RESEARCH ARTICLE

Prolactin Receptor Gene Exon 9 Polymorphism in Surti and Jaffarabadi Buffaloes

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Abstract

Molecular genetic analysis of quantitative traits leads to a better understanding of genes governing milk yield and the composition of traits. Prolactin (PRL) acts through its receptor PRLR via both endocrine and local paracrine/autocrine pathways to regulate biological processes including production and reproduction. The present study was undertaken with the objective to amplify Prolactin Receptor (PRLR) gene exon nine regions using bovine specific primers using Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Jaffarabadi buffaloes. A total of 50 Surti and 50 Jaffarabadi buffaloes were screened for polymorphism in 582 bp exon 9 of PRLR gene and observed three genotypes AA (582 bp), heterozygous AG (582,399,183 bp) and GG (399, 183 bp) in Surti buffaloes with genotypic frequencies of 0.48, 0.44 and 0.08 for AA, AG and GG genotypes and gene frequencies for A and G alleles were 0.70 and 0.30, respectively. The Jaffarabadi buffaloes PCR-RFLP pattern revealed two genotypes with genotypic frequencies 0.44 and 0.56 for AA and AG genotypes with absence of GG genotype and gene frequencies for A and G 0.72 and 0.28, respectively. Association study of PRLR/DraIII genotypes with milk production traits gave non-significant results.

Keywords: Jaffarabadi, Polymorphism, Prolactin receptor, Surti.

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Introduction

India has a very rich reservoir of genetic diversity and possesses some of the best breeds of cattle and buffaloes in the world. Buffalo milk has relatively lower cholesterol, more calories and fat, and can be well-preserved naturally for a longer time compared to cow's milk. Buffalo milk produces thick and creamy dairy products suitable for the manufacture of traditional milk products (Anonymous, 2020; Kumaraswamy, 2020). In India, buffaloes are preferred over cattle as a dairy animal and are called "The black gold of India" because of high milk fat content, fetching higher market price (Vignal *et al.,* 2002).

Prolactin (PRL), secreted from the anterior pituitary gland, plays an important regulatory function in mammary gland development, maintenance of milk secretion, synthesis of milk and milk components (Hayes *et al*., 1996). Prolactin (PRL) acts through its receptor, Prolactin Receptor gene (PRLR), which belongs to the hematopoietic receptor superfamily (Kossiakoff *et al*., 1994). The PRLR has been detected in various tissues including ovary, placenta and uterus in several mammalian species. In buffalo, PRLR is mapped on chromosome 19 (Vitala *et al*., 2006; Amaral *et al*., 2008). PRLR play crucial role in signal transduction from lactogenic hormones to milk protein gene promoter (Gao *et al.,* 1996; Bole-Feysot *et al*., 1998). PRLR is a strong candidate gene that plays an important role in the milk production and reproduction (Parihar *et al.,* 2017).

Gujarat has four recognized breeds of buffalo, *viz.* Surti, Jaffarabadi, Mehsani and Banni. Surti and Jaffarabadi breeds of buffalo are premier dairy animals of Gujarat and India. Both are the best milch purpose breeds of India and are divergent in relation to body size and fat percent (Nivsarkar *et al.,* 2000). A study of PRLR gene polymorphism and its effects on milk production traits explore the possibilities of PRLR gene being used as a candidate marker gene for milk production traits (Al-Samarai and Al-Kazaz 2015; Athe *et al*., 2018).

Several techniques can identify genetic polymorphism. Several single nucleotide polymorphisms (SNPs) studies in

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Table 1: Primers and restriction enzyme of PRLR gene

SNP	*Primer sequence of PRLR gene*	*Region and PCR product size*	*Restriction Enzyme*	*Annealing temperature*
A>G	F-5'CAACATTGCTGACGTTGTGTG3' R- 5'CAATTGAACCCAT CCTTCCA3'	Exon-9 Size 582 bp	DraIII	60℃

Table 2: PCR protocol of PRLR gene

Step	*Temperature (°C)*	*Time (min)*
Initial denaturation	94	5
Denaturation	94	1
Annealing	60	1
Extension	72	1
Repeat step 1 to 3 for 35 cycles		
Final extension	72	10

Table 3: Protocol of restriction enzymatic digestion DraIII on PCR products

Restriction Enzyme	*Incubation*		*Inactivation*	
DraIII	37°C	05 min	85°C	20 min.

