ASSOCIATION OF SNP IN THE EXON 2 OF *OVOCALYXIN-32* GENE WITH PRODUCTION TRAITS IN IWN STRAIN OF WHITE LEGHORN

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ABSTRACT

An experiment was conducted using randomly selected 150 hens of IWN strain of White Leghorn maintained at AICRP on Poultry Improvement, Mannuthy to identify the SNP in the exon 2 of *ovocalyxin-32* gene and its association with the production traits. PCR-RFLP analysis was done to find the genotypes TT, TG and GG in exon 2. The genotypic frequencies of TT, TG and GG were 0.900, 0.047 and 0.053, respectively and the allelic frequencies of T and G were 0.92 and 0.08, respectively. The frequency of normal allele T was much higher than the mutant G. The result of this study revealed no significant association of c.267T>G with body weight, egg weight, egg number, shell thickness and age at sexual maturity. Present study indicates that the SNP in the exon 2 of *ovocalyxin-32* (*OCX-32*) can't be used as a marker for selection of IWN strain of White Leghorn for eggs.

Keywords: PCR, RFLP, *ovocalyxin* gene, exon 2, c.267T>G

INTRODUCTION

Selection based on genetic markers can make accurate, efficient and also faster improvement in production traits at earlier age. Favourable SNPs of genes associated with production traits can be utilized for Marker assisted selection (MAS). *Ovocalyxin-32 (OCX-32)* is a candidate gene influencing both egg production and eggshell quality (Uemoto *et al*., 2009 and Takahashi *et al.,* 2010). The present study was aimed to explore the SNP in the exon 2 of *ovocalyxin-32 (OCX-32)* gene and its association with production traits of White Leghorn layers.

MATERIALS AND METHODS

Study was carried out using 150 randomly selected IWN strain of White Leghorn birds maintained at All India Co-ordinated Research Project (AICRP) on Poultry Improvement, Mannuthy, which have undergone 27 generations of intense intra-population selection. All the birds were reared under uniform management conditions. The production parameters of birds including body weight at 16, 40 and 64 weeks of age, egg weight at 28, 40 and 64 weeks of age, egg number at 40 and 64 weeks, shell thickness at 72 weeks and age at sexual maturity were recorded.

Genomic DNA was isolated from the blood using genomic DNA isolation kit. The quality of isolated DNA was checked

by agarose gel electrophoresis and the purity of DNA samples were checked using NanoDrop spectrophotometry. DNA samples showing optical density ratio 260/230 between 1.7 and 1.9 indicating good quality was used for further study. The PCR was carried out to amplify 405bp fragment of exon2 using following primers, F1-GGCCCACTGGTCAGAAAAGAA and R1- CCTGCAAGGAAAAAGCTG.

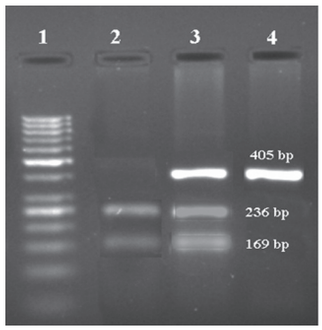
Diluted primer (10 pM/µl) was added to the template DNA [working solutions prepared from stock solution by diluting with sterile distilled water (Millipore) to get a final concentration of 100 ng/µL] and 2X PCR Smart Mix (origin) in a PCR tube and made upto the final volume of 20 µL using ultra-filtered 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. The thermal cycler was pre-programmed for the initial denaturation temperature of 95^0C for 3minutes, followed by 35 cycles of denaturation at 95^0C for 30 seconds, annealing at 59^0C for 30 seconds and extension at 72^0C for 30 seconds followed by final extension at 72^0C for 5 minutes.

The RFLP analysis was done on PCR amplicons to identify the SNP c.267T>G in exon 2 of *ovocalyxin-32* gene. The restriction digestion of amplified fragments was done by adding 5 µL of the PCR product with 5U of *TaiI* enzyme, followed by incubation at 37^0C. The digested PCR product was 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.

The production traits of different genotypes of exon 2 viz., body weight (BW) at 16, 40 and 64 weeks of age, egg weight (EW) at 28, 40 and 64 weeks of age, egg number (EN) at 40 and 64 weeks of age, shell thickness (ST) at 72 weeks of age and age at sexual maturity (ASM) were given in table 1. The association between SNPs and production traits was evaluated using one way ANOVA.

RESULTS AND DISCUSSION

RFLP analysis revealed three genotypes (TT, TG and GG) and two alleles (T and G) in exon 2. The genotypic frequencies of TT, TG and GG were 0.900, 0.047 and 0.053, respectively and the allelic frequencies of T and G were 0.92 and 0.08, respectively. The frequency of T allele was more in the population than the mutant G. Contrary to this finding, Uemoto *et al.* (2009) found these genotypic frequencies as 0.309, 0.357 and 0.335, respectively in F2 generation of White Leghorn X Rhode Island Red cross. Lee *et al*. (2014) found varying frequencies in different breeds of Korean native chicken.



TT TG GG

PCR-RFLP analysis of exon 2 of *OCX-32* gene on 3% agarose gel

The genotypes of exon 2 did not have any correlation with the body weight (BW 16, BW 40 and BW 64), egg weight

Table 1. Production parameters of genotypes of exon 2 of ovocalyxin-32 gene in IWN strain of White Leghorn

Geno-type	BW16	BW40	BW64	EW28	EW40	EW64	EN40	EN64	ST	ASM
TT	1026.93 ±7.22	1587.93 ±15.95	1486.00 ±15.89	48.53 ±0.25	52.54 ±0.28	51.60 ±0.31	122.57 ±1.47	268.21 ±2.32	0.285 ±0.002	147.84 ±1.26
TG	1063.57 ±43.76	1632.14 ±63.01	1485.00 ±46.03	49.49 ±0.57	53.99 ±0.98	54.57 ±0.68	121.00 ±5.45	249.00 ±20.79	0.277 ±0.008	143.00 ±3.82
GG	1018.13 ±17.78	1547.50 ±40.55	1455.00 ±40.32	49.31 ±1.71	53.04 ±1.26	51.94 ±4.27	121.25 ±6.48	271.63 ±8.90	0.290 ±0.009	151.00 ±6.88

(EW 28, EW 40 and EW 64), egg number (EN 40 and 64), shell thickness (ST 72) and age at sexual maturity (ASM). Similarly Uemoto *et al.* (2009) reported no significant association between SNP of exon 2 with body weight, egg weight, shell thickness and age at sexual maturity, but found significant association with egg production. Lee *et al*. (2014) also found no association between exon 2 and egg weight, egg production and age at sexual maturity in different Korean native chicken breeds. In contrary, Research accomplishment and recommendations of Anand Agricultural University reported that SNP of exon 2 of *OCX-32* gene had significant association with egg production without affecting the egg weight (AAU, 2012).

SUMMARY

Results of this study showedno significant association between SNP c.267T>G and production traits like body weight, egg weight, egg number, shell thickness and age at sexual maturity. Hence, this study concludes that SNP of exon 2 may not be utilized as marker in selection and breeding of layers.

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