prolactin* gene and based on the polymorphic patterns, genotypes were designated as II, ID and DD. Out of 200 birds of White Leghorn 193 birds were observed with II genotype, 7 birds with ID genotype and no birds were revealed with DD genotype. The genotype frequencies of II, ID and DD were 0.965, 0.035 and 0.000, respectively. As there was no DD genotype in the population, the birds with II and ID genotype alone were taken for further comparison with production traits viz., broodiness, age at sexual maturity (ASM), egg weight (EW) at 28, 32 and 40 weeks of age and egg number (EN) up to 40 weeks of age. These production traits did not have any significant association with the genotypes of 24-bp indel polymorphism.

Keywords: *Prolactin* gene, polymorphism, production traits, White Leghorn

INTRODUCTION

In conventional poultry breeding programme, the selection is mainly based on extensive pedigree records, data on production traits and predicted breeding value. In All India Co-ordinated Research Project (AICRP) on Poultry for eggs, Mannuthy, Osborne index method of selection for egg number at 64 weeks of age is followed for 28 generations in White Leghorn population. The marker assisted selection (MAS) is an effective and indirect method of selection, which reduces the generation interval and achieve genetic gain very rapidly. MAS can be done using different types of molecular markers namely, Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Polymerase chain reaction (PCR)

and Single Nucleotide Polymorphisms (SNPs). With this background, the present study was aimed at identification of a 24-bp indel (at the site of -358) polymorphism in the promoter region of *prolactin* gene.

MATERIALS AND METHODS

A total of 200 birds of White Leghorn (IWN strain) which had undergone 28 generations of continuous selection were randomly selected from All India Co-ordinated Research Project (AICRP) on Poultry for eggs, Mannuthy. From each bird, 0.5-1 ml of blood was collected from the wing vein using 2.5 ml disposable syringe in EDTA vial under aseptic condition. The samples were brought to the laboratory at 4°C in ice pack. Isolation of genomic DNA was done according to the standard procedure for randomly collected blood samples using Origin genomic DNA isolation kit. The isolated samples of DNA were assessed on Nano drop 2000C spectrophotometer as well as on 0.8 per cent agarose gel by electrophoresis to determine the concentration and quality of DNA, respectively. All the isolated samples of genomic DNA were subjected to PCR using a specific set of forward primer 5'-TTTAATATTGGTGGGTGAAGAGA CA-3' and reverse primer 5'- ATGCCAC TGATCCTCGAAAACTC-3' to amplify the *prolactin* gene fragment containing a 24-bp insertion-deletion at the site of -358 on a thermal cycler with the following cycle: initial denaturation of 3 min at 95°C, 35 cycles of 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72° C for 30 second with a final elongation of 5 min at 72° C. PCR products were checked by 2 per cent agarose gel in 1X buffer with 50bp DNA size marker. The polymorphic pattern was visualized under UV trans-illuminator and documented in gel documentation system and designated with different genotypes, accordingly (Fig.1.).

The production traits like broodiness, age at sexual maturity (ASM), egg weight (EW) and egg number (EN) were measured in the randomly selected birds and their association with polymorphisms of *prolactin* gene was analyzed by one-way ANOVA by using the software SPSS (Version 21.0).

RESULTS AND DISCUSSION

The 24-bp insertion-deletion at the site of -358 was shown to be polymorphic in the examined sample with two alleles, I and D. The observed genotype frequencies of II, ID and DD were 0.965, 0.035 and 0.000, respectively. The allelic frequency of I and D was 0.9825 and 0.0175, respectively. Jiang *et al.* (2005), Cui *et al.* (2006) and Liang *et al.* (2006) also reported similar finding in White Leghorn population.

The non-broody behaviour of White Leghorn birds is due to the 24bp insertion (II) which lead to presence of ecotropic viral integration site-1 encoded factor (Evi-1)

Table 1. Number of birds belonging to different genotypes of 24bp indel (at -358 site) polymorphism in the promoter region of *prolactin* gene in IWN strain of White Leghorn

Promoter region	Genotype	No. of birds	Total
24-bp indel (at -358 site)	II	193	200
	ID	7	
	DD	0	

Table 2. Mean ± SE of different production traits for the respective genotypes of 24bp indel (at -358 site) polymorphism in the promoter region of *prolactin* gene

Production traits	Genotype	Mean ± SE
Broodiness (days)	II	0
	ID	0
ASM (days)	II	145.96 ±0.540
	ID	143.43 ±2.716
EW at 28 weeks, g	II	48.83 ±0.213
	ID	48.39 ±0.859
EW at 32 weeks, g	II	45.56 ±0.248
	ID	46.80 ±1.289
EW at 40 weeks, g	II	52.93 ±0.230
	ID	52.64 ±1.367
EN up to 40weeks	II	124.33 ±0.680
	ID	124.57 ±3.449

binding site in the 5' flanking region of the chicken *PRL* gene which suppresses the expression of *PRL* gene in White Leghorn birds by binding the Evi-1 binding site and leads to prevention of broodiness (Jiang *et al*., 2005).

The investigation on association of different genotypes of promoter region of *prolactin* gene with production traits (broodiness, age at sexual maturity, egg weight and egg number) in White Leghorn (IWN strain) revealed that a 24-bp indel polymorphic site (-358) did not have significant association with these production traits. This may be due to the intense selection of birds by AICRP on poultry for eggs, Mannuthy.

SUMMARY

This study showed that a 24-bp indel polymorphism of *prolactin* gene in White Leghorn (IWN strain) did not have any significant association with the observed production traits which may be due to the intense selection for past thirty five years followed in AICRP on Poultry improvement, Mannuthy. Hence, further studies on *prolactin* gene may be done in other breeds of chicken and also in unselected population of White Leghorn to find its relationship with production traits.

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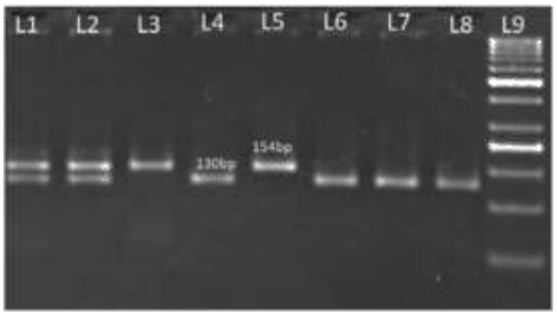

Fig.1. PCR amplification of 130-154 fragment (24-bp indel) of promoter 1 of *prolactin* gene on 2% agarose gel

Lane 1, 2: 130 and 154bp fragments of ID genotype

Lane 3, 5: 154bp fragment IIgenotype

Lane 3, 6, 7, 8: 130bp fragment of DD genotype

Lane 9 : 50bp ladder

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