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Prolactin gene polymorphism and its association with milk production in Malvi, Nimari and Frieswal cattle

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ABSTRACT

The prolactin (PRL) *Rsa* I genotypes were analysed using the PCR-RFLP method. Three different RFLP patterns were obtained at 2 restriction sites corresponding to AA, AB and BB genotypes. Genotypic frequencies among all the 3 breeds for AA genotype ranged from 0.000 to 0.315, for AB genotype from 0.629 to 0.900 and for BB genotype from 0.000 to 0.100 while allelic frequencies for A allele ranged from 0.450 to 0.630 and B allele from 0.370 to 0.550 among 3 breeds of cattle. The significant chi-square value for all the genotypes in Malvi, Nimari and Frieswal cattle breeds showed that the population was not in Hardy-Weinberg equilibrium. It is concluded from the results of this study that the animals of AB genotype for higher lactation length and lactation yield and animals of AA genotype for minimum service period may be selected for future breeding.

Key words: Lactation length, lactation yield, PCR-RFLP, Prolactin (PRL), Polymorphism, Service period

Prolactin (PRL) is one of the most versatile hormones of the pituitary gland in terms of biological functions. It is the best known hormone for the multiple effects it exerts on the mammary gland. The varied effects of PRL on the mammary gland include growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis) and maintenance of milk secretion (galactopoiesis). Hence, PRL gene is a potential quantitative trait locus and genetic marker of production traits in dairy cattle (Brym *et al.* 2005).

Bovine PRL gene has been located on chromosome 23 and is composed of 5 exons and 4 introns with an overall length of approximately 10 kb encoding the 199 amino-acid mature protein (Camper *et al.* 1984). A silent A→G transition mutation at the codon for amino acid 103 in exon-3 of size 156bp bovine PRL gene gives rise to a polymorphic *Rsa* I (*Rhodopseudomonas sphaeroides*). This site has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP analysis (Chrenek *et al.* 1998, Dybus 2005). Many workers have reported that PRL gene is highly polymorphic and has been found associated with milk production (Alipanah *et al.* 2008, Maksymiec *et al.* 2008 and Ghasemi *et al.* 2009).

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MATERIALS AND METHODS

Resource populations and DNA isolation: The present study comprised of 50 blood samples of Malvi lactating cows, 32 of Nimari lactating cows and 54 of Frieswal lactating cows collected from Government cattle breeding farm, Agar Malwa (Distt. Shajapur), Government cattle breeding farm, Rodia (Distt. Khargone) and military dairy farm, Jabalpur, respectively along with their lactation length, lactation yield and service period records.

Genomic DNA was extracted from 5 ml venous blood sample by the method of John *et al.* (1991). Purity and concentration of genomic DNA was determined by using Nano-drop spectrophotometer. Genomic DNA quality was assessed by using 0.8% horizontal submarine agarose electrophoresis. PCR products were amplified using Oligonucleotide primers specific to bovine PRL gene locus were custom synthesized in accordance with Lewin *et al.* (1992) primer sequences: forward 52 -CGAGTCCTTATGAGCTTGATTCTT-32) and reverse (52 -GCCTTCCAGAAGTCGTTTGTTTTC-32) primers. Polymerase chain reaction was carried out in a final reaction volume of 25µl. PCR reaction mixture used for amplification of DNA was 2X PCR master mix 12.5 µl, forward primer (10 pmol/µl) 1.0 µl, reverse primer (10 pmol/µl) 1.0 µl, genomic DNA 3.0 µl and DNAase free water 7.5 µl. Amplification was performed in PCR thermal cycler programmed for 32 cycles with an initial denaturation at 95°C

for 10 min, denaturation at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min with a final extension at 72°C for 10 min. Total 5 µl of amplified PCR product of each sample was mixed with 1 µl of 6× gel loading dye buffer from each tube. The samples were loaded on 2% agarose gel containing ethidium bromide (1% solution @ 5µl/100 ml) along with 80 bp DNA ladder at a constant voltage of 80 V for 30 min. in 0.5 × TBE buffer.

