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Effect of broodiness on production traits and its association with polymorphism in 5' regulatory region of dopamine D2 receptor gene in Ghagus breed

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ABSTRACT

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Ghagus breed was characterized for broodiness, its effect on production traits and polymorphisms at 5' regulatory region of dopamine D2 receptor (DRD2) and prolactin genes for their association with production and broody traits. A high incidence (85.4%) of broodiness was observed and it had a significant effect on production traits. The broody hens were (P<0.001) lesser in bodyweight than non-broody hens and the bodyweight decreased with the increasing duration of broodiness (DB). Non-broody hens produced more (P<0.001) eggs than the broody hens. The number of clutches and pauses was (P<0.001) higher in non-broody hens as compared to broody hens. However, the average pause size was significantly (P<0.001) lesser in non-broody hens. The number of clutches and pauses reduced while pause size increased significantly as DB increased. Insertion-deletion (InDel) genetic marker at 5' region of the DRD2 gene was associated (P<0.05) with the age at first broody cycle, duration of the first broody cycle (P<0.01), egg production, and 40 weeks body weight (P<0.05) while SNP at 5' region of DRD2 gene had significant (P<0.004) influence on 40 weeks body weight. The study concluded that broodiness had a significant influence on production traits and InDel and SNP markers at the 5' regulatory region of DRD2 gene had significantly (P<0.004) affected the bodyweight at 40 weeks. The findings of the study could be useful for the development of breeding programs for the improvement of indigenous chickens like Ghagus.

Keywords: Broodiness, Body weight, Ghagus, Indigenous chicken, Marker

INTRODUCTION

Indigenous chickens are the backbone of poultry production in tribal and rural areas in India. They are known for unique characteristics like hardiness, aggressiveness, broodiness, mothering ability, the capacity to adapt to harsh tropical environments and suboptimal conditions of rearing, etc. Meat and eggs of indigenous chicken command premium prices due to their perceived health benefits. The demand for meat and eggs of indigenous chickens is continuously increasing particularly in urban and semi-urban areas. Therefore, they are now also being reared in semi-intensive and intensive systems in large numbers. However, indigenous chickens are known for slow growth and poor production potential. Therefore, the rearing of low-producing indigenous chickens is unable to meet the ever-increasing demand. Improvement of growth and production performance of indigenous chicken breeds through genetic selection helps in increasing the productivity of backyard, free-range and semi-intensive systems without affecting the cost of production and biodiversity (Magothe *et al*., 2012). So, characterization for growth, production and broodiness traits of indigenous chicken breeds is the need of the hour before initiating the improvement through selective breeding.

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Unlike in modern commercial layers, broodiness in indigenous chickens is desirable for self-propagation of chicks through natural hatching in the backyard or free-range poultry production, and therefore it is an important trait that needs to be retained in the them (Pym, 2010). Broodiness was observed in different indigenous chickens throughout the world (Jiang *et al*., 2005; Liang *et al.*, 2006; Begli *et al*., 2010). However, little information is available on broodiness characters in chicken breeds indigenous to India. One study has reported the low incidence (8.42%) of broodiness in the Aseel while no broodiness in Kadaknath breed reared under the intensive system (Haunshi *et al*., 2011). Similarly, no broodiness behaviour was reported in the Nicobari breed of chicken. Ghagus is one of the indigenous chicken breeds of India. This breed is originated from the southern part of India (Haunshi *et al*., 2015). It is a medium-sized dual-purpose bird with high broodiness and good mothering ability.

Various factors affect the reproduction and production potential of domestic chickens. Broodiness is one such factor that significantly influences egg production. Genetic control of broodiness trait in chickens is well established (Romanov, 2001; Romanov *et al*., 2002). Both major and minor autosomal genes were involved in the control of broodiness trait. Prolactin

and dopamine D2 receptors (DRD2) genes are considered as candidate genes of interest for the molecular study of broodiness trait (Xu *et al*., 2010). Prolactin is the main reproductive hormone that is involved in the onset and continuation of broodiness (Jiang *et al*., 2005). A 24bp insertion-deletion (InDel) marker in the promoter region (at -377 to -354 bp) of prolactin gene in Blue-shell Chinese local chicken breed is reported to be associated with broodiness (Jiang *et al*., 2005) and bodyweight and egg weight in Silkie fowl (Rahman *et al*., 2014). Similarly, it was found to influence the egg production in Nongdahe × Taihe Silkies chickens cross (Cui *et al*., (2006) and in native fowl of Yazd province of Iran (Begli*et al*., 2010). However, no significant association of this InDel marker with egg production and bodyweight traits in female birds of Japanese Silkie fowl was reported (Rahaman *et al*., 2014).

