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**Original Research Article**

# Polymorphism at Prolactin Promoter Region and its Relation with Production Performance in Aseel Birds

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**A B S T R A C T**

Present study was carried out to study egg production performance and polymorphism of Prolactin gene at promoter region (PRL24). Study was conducted on the Aseel hens kept for laying purpose at University poultry farm. Egg production performances were recorded as age at first laying (AFE), Body Weight at First Egg (WFE), Mean Egg Weight (MEW) and Total No. of Eggs at 90 days of laying (TEN). DNA was isolated from 2- 3 of Blood of 40 birds collected from wing vein. PRL24 locus of Prolactin was amplified by PCR and the product was resolved on 6% native PAGE for genotyping. The prolactin gene locus PRL24 showed three genotypes: II, ID & DD. The frequencies of these genotypes were 0.30, 0.45 and 0.25 respectively for II, ID and DD. The II genotype was associated with WFE; ID genotype with TEN and DD genotype was closely associated with MEW.

## Introduction

The Asil or Aseel is a breed of chicken originating from South Punjab / Sindh area of Pakistan and India. The Aseel breed is known for its stamina, pugnacity, majestic gait, and dogged fighting qualities (Panda and Mahapatra, 1989). This breed is characterized by its hardiness and ability to thrive under adverse climatic conditions, and its meat is considered to have a desirable taste and flavor. The superiority of Aseel on other indigenous breeds is due to its hardiness, resemblance to Cornish and larger body size (Bhatti *et al.,* 1991). Almost all the varieties of Aseel are characterized with heavy body weight and poor egg production potential thus leading to low progeny size due to erratic ovulation, short or erratic clutches and broodiness. Prolactin (PRL) is a polypeptide hormone which plays a key role in egg production. Onset of incubation behavior is induced by an increase in PRL secretion, which results in regression of ovary & loss of egg production (Sharp, 1997). Polymorphism in the promotor region specially those that result in change of promoter binding sites, most likely influence mRNA expression and thus influence incubation behaviour and egg production (Cui *et al.,* 2006).

Due to different biological activities attributed to PRL, they can be used as the major candidate genes in molecular animal breeding programs. Thus, present study was

planned to study the polymorphism at prolactin promoter region PRL24 and its relation with egg production performance in Aseel birds.

## Materials and Methods

### Birds and production data

The Aseel birds used in the current study were selected from the poultry farm of College of Veterinary Science & Animal Husbandry, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad. A flock of 40 female birds of Aseel were taken that were nearing their age of laying. Separate cages for each bird were use for the ease of sample & data collection and managemental conditions. Birds were fed *ad libitum*. The egg production performance were recorded as weights at first egg (WFE) in kg; Age at first egg (AFE) in days; Mean weight of eggs (MEW) in grams as average of daily egg weights over a period of 90 days of laying and Total number of eggs (TEN) as the number of eggs laid over the study period of 90 days.

### Blood collection and DNA isolation

EDTA containing vacutainer tubes were used to collect 2 to 3 ml of blood from wing vein of each bird. DNA was isolated from blood using High salt method of Montgomery and Sise (1990) with slight modifications.

### Polymerase chain reaction

Polymerase chain reaction was carried out in a Bio-Rad $CFX_{96}$ Real Time system. Primer pair for PRL24 was used as described by Rashidi et al. (2012), to amplify the fragment (130 or 154 bp) containing the 24 bp insertion or deletion (indel) at the site of 358 in promoter region of Prolactin gene.

The sequence of primers is as follows—
Forward: 5'-GGCTCTCCATGGGTATTAGGA-3'
Reverse: 5'-GGCTCTCCATGGGTATTAGGA-3'

PCR was performed in a final volume of 50 µl containing:100 ng of genomic DNA, 0.5 µM of each primer, 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$, 1.0 U Taq DNA polymerase and 1× reaction buffer. The cycle conditions for PCR included-- Initial denaturation of 5 min at 94°C; followed by 35 cycles of 94°C for 30s, annealing at 54°C for 60 s, extension at 72°C for 60 s followed by a final extension of 5 min at 72°C. The PCR product was resolved on to a native 6% PAGE.

