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Genetic evaluation of Gir bulls under associated herd progeny testing programme

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Gir is one of the important cattle breeds of India known for its comparatively higher milk production, better resistance to most of the tropical diseases, and ability to survive under poor nutrition and extreme climatic conditions prevalent in its native tract (Gaur *et al.* 2003). Considering the importance of indigenous cattle breeds, efforts are being taken by ICAR for their genetic improvement through All India Coordinated Research Project (AICRP) on Cattle. Gir breed of cattle is one among the 3 important cattle breeds included under the Indigenous Breeds Project (CIRC Annual Report 2016–17). The project is coordinated by ICAR-CIRC, Meerut in collaboration with the Junagadh Agricultural University, Junagadh, Gujarat. Under this programme, Cattle Breeding Farm, Junagadh has been identified as bull mother or germplasm unit from which the young bulls born to elite females are identified as bulls for progeny testing. The farmer's herds, *Gaushalas* and herds maintained by NGOs are utilized as data recording units where the semen of young bulls is used for insemination of Gir animals.

Evaluation of breeding bulls is highly essential for selection and dissemination of genetically superior germplasm to improve the milk production. Small herd size, lack of large commercial cattle herds and lack of proper recording system are the major limiting factors preventing the evaluation of breeding bulls extensively. These difficulties are overcome by the concept of associated herd progeny testing programme under which the bulls are evaluated utilizing large number of farmers' herds as field units so as to get enough number of daughter records for testing. Since no scientific studies have been reported so far on the genetic evaluation of Gir bulls through associated herd progeny testing programme, the present study was conducted to predict the expected breeding value (EBV) of the bulls by best linear unbiased prediction method utilizing the first lactation 305-days milk records of daughters.

For the study, information on 222 Gir cows born to six sires of first set, calved during 2013–17 and maintained under different data recording units of the project were utilized. The basic information on pedigree of Gir animals was collected. Fortnightly milk records collected at 15 days interval were utilized to predict the first lactation 305-days milk yield. Abnormal records, viz. abortion, stillbirth, mastitis etc. were excluded from the study. Information on animals having incomplete lactation records due to early disposal was also excluded. Finally, animals having lactation length more than 100 days and milk yield above 800 kg were included in the analysis.

The home tract of the Gir breed is the Gir hills and Kathiawar forest and presently the Gir cattle population is concentrated in Junagadh, Porbander, Amreli and Bhavnagar districts of Gujarat. The home tract of Gir breed lies between 20°5' and 22°6' north latitude and 70° and 72° east latitude (Gaur *et al.* 2003). The breeding tract lies about 400 meters above MSL with a range of 125–600 meters and enjoys the typical tropical climatic conditions. Based on agro-climatic conditions of the region, the whole year was divided into three seasons, viz. summer (March–June), monsoon (July–October) and winter (November–February) as reported by Dangar and Vataliya (2014). The information on centre, year and season of calving were utilized to generate the herd-year-season (HYS) effect which was considered as fixed effect while the effect of sire was considered as random. For sire evaluation, bulls having 5 and more daughters were only included. The expected breeding values (EBV) of bulls were estimated by best linear unbiased prediction (BLUP) method as given by Henderson (1973, 1975) using the LSMLMW Model VIII (Harvey 1990).

The general model of BLUP estimation was considered as follows:

$$Y_{ijk} = Xh_i + Zs_j + e_{ijk}$$

where Y_{ijk}, observation vector of trait with dimension (n× 1); X, design matrix or incidence matrix for fixed effects with dimension (n × p); h_i, vector for fixed effect of dimension (p × 1); Z, design matrix or incidence matrix for random effects with dimension (n × q); s_j, vector of random effect with mean zero and variance $G\sigma_s^2$ with dimension (q × 1); e_{ijk}, random error vector with dimension (n × 1) with mean zero and variance I σ_e^2.

The descriptive statistics for first lactation 305-days milk yield of Gir cattle was calculated. The first lactation 305-

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days milk yield of Gir cattle ranged between 865–5148 kg with an overall average of 2572.18 kg. The coefficient of variation was 23.76%.

The EBVs of Gir bulls were estimated by BLUP method using the LSMLMW package of Harvey and the results are presented in Table 1. The average number of daughters/bull ranged between 5 (for Bhola) to 59 (for Bhavik). The average number of daughters/sire was estimated as 29.89. The variation in the number of daughters/sire was due to the loss of daughters data. Since the bulls were evaluated on the basis of daughters born in associated herds covering the farmer herds the loss of data due to disposal of animals before completion of first lactation could not be avoided.

Table 1. Expected breeding values (EBVs) of first set Gir bulls

Bull	No. of daughters	FL305 DMY (kg)	EBV (% of genetic superiority over population)	Rank
Overall	222	2563.793		
Bhavik	59	2570.081	+ 6.288 (+0.24)	2
Bhola	05	2539.085	–24.708 (–0.96)	4
Murari	48	2541.141	–22.652 (–0.88)	3
Pankaj	51	2714.503	+150.710 (+5.87)	1
Raj	18	2525.286	–38.507 (–1.50)	5
Rupak	41	2492.661	–71.132 (–2.77)	6

The overall average breeding value of the Gir bulls for first lactation 305-days milk yield was estimated as 2563.79 kg which was comparatively higher than the breeding values reported for Sahiwal cattle by Banik and Gandhi (2006), Raja (2010) and Dongre and Gandhi (2014). The estimate was also higher than the EBV of 2137.17 kg reported by Pandey *et al.* (2013) in Vrindavani cattle. The breeding values of Hariana and Ongole bulls of Indigenous cattle were estimated and also reported by Singh *et al.* (2006, 2008 and 2012).

The EBVs of Gir bulls ranged between –71.132 kg for Rupak to +150.710 kg for Pankaj. The wider variation in EBVs indicate that the BLUP method discriminates the bulls to a larger extent so that this method can be used for selection of high genetic merit bulls for improving the milk yield in Gir cattle. Among the 6 bulls, 2 bulls (33.33%) had breeding values above the overall average while rest 4 bulls (66.67%) had breeding values lower than the overall average. Contrary to the present finding, Pandey *et al.* (2013) reported that 50% of sires had breeding values equal and above the overall average breeding value in Vrindavani cattle and this variation might be due to the variations in number of breeding bulls included for evaluation.

Based on the EBV estimates and sire rankings, use of frozen semen doses of two Gir bulls, viz. Pankaj and Bhavik is recommended to breed the Gir cows for increasing the milk production in subsequent generations.

SUMMARY

A study was conducted to predict the expected breeding values of 6 Gir bulls inducted under the All India Coordinated Research Project on Cattle. A total of 222 first lactation 305 days records of Gir daughters born between 2013 and 2017 were analyzed by Best Linear Unbiased Prediction (BLUP) method using Model VIII of LSMLMW software. The BLUP model included the herd-year-season effect as fixed factor and sires as random factor. The overall average expected breeding value (EBV) was 2563.79 kg. The breeding values of Gir sires ranged between –71.13 to +150.71 kg. The results of the study revealed that the BLUP method discriminated the sires for their breeding values to a larger extent so that the genetically superior bulls can be discriminated from the poor bulls. Based on the results, use of frozen semen doses of two Gir bulls, viz. Pankaj and Bhavik is recommended to breed the Gir cows for increasing the milk production in subsequent generations.

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Polymorphism of prolactin (*PRL*) gene in indigenous ducks of Asom

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Genetic improvement of farm animals, including poultry, is generally aimed at maximizing economic performance and production potential. Identification and utilization of potential candidate genes with significant effects on economically important traits have become increasingly important in poultry breeding programmes.

Prolactin (*PRL*) is a single-chain polypeptide hormone that belongs to the growth hormone family and is synthesized mainly in the anterior pituitary of all vertebrates. Prolactin (*PRL*) gene has significant effect on egg production (Reddy *et al*. 2002, Wang *et al.* 2011). In birds, *PRL* inhibits the growth and development of ovarian follicles (Chen *et al.* 2011). *PRL*-encoding gene is found on chromosome 2 in birds (Miao *et al.* 1999, Alipanah *et al.* 2011). *PRL* gene in duck is 10 kb in size and is composed of 5 exons and 4 introns, encoding 229 amino acids. The onset of incubation behaviour (broodiness) of poultry is induced by an increase in *PRL* secretion (Ishida *et al.* 1991, Shimada *et al.* 1991, Wong *et al.* 1991, Talbot and Sharp 1994). Elevated levels of *PRL* decrease the egg sequence lengths (clutch length) by increasing the inter-sequence pauses between the sequences of egg laying. This is particularly pronounced in native birds (Reddy *et al.* 2002, 2006).

Duck population constitutes only 3% of the total poultry population in India and the duck population has decreased by 15% from 2007 to 2012 (Livestock Census 2012). Indigenous ducks of Asom are reared by farmers under traditional systems. This duck population is most common in the Brahmaputra valley. These ducks have innate potential to produce eggs and meat with less input, and are good sources of protein. As an important poultry species, duck egg has become an important source of protein in human diet, but the egg performance of some native duck breeds needs to be improved.