DRD2 gene, which is involved in the secretion of prolactin hormone at the pituitary level, was associated with broodiness. SNP (A-16105G) at 5' regulatory region of DRD2 was reported to be associated with broody frequency in the Chinese native chicken population (Xu et al. 2010). Furthermore, 22 bp InDel marker at 5' regulatory region of DRD2 gene was also reported to be associated with bodyweight at 50 days in Japanese Taihe Silkie fowl (Rahman *et al.*, 2014) and bodyweight at 7 and 110 days in Russian Phuskin breed of chicken (Mitrofanova *et al*., 2017).

Therefore, the objective of the present study was to characterize the Ghagus, breed for broody traits, to study the effect of broodiness on production traits, and to investigate the association of polymorphisms of InDel marker located at the promoter region of prolactin gene and, SNP (A-16105G) and InDel (I-13387D) markers located at 5' regulatory region of DRD2 gene on production and broodiness traits in Ghagus breed of chicken.

MATERIALS AND METHODS

Experimental population

A total of 900 healthy chicks of Ghagus were hatched, vaccinated for Marek's disease, and reared on the floor up to 20 weeks. The brooding of chicks was carried out in poultry sheds with open sides having curtains. Maize-soybean based starter ration [metabolizable energy (ME) 2600 kcal/kg and crude protein (CP) 18%] up to eight weeks and grower ration (ME 2500 kcal/kg and CP 16%) from 9-20 weeks was given in ad libitum quantity. Water was provided continuously to all growing chicks. At 20 weeks, hens (215 Nos.) were selected randomly and housed in laying cages (individual) in a layer house with open sides. Ad libitum layer ration (ME 2600 kcal/kg and CP 16%) was provided daily. Hens were given 16 h light (natural daylight and artificial light) from the start of egg production.

Traits studied

Production traits: Each hen was evaluated for below-mentioned traits. The bodyweight was assessed at 20 and 40 weeks using a digital balance (0.1 g precision). Shank length was measured at 20- and 40-weeks using Vernier calibres (0.1 mm precision). Age at first egg (AFE) laid by each hen was determined by counting the number of days from the date of the hatch to the date of the first egg laid and averaged over the population. Hen housed (HHEP) and hen day egg production (HDEP) were recorded up to 40 weeks.

Broodiness traits

Hens in cages were observed every day to record broodiness behavior from 26 to 40 weeks. The weekly incidence of broodiness (from 26 to 40 weeks) was determined by calculating the percentage of broody hens to the total population every week. Other broody traits recorded were age at first broody cycle (AFB), and duration of the first broody cycle (DFB). Clutch size, pause size, the average number of clutches, and pauses from age at the first egg to 40th week were also recorded.

DNA extraction and PCR amplification

About 1 ml blood was collected from the brachial vein at 30 weeks in tubes containing 0.5 M EDTA. Phenol-chloroform procedure was used to extract the high-quality genomic DNA (Sambrook and Russell, 2001). The quantity and quality of DNA were checked by the spectrophotometer method. Fragments at the 5' regulatory region of DRD2 and at the promoter region of prolactin genes were amplified from genomic DNA using a specific set of primers to study the polymorphism. The fragment at 5' regulatory region having 22 bp InDel (I-13387D) marker (187/165 bp) of DRD2 gene was PCR amplified using 5'-TGCACTTCAATCCTTCCCAGCTT-3' (forward) and 5'-TTGCGCTGCCCATTGACCA-3' (reverse) primers (Rahman *et al*., 2014). The 233 bp fragment of 5' regulatory region having SNP (A-16105G) was amplified using 5'-CCCCCGGCAGGCAGAGCAC-3' (forward) and 5'-ACGCGATCTGGGAGCAAACCTTC-3' (reverse) primers (Xu *et al.*, 2010). The fragment of promoter region having 24 bp InDel marker (154/130 bp) located at -377 to -354 bp of prolactin gene was amplified using 5'-GGTGGGTGAAGAGACAAGGA-3' (forward) and R: 5'-TGCTGAGTATGGCTGGATGT-3' (reverse) primers (Jiang *et al.*, 2005; Rahman *et al.*, 2014). Three fragments of two genes were amplified in a thermal cycler (Veriti, Applied Biosystems, Foster City, CA, USA). PCR was done in 0.2 ml PCR tubes (25 ìl volume) having Taq buffer with $MgCl_2$ (25 mM), dNTPs (200 ìM), forward and reverse primers (0.3 ìM each) and Taq polymerase (1 unit).The PCR cyclic conditions followed were initial denaturation at 95^{o}C for 10 min followed by 35 cycles of denaturation at 95^{o}C for 25 s, primer annealing (at 52^{o}C for InDel marker of DRD2, 68^{o}C for

SNP marker of DRD2, and 51°C for InDel marker of prolactin gene) for 25 s and extension at 72°C for 25 s and final extension of 72°C for 10 min.