Amplified DNA was digested with the *Rsa* I enzyme. Restriction digestion of the PCR product was performed in a total 30 µl. Content having 10×buffer tango 2 µl, PCR reaction mixture 10 µl, restriction enzyme (10units/µl) 1 µl and 17 µl nuclease free water. The reaction mixture was spinned for few seconds for uniform mixing and then incubated at 37°C for 4 hrs and 30 min in the water bath.

After restriction digestion, the restriction product mixtures were electrophoresed on 3.5% agarose gel containing 1% ethidium bromide @ 5 µl/100 ml at constant voltage of 80 V for 80 min using 0.5 × TBE buffer. Gel loading dye (6 ×) was used to load the digested PCR samples. 100 bp DNA ladder (Range 100–1000 bp) was used as a molecular size marker. The DNA PCR-RFLP bands were visualized under UV light and documented by gel documentation system. The band size was judged by comparing with molecular size marker and recorded. Genotyping of PRL gene locus was carried out according to the band pattern of respective genotypes.

The frequency of PRL gene allele A and B and genotype in each breed were estimated using the POPGENE software package (Yeh *et. al.* 1999) Frequencies of distribution of alleles within the herds were compared using the Chi-square test and the association of various polymorphic variants of bovine PRL gene with economic traits were analyzed by mixed model least squares and maximum likelihood computer program PC-2 (Harvey 1990).

$$Y_{ijk} = \mu + a_i + B_j + e_{ijk}$$

where, Y_{ijk} is the milk production of k^{th} cow of the j^{th} breed for i^{th} genotype, µ is overall mean; a_i, set of random cross classified effects due to genotypes; B_j, set of fixed effect due to breeds and e_{ijk}, random error.

RESULTS AND DISCUSSION

Present study revealed amplified PCR product on 2% agarose gel as a single compact band of 156 bp size in Malvi, Nimari and Frieswal cattle breeds under UV transilluminator and documented by gel documentation system. The PCR product of the same bp size was also reported by Kumari *et al*. (2008) in exotic and Zebu cattle, Sacravarty *et al.* (2008) in Kankrej cattle, and Ghasemi *et al*. (2009) in Montebeliard cows.

Restriction digestion with *Rsa* I restriction enzyme revealed restriction fragments of 156, 82 and 74 bp sizes among the 3 breeds of cattle. The different restriction fragments generated three different RFLP patterns. The following DNA restriction fragments were obtained for the PRL-*Rsa* I polymorphism: 156 bp (no digestion) for the AA genotype156, 82 bp and 74 bps for the AB and, 82 and 74 bps for the BB genotype (Fig. 1 - a, b and c). These 3 different RFLP patterns were obtained at two restriction sites corresponding to AA, AB and BB genotypes. Similar RFLP patterns using *Rsa* I restriction enzyme were reported by Kumari *et al*. (2008) in exotic and zebu cattle, Sacravarty *et al.* (2008) in Kankrej cattle and Ghasemi *et al*. (2009) in Montebeliard cows.

The genotypic frequencies ranged from 0.000 to 0.90 for AA genotype, from 0.000 to 0.843 for AB genotype and from 0.056 to 0.629 for BB genotype whereas, allelic frequencies ranged from 0.450 to 0.630 for A allele and from 0.370 to 0.550 B allele in Malvi, Nimari and Frieswal cattle breeds respectively (Table 1.)

The significant ($P<0.05$) chi-square value for all the genotypes in Malvi, Nimari and Frieswal cattle showed that the population was not in Hardy-Weinberg equilibrium. Similar results were reported by Klauzinska *et al*. (2004) in Polish Red and Polish Black and White cattle, Brym *et al*. (2005) in Jersey and Black and White cattle and Skinkyte *et*

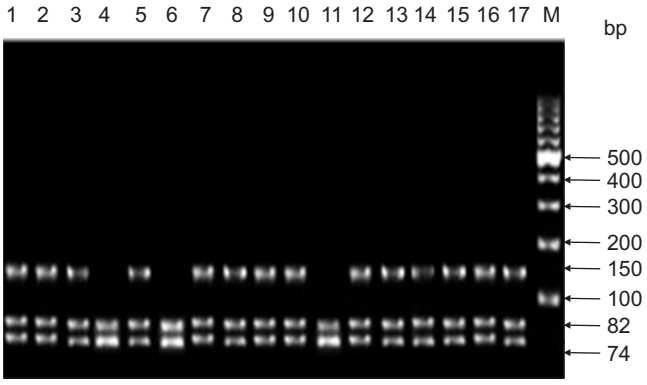

Fig.1 (a) Prolactin genotypes of Malvi cattle.

