Prolactin Gene and Laying Performance in Aseel Birds

Abstract

Prolactin gene polymorphism at exon 5 of prolactin receptor gene (PRLR5) was studied in Aseel birds along with its interaction with laying performance. Laying 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 ml not clear of Blood from 40 birds collected from wing vein. PRLR5 locus was amplified by PCR and the product was restriction digested with Bam HI and resolved on 2% Agarose gels for genotyping. The AFE (d), WFE (Kg), MEW (g) and TEN of Aseel birds in the present study were found to be 188.40±0.22, 1.57±0.03, 43.61±0.36 & 35.48±0.40 respectively. The prolactin gene locus PRLR5 showed two alleles A & B. The frequencies of A and B alleles at this locus were 0.60 and 0.40 respectively. There was no interaction of various laying performances found with alleles of PRLR5.

Keywords: Egg production performance; Kadaknath; Prolactin; Polymorphism; Birds

Abbreviations: MEW: Mean Egg Weight; WFL: Weight at First Laying; AFH: Age of Hens; MEW: Mean Eggs Weight; TEN: Total Number of Eggs

Introduction

Egg productivity is the most important economic trait in egg-laying poultry. Endocrine and environmental factors such as length of photoperiod and feeding allowance can influence egg production [1]. However, a genetic factor would be a prerequisite. Egg production is a polygenic inheritance trait with low to moderate heritability depending on the period involved, a major opportunity for improvement in this trait lies in the period after 40 weeks of age [2,3]. In traditional breeding, genetic improvement based on estimated breeding values requires extensive data from a variety of sources. 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 [4]. 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 [5]. The Asil or Aseel is a breed of chicken originating from South Punjab/Sindh area of Pakistan and India. The Asiel breed is known for its stamina, pugnacity, majestic gait, and dogged fighting qualities [6]. 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 [7].

Materials and Methods

Birds and production data

The birds from the poultry farm of College of Veterinary Science & Animal Husbandry, Narendra Deva University of Agriculture & Technology, Kumarganj, and Faizabad were used for the present study. Forty female birds of Aseel breed nearing their age of laying were taken for the present study. Birds were kept in separate cages for the ease of sample & data collection and were fed ad libitum. The weights at first egg (WFE) were recorded in kg with a balance on the day when they gave their first egg. Age at first egg (AFE) was calculated from the records. Mean weight of eggs (MEW) was taken as average of daily egg weights over a period of 90 days of laying and recorded in g with the help of a monopan balance. Total no. of eggs (TEN) represented the number of eggs laid over the study period of 90 days.

Blood Collection and DNA isolation

Two to three ml of blood was collected from wing vein of each bird in a vacutainer tube containing EDTA. DNA was isolated from 2-3 ml of blood using High salt method of Montgomery and Sise [8] with slight modifications.

Polymerase chain reaction

Polymerase chain reaction was carried out in a Bio-Rad CFX96 Real Time system. Primer pair for PRLR5 was used as described by Rashidi et al. [9], to amplify a fragment of 250 bp from exon 5 of prolactin receptor gene. The sequence of primers is as follows:

Forward: 5’-TTGTCTGCTTTGATTCATTTCC-3’
Reverse: 5’-TGCATTTCATTCTTCCCTTTT-3’

PCR was performed in a final volume of 50 μl containing: 100 ng of genomic DNA, 0.5μM of each primer, 0.2 mm of each dNTPs, 1.5 mM MgCl2, 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 59 °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 a 2% Agarose gel.

**Restriction digestion**

The fragment amplified by PRLR5 contains GGATC sequence for BamHI endonuclease. Thus, restriction enzyme BamHI was used. The restriction digestion was performed in 20μl volume having 1 X restriction enzyme buffer and 4-5 units of enzymes. The reaction tubes were incubated overnight at 37 °C.

**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:

\[
\text{Frequency of an allele} = \frac{2 \times \text{No. of Homozygote}}{2 \times \text{Total no. of Individuals} (N)} - \frac{\text{No. of Heterozygote}}{N}
\]

Alleles frequency and their accordance to Hardy-Weinberg equilibrium were calculated from Graphpad Prism software version 5.0. The following linear equation was applied to analyze the genetic effects of PRL24:

\[
Y_{ij} = \mu + G_i + H_j + e_{ij}
\]

Where \(Y_{ij}\) is the average performance of ith genotype in jth hatch, \(\mu\) is mean of the population, \(G_i\) is fixed effect of ith genotype \((i=1,2,3)\), \(H_j\) is fixed effect of jth hatch \((j=1,2,3)\), and \(e_{ij}\) is random residual error.

**Results and Discussion**

The AFE in days in Aseel hens ranged from 184 to 194, whereas the Mean±SEM was found to be 188.40±0.22. The weight at first laying (WFE) in Kg among Aseel hens ranged from 1.20 to 2.20; the Mean±SEM was found to be 1.57±0.03. The mean egg weight (MEW) and TEN in the present study, were better than the studies of Mohan et al. [12], in Peshawari variety of Aseel; whereas, mean eggs weights (g) to be 1133±1.52 at an age of 6 months. This difference might be attributed to breed of birds used in the study. Based on the findings of current study, it can be concluded that though PRLR5 shows polymorphisms in Aseel hens, statistically there is no association of laying performance with polymorphism at this locus of Prolactin.

**Conclusion**

Statistically, there was no significant difference was found between the means of various laying traits with genotypes and this finding is in contrary to the findings of Zhang et al. [15], where they found that polymorphism of the PRLR gene is significantly associated with egg production traits in the Erlang Mountainous chicken. This difference might be attributed to breed of birds used in the study. Based on the findings of current study, it can be concluded that though PRLR5 shows polymorphisms in Aseel hens, statistically there is no association of laying performance with polymorphism at this locus of Prolactin.

**Acknowledgement**

None.

**Conflict of Interest**

Author declares there is no conflict of interest.

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