

Microsatellite BM1500 polymorphism and milk production traits in Vechur and crossbred cattle of Kerala

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ABSTRACT

Vechur (*Bos indicus*) is the only native cattle breed of Kerala. The present study was aimed at leptin gene polymorphisms and their associations with milk production traits in 74 heads of Vechur and 108 crossbred cattle (*Bos taurus* × nondescript cattle) from Kerala. Microsatellite analysis was performed at the BM1500 marker, located 3.6 kb downstream from the leptin gene by polymerase chain reaction and denaturing polyacrylamide gel electrophoresis (6%). The alleles for the marker BM1500 in Vechur cattle were 126 bp, 132 bp, 136 bp, 144 bp and 150 bp whereas in crossbreds, the alleles were 126 bp, 132 bp, 136 bp, 138 bp and 144 bp. The 132 bp allele was the most abundant in Vechur and the crossbred cattle population with frequencies of 0.40 and 0.46, respectively. The 138 bp allele was absent in Vechur as against 150 bp allele in crossbreds. In comparison to earlier studies on BM1500 locus in cattle, two novel alleles of 126 and 132 bp were detected in the present study. Allele frequency was highest for 132 bp in Vechur (0.40) as well as the crossbred cattle (0.46) under study. The polymorphic information content (PIC) of BM1500 marker in Vechur was 0.6595 while 0.656 in crossbred cattle. In the association study, the highest averages of 305 day milk yield and daily milk yield were observed for Vechur and crossbred cattle possessing 136 bp allele. The highest milk fat percentages were recorded in 136 bp allele bearing Vechur animals and crossbreds possessing 138 bp allele.

Key words: Vechur, leptin gene polymorphism, crossbred cattle of Kerala, BM1500 marker, milk production

Introduction

According to the National Bureau of Animal Genetic Resources (NBAGR) there are 30 recognized cattle breeds in India, of which Vechur (*Bos indicus*) is the only one from Kerala. Vechur cattle owe their name to the village of its origin, Vechur, a place near Vaikom of Kottayam District in Kerala, on the banks of the fresh water lake 'Vembanade'. Regardless of its low milk production, the smaller sized and docile natured Vechur

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cattle are famous as a household animal. This breed is considered as one of the smallest cattle breeds in the world. Vechur bull and cow measure an average of 99 and 89 cm in height and 104 and 93 cm in length, respectively (IYPE and VENKATACHALAPATHY, 2001). The characteristic features of Vechur cattle include high milk fat percentage (4.7), smaller sized milk fat globules (Vechur: 3.21 μm , crossbred cattle of Kerala: 4.87 μm and Murrah buffalo: 5.85 μm), low level of feed requirements and high disease resistance (VENKATACHALAPATHY, 1996; RAGHUNANDANAN, 2006). Milk fat percentage, as an important criterion that determines milk pricing, the genetic basis of which needs to be investigated, shall aid the proper selection and further improvement of the breed.

Various genes are found to be associated with production traits in cattle and the leptin gene is one among them. Leptin is involved in the regulation of feed intake, energy metabolism, fertility and immune response (HOUSEKNECHT et al., 1998) which renders it a potential candidate for quantitative trait loci (QTL) studies and marker assisted selection (MAS). If association between leptin gene polymorphism and milk production traits exists in the indigenous or crossbred cattle population of Kerala, it will be useful for selection based on molecular markers. The present study was focused on the associations of polymorphisms of BM1500 marker at leptin locus with milk production traits in Vechur and crossbred cattle of Kerala.

