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Association of Prolactin promoter (PRL5) Gene with Egg Production Performance in Kadaknath Hens

Shiv Kumar Yadav, S. K. Maurya, Alok Kumar Yadav, Anand Kumar, Kamalesh Yadav

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Abstract A study was carried out to study egg production performance and polymorphism of prolactin gene at promoter region (PRL5). Primer pair PRL5 was used to amplify the 439 bp fragment of the chicken PRL gene containing AGCT sequence for *AluI* restriction enzyme. PRL5 locus for polymorphism was amplified by PCR and the product was resolved by electrophoresis and performed in 2% agarose gel; 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 20 birds collected from wing vein. The AFE (d), WFE (Kg), MEW (g) and TEN of Kadaknath hens in the present study were found to be 188.00 ±0.71, 1.26 ±0.03, 42.83 ± 0.21 and 37.75 ± 0.59 respectively. The prolactin gene locus PRL5 showed two alleles C and T and three genotypes : CC, CT and TT. The frequencies of C and T alleles at this locus were 0.50 and 0.50 respectively. The frequencies of CC, CT and TT genotypes were 0.30, 0.40 and 0.30 respectively. The birds of C allele had a significantly (p<0.05) better MEW and TEN than birds of T allele.

Keywords Egg poduction performance, Kadakath, Prolactin, Polymorphism.

Introduction

Egg productivity is the most important economic trait in egg-laying poultry [1]. Endocrine and environmental factors such as length of photo period and feeding allowance can influence egg production [2]. 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 [3].

Shiv Kumar Yadav*[1], S. K. Maurya[1],
Anand Kumar[1], Kamalesh Yadav[1],
Alok Kumar Yadav[2]

[1]College of Veterinary Science and Animal Husbandry,
Narendra Deva University of Agriculture and Technology,
Kumarganj, Faizabad 224229, UP, India
[2]PhD Scholar, AGB Division, ICAR-NDRI,
Karnal, Haryana, India
e-mail : dr.shivkumarjnp@gmail.com
*Correspondence

Kadaknath is a native Indian breed of poultry. Meat of Kadaknath is dark colored and delicious, also attributed with medicinal qualities. Locally it is known as KALAMASI and used for traditional treatment of many disease in human beings and is also considered an aphrodisiac. Kadaknath do not lay eggs at particular time and strat egg laying 6 months onwards. Eggs are in 2-3 clutches in a year with 25-30 eggs/clutch. Characteristics of Kadaknath poultry breed include black colored meat, black blue plumage, black hued internal organs. The skin, shank, toes and soles is dark gray colored where as tongue is dark gray or light black in color. Most of the internal organs show intense black coloration due to deposition of melanin pigment in the organs. Its Unique breed characteristics have been established through many generations of selection and fixation of genes. It tolerates extreme climatic conditions of summer heat and cold winter stress and thrives very well under minimal management inputs like poor housing, no health care or supplementary feeding while exhibiting appreciable degree of resistance to diseases compared to other exotic breeds of fowl [4]. The kadaknath lays around 80-90 eggs annually and is not a good brooder. The meat and eggs are also reckoned to be a rich source of protein.

Prolactin (PRL) is a polypeptide hormone which plays a key role in egg production. On set of incubation behavior in induced by an increase in PRL secretion, which results in regression of ovary and loss of egg production. With development of molecular biological approaches, genetic basis of egg production has been performed through prolactin (PRL) gene. Chicken PRLgenomic sequence is 6163 bp long [5]. The exois are 28,182, 108, 180, 192 bp long. The 4 introns are 1,520, 408, 1348, 1909 bp long. Most of the sequence polymorphism in the chcken PRL gene occurs in 5 flanking region, 3 flanking region, and coding region of single peptide [6,7]. 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. PRL is involved in more than 300 biological activities ranging from reproduction, growth, and metabolism to behavior and immune responses. Its binding sites or receptors are widely distributed throughout vertebrates. Injection of PRL induced incubation behavior where as active immunization against PRL inhibit development of broodiness. The research on prolactin promoter gene polymorphism and its relation to egg production in this indigenous breed of hen is scarce. Hence the present work was planned to explore the polymorphism in PRL 5 and egg production in Kadaknath.