### Genotyping and statistical analysis

Genotypes were manually scored based on the bands resolved on the gel.

Frequencies of various alleles were calculated using the following formula—

Frequency of an allele =

$$\frac{(2 \times No.\ of\ Homozygote) + (No.\ of\ Heterozygote)}{2 \times Total\ no.\ of\ Individuals\ (N)}$$

Alleles frequency and their accordance to Hardy-Weinberg equilibrium were calculated from Graphpad Prism software version 5.0.

## Results and Discussion

Primer pair PRL24 was used to amplify the fragment (130 or 154 bp) containing the 24 bp insertion or deletion (indel) at the site of-358 in promoter region of prolactin gene. The insertion (I) allele was of 154 bp and the deletion (D) allele was shown by a 130 bp band. The allele frequencies and interaction of prolactin promoter gene at

locus PRL24 on egg production performance of Aseel is presented in Table 1.

The three genotypes obtained were II, ID and DD. The frequencies of these three genotypes at this locus were found to be 0.30, 0.45 and 0.25 for II, ID and DD genotypes respectively. The frequency of ID genotype was higher than other genotypes. The frequencies of the two allele I and D were found to be 0.55 and 0.45 respectively.

In the present study, the frequency of I (0.55) and D (0.45) alleles were approximately close to each other but the frequency of heterozygous genotype (ID) was higher compared to other homozygous genotypes. These results support the findings of Cui et al. (2006); Yousefi et al. (2012); Rashidi et al. (2012) and Fathi and Zarringhobayi (2014); where they found the higher frequencies of ID genotypes than other genotypes.

The age of hens at their first laying (AFE) in the genotypes II, ID and DD were 187.7±0.98, 187.4±0.79 & 186.8±1.03 respectively. There was no significant difference (P<0.05) between the means of AFE in various genotypes.

The mean body weight at 1st laying of hens (WFE) having genotypes II, ID and DD were 1.62±0.07, 1.57±0.06 & 1.52±0.06 respectively. There was no significant difference (P<0.05) between the means of WFE in various genotypes.

**Table.1** Allele frequencies and interaction of PRL24 on egg production performance of Aseel

| Genotype | Frequency | Traits (Mean± SE) | | | |
|---|---|---|---|---|---|
| | | AFE(d) | WFE(Kg) | MEW(g) | TEN |
| II | 0.30 | 187.7±0.98 | 1.62±0.07 | 43.39±0.64 | 35.36±0.82 |
| ID | 0.45 | 187.4±0.79 | 1.57±0.06 | 43.72±0.53 | 35.69±0.63 |
| DD | 0.25 | 186.8±1.03 | 1.52±0.06 | 43.72±0.86 | 35.30±0.59 |

Values with different superscripts in a column differ significantly: (P<0.05)

Birds showing mean egg weight (MEW) having genotypes II, ID and DD were 43.39±0.64, 43.72±0.53 & 43.72±0.86 respectively. There was no significant difference (P<0.05) between the means of MEW in various genotypes.

All birds showing mean total no. of egg (TEN) having genotypes II, ID and DD were 35.36±0.82, 35.69±0.63 & 35.30±0.59 respectively. The mean TEN did not differ significantly (P<0.05) among various genotypes.

In the present study Aseel hens with ID genotypes had much more egg production performance but statically, there was no significant difference in various genotypes. The finding of no interaction with any production trait in present study does not support earlier studies of Emamgholi-Begli et al. (2010) and Fathi and Zarringhobayi (2014). All these researchers found some genotypes associated significantly with certain traits of egg production. However, Fathi and Zarringhobayi, 2014 also found no significant difference in MEW, among the

three genotypes in the turkey population. These differences may be due to difference in sampling, breeds of birds etc.

In the present study, genotype DD showed higher egg number and egg weights. But in their study, Kulibaba and Podstreshnyi (2012) found that the frequency of I alleles were higher in the egg line chickens than meat line chickens and thus the I allele could be attributed for a better egg production. This may be due to the difference in the breed in the present study.

Based on the findings of current study, it can be concluded that though PRL24 shows polymorphisms in Aseel birds, statistically there is no association of laying performance with polymorphism at this locus of Prolactin promoter gene.

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