Polymorphism of Prolactin gene in relation to egg production performance in Kadaknath hens

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ABSTRACT

Present study was carried out to study egg production performance and polymorphism of Prolactin gene at 24 bp indel locus at promoter region (PRL24). Egg production performances were recorded as age at first egg (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 blood of 20 Kadaknath birds collected from wing vein. PRL24 locus for indel polymorphism was amplified by PCR and the product was resolved on native PAGE for genotyping. 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 PRL24 showed two alleles I and D and three genotypes: II, ID and DD. The frequencies of I and D alleles at this locus were 0.55 & 0.45 respectively. The birds of D allele had a significantly (P<0.05) better TEN than birds of I allele.

Key words: Egg production performance, Kadaknath, Prolactin, Polymorphism.

INTRODUCTION

Prolactin (PRL) is a polypeptide hormone which plays a key role in egg production. An increase in PRL secretion causes onset of incubation behavior, which results in loss of egg production (Sharp, 1997). Chicken PRL genomic sequence is 6163 bp long and has 5 exons and 4 introns (Kansaku et al., 2005). The exons are 28, 182, 108, 180, 192 bp long. The 4 introns are 1,520, 408, 1348, 1909 bp long. Polymorphism in the promoter 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). It was found that the presence of a 24 bp insertion in the promoter region of the avian Prolactin gene (PRL24) is correlated with the intensity of egg-laying activity in birds and broody behavior (Kulibaba and Podstreshnyi, 2012; Jing et al., 2009).

Egg production is the most important economic trait in poultry birds. Endocrine and environmental factors such as length of photoperiod and feeding allowance can influence egg production (Lewis and Gous, 2006). However, genetic factor also plays an important role. Egg production is a polygenic trait with low to moderate heritability and a major opportunity for improvement in this trait lies in the polygenic trait with low to moderate heritability and a major opportunity for improvement in this trait lies in the PRL24 sequence. The promoter region of the PRL gene is rich in TATA box and this implies that PRL is a TATA binding protein-dependent gene (Sakaguchi and Bell, 1988).

Kadaknath is a native Indian breed of poultry. Meat of Kadaknath is dark coloured and delicious and attributed with medicinal qualities. Kadaknath birds can tolerate 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 (Thakur et al., 2006). 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 PRL24 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 near to their age of laying were taken for the present study. Birds were kept in separate cages for the ease of sample and data collection and were fed ad libitum. The weights at first egg (WFE) were recorded in Kilograms 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 grams with the help of a monopan balance. Total number 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 using High salt Method of Montgomery and Sise (1990).
Polymerase chain reaction: Polymerase chain reaction was carried out in a Bio-Rad CFX™ Real Time system. Primer pair for PRL24 was useful 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 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 30 s, 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 6% native Polyacrylamide gel.

Genotyping and statistical analysis: Genotypes were manually scored based on the bands resolved on the PAGE. Frequencies of various alleles were calculated using the following formula—

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

Allelic 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 PRL24:

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

Where \(Y_j\) is the average performance of \(i^{th}\) genotype in \(j^{th}\) hatch, \(\mu\) overall mean, \(G_i\) is fixed effect of \(i^{th}\) genotype \((i=1,2,3)\), \(H_j\) is fixed effect of \(j^{th}\) hatch \((j=1,2,3)\), and \(e_{ij}\) is random residual error \((\text{NID}, 0, \sigma^2_j)\).

RESULTS AND DISCUSSION

The Mean ± S.E. and range of various egg production performance of Kadaknath are presented in Table 1.

The AFE in Kadaknath birds ranged from 182 to 194 days. whereas the Mean ± S.E. was found to be 188 ± 0.71 days. The weight at first laying (WFE) for Kadaknath hens ranged from 1.0 to 1.65 Kg; the Mean ± S.E. being 1.26 ± 0.03 Kg. The mean egg weight (MEW) ranged from 41.07 gm to 45.03 gm. Total number of eggs (TEN) found to be 42.83 ± 0.21 gm. Total number of eggs (TEN) varied from 31 to 41; the mean being 37.75 ± 0.59.