Materials and methods

Vechur and crossbred cattle maintained in the Vechur conservation trust, University livestock farm, Mannuthy and Cattle breeding farm, Thumboormuzhi of Kerala Agricultural University were selected for the present study. The crossbred cattle population of Kerala was developed by a crossbreeding programme implemented in the state to enhance milk production. Exotic breeds used for crossbreeding include Holstein Friesian, Brown Swiss and Jersey. In the present scenario, the crossbred cattle population of Kerala possess a mosaic genetic make up of exotic and indigenous cattle. DNA was isolated from blood samples of 74 Vechur and 108 crossbred cattle of Kerala using phenol chloroform extraction procedure (SAMBROOK and RUSSEL, 2001). The quality and molecular weight of DNA were measured electrophoretically using 0.8% agarose. Template DNA for PCR was prepared by diluting the DNA stock solution with sterile triple distilled water to a concentration of 50 ng/ μL . The BM1500 marker region was amplified using a custom synthesized primer (STONE et al., 1996). The forward primer was radiolabelled using DNA-end-labelling kit (Bangalore Genei) at the 5' end with γ ^{32}P ATP to visualize the PCR products by autoradiography. The PCR cocktail of 10 μL included template DNA (50 ng), dNTPs (200 μM), MgCl_2 (1 mM), forward and reverse primers (5 pmol), *Taq* DNA polymerase (0.3 U) and 10X buffer (1 μL). Temperature and cycling conditions for PCR were as follows: 94 °C for 5 min, 94 °C for 1 min, 57.4 °C for 1 min,

72 °C for 1 min and final extension at 72 °C for 5 min. The PCR products were subjected to denaturing polyacrylamide gel electrophoresis (6%) along with M13 Bacteriophage DNA as a marker. The gels were electrophoresed at 40 W for three hours, transferred to a filter paper, covered with cling film, dried in a vacuum heated gel drier (Scie-Plas, GD-4534) at 80 °C for one and a half hours and visualized by autoradiography. The sizes of alleles were determined by comparing with G, A, T and C sequences of M13 phage DNA. Heterozygosity of BM1500 marker was calculated in Vechur and crossbred cattle population by the method of OTT (1992) using the formula

$$He = 1 - \sum_{i=1}^k p_i^2$$

where p_i is the frequency of i^{th} allele AND k is the number of alleles at BM1500 locus.

The polymorphic information content of BM1500 microsatellite was calculated for Vechur and crossbred cattle population using the formula

$$PIC = 1 - \left[\sum_{i=1}^k p_i^2 \right] - \sum_{i=1}^{k-1} \sum_{j=i+1}^k 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of i^{th} and j^{th} alleles, respectively and k is the number of alleles (BOTSTEIN et al., 1980).

Thirty milliliters of milk were collected consecutively for two days from animals in 65-90 days of lactation. Milk fat percentage was estimated by the Gerber method as described in IS: 1224 (1977) part one. The information regarding the first lactation milk yield, lactation length and daily milk yield were collected from the records maintained on the farms. The lactation milk yield was standardized for 305 days by multiplying with correction factors to obtain 305 day milk yield (RICE et al., 1970).

The effect of BM1500 polymorphism on the 305 day milk yield, daily milk yield, lactation length and milk fat percentage were determined by t- test and univariate analysis of variance (ANOVA) in Vechur and crossbred cattle (SNEDECOR and COCHRAN, 1994).

Results

Microsatellite analysis at the BM1500 marker revealed five alleles each in Vechur (126, 132, 136, 144 and 150 bp) as well as in the crossbred cattle population (126, 132, 136, 138 and 144 bp) in the present study (Fig. 1). The frequencies of each allele and genotype are summarized in Table 1. The highest frequency at BM1500 locus was observed for 132 bp allele in Vechur (0.40) and crossbred cattle (0.46) under study.