PRLR gene have been reported in different breeds, including Polish Black-white cow and Jersey cow (Brym *et al*., 2005), *Bos taurus* (Amaral *et al*., 2008), Chinese Holstein cow (Aijun and Xiucai, 2010), Murrah river buffalo (Javed *et al*., 2011[a]), Murrah and Mehsana (Javed *et al*., 2011[b]), Indigenous Grey cattle breeds of India (Deepika and Salar, 2014), Reggiana cattle (Scotti *et al.,* 2016), Murrah, Nili-Ravi, crossbred buffaloes and Holstein cows (Abakar *et al.,* 2018) and Italian Mediterranean river buffaloes (Cosenza *et al.,* 2018). It is a powerful method for identifying nucleotide sequence variation in amplified DNA (Zhang *et al*., 2008). However, there is no report available in Surti and Jaffarabadi buffalo in India. Keeping this in view, the present study was undertaken to detect the PRLR polymorphism using PCR-RFLP assay and its association with milk production traits in Surti and Jaffarabadi buffalo breeds.

Materials and Methods

Animals and Genomic DNA Isolation

Samples were collected from a total of 100 healthy adult female of Surti (n=50) buffalo breed maintained at Livestock Research Station under Navsari Agricultural University, Navsari and adult female of Jaffarabadi (n = 50) buffalo breed maintained at Patel Dairy Farm, Navsari.

About 5 mL of the blood was collected from each animal from the jugular vein of buffalo in 10 ml of sterile vacutainer tube containing 0.5M EDTA (pH 8.0) as anti-coagulant. Genomic DNA was isolated from whole blood samples using the standard phenol-chloroform extraction method in the laboratory (Sigma-Alderich and Himedia Ltd.) by John *et al*. (1991). The DNA concentration and purity was checked by using a Nanodrop spectrophotometer (ND-2000c) at optical density (OD) 260 nm and 280 nm. The quality of DNA was checked by running a genomic DNA sample with 0.8% agarose gel electrophoresis, and the gel was visualized under UV transilluminator after staining with ethidium bromide (EtBr).

Polymerase Chain Reaction (PCR)

The specific oligonucleotide primers for the buffalo PRLR gene were synthesized and supplied by Eurofins Genomics. A>G SNPs containing regions of PRLR gene fragments had been amplified from isolated DNA using primer pairs described (Table 1) as per Parihar *et al*. (2017).

PCR reaction was carried out in total volume of 25 µl that included 12.5 µl of 2X master mix (by Takara Ltd. containing 1.5 mM Mgcl2, 0.2 mM of each dNTP and 0.5 U Taq DNA polymerase), 3 µl of genomic DNA (90 ng), 1 µl of each forward and reverse primer (10 pmole) and and 7.5 µl of nuclease free water. PCR of PRLR gene was performed as per protocol mentioned in Table 2.

Restriction Fragment Length Polymorphism (RFLP)

The restriction enzyme DraIII used in the present study was supplied by Thermofisher. Restriction digestion of the amplified PCR products were carried out in a total reaction volume of 20 µL containing 10 µL PCR products, 2 µL 10X RE buffer, 7 µL nuclease-free water and 1 µL restriction enzyme. Incubation and inactivation protocol of PCR products for restriction enzymatic digestion DraIII is presented in Table 3.

For restriction fragment analysis, digested products were checked on 2.0% agarose gel in 0.5 X TBE buffer for 90 minutes at 5 V/cm and visualized under UV light.

Statistical Analysis and Association Study

The gene and genotypic frequencies of different PRLR genotypes were estimated by standard procedure (Falconer and Mackay, 1996). The chi-square (χ^2) test ($p < 0.05$) was performed to test whether the distribution of the gene and genotypic frequencies were in the Hardy-Weinberg equilibrium.

The association study of PRLR genotypes with the milk production traits in Surti and Jaffarabadi buffaloes included Total milk yield (TMY), Standard milk yield in 300 days (MY300), and Peak milk yield (PMY). Statistical analysis of milk production traits in relation to PRLR genotypes was carried out using the General Linear Model (GLM) using 'R' software version 4.0.3. The following linear model was applied:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where Y_{ij}– observed trait value in an animal; µ–mean trait value; G_i – effect of genotype; eij-random error. Significant differences among least-square means of different genotypes were calculated using Duncan's multiple range test.

