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PCR-RFLP of GH gene and its associations with milk-production traits in Malvi, Nimari and Frieswal cattle

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ABSTRACT

A study was caried out to detect polymorhism in exon V of the bovine GH gene and to find out association of polymorphism with economic traits in Malvi, Nimari and Frieswal cattle. Association was analyzed between the GH gene polymorphism at exon V (GH1 locus) and milk production traits in Malvi, Nimari and Frieswal cattle. Blood samples along with records of lactation length and lactation yield were collected randomly from 50 Malvi, 25 Nimari and 50 Frieswal lactating cows. PCR-RFLP was performed for genotyping of animals. PCR exposed 427 bp long amplicon whose restriction digestion was performed by using restriction enzyme *Alu*I. Three genotypes, viz AA, AB and BB, were found with frequency of 0.88, 0.10 and 0.02 in Malvi; 0.76, 0.24 and 0.00 in Nimari and 0.26,072 and 0.02 in Frieswal respectively. Frequencies for gene A were 0.93, 0.88 and 0.62 and for gene B were 0.07, 0.12 and 0.38 respectively in Malvi, Nimari and Frieswal respectively. Least square analysis revealed superiority of genotype AB over AA for lactation length (301.2±6.05 vs 285.17±5.32) and lactation yield (1542.98±77.33 vs 1438.12±68.04) among all the 3 breeds.

Key words: PCR-RFLP, Growth hormone (GH) Gene, Lactation length, Lactation yield

Molecular markers that reveal polymorphism at DNA level are now playing a crucial role in animal genetics. Recently several potential candidate gene were recognized. Variation in different regions of candidate gene may influence the diversification of milk yield and milk composition. The growth hormone (GH) gene is considered one of the most important candidate genes that can influence economic traits because of its crucial function in growth, nutrient utilization, milk composition, galactopoesis, aging, reproduction and metabolism (Bauman and McCutcheon 1986, Gluckman *et al*.1987, Etherton and Bauman 1998). GH gene was located on chromosome 19 (Hediger *et al*.1990) and was sequenced by Gordon *et al*. (1983). Polymorphism in GH gene was widely studied by restriction fragment length polymorphism (RFLP) or sequencing. RFLP was characterized in the exons and introns of bovine GH gene and it is suggested that the alleles identified were linked to milk composition, milk yield and ovarian cyclicity (Dybus *et al*. 2004, Dario *et al*. 2008, Balogh *et al*. 2009). PCR-RFLP was also studied in Polish black and white cattle and it was suggested that an allele present at GH1 locus might be linked to milk, fat and protein yield (Dybus *et al.* 2004). PCR-RFLP analysis of bovine GH gene in Kankrej, Gir and Holstein cattle suggested the role of B allele at GH1 locus for higher lactation yield (Pawar *et al.* 2007).

The present study was undertaken to detect polymorphisms in the exonV of the bovine GH gene (GH1 locus) using the PCR-RFLP method and to analyze the associations of these polymorphisms with economic traits in Malvi, Nimari and Frieswal cattle.

MATERIALS AND METHODS

Resource populations and DNA isolation: Blood samples from 50 lactating Malvi cows, 25 lactating Nimari cows and 50 lactating Frieswal cows selected randomly were collected from Government cattle breeding farm, Agar (Shajapur), Government cattle breeding farm, Rodia (Khargone) and Military dairy farm, Jabalpur, respectively, along with their records. Blood sample (5 ml) of each cow was collected aseptically from jugular vein in sterile EDTA-coated vacutainer. After proper labeling, the samples were transported to the laboratory in an icebox for further analysis. The blood samples were kept at –20°C till further analysis. DNA was isolated as per the standard method (John *et al.* 1991) with slight modifications. The purity and quantity of the extracted DNA was checked by Nanodrop spectrophotometer and quality of DNA was assessed on 0.8%

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horizontal submarine agarose gel electrophoresis.

PCR-RFLP of exon V: exon V of bovine GH gene was amplified using a set of primers (Forward) 5'CCGTGTCTATGAGAAGC3' and (Reverse) 5'GTTCTTGAGCAGCGCGT3' (Lucy *et al.* 1993). The PCR conditions used comprised initial denaturation at 94°C for 10 min followed by 31 cycles of denaturation at 94°C for 1 min then annealing at 56°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 10 min. The 427 bp amplified PCR product digested with *AluI* restriction endonuclease (RE). 10 µl PCR product of each sample was digested with 1 µl of *AluI* in a final reaction volume of 30 µl. The reaction mixture was incubated at 37°C for 4 h in waterbath. Restriction digests were electrophoresed on 2% of agarose gel along with 100 bp DNA ladder as a molecular size marker.

The genotypic frequencies were calculated using various RFLP patterns among all the 3 breeds. The allelic frequencies were calculated using genotypic frequencies. The allelic frequencies were calculated by observing the presence or absence of restriction sites at different alleles. Genotype frequencies, gene frequencies and genetic equilibrium at different loci were estimated using software POPGENE 32 version 1.32, the user-friendly software for population genetic analysis. (Yeh *et al.* 1999) and the association of various polymorphic variants of bovine GHI gene with economic traits were analyzed by mixed model least squares and maximum likelihood computer program PC-2 (Harvey 1990).

RESULT AND DISCUSSION

The 427 bp amplicon of bovine GH gene on digestion with *Alu*I revealed 3 different patterns (AA, AB and BB). The 3 different RFLP patterns revealed restriction fragments of 264bp, 147bp, 96bp and 51bp sizes. An allele with presence of 3 site at 264bp, 96bp and 51bp was considered as 'A' allele and presence of 2 restriction sites at 264 and 147bp was considered as 'B' allele.The estimated genotypic and allelic frequencies are presented in Table 1. Genotypic frequencies in Malvi, Nimari and Frieswal cattle for AA genotype were 0.88, 0.76 and 0.26; for AB genotype 0.10, 0.24 and 0.72 and for BB genotype 0.02, 0.00 and 0.02 respectively. The allelic frequencies in Malvi, Nimari and Frieswal for A allele were 0.93, 0.88 and 0.62 and for B allele 0.07, 0.12 and 0.38 respectively. The highly significant Chi-square value in Frieswal showed that the population was not in Hardy Weinberg equilibrium, however non-significant Chi-square value in other 2 breeds showed that the population was in equilibrium.

Association of growth hormone gene polymorphic variants with milk production: The mean genotypic values for lactation length and lactation yield are presented in Table 2. Mean and standard error for lactation length (days) of genotype AA for Malvi, Nimari and Frieswal was 283.06±4.55, 275.29±11.1 and 314±10.09 respectively; for genotype AB 303.53±15.49, 305.22±20.69 and 317.75±4.84 respectively; for genotype BB 299.01±11.1 in Malvi and 299.5±19.73 in Frieswal, whereas BB genotype was not found in 25 animals of Nimari breed. Mean and standard error for lactation yield (kg) of genotype AA for Malvi, Nimari and Frieswal was 843.8±27.05,426.83±32.51 and 2818.05±14.82 respectively; for genotype AB 792.39±62.67,530.70±73.44 and 3031.01±93.7 respectively; for genotype BB 913.74±34.73 in Malvi and 2 525.27±14.08 in Frieswal, where as BB genotype was not found in 25 animals of Nimari breed. Lactation yield of AB genotype differ significantly from genotype BB, which gives idea about superiority of AB genotype to other 2 for lactation length and lactation yield and relatedness of allele B for higher milk production this result is in accordance with the study of Pawar *et al*. (2007).