Genotyping

The size of PCR amplified products of InDel markers of DRD2 and prolactin genes was determined on 3% agarose gel by horizontal electrophoresis. 100 bp and 50 bp ladders were used as standard markers to size the PCR products. PCR products of the DRD2 gene were initially resolved on 1.5% agarose gel electrophoresis and documented using gel documentation system (Syngene, Cambridge, U.K.) to confirm the size of the fragment (233 bp). Single Strand Conformational Polymorphism (SSCP) study was done to know the SNP polymorphism (Bassam *et al.,* 1991). Briefly, formamide dye (12 ìl) was added to the PCR product (6 ìl) in PCR tubes, mixed, centrifuged, and denatured at 95°C for 10 min. Subsequently, the tube was snap cooled -20°C for 15-20 min. Denatured PCR products were resolved on non-denaturing polyacrylamide gel (12%) by vertical electrophoresis. The silver staining technique was used to visualize the banding patterns on the gels. The common band pattern was identified and coded as 11 and 12.

Sequencing

The sequencing of representative samples of 11 and 12 SSCP patterns of the DRD2 gene was carried out to determine the SNP. PCR products were resolved on 3% agarose gel and a QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany) was used to extract the desired fragments. The gel eluted fragments were sequenced (both directions) through Big Dye terminator sequencing (ABI 3730 sequencer, Chromous Biotech Pvt. Ltd., Bengaluru). BioEdit software was used to align the forward and reverse sequences to arrive at a consensus sequence. Clustal W functions (BioEdit software) were used to determine the SNPs in the DRD2 gene. The sequencing results revealed that hens with 11 SSCP patterns had the AA genotype while those with 12 patterns possessed the AG genotype.

Statistical analysis

The descriptive statistics function of MS Excel was used to determine the means and standard errors of different traits. Pearson correlation coefficients between broody and production traits were calculated and significance was tested. GenAlEx software version 6.502 was used to determine the number of effective (Ne) and observed (Na) alleles, allele frequency, expected (He), unbiased expected (uHe), and observed heterozygosity (Ho) of different markers (Peakall and Smouse 2012). AMV function of GenAlEx software was used to test deviation from Hardy–Weinberg equilibrium (HWE), if any. The association analysis of polymorphisms of three markers (InDel/SNP) with various traits like shank length, bodyweight was done by general linear model (GLM) by considering genotype of the marker as fixed effect and hatch as a random effect. The association of markers with traits having count data such as AFE, age at first broody cycle, duration of the first broody cycle, and egg production was carried out using the generalized linear model (SPSS, Version 12.0). Comparisons for the frequency of broodiness and non-broodiness between different genotypes within marker (groups) were assessed by H^2 tests.

RESULTS AND DISCUSSION

The performance of broody and non-broody hens with respect to production traits is given in Table 1. Broody hens were significantly ($P < 0.001$) lesser in 40 weeks body weight than the non-broody hens. However, there was no difference in body weight recorded at 20 weeks and in shank length measured at 20 and 40 weeks. Lower 40 weeks body weight observed in broody hens might be due to a lesser feed intake as they were continuously sitting/brooding. Non-broody hens produced more ($P < 0.001$) numbers of eggs up to 40 weeks (EP 40w) than the broody hens even though they did not differ in AFE. Similarly, no difference in AFE between broody and non-broody hens but higher egg production in non-broody hens was reported in Chinese Qingyuan indigenous chickens (Jiang *et al.,* 2010).The low egg production recorded in the Ghagus is in conformity with the production status of other indigenous chicken breeds of India (Haunshi *et al.,* 2011; 2015). However, the broodiness is perhaps the main reason for lesser egg production observed in this breed. The intensity of broodiness in the Ghagus breed is so high that this behaviour was observed in hens kept in cages. Earlier studies also reported the display of broodiness behavior by indigenous chickens reared in cages (Yang and Jiang, 2005; Jiang *et al.,* 2010).

Table 1: Comparison of broody and non-broody hens for growth and production traits.

Traits	Non-broody	Broody	SEM
Number of hens	32	173	
Bodyweight at 40 wks (g)	1903±24.4[a]	1618±52.7[b]	0.001
SL at 40 wks (mm)	99.26±0.80	98.97±0.37	0.760
AFE (d)	178.0±2.51	175.4±1.00	0.320
EP 40 wks (Nos.)	53.28±3.76[a]	30.30±1.03[b]	0.001
Number of clutches	16.62±1.23[a]	7.90±0.290[b]	0.001
Avg. clutch size (d)	3.60±0.380	4.25±0.140	0.078
Number of pauses	16.56±1.23[a]	7.80±0.280[b]	0.001
Avg. pause size (d)	3.80±0.550[a]	12.90±0.72[b]	0.001

[a,b]Means with different superscripts row wise differ significantly. AFE: age at first egg; EP 40 wks: Egg production up to 40 weeks of age; SL: Shank length, SE: Standard error

One of the interesting observations made was that the numbers of clutches and pauses were higher

(P<0.001) in non-broody hens than in broody hens. Nevertheless, there was no difference in clutch size amongst these two groups of hens. The average pause size was higher (P<0.001) in broody hens, and this has contributed to the lesser egg production in broody hens. Non-broody hens could pause for a shorter duration (1, 2, or 3 days) on frequent occasions, hence a greater number of pauses and clutches were observed in non-broody hens. Broody hens taken long pauses and that resulted in a lesser number of pauses/clutches. Lesser EP 40w observed in hens with higher DB may be due to the termination of ovulation and cessation of egg-laying because of regression of ovaries in hens (Sharp, 1997).