Lane 1-3,5,7–10,12–17 - AB type; Lane 4,6,11 - BB type; M - 100bp DNA ladder

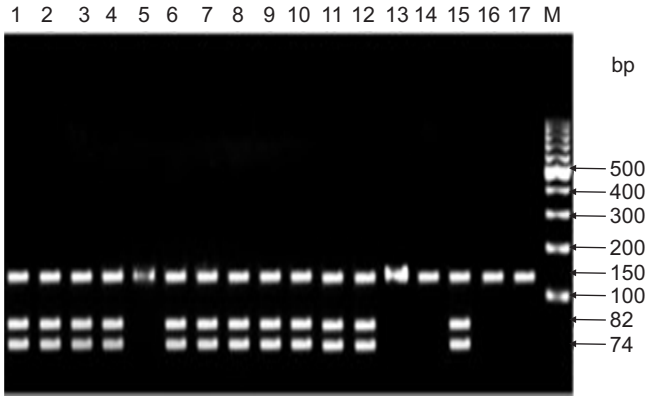

Fig. 1(b) Prolactin genotypes of Nimari cattle.

Lane 5, 13–14, 16–17, AA type; Lane 1–4, 6–12, 15, AB type; M, 100bp DNA ladder

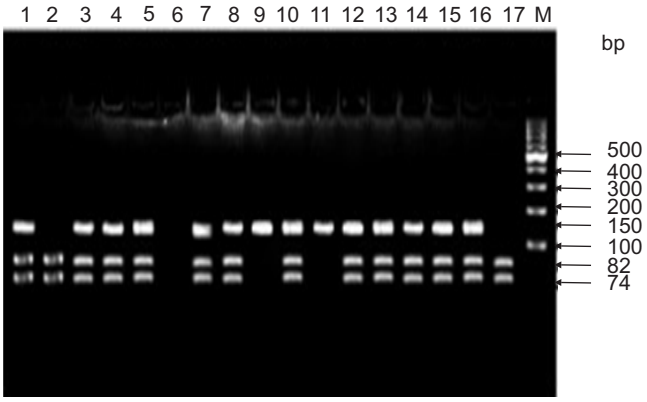

Fig. 1 (c) Prolactin genotypes of Frieswal cattle.

Lane 9, 11, AA type; Lane 1,3–5,7–8,10,12–16, AB type; Lane 2, 17, BB type; Lane 6, Blank; M, 100bp DNA ladder

Table 1. Frequency of genotypes and alleles at prolactin locus

Genotype/allele	Breeds		
	Malvi (50)	Nimari (32)	Frieswal (54)
AA	0.000	0.157 (5)	0.315 (17)
AB	0.900 (45)	0.843 (27)	0.629 (34)
BB	0.100 (5)	0.000	0.056(3)
A	0.450	0.578	0.630
B	0.550	0.422	0.370
Chi-square value	33.48**	17.13**	6.60*

**,Highly significant (P<0.01). *,Significant (P<0.05). Figures in parentheses are number of observations.

al. (2005) in Lithuanian Black and White and Lithuanian Red cattle. The results obtained in the present study are not in agreement with those reported by Alipanah *et al.* (2007a) in Red Pied cattle, Alipanah *et al.* (2007b) in Russian Black Pied and Red Pied cattle and Alipanah *et al.* (2008) in Russian Black Pied and Red Pied cattle.