Materials and Methods

Birds and production data

The birds from the poultry farm of College of Veterinary Science and Animal Husbandry, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad were used for the present study. Twenty female birds of Kadaknath breed nearing their nearing their age of laying were taken for the present study. Birds were kept in separate cages for the case of sample and 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 CFX_{96} Real Time system. Primer pair PRL5 was used to amplify the 439 bp fragment of the chicken PRL gene containing AGCT sequence for *Alul* restriction enzyme. The electrophoretic profiles of RFLP analysis of the fragment obtained from primer

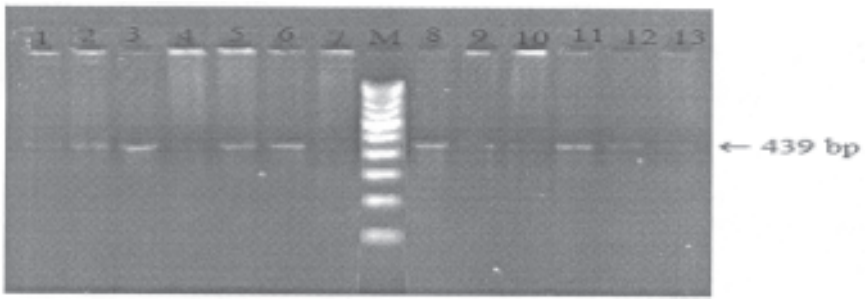

Fig. 1. 2% Agarose gel electrophoresis of PRL5.

pair PRL5 for 5 -flanking region of prolactin gene showed three genotypes as a result of single nucleotide polymorphism for C-2402T (*AluI*) at this site (Fig. 1). Individuals with 4 bands (160, 144, 81, 54 bp) were considered as of CC genotypes; individuals with 3 bands (304, 81, 54 bp) were considered as TT genotypes and individuals with 5 bands (304, 160, 144, 81, 54 bp) were considered as of CT genotypes. Primer pair for PRL5 was used as described by Rashidi et al. [9].

The sequence of primers is as follows—
Forward 5′-CTAAAGGACCTGGAAGAAGGG-3′

Reverse 5′-AACTTGTCGTAGGTGGGTCTG-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 nM of each dNTPs, 1.5 mM $MgCl_2$, 1.0 U Taq DNA polymerage and l× 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 62°C for 60s, extension at 72°C for 60s followed by a final extension of 5 min at 72°C . The PCR product was resolved on to a 2% agarose gel.

Genotyping and statistical analysis

Genotypes were manually scored based on the bands resolved on the agarose gel. Frequencies of various alleles were calculated using the following formula—

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

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 analyse the genetic effects of PRL5 :

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

Where Yij is the average performance of ith genotype in jth hatch, µ is mean of the population, Gi is fixed effect of ith genotype (i =1,2,3), hj is fixed effect of jth hatch (j=1, 2,3), and eji is random residual error.

Results and Discussion

The mean ± SEM and range of various egg production performance of Kadaknath are presented in Table 1.

The AFE in days in Kadaknath ranged from 182 to 194, whereas the Mean ± SEM was found to be 188 ± 0.71. The weight at first laying (WFE) in Kg among Kadaknath hens ranged from 1.0 to 1.65; the

Table 1. Production Performance of Kadaknath.

Parameter	Range	Mean ± SEM
AEF (day)	182.00 - 194.00	188.00 ± 0.71
WFE (Kg)	1.00 - 1.65	1.26 ± 0.03
MEW (gm)	41.07 - 45.03	42.83 ± 0.21
TEN (90 days)	31.00 - 41.00	37.75 ± 0.59

mean ± SEM being 1.26 ± 0.03. The mean weight (MEW) ranged from 41.07 g to 45.03 g and the mean ± SEM values were found to be 42.83 ± 0.21. Total number of eggs (TEN) varied from 31 to 41 ; the mean being 37.75 ± 0.59.

In a study, Biswas et al. [10] reported the age at first laying(d) in Kadaknath hens to be 158 .20 ±2.10. which is less than the age at first laying (AFE) in present study.

The body weights of Kadaknath hens in the present study were better than the study of [4]; where they reported the body weights to be 1026 ± 6.20 at an age of 6 months. The body weights in this study were also higher than the study earlier [10]. They found the body weights of Kadaknath birds to be 1129 g in their study. This difference might be due to the fact that they studies this breed reared by the farmers/ localities as unorganized farms; whereas in the present study, birds were kept in organized farm of the University.