To assess the effect of duration of broodiness (DB) on various traits, broody hens were categorized into three groups based on the DB observed up to 40 weeks (1-30, 31-60, and 61-89 days) and compared for various traits (Table 2). It was evident that as the DB increased the bodyweight of hens decreased (P<0.001). Similarly, as the DB increased the EP 40w decreased (P<0.001). AFE decreased as DB increased although non-significantly (P<0.122). Number of clutches and pauses also reduced (P<0.001) as the DB increased. As the DB increased the average pause size increased (P<0.01). Nevertheless, there was no relationship between average clutch size and DB.

Relationship among broodiness, growth, and production traits

The relationship between the incidence of broodiness at weekly intervals and egg production recorded in terms of HDEP and HHEP was studied. It was interesting to observe that the manifestation of broodiness started when the flock reached peak egg production (27th week) and egg production declined to the lowest point when the broodiness reached the highest proportion (36th week) demonstrating the inverse relationship (r=-0.53, P<0.05) between egg production and incidence of broodiness traits (b=-0.27±0.11, P<0.024). Hens started exhibiting the behaviour of broodiness at the age of 27th week, just after the four weeks of the onset of egg production. The percentage of broody hens continued to increase till the age of 36th week at that age the peak incidence of broodiness (65.53%) was observed and thereafter the number of hens exhibiting broodiness started decreasing. Egg production started during the 23rd week and HDEP (51.51%) and HHEP (50.13%) peaked at 27th week, i.e. four weeks after the onset of egg production in the population. Subsequently, both HDEP and HHEP started decreasing and the decline was continued up to the age of 36th week (Fig. 1).

The incidence of broodiness in Ghagus is perhaps highest (85.4% of birds exhibited broodiness behaviour) among indigenous breeds of India. Lesser incidence of broodiness (8.42%) was reported in Aseel hens while no broodiness in hens of Kadaknath breed reared under the intensive system (Haunshi *et al.*, 2011). In indigenous chickens of China, 10-80% of the incidence of broodiness was reported (Xu *et al*., 2010).

The relationship among broody, growth, and production traits was studied (Table 3). The bodyweight was negatively correlated with broody traits. The DFB and AFB were correlated positively. Surprisingly, the 40 weeks bodyweight was positively correlated with EP 40w, clutch and pause numbers while it was negatively correlated with the average pause length (APL). The positive correlation between EP 40w and bodyweight might be due to the fact that hens which produced more eggs were non-broody and they consumed sufficient feed

