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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- 3ml 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

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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 behavior and egg production.[5] 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.[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] 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. Due to different biological activities attributed to PRL and PRLR, they can be used as the major candidate genes in molecular animal breeding programs. There is scarce data in case of breeds like Aseel, thus present study was planned.

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 2-3ml 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 250bp from exon 5 of prolactin receptor gene. The sequence of primers is as follows:

Forward: 5'-TTGTCTGCTTTGATTCATTTCC-3'

Reverse: 5'-TGCATTTCATTCTTCCCTTTTT-3'

PCR was performed in a final volume of 50µl containing:100ng of genomic DNA, 0.5µM of each primer, 0.2mm of each dNTPs, 1.5mM MgCl2, 1.0U Taq DNA polymerase and 1× reaction buffer. The cycle conditions for PCR included-Initial denaturation of 5min at 94°C; followed by 35 cycles of 94°C for 30s, annealing at 59°C for 60s, extension at 72°C for 60s followed by a final extension of 5min at 72°C. The PCR product was resolved on to 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 1X 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:

$$\text{Frequency of an allele} = \frac{(2\,x\ No.of\ Homozygote) + (No.of\ Heterozygote)}{2\,x\,Total\ no.of\ Individuals\,(N)}$$

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:

$$Yij = \mu + Gi + Hj + eij,$$

Where Yij is the average performance of ith genotype in j^{th} hatch, µ is mean of the population, Gi is fixed effect of i^{th} genotype (i=1,2,3), hj is fixed effect of jth hatch (j=1,2,3), and eij 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 being 1.57±0.03. The mean egg weight (MEW) ranged from 39.97g to 49.62g and the mean±SEM values were found to be 43.61±0.36. Total number of eggs (TEN) varied from 29 to 41; the mean being 35.48±0.40. The age at first laying (AFE) in the present study was higher than the studies of Mohan et al.[10] (2008a, b); where they reported it to be 154d. The body weights of Aseel hens (WFE) in the present study were better than the study of Gurung & Singh;[11] where they reported the body weights (g) to be 1133±1.52 at an age of 6 months. This difference might be due to the fact that they studied this breed reared by the farmers/ localities as unorganized farms; whereas in the present study, birds were kept in organized farm of the University.

Similar findings regarding WFE were reported by Ahmad et al.,[12] in Peshawari variety of Aseel; whereas, mean eggs weight (MEW) and TEN in the present study, were better than previous study.[11,13,14] These differences might be due to the difference in breed of Aseel hens and managemental conditions. There was present of only two alleles: one where the restriction site was present and other where restriction site was absent. The individuals showing no restriction site for the enzyme were designated as of BB genotypes; whereas AA genotypes were cut by the enzyme. The frequency of A allele was higher (0.60) than B allele (0.40) at PRLR5 marker site. These results are in concurrence with the findings of Rashidi et al.[9] where they reported the frequencies of AA and BB genotypes to be 0.72 and 0.28 respectively. The age of hens at their first laying (AFE) in the genotypes AA & BB were 186.8±0.69 & 188.1±0.79 respectively. The mean body weight at 1st laying of hens (WFE) having genotypes AA & BB were 1.60±0.05 & 1.53±0.04 respectively. Birds showing mean egg weight (MEW) having genotypes AA & BB were 43.20±0.45 and 44.11±0.60 respectively. All birds showing mean total no. of egg (TEN) having genotypes AA & BB were 36.14±0.48 & 34.67±0.63 respectively.

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.

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None.

Conflict of interest

The author declares no conflict of interest.

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