The MEW and TEN of the present study support the earlier findings [10]; where they reported almost equal egg weights and total eggs. Based on these production performance of Kadaknath in present study; it can be said that the birds of the farms are better in terms of WFE, MEW and TEN; though their AFE is some what higher. Biswas et al. [10] studied various production performances of Kadaknath hens and found that in their study, the weights (g) were 1.129 .05 ± 20.52. In a study, Biswas et al. [10] found the mean egg weight (g) in Kadaknath hens to be 41.84 ± 1.23. Biswas et al. [10] reported the egg number in Kadarknath birds and found it to be 19.50 ± 1.44 for 6 weeks/birds.

M : 100 bp DNA Ladder

The PRL5 locus produced two alleles “C” and “T” .

Table 2. Interaction of prolactin gene PRL5 on egg production performance of Kadaknath. Values with different superscripts in a column differ significantly (p<0.05).

Geno-type	Traits (Mean ± SE) AFE (D)	WFE (Kg)	MEW (g)	TEN
CC	187.3 ± 0.99	1.26 ± 0.09	43.17 ± 0.45	37.50 ± 1.23
CT	187.9 ± 1.35	1.25 ± 0.04	42.56 ± 0.28	38.63 ± 0.68
TT	188.7 ± 1.30	1.29 ± 0.04	42.85 ± 0.38	36.83 ± 1.30

These alleles producted three genotypes : “CC”, “CT” and “TT”. The frequencies of these three genotypes were 0.30, 0.40 and 0.30 respectively. The allelic frequencies of the two alleles C and T were same 0.50 in the present study. These findings are in concurrence with the studies earlier [9] in terms of number of genotypes, where they find the same numbers of genotypes. Though the frequenceies of various genotypes in the present studies were different than those earlier [9], where they found the frequencies of CC, CT and TT alleles to be 0.10, 0.84 and 0.06 respectively. These differences in the frequencies may be because of the difference in the breed studied. Present study was conducted in Kadaknath, while the study earlier [9] was on indigenous chicken of Iran. The allelic frequencies obtained for 5′ flanking region of prolactin gene by primer pairs of PRL5 in the present study differ from those reported by Cui et al. [7]. They reported that the frequency of C allele at nucleotide -2402 of 5′ flanking region of prolactin gene varied from 0.02 (Taihe Silkies F0 generation), 0.05 (Yangshan), 0.23 (Taihe Silkies F1 generation), 0.35 (White Rock), 0.42 (Nongdahe) in indigenous chickens to 1.0 in commercial White Leghorn chickens. This difference can be attributed to breed difference, as in their study earlier [7] also foud a huge difference in frequencies in different breeds of hens. In the present study, interactions of alleles with egg production parameters were investigated. The result of interaction of these alleles with egg production performance is represented in Table 2.

The age of hens at their first laying (AFE) in the genotypes CC, CT and TT were 187.3 ± 0.99, 187.9 ± 1.35 and 188.7 ± 1.30 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 CC,

CT and TT were1.26 ±0.09, 1.25 ±0.04 and 1.29 ± 0.04 respectively. There was no significant difference (P<0.05) between the means of WFE in various genotypes. Birds showing mean egg weight (MEW) having genotypes CC, CT and TT were 43.17 ± 0.45, 42.56 ± 0.28 and 2.85 ± 0.38 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 CC, CT and TT were 37.50 ± 1.23, 38.63 ± 0.68 and 36.83 ± 1.30 respectively. The mean TEN did not differe significantly (p<0.05) among various genotypes.The mean egg weight shown by CC genotype were higher than other genotypes in the present study, whereas, the egg number was higher in CT genotypes. This finding is in accordance with the earlier findings [7], where they also found that the frequency of C alleles was higher in the birds that were good layer. Present findings also support the work earlier [11] where they also found that the frequency of C alleles were higher in the egg line chickens than meat line chickens. Same findings have also been reported by Sarvestari et al. [12] , where they also found an association between egg productions with C allele.

Based on the above findings, it can be concluded that PRL5 shows polymorphisms in Kadaknath hens. The “C” allele is associated with higher number of eggs and higher egg weights and thus, PRL5 can be used as molecular markers for selection of egg producing birds of Kadaknath.

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