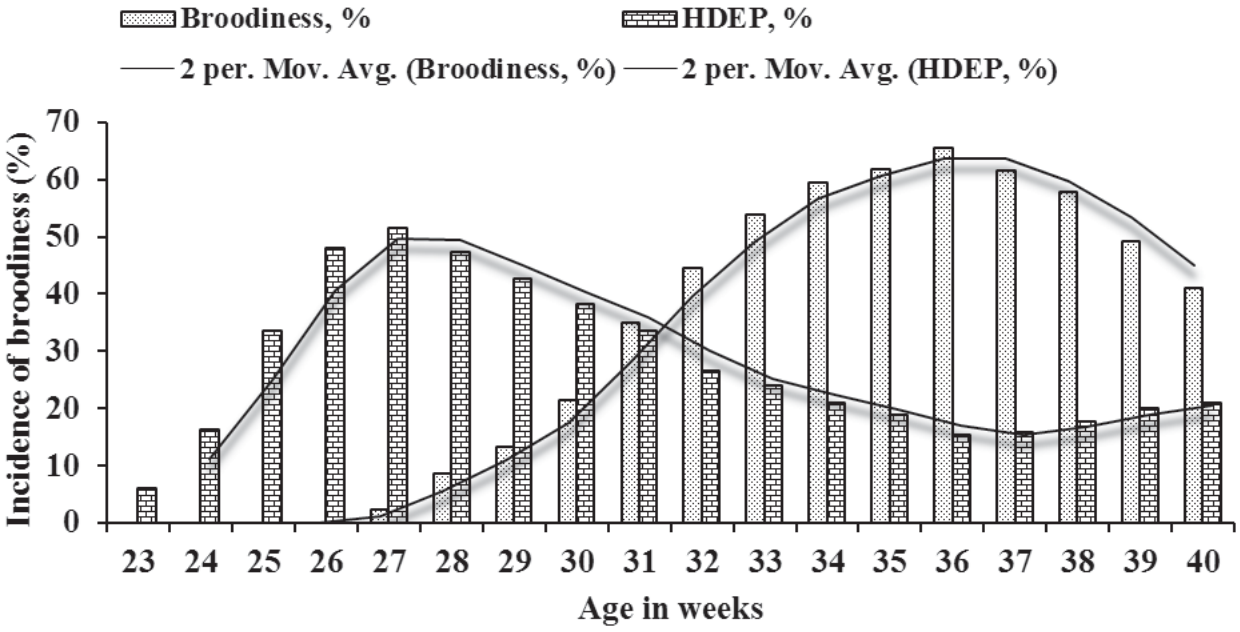

Fig. 1: Trend of incidences of broodiness and hen day egg production at weekly intervals up to 40 weeks of age in Ghagus breed.

Table 2: Comparison of broody and non-broody hens and effect of duration of broodiness on growth and production traits.

Trait	Duration of broodiness (Mean±SE)			P value
	1 to 30 d	31 to 60 d	61 to 89 d	
Number of hens	46	77	50	
Bodyweight at 40 wks (g)	1874±43.0[a]	1607±32.0[b]	1399±30.0[c]	0.001
SL at 40 wks (mm)	99.0±0.70	99.6±0.51	97.9±0.73	0.470
AFE (d)	179.4±2.4	175.5±1.4	171.6±1.4	0.122
EP 40 wks (Nos.)	40.9±1.95[a]	29.9±1.48[b]	21.0±0.84[c]	0.001
Number of clutches	10.6±0.52[a]	8.0±0.38[b]	5.2±0.40[c]	0.001
Avg. clutch size (d)	4.1±0.25	3.9±0.18	4.8±0.31	0.439
Number of pauses	10.6±0.51[a]	7.9±0.38[b]	5.2±0.40[c]	0.001
Avg. pause size (d)	6.5±0.486[c]	11.3±0.85[b]	21.1±1.5[a]	0.010

[a,b]Means with different superscripts row wise differ significantly. AFE: age at first egg, EP 40 wks: Egg production up to 40 weeks of age, SL: Shank length.

Table 3: Correlation between the broodiness, production and growth traits up to 40 weeks of age.

	Bwt 40w	AFE	EP 40w	AFB	DFB	Clutch Nos.	Pause Nos.	ACL	APL
Bwt 40w	1								
AFE	-0.11	1							
EP 40w	0.44***	-0.28***	1						
AFB	0.30**	0.01	0.37***	1					
DFB	-0.61***	-0.16*	-0.66***	-0.48***	1				
Clutch Nos.	0.44***	-0.03	0.63***	0.45***	-0.64***	1			
Pause Nos.	0.43***	-0.03	0.62***	0.45***	-0.64***	0.99***	1		
ACL	-0.06	-0.27***	0.22***	-0.22	0.16*	-0.49***	-0.49***	1	
APL	-0.42***	-0.15*	-0.57***	-0.48***	0.67***	-0.71***	-0.71***	0.48***	1

Bwt40w: Body weight at 40 weeks, AFE: Age at first egg, AFB: Age at first broody cycle, DFB: Duration of first broody cycle, ACS: Average clutch length, APS: Average pause length, * P<0.05, ** P<0.01, *** P<0.001.

and hence maintained adequate bodyweight whereas, broody hens which continuously sat consumed less feed and hence resulted in a reduction in their body weight. This is further supported by the fact that body weight was negatively correlated with DFB and APL and it was positively correlated with clutch and pause numbers. Hens with higher AFB had higher body weight as they spent less time on brooding. A negative correlation observed between AFE and EP 40w was on the expected line. The negative correlation of AFE with DFB, ACL and APL is attributed to the fact that the higher the AFE lesser was the broodiness duration. APL will be obviously higher in less producing hens owing to higher AFE.

Hens with higher AFB produced more eggs as they spent less time in brooding which was evident with the lesser DB. A positive correlation observed between egg production with clutch and pause numbers and ACL can be explained based on the fact that high producing hens took more number of small breaks with higher clutch size and lesser pause size. The negative correlation of AFB with DFB and APL is obvious as the lesser the age at the start of broodiness higher were the DB and pause size. Similarly, hens with higher AFB had higher clutch and pause numbers with lesser pause size. A negative correlation between AFB and DFB was reported previously in Chinese indigenous chickens (Jiang *et al*., 2010). The DFB was negatively correlated with clutch and pause numbers and positively correlated with clutch and pause size. This is due to the fact that birds with higher DB will have lesser egg production and lesser egg-producing hens tend to have longer pause duration and consequently lesser pause and clutch numbers. A negative correlation observed between clutch number and clutch length was previously reported in WLH and RIR breeds (Wolc *et al*., 2018).