Table 1. Breed-wise genotypic and allelic frequencies obtained at bovine GH1 locus

Genotype	Breed		
	Malvi	Nimari	Frieswal
AA	0.88(44)	0.76(19)	0.26(13)
AB	0.10(5)	0.24(6)	0.72(36)
BB	0.02(1)	0.00(0)	0.02(1)
Allele	Malvi	Nimari	Frieswal
A	0.93	0.88	0.62
B	0.07	0.12	0.38
Chi-square value	0.183^{NS}	1.3^{NS}	13.03^{**}

NS, Non-significant; ** highly significant (P<0.01); figures in the parentheses show number of animals for each genotype.

The association between various polymorphic variants/ genotypes with lactation length, lactation yield, service period and age at first calving was studied by least squares analysis of variance. The result of least squares analysis of variance showed highly significant differences among breeds and genotypes. The effect of breed (X) genotype interaction was not applied because in Nimari only 2 genotypes (AA and

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

Breed	Genotype	Lactation length (days)	Lactation yield (kgs)
Malvi	AA	283.06±4.55(100)	843.8±27.05(100)
	AB	303.53±15.49(15)	792.39±62.67(15)
	BB	299.01±11.1(2)	913.74±34.78(2)
Nimari	AA	275.29±11.1(24)	426.83±32.51(24)
	AB	305.22±20.69(9)	530.70±73.44(9)
Frieswal	AA	314.12±10.09(28)	2818.05±14.8(28)
	AB	317.75±4.84(98)	3031.01 ± 93.7^{a}(98)
	BB	299.5±19.73(4)	2525.27 ± 148.08^{b}(4)

Different superscript among mean showed significant difference (P<0.05); figures in parentheses are number of observations.

Table 3. Least squares means and standard errors of genotypes for lactation length, lactation yield, age at first calving and service period

Genotypes	Lactation length (days)	Lactation yield (kg)	Service period (days)	Age at first calving (days)
AA	285.17±5.32[a](152)	1438.12±68.04[a](152)	183.86±16.19[a](130)	1523.86±36.49[a](152)
AB	301.2±6.05[b](122)	1542.98±77.33[b](122)	205.81±17.42[a](115)	1535.51±41.47[b](122)
BB	286.74±18.9[ab](6)	1223.23±241.2[ab](6)	201.43±53.43[a](5)	1521.65±129.68[ab](6)

Figures in parentheses show number of observations in each genotype, values with different superscript in columns differ significantly (P≤0.05).

AB) were found. The effect of genotypes for age at first calving, lactation length, lactation yield and service period was found non-significant in Frieswal and Nimari, whereas significant effect was found for service period in Malvi (P≤0.05). The breed-wise genotypic least squares means for lactation length, lactation yield, service period and age at first calving for each genotype among all the 3 breeds are presented in Table 3. Least square analysis showed that least square mean of genotype AB is significantly (P≤0.05) superior from genotype AA for lactation length (301.2±6.05 vs 285.17±5.32) and lactation yield (1542.98±77.33 vs 1438.12±68.04) and non-significantly superior from genotype BB for these 2 traits among all the 3 breed.

Significant (P≤0.05) differences among least square means of different genotypes were found for lactation length, lactation yield and service period. Significant (P≤0.05) difference among least squares means of different genotypes were found for lactation length, lactation yield and age at first calving.

The result revealed in the present study was in accordance with van der velf *et al*. (1996) in Holstein and Brown Swiss cattle, Vukasinovik *et al*. (1999) in Holstein cattle, Grochowska *et al*. (1999) in Polish Holstein and Polish black and white cattle, Grochowska *et al*. (2001) in Holstein, Danish red and Jersy cattle, Katami *et al*. (2005) in Holstein cattle and Pawar *et al*. (2007) in Kankrej, Gir and Holstein cattle.

The significant differences between least squares means of genotype AA and AB were observed for lactation length. Lactation yield and age at first calving, lactation length and lactation yield of genotype AB was superior to other. Hence it is recommended that cattle having AB genotype may be selected as parents for future breeding.

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