Results and Discussion

The amplified fragments of the PRLR gene exon nine region revealed about 582 bp PCR product in both breeds (Plates 1 and 2). In the present study, the PRLR/DraIII PCR-RFLP assay

revealed three fragments (Genotypes) one of them was AA genotype (582 bp); second of AG genotype (582, 399, 183 bp), and third of GG genotype (399, 183 bp) in Surti buffalo breed (Plate 3). Jaffarabadi buffalo breed PCR-RFLP pattern revealed two genotypes AA (582bp); AG (582,399,183bp), while GG genotype was not observed (Plate 4).

The genotypic frequencies for AA (0.48), AG (0.44), GG (0.08) and gene frequency for A allele (0.70) and for G allele (0.30) observed in Surti buffaloes, and the genotypic frequencies for AA (0.44), AG (0.56) and gene frequencies for A (0.72) and G (0.28) observed in Jaffarabadi buffalo are presented in Table 4. Genotype GG was absent in Jaffarabadi buffaloes. In the present study, higher heterozygous genotypic frequency was observed in Jaffarabadi buffaloes as compared to Surti buffaloes.

Chi-square test revealed that χ^2cal (0.107) in Surti buffalo and χ^2 cal (3.53) in Jaffarabadi buffalo was < χ^2 tab (3.84) at 5% level of significance for 2 degrees of freedom, indicating

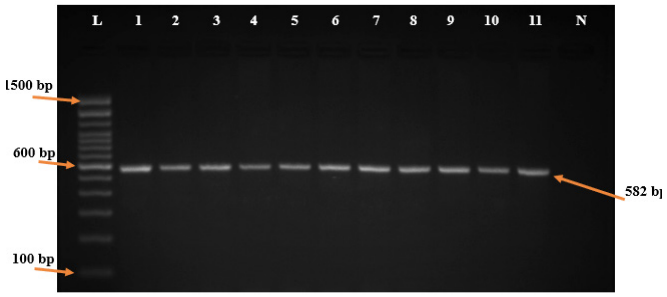

Plate 1: PCR products of Exon 9 PRLR 582 bp fragments of Surti buffaloes
Lane: L-100 bp Ladder; 1 to 11 -582 bp PCR products, N-Negative control

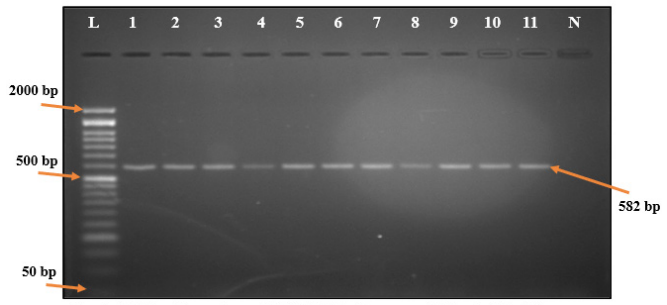

Plate 2: PCR products of Exon 9 PRLR 582 bp fragments of Jaffarabadi buffoloes
Lane: L-50 bp Ladder; 1 to 11 -582 bp PCR products, N-Negative control

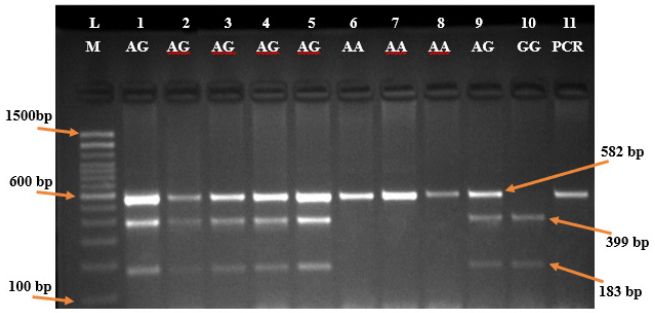

Plate 3: Restriction patterns of Exon 9 PRLR 582 bp fragments of Surti buffaloes
Lane: L-100 bp Ladder; 1 to 10-582 RFLP products, L 11- PCR product

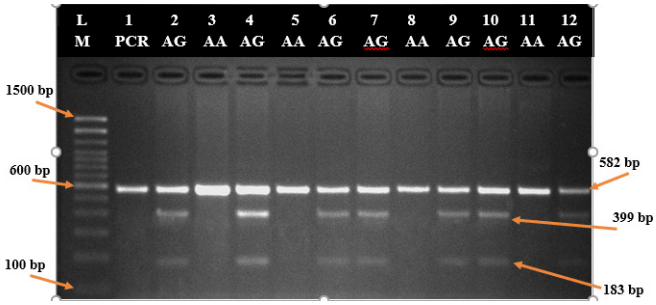

Plate 4: Restriction patterns of Exon 9 PRLR 582 bp fragments of Jaffarabadi buffoloes on digestion with DraIII.
Lane: L-100 bp Ladder; L1- PCR product, 2 to 12-582 RFLP products