Genotype and allele frequency

The genotype and allele frequency of InDel and SNP markers present at 52 regulatory region of DRD2 gene and InDel marker present at the promoter region of prolactin gene were investigated. Two fragments 187 bp and 165 bp of InDel marker (22 bp size) at 52 regulatory region of DRD2 gene representing insertion and deletion respectively were detected. The genotype frequency of In/In, Del/Del, and In/Del genotypes was 0.15, 0.40 and 0.45, respectively. The allele frequency of insertion and deletion was 0.37 and 0.63, respectively. SNP (A-

16105G) in the 52 regulatory region of the DRD2 gene revealed two genotypes, AA and AG. The frequency of the AA genotype was 0.89 whereas that of the AG genotype was 0.11. The allele frequency of A was 0.94 while a very low frequency of 0.056 was detected for the G allele of this SNP. The 24 bp InDel (154/130bp) polymorphism at the promoter region (-377 to -354 bp) of the prolactin gene was detected in Ghagus hens. The frequency of In/In, Del/Del, and In/Del genotypes were 0.12, 0.34, and 0.54, respectively. The allele frequency of insertion (0.38) allele was lesser than that of deletion (0.62) allele. The allele frequency of insertion allele of In/Del marker of DRD2 gene found in the Ghagus was higher than that observed in Red Jungle Fowl and lower than that reported in Chinese native chickens (Xu *et al*., 2010) and comparable to those observed in Russian Phuskin breed of chicken (Mitrofanova *et al*., 2017). Similar to the finding (0.056 low frequency of allele G) of the present study very low frequency (0.061) of allele G (of A-16105-G SNP) at 52 regulatory region of DRD2 gene was reported in the Red Jungle fowl (Xu *et al*., 2010).

The allele frequencies of insertion (0.38) and deletion (0.62) allele of InDel polymorphism at the promoter region of prolactin gene were almost similar to those reported in Hy-Line layer, Avian broiler, and Chinese blue-shelled chicken breed (Jiang *et al*., 2005), Yuehuang (Chinese) breed (Liang *et al*., 2006), and Taihe Silkies (Chinese) and White Rock breeds (Cui *et al*., 2006). However, a higher frequency of insertion allele and lower frequency of deletion allele was reported in native fowl of Yazd province (In-0.761 and Del-0.239) of Iran (Begli *et al*., 2010). The genotype frequency of In/In (0.12) and In/Del (0.54) genotypes of prolactin gene detected in the present study were higher than those found in Chinese native breeds (Jiang *et al*., 2005; Liang *et al*., 2006).

Results of the total number and the effective number of alleles for each locus along with observed heterozygosity are given in Table 4. The fixation index (F) (indicating the inbreeding level) was 0.06 for the InDel marker of DRD2 while it was negative (higher heterozygosity) for the InDel marker of prolactin and SNP marker of DRD2 gene. Thus, indicating the lesser inbreeding in the population of Ghagus. The results of all three markers indicated that the population of the Ghagus breed did not deviate from the HWE indicating the stability in the population.

Analysis of marker-trait association

The analysis of association of InDel and SNP markers with broodiness and production traits (Table 5) revealed that InDel genotypes at 52 regulatory region of DRD2 gene were significantly (P<0.05) associated with the 40 weeks bodyweight. Hens with In/In genotypes were higher in body weight than those of Del/Del genotypes. When association analysis was carried out among hens that exhibited broodiness behaviour, a similar trend was noticed with respect to 40 weeks body weight. The EP 40w was significantly (P<0.05) lesser in hens with Del/Del genotypes as compared to In/In and In/Del genotypes of DRD2 marker. The AFB was higher (P<0.003) in In/In genotypes than In/Del and Del/Del genotypes of DRD2 marker while DFB was higher (P<0.006) in Del/Del genotypes than In/In and In/Del genotypes. There was no significant association of different genotypes with AFE, EP 40w, 40 weeks shank length, average clutch size, and the number of clutches. Similarly, there was no significant association between different genotypes with the incidence of broodiness (Broody frequency).

The SNP marker at the 5' region of the DRD2 gene was significantly (P<0.001) associated with 40 weeks bodyweight. Hens with the AA genotype had lower bodyweight than AG genotypes. Hens with AG genotypes produced numerically a greater number of eggs compared to AA genotypes (P<0.08). A similar trend was noticed for bodyweight and egg production traits when association analysis was done within broody hens. However, there was no significant association between the SNP marker of DRD2 and broody traits although hens with AA genotypes exhibited numerically higher DFB with lesser AFB as compared to hens with AG genotypes.

Association analysis of InDel and SNP markers with various traits revealed that InDel genotypes at 52 regulatory region of DRD2 gene were significantly associated with the 40 weeks bodyweight, EP 40w and AFB. Perhaps ours is the first study to find the association of the InDel marker of the DRD2 gene with 40 weeks bodyweight. However, it was reported to be associated with the110 days bodyweight in hens (but no association with bodyweight at 49 days of age) but this association

Table 4: Effective number of alleles, heterozygosity and fixation index.

Gene	Na	Ne	Ho	He	uHe	F	$\div^2$ (HWE)
DRD2 (InDel)	2	1.87	0.44	0.47	0.47	0.06	0.71
DRD2 (SNP)	2	1.12	0.11	0.11	0.11	-0.06	0.72
PRL (InDel)	2	1.89	0.52	0.47	0.47	-0.11	2.47

Na= Observed number of different alleles, Ne: Effective number of alleles, Ho: Observed heterozygosity, He: Expected heterozygosity, uHe: Unbiased expected heterozygosity, F: Fixation index, HWE: Hardy Weinberg Equilibrium.DRD2: Dopamine receptor D2, PRL: Prolactin.

Table 5: Association of genotypes of polymorphic markers with production and broody traits in Ghagus breed.

Gene /marker	Genotype	No.	Production traits (Mean±S.E)		No.	Production and broody traits in broody hens (Mean±S.E)			
			B wt 40 w (g)	EP 40w (Nos.)		B wt 40 w (g)	EP 40w (Nos.)	AFB (d)	DFB (d)
DRD2 (InDel)	InIn	30	1765±59.9^{a}	35.8±3.13	27	1758±61.3^{a}	31.5±6.2^{ab}	232.3±3.7^{a}	35.5±4.2^{b}
	InDel	93	1674±33.9^{ab}	35.3±1.77	78	1630±35.9^{ab}	32.7±3.7^{a}	220.4±2.2^{b}	41.1±2.4^{b}
	DelDel	82	1626±36.3^{b}	32.1±1.90	69	1576±38.4^{b}	27.7±3.4^{b}	217.2±2.3^{b}	49.8±2.6^{a}
	P value		0.05	0.410		0.05	0.05	0.003	0.006
DRD2 (SNP)	AA	182	1637±23.6^{b}	33.2±1.2	156	1600±73.2^{b}	30.3±1.02	220.1±1.6	44.9±1.8
	AG	23	1920±66.2^{a}	39.6±4.9	18	1890±24.9^{a}	33.9±4.39	229.0±4.6	33.5±5.2
	P value		0.001	0.08		0.004	0.412	0.133	0.115

^{a,b}Means in the same column within each gene/marker having different superscripts differ significantly ($P<0.05$). DRD2: Dopamine receptor D2, EP 40w: Egg production up to 40 weeks of age, B wt 40 w: Body weight at 40 weeks of age, DFB: Duration of first broody cycle, AFB: Age at first broody cycle.

was inconsistent among male and female birds of Russian native chickens (Mitrofanova *et al*., 2017).

The SNP marker at the 5' region of the DRD2 gene was associated with bodyweight at 40 weeks. Hens with AA genotype had lower bodyweight than AG genotypes of DRD2 gene. This association might be due to the fact that hens having AA genotype exhibited a relatively higher DB and hence might have taken less feed during the period and in turn that affected their bodyweight negatively. However, there was no significant association between the SNP marker of the DRD2 gene and broody traits. Similarly, no association between genotypes of DRD2 SNP and DB was reported although these genotypes were associated with broody frequency in Chinese Ningdu Sanhuang chickens (Xu *et al*., 2010).

Prolactin is the main candidate gene responsible for the onset and maintenance of broodiness (Jiang *et al.,* 2005). The production of prolactin hormone is governed by regulatory factors involved in the expression of the prolactin gene. Therefore, polymorphism (InDel) at the promoter region of the prolactin gene may affect the binding of transcriptional factors and thus alters the expression of the prolactin gene. Previous studies show inconsistent results of the effect of this InDel marker of prolactin gene on broody traits and egg production. Some studies showed a significant effect of InDel marker on broody frequency and DB (Jiang *et al.*, 2005), and egg production (Begli *et al*., 2010) while other studies did not report any effect on the incidence of broodiness (Liang *et al*., 2006), egg production (Rahaman *et al*., 2014), bodyweight of hens and cocks (Mitrofanova *et al*., 2017) and AFE (Begli *et al*., 2010). Similarly, no association with egg production was reported in five pure breeds of chicken although association with the egg production in the cross of Nongdahe × Taihe Silkies chickens was reported (Cui *et al*., 2006). In the Ghagus breed also no significant association of the InDel marker of prolactin gene with production and broody traits was observed. These results suggest that besides the InDel marker of the prolactin gene various other markers and/or genes play a prominent role in broody and egg production traits. Broodiness is a complex trait that is influenced by the breed, rearing environment, reproductive age of hens, genes, etc.

The findings of the present study suggest that broodiness was higher in the Ghagus breed and it has a considerable effect on production traits. The DB had a significant influence on production traits. Polymorphism in DRD2 and prolactin genes was observed and genetic diversity study revealed no significant deviation from the Hardy Weinberg equilibrium in the population of Ghagus. InDel and SNP markers at the 5' regulatory region of DRD2 genes had a significant influence on broody and production traits. The findings of the study could be useful for the development of breeding programs for the improvement of low-producing indigenous chickens like Ghagus.

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REFERENCES

Bassam, B.J., Caetano-anollés, G. and Gresshoff, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, **196**(1): 80-83.

Begli, H.E., Zerehdaran, S., Hassani, S., Abbasi, M.A., and Ahmadi, A.K. 2010. Polymorphism in prolactin and PEPCK-C genes and its association with economic traits in native fowl of Yazd province. *Iranian journal of Biotechnology*, **8**(3): 172-203.

Cui, J.X., Du, H.L., Liang, Y., Deng, X.M., Li, N., and Zhang, X.Q. 2006. Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poultry Science*, **85**(1): 26-31.

Haunshi, S., Niranjan, M., Shanmugam, M., Padhi, M.K., Reddy, M.R., Sunitha, R. and Panda, A.K. 2011. Characterization of two Indian native chicken breeds for production, egg and semen quality, and welfare traits. *Poultry Science*, **90**(2): 314-320.

Haunshi, S., Shanmugam, M., Rajkumar, U., Padhi, M.K. and Niranjan, M. 2015. Characterization of Ghagus breed *vis-a-vis* PD–4 birds for production, adaptability, semen and egg quality traits. *Indian Journal of Animal Sciences*, **85**: 1338-1342.

Jiang, R.S., Chen, X.Y. and Geng, Z.Y. 2010. Broodiness, egg production, and correlations between broody traits in an indigenous chicken breed. *Poultry Science*, **89**(6): 1094-1096.

Jiang, R.S., Xu, G.Y., Zhang, X.Q. and Yang, N. 2005. Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. *Poultry Science*, **84**(6): 839-845.

Liang, Y., Cui, J., Yang, G., Leung, F.C. and Zhang, X. 2006. Polymorphisms of 52 flanking region of chicken prolactin gene. *Domestic Animal Endocrinology*, **30**(1): 1-16.

Magothe, T.M., Okeno T.O., Muhuyi W.B. and Kahi, A.K. 2012. Indigenous chicken production in Kenya: II. Prospects for research and development. *World's Poultry Science Journal*, **68**: 133–144.

Mitrofanova, O.V., Dementeva, N.V., Krutikova, A.A., Yurchenko, O.P., Vakhrameev, A.B. and Terletskiy, V.P. 2017. Association of polymorphic variants in MSTN, PRL, and DRD2 genes with intensity of young animal growth in Pushkin breed chickens. *Cytology and Genetics*, **51**(3): 179–184.

Peakall, R. and Smouse, P.E. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, **28**: 2537-2539.

Pym, R. 2010. Poultry genetics and breeding in developing countries. Food and Agriculture Organization of the United Nations, Poultry Development Review pp. 4.

Rahman, M.M., Matsuda, R., Matsuda, T., Nishiyama, Y., Jozaki, K., Anann, K. and Wada, Y. 2014. Relationship between the production traits and three candidate genes in the prolactin's In/Del× In/Del population of Silkiefowl. *Journal of Poultry Science*, **51**(2): 138-143.

Romanov, M.N. 2001. Genetics of broodiness in poultry a review. *Asian Australasian Journal of Animal Sciences*, **14**: 1647-1654.

Romanov, M.N., Talbot, R.T., Wilson, P.W. and Sharp, P.J. 2002. Genetic control of incubation behaviour in the domestic hen. *Poultry Science*, **81**(7): 928-931.

Sambrook, J. and Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual" 3rd Ed. Cold Spring Harbor Laboratory Press, New York.

Sharp, P. 1997. Immunological control of broodiness. *World's Poultry Science Journal*, **53**(1): 23-31.

Wolc, A., Jankowski, T., Arango, J., Settar, P., Fulton, J.E., O'Sullivan, N.P. and Dekkers, J.C.M. 2018. Investigating the genetic determination of clutch traits in laying hens. *Poultry Science*, **98**(1): 39-45.

Xu, H.P., Shen, X., Zhou, M., Luo, C.L., Kang, L., Liang, Y. and Zhang, X.Q. X.Q. 2010. The dopamine D2 receptor gene polymorphisms associated with chicken broodiness. *Poultry Science*, **89**(3): 428-438.

Yang, N. and Jiang, R.S. 2005. Recent advances in breeding for quality chickens. *World's Poultry Science Journal*, **61**: 373–381.