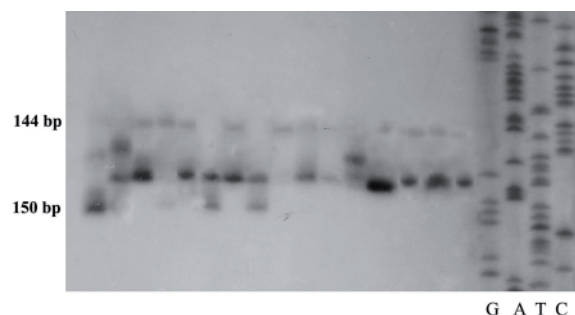


Fig. 1. Autoradiograph of BM1500 polymorphism in crossbred cattle of Kerala

In Vechur cattle population five genotypes (132/132, 136/126, 136/132, 144/132, 150/144) were observed as against seven genotypes (126/126, 132/132, 136/126, 136/132, 138/132, 144/132 and 144/136) in crossbred cattle population. The highest frequency was observed for the 144/132 genotype in Vechur (0.44) as well as crossbred cattle (0.38). The lowest frequency was observed for 132/132 and 136/126 (0.07) in Vechur as opposed to 136/132 (0.05) in crossbred cattle.

In the present study, the PIC value of BM1500 marker was obtained as 0.6595 in Vechur and 0.6560 in crossbred cattle. The heterozygosities in Vechur and crossbred cattle were 0.7082 and 0.6984, respectively.

Table 1. Allele and genotype frequencies of BM1500 polymorphism in Vechur and crossbred cattle of Kerala

Population	Allele Frequencies						Genotype Frequencies							
	126 bp	132 bp	136 bp	138 bp	144 bp	150 bp	126/126	132/132	136/126	136/132	138/132	144/132	144/136	150/144
Vechur	0.05	0.40	0.13	-	0.32	0.10	-	0.07	0.07	0.22	-	0.44	-	0.2
Crossbred cattle	0.12	0.46	0.12	0.06	0.24	-	0.07	0.1	0.1	0.05	0.12	0.38	0.2	-

The highest 305 day milk yield and daily milk yield were recorded in Vechur (378.39 ± 42.44 and 1.43 ± 0.12 kg) and crossbred cattle (2142.61 ± 104.4 and 7.31 ± 0.38 kg) possessing 136 bp allele, but this increase was not statistically significant. The highest milk fat percentage was noticed in crossbred cattle carrying 138 bp allele (3.56 ± 0.13) compared to animals carrying 136 (3.51 ± 0.11), 132 (3.42 ± 0.08) and 144 bp alleles (3.35 ± 0.09).

In Vechur cattle, 136/132 genotyped animals had the highest averages for 305 day milk yield (363.20 ± 53.78 kg), daily milk yield (1.51 ± 0.14 kg) and milk fat percentage

(4.55 ± 0.37) whereas in crossbreds 132/132 genotyped animals had the highest averages for 305 day milk yield (2028.55 ± 134.5 kg) and daily milk yield (6.72 ± 0.64 kb). In the present study milk fat percentage (3.58 ± 0.14) in 138/132 genotyped crossbred cattle was significantly higher compared to 132/132 genotyped animals (3.41 ± 0.08).

Discussion

At the BM1500 locus, various researchers have reported varied numbers of alleles and genotypes in a diverse panel of cattle, four alleles and five genotypes in beef breeds comprising Angus, Charolais, Hereford and Simmental (FITZSIMMONS et al., 1998), three alleles and six genotypes in Holstein Friesian (LIEFERS et al., 2002), six alleles in Angus and four alleles in Charolais (ALMEIDA et al., 2007).

In comparison with the alleles reported by various researchers, 136 and 144 bp by TESSANE et al. (1999), 136, 144 and 146 bp by LIEFERS et al. (2002) and 136, 138, 142, 144, 146, 148 and 150 bp by ALMEIDA et al. (2007), two novel alleles of size 126 and 132 bp were detected at the BM1500 locus in the present study.

In contrast to present results, LIEFERS et al. (2003) reported the highest frequency for 144 bp allele (0.45) in Holstein Friesian cattle while ALMEIDA et al. (2007) recorded the highest frequency for 142 bp allele (0.46) in Charolais breed.