Table 4: Genotypic and gene frequencies for PRLR locus in Surti and Jaffarabadi buffalo

Breed	*Observed no. of genotypes*			*Genotypic Frequency*			*Gene Frequency*		*Expected no. of genotype*		
Surti (n=50)	AA	AG	GG	AA	AG	GG	A	G	AA	AG	GG
	24	22	04	0.48	0.44	0.08	0.7	0.3	24.5	21	4.5
Jaffarabadi (n=50)	22	28	00	0.44	0.56	0.00	0.72	0.28	25.92	20.18	4.00

Table 5: Association study of PRLR/DraIII genotypes with milk production traits in Surti and Jaffarabadi buffalo

Breed	*Genotype*	*Total lactation yield*	*Standard lactation yield*	*Peak milk yield*
Surti (n=50)	AA	1213.08 ± 58.53	1150.42 ± 63.31	25.55 ± 1.41
	AG	1170.53 ± 64.71	1128.47 ± 64.72	24.55 ± 1.45
	GG	1112.2 ± 71.35	1064.4 ± 73.13	25.06 ± 3.3
	P Value	0.7295	0.8356	0.8906
Jaffarabadi (n=50)	AA	1031.77 ± 27.25	985 ± 27.55	37.57 ± 1.02
	AG	1035.98 ± 25.6	993.96 ± 25.69	39.01 ± 0.62
	P Value	0.9115	0.814	0.2133

*Significant at p ≤0.05, **highly significant at p ≤0.01, n = Number of observations

that screened breeds of buffalo population was in Hardy-Weinberg equilibrium for locus exon 9 of PRLR gene.

In accordance with the present study, PCR product of similar size had also been reported in breeds of cattle and buffalo like Sahiwal and Hariana cattle (Parihar *et al.,*2017) and in Italian Mediterranean river buffaloes (Cosenza *et al*., 2018) by amplification of this region of the PRLR gene.

The present RFLP pattern for exon 9 region of PRLR gene with three genotypes AA, AG and GG is in agreement with the findings of Javed *et al.* (2011[b]) in Indian buffalo as AA (65%), AG (30.0%) and GG (3.9%) and Parihar *et al.* (2017) in Sahiwal and Hariana cattle which showed the genotype frequencies 0.16; 0.72; 0.12 and allelic frequencies for A and G as 0.52 0.48, respectively, in Sahiwal cattle, however in Hariana cattle genotypic frequencies 0.18; 0.62; 0.20 and allelic frequencies 0.49 and 0.51, respectively, were noted.

In association study of PRLR/DraIII polymorphism with milk production traits, there was a non-significant difference among three genotypes for Total milk yield, Standard milk yield and Peak milk yield in both Surti and Jaffrabadi buffalo breeds. The means and standard errors for each trait related to PRLR/DraIII genotype are presented in Table 5.

In accordance with the present study, there was no significant difference observed for the milk production and reproduction traits, *i.e.,* Lactation period, Total milk yield, Milk yield in 300 days, Dry period, Calving interval, Peak yield, and Days to reach peak yield among all the PRLR/DraIII genotypes in Sahiwal and Hariana cattle for exon 9 PRLR gene polymorphism by Parihar *et al.* (2017). Javed *et al.* (2011[b]) investigated this polymorphism in Indian buffalo, but an association study was not done with milk production traits.

Conclusions

In the current study, PRLR exon nine regions was found to be polymorphic in both Surti and Jaffarabadi buffalo breeds. In Surti buffalo, the frequency of genotype AA, AG and GG were 0.48, 0.44 and 0.08 The gene frequency of A and G observed was 0.7 and 0.3, respectively. In Jaffarabadi buffalo population, the frequency of genotypes AA and AG was 0.44 and 0.56, respectively, with GG genotype. The gene frequency of A and G observed were 0.72 and 0.28, respectively. Polymorphism in PRLR exon nine regions in both breeds using PCR-RFLP indicates that the molecular method used is an appropriate tool for detecting genetic polymorphism. The present study was the first report of PRLR exon nine polymorphism and their association with milk production traits, however, there were non-significant associations of PRLR exon nine polymorphism with milk production traits in Surti and Jaffarabadi buffalo breeds.

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