The significant (P<0.05) Chi-square value among the three breeds indicates that the three breeds differ in their genotypic distribution from each other in respect to gene frequency. The deviation in observed and expected genotypic frequencies among all these breeds may also be due to small population size and selective breeding. The effect of genotypes for lactation yield and lactation length was found non-significant in Malvi and Frieswal, whereas significant (P<0.05) effect of genotypes was obtained for service period in Nimari breed of cattle. Nonsignificant differences between the means of various genotypes were observed for lactation yield and lactation length among the 3 breeds, whereas means of various genotypes for service period differed significantly (P<0.05) in Nimari and Frieswal cattle breeds (Table 2). Less number of days for service period was recorded (161.83 days) in Nimari and (117.45 days) in Frieswal cattle breeds for AA genotype as compare to other genotypes.

Least squares means among breeds showed significant (P<0.05) differences in lactation length, whereas non-significant differences among genotypes and parity were observed for lactation length. Though the differences among genotypic least squares means are nonsignificant but genotype AB (295.29 days) seems to be superior for lactation length among the 3 breeds (Table 3). Nonsignificant association of lactation length was reported by Aravindakshan *et al.* (2004) in Vechur and Kassargode cattle.

Least squares means among breeds and genotypes showed significant (P<0.05) differences for lactation yield. The highest least squares means 1542 kg was observed for AB genotype as compare to other genotypes, which indicates the superiority of AB genotype among the 3 breeds. The results revealed in the present study are in accordance as reported by Alipanah *et al.* (2007a) in Russian Red Pied cows, Alipanah *et al.* (2007b) in Russian Black Pied and Russian Red Pied cows, Alipanah *et al.* (2008) in Russian Black Pied and Russian Red Pied cows, and Ghasemi *et al.* (2009) in Montebeliard cows. Nonsignificant association of lactation yield was reported by Aravindakshan *et al.* (2004) in Vechur and Kassargode cattle, Khatami *et al.* (2005) in Russian and German Black and White and Yaroslavl cattle breeds and Sacravarty *et al.* (2008) in Kankrej cattle.

Least squares means among breeds and genotypes showed significant (P<0.05) differences for service period. The genotype AA (143.12 days) seems to be superior for service

Table 2. Mean performance of genotypes (mean ± SE) for economic traits in Malvi, Nimari and Frieswal cattle breeds

Breed	Genotype	Economic traits		
		Lactation length (days)	Lactation yield (kg)	Service period (days)
Malvi	AA	-	-	-
	AB	286.95±4.01[a]	877.59±24.31 [a]	171.52±11.35 [a]
	BB	286.28±12.26 [a]	841.18±47.70 [a]	119.31±17.33 [a]
Nimari	AA	269.86±30.60 [a]	367.84±61.16 [a]	161.83±71.29[b]
	AB	290.27±10.70 [a]	478.94±40.54 [a]	388.50±40.19[a]
	BB	-	-	-
Frieswal	AA	306.21±5.96 [a]	2878.00±116.51 [a]	117.45±12.57[a]
	AB	315.90±4.93 [a]	3161.19±90.86 [a]	159.62±13.63[b]
	BB	329.14±18.44 [a]	2536.87±276.89 [a]	172.50±35.10[ab]

Different superscripts among least-squares means showed significant differences (P<0.05).

Table 3. Least squares means and standard error of genotypes of lactation for lactation length (days), lactation yield (kg) and service period (days)

Genotype	Traits		
	Lactation length	Lactation yield	Service period
AA	283.13±7.87 [a]	1336.04±103.63 [a]	143.12±21.30[a]
AB	295.29± 4.60 [a]	1542.90± 60.60 [b]	212.54±14.08[bc]
BB	293.83± 12.56 [a]	1291.03±165.27 [b]	173.08±33.19[ac]

Different superscripts among least-squares means showed significant differences (P<0.05).

period among the 3 breeds. Nonsignificant association of service period was reported by Sacravarty *et al.* (2008) in Kankrej cattle.

The significant (P<0.05) differences between least squares means among the 3 breeds may be due to breed differences. The significant (P<0.05) differences between least squares means of different genotypes was observed for lactation yield and service period. The maximum least squares means for lactation yield (1542.90 kg) was observed for AB genotype and minimum numbers of days for service period (143.12 days) was observed in AA genotype, which clearly indicated the superiority of AB genotype for lactation yield and AA genotype for service period as compare to other genotypes. Hence, it can be concluded that animals of AB genotype for higher milk production and animals of AA genotype for less service period may be used for future breeding.

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