The body weights of Kadaknath hens in the present study were better than the study of Thakur et al. (2006); where they reported the body weights (gm) to be 1026 ± 6.20 at an age of 6 months. The body weights in this study were also higher than the study of Biswas et al. (2010). 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 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.

The age at first egg (AFE) in the present study was higher than the studies of Biswas et al. (2010); where they reported it to be 158.20 ± 2.10 days.

The MEW and TEN of the present study support the findings of Biswas et al. (2010); 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 somewhat higher.

The gel photograph of PAGE showing various genotypes of birds is shown in Figure 1. The PRL24 locus produced two alleles “I” and “D”. These alleles produced three genotypes: “II”, “ID” and “DD”. 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 insertion (I) allele was of 154 bp and the deletion (D) allele was shown by a 130 bp band. The frequencies of the two allele I and D were found to be 0.55 and 0.45 respectively.

These results support the findings of Cui et al. (2006); Yousefi et al. (2012) and Rashidi et al. (2012) where they found the higher frequencies of ID genotypes than other genotypes. In a study on Iranian native fowl population from

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
<th>AFE(day) Mean± SE</th>
<th>WFE(Kg) Mean± SE</th>
<th>MEW(gm) Mean± SE</th>
<th>TEN(90 days) Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.55</td>
<td>188.3±0.69</td>
<td>1.28±0.02</td>
<td>42.66±0.18</td>
<td>37.00±0.63*</td>
</tr>
<tr>
<td>D</td>
<td>0.45</td>
<td>187.5±0.71</td>
<td>1.24±0.04</td>
<td>43.04±0.23</td>
<td>38.67±0.42b</td>
</tr>
</tbody>
</table>

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

Table 1: Interaction of Prolactin gene PRL24 on egg production performance of Kadaknath.
Yazd province, Emamgholi-Begli et al. (2010) reported higher frequency of 0.566 for II genotypes at the 24 bp indel site of prolactin promoter.

The age of hens at their first egg (AFE) in the alleles “I” & “D” was 188.3±0.69 and 187.5±0.71 respectively. There was no significant difference (P<0.05) between the means of AFE in various alleles.

The mean body weight at 1st laying of hens (WFE) having alleles “I” & “D” were 1.28±0.02 and 1.24±0.4 respectively. There was no significant difference (P<0.05) between the means of WFE in various alleles.

Birds showing mean egg weight (MEW) having alleles “I” & “D” were 42.66±0.18 and 43.04±0.23 respectively. There was no significant difference (P<0.05) between the means of MEW in various alleles.

Mean ± S.E. of total number of egg (TEN) having alleles “I” & “D” were 37.00±0.63 and 38.67±0.42 respectively. There was a significant difference (P<0.05) in the total no. of eggs between “I” & “D” alleles.

In the present study, “D” allele was found to be associated with an increased no. of eggs & better egg weights; which do not support some of the earlier findings. Researchers have found the association of “I” allele with better number of eggs in eggs in Iranian fowl (Emamgholi-Begli et al. 2010) and in Turkey (Fathi and Zarringhobayi 2014). The “I” allele has also been shown to be found in higher frequencies in egg line chickens than meat line chickens and thus the I allele could be attributed for a better egg production (Kulibaba and Podstreshnyi 2012).

Although, the 24 bp indel site of Prolactin promoter may not be associated with any production traits (Rashidi et al., 2012). Insertion of this sequence in the promoter may inhibit a transcriptional factor binding site for PRL gene and, hence, decrease the expression of PRL, which contributes to non broodiness in hens (Jiang et al., 2005).

Based on the above findings, it can be concluded that PRL24 shows polymorphisms in Kadaknath hens. The “D” allele is associated with higher number of eggs and higher egg weights and thus, PRL24 can be used as molecular markers for selection of egg producing birds of Kadaknath.

REFERENCES


Fig 1: 6% Native PAGE of PRL24 showing different genotypes.