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However, sufficient attention has not been paid towards the improvement of these native ducks by the scientific community. So far, very few systematic studies on these ducks have been carried out. Keeping all these facts in view, this study was undertaken to study occurrence of polymorphism in sequences of *PRL* gene and to analyze the sequences for determining gene and genotypic frequencies in indigenous ducks of Asom.

The study was conducted on indigenous ducks from different districts of Asom, viz. Dhubri, Bongaigaon, Barpeta, Nalbari, Kamrup, Morigaon, Nagaon, Karbi Anglong, Sivasagar. A total of 101 indigenous ducks randomly chosen were used for the study.

Isolation of genomic DNA: Blood (1–2 ml) from each bird was aseptically collected from the wing vein in vacutainer tube containing EDTA (2.7%) as anticoagulant. Genomic DNA was isolated from 20 µl of whole blood using DNeasy blood and tissue kit (QIAGEN).

Gene amplification by polymerase chain reaction: A pair of specific forward (F: TCCCTGTCTTCTTAACAG-TAGG) and reverse (R: GAGGGAAGCACGGTCATTCG) primers was designed using sequence template downloaded from NCBI (Accession no. DQ345782) Polymerase chain reaction was performed to amplify the 457 bp fragment of *PRL* gene.

The PCR reactions were carried out in 25 µl reaction mixture containing 1.0 µl DNA template, 0.5 µl each of forward and reverse primers of concentration 10 pmol/µl, 12.5 µl Master Mix (Dream taq, 2×) (Thermo Scientific) and 10.5 µl nuclease free water. The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler under the following condition: initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 45 sec, annealing at 50°C for 45 sec, extension at 72°C for 60 sec with a final extension step at 72°C for 10 min.

Polymorphism detection by PCR-RFLP: PCR product (4 µl) was digested with 1 µl of *DraI* enzyme in 1× REs buffer followed by incubation at 37°C for 12 h. The digested products were analyzed by 12% neutral polyacrylamide gel electrophoresis (PAGE) and the gel was visualized in gel documentation system. The amplified PCR products were sequenced by outsourcing (First Base, Malaysia) using both

forward and reverse primers for 15 samples (5 for each genotype, viz. AA, AB and BB). The sequences obtained were analysed by NCBI-BLAST.

Sequencing and alignment: The identity of the sequences were confirmed by using NCBI-BLAST tool and the results of sequencing were analysed by using BioEdit.

Genotype and allele frequencies: Gene and genotypic frequencies were calculated as described by Falconer and Mackay (1996). Chi-square (χ^2) test was performed to test if the population was in Hardy-Weinberg equilibrium.

The yield and purity of extracted DNA samples were estimated using UV Spectrophotometer (Nanodrop Spectrophotometer, Model-UV/VIS 916). The OD at 260 nm and 280 nm were measured and used for estimating purity and concentration of DNA samples. The yield of DNA extracted from 20 µl of whole blood ranged from 361.2 ng/µl to 502.0 ng/µl with a mean of 431.6 ng/µl. The OD ratio was in the range of 1.7 to 1.9 indicating purity of the extracted DNA. After quantification of each DNA sample, a uniform final concentration of 100 ng/µl was prepared by further dilution of the entire sample in 2.7% Tris EDTA (TE) buffer. Agarose gel electrophoresis (1%) of the isolated DNA yielded distinct bands with no smearing. By PCR amplification, a 457 bp amplified product was obtained in all samples from indigenous ducks of Asom. The PCR-RFLP studies on *PRL* gene in indigenous ducks of Asom using *DraI* revealed three types of fragment patterns, arbitrarily designated as AA, AB and BB genotype. Following digestion of the PCR products, AA genotype yielded two fragments (141 and 316 bp), AB genotype yielded three fragments (141, 316 and 457 bp), and BB genotype yielded one fragment (457 bp). This finding was in conformity with earlier observations in Gaoyou ducks (Li *et al.* 2009). They also observed three genotypes, viz. AA, AB and BB of the *PRL* gene in their study. Three alleles (designated as A, B and C) and 5 genotypes of the *PRL* gene has been observed in 4 strains of White Leghorn chickens, viz. IWH, IWI, IWK and layer control (Bhattacharya *et al*. 2011).

A nucleotide transition (T→G) was observed at position 110 bp. In the population studied, 3 groups of ducks exhibiting polymorphism of *PRL* gene could be distinguished (AA, AB and BB). Notably, there were greater number of AA homozygotes in comparison to BB homozygotes. The frequencies of AA, AB and BB genotypes were 0.812, 0.069 and 0.119, respectively and the frequencies of 'A' and 'B' alleles were 0.847 and 0.154, respectively (Table 1). Gene frequencies showed that the *PRL* 'A' variant is predominant in indigenous ducks of Asom.

Table 1. Genotype and gene frequencies for *PRL* genes

Loci	Genotype frequency			Gene frequency	
	AA	AB	BB	A	B
PRL	0.812 (82)	0.069 (7)	0.119 (12)	0.847	0.154

The Chi-square (χ^2) test revealed that the calculated value for *PRL* gene (54.188) was higher than the tabulated value at 1% level of significance with 2 degrees of freedom (Table 2). Hence the population under study was not in Hardy-Weinberg equilibrium for this gene.

Table 2. Chi-square test for Hardy-Weinberg equilibrium in *PRL* genotypes

Loci	Genotype	Observed	Expected	$(O-E)^2/E$	χ^2
PRL	AA	82	72.387	1.277	54.188**
	AB	7	26.249	14.117	
	BB	12	2.384	38.796	

Polymorphism of *PRL* gene was also observed among White Leghorns, Hy-Line brown egg layers, Avian broilers, and Chinese local breeds of chicken including Shou-guang, Beijing-you and Silkie (Jiang *et al.* 2005); muscovies and Baigai ducks of China (Wang *et al.* 2009); native fowl of Yazd province (Begli *et al.* 2010); Gaoyou, Jinding, Liancheng White, Putian Black, Beijing, Shaoxing, Youxian Sheldrake and Jianchang ducks of China (Wei-Tao *et al.* 2010); White Leghorn chickens (Bhattacharya *et al.* 2011); Chinese native duck breeds (Wang *et al.* 2011); indigenous chickens of Mazandaran province (Rashidi *et al.* 2012); West-Azarbaijan Native chicken (Abdi *et al.* 2014); Poltava clay chicken (Kulibala 2015) and Khaki Campbell ducks (Chauckwon and Boonlum 2016).

However, all duck samples of Pekin, Mojosari, and Pekin × Mojosari crossbreds had been reported to be homozygous and monomorphic (Irma *et al.* 2014).

In the sequence of *PRL* gene, one *DraI* restriction site was detected at position 141 yielding two fragments of 141 bp and 316 bp in 'A' allele of the *PRL* gene. Whereas, the other allele 'B' of the same gene was not having any *DraI* restriction site because of T→G mutation, which was confirmed by the non-digestion of the product.

Gene and genotypic frequencies were calculated as per the method described by Falconer and Mackay (1996). The frequencies of A and B alleles of *PRL* gene were 0.847 and 0.154; those of AA, AB and BB genotypes were 0.812, 0.069 and 0.119, respectively. The present findings revealed a higher frequency of AA genotype followed by BB and AB for *PRL* gene. Frequency of AA genotype was the highest. These data were in conformity with those of Chauckwon and Boonlum (2016), who identified three genotypes namely GG, GT and TT, coded by two different alleles G and T in *PRL* gene in Khaki Campbell ducks, where the frequencies of genotype GG and allele G were found to be the highest (0.56 and 0.74, respectively).

Chi-square (χ^2) test revealed that the calculated value for *PRL* gene was more than the tabulated value at 1% level of significance with 2 degrees of freedom. Hence, the population under study deviated from Hardy-Weinberg Equilibrium for *PRL* gene and it was influenced by selection. The finding was in consistency with those determined in Mazandaran native fowls (Rashidi *et al.*

2012), Turkey (Fathi *et al.* 2014), and in Muscovy, Pekin and Mulard ducks (Mazurowski *et al.* 2016).

SUMMARY

The present study was conducted to investigate the polymorphism of prolactin (*PRL*) gene in 101 indigenous ducks of Asom. PCR-RFLP analysis of *PRL* gene using restriction enzyme *DraI* revealed three genotypes, arbitrarily designated as AA, AB and BB. Following digestion, the AA genotype yielded two fragments (141 and 316 bp), AB genotype yielded three fragments (141, 316 and 457 bp), and BB genotype yielded one fragment (457 bp). The frequencies of A and B alleles were 0.847 and 0.154 respectively and the genotypic frequencies for AA, AB and BB genotypes were 0.812, 0.069 and 0.119, respectively. It was found that the A variant of *PRL* gene was predominant in the indigenous ducks of Asom with the highest frequency of AA genotype followed by BB genotype (AA>BB>AB). Chi-square (χ^2) test revealed that the population under study was not in Hardy-Weinberg equilibrium for *PRL* gene.

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