The frequencies of alleles at the BM1500 locus observed in the present study are comparable with previous reports. ALMEIDA et al. (2007) reported frequencies of 0.25 and 0.31 for 136 bp and 144 bp alleles, respectively, whereas TESSANE et al. (1999) reported higher frequencies of 0.49 and 0.51, respectively in the Angus breed. The frequency of 150 bp allele in the Charolais breed was reported as 0.07, which is comparable with the frequency of 150 bp allele in Vechur cattle. In Holstein Friesian, one of the purebred *Bos taurus* cattle used for the breeding programme in Kerala, allele frequencies of 0.41 (136 bp), 0.45 (144 bp) and 0.14 (146 bp) were reported (LIEFERS et al., 2003).

ALMEIDA et al. (2007) and LIEFERS et al. (2003) reported alleles of size 142 and 146 bp at the BM1500 locus in Angus and Holstein Friesian, respectively, which were not detected in the present study. The reason for the identification of 142, 146 and 148 bp alleles among crossbred cattle of Kerala could be attributed to the reduced sample size or on-going selection against these alleles in the population.

While examining the genotypes reported by FITZSIMMONS et al. (1998), LIEFERS et al. (2003) and ALMEIDA et al. (2007), six new genotypes (126/126, 132/132, 136/126, 136/132, 138/132 and 144/132) were identified at the BM1500 locus in the present study. In Vechur cattle the highest genotype frequency was observed for 144/132 (0.44) while the lowest was 132/132 as well as 136/126 with a frequency of 0.07. The 144/132 genotype topped with a frequency of 0.38, whereas the 136/132 genotype (0.05) showed the lowest frequency in crossbred cattle. None of the genotypes, other than 144/136 (LIEFERS et al.,

2003) observed in the crossbred cattle population, have been reported on yet. Differences in numbers and frequencies of alleles and genotypes at a microsatellite marker are indicative of genetic variability and form the basis of all genetic diversity indices.

In the Vechur cattle population the 144 bp allele showed a frequency of 0.32, but no homozygous animals were detected for the said allele. The process of natural or artificial selection going on against the 144/144 genotype may be the reason for this.

Since, BOTSTEIN et al. (1980) opined that a marker was highly informative if its PIC was greater than 0.5, the BM1500 marker is considered as highly informative in the Vechur and crossbred cattle population.

The increased milk yield in animals possessing 136 bp allele may be due to lower leptin concentration associated with 136 bp allele, as reported by LIEFERS et al. (2003). ALMEIDA et al. (2007) detected a positive correlation of 136 bp allele of the BM1500 marker with increased average weight gain in beef cattle and live weight in Holstein Friesian cattle. LIEFERS et al. (2002) could not find a significant association between polymorphism at the BM1500 marker and milk production traits in Holstein Friesian cattle.

FITZSIMMONS et al. (1998) reported an association of 138 bp allele with fat deposition in beef cattle, which is similar to the present findings. The average lactation length was highest in 138 bp allele carrying crossbred cattle (304 ± 6.72 days) while in Vechur cattle the highest value was observed in 150 bp allele carrying animals (246.83 ± 25.73 days). The crossbred cattle possessing 144 bp allele had a higher average for 305 day milk yield (1943.71 ± 128.6 kg) and daily milk yield (6.49 ± 0.45 kg) compared to animals bearing 132 and 138 bp alleles.

Conclusions

Analysis of microsatellite marker polymorphism at BM1500 locus revealed that allele 136 bp was associated with higher 305 day milk yield, daily milk yield and milk fat percentage in Vechur. Higher values for the said parameters were observed in animals with 136/132 genotype. It was also observed that the highest average lactation length was observed for animals possessing 150 bp allele. In crossbred cattle also animals carrying 136 bp allele topped for 305 day milk yield and daily milk yield. Average lactation length and milk fat percentage were higher in 138 bp allele bearing crossbred animals. Extension of research on leptin gene polymorphism to a greater number of animals and exploration of the association of different molecular markers on the leptin gene may complement traditional selection methods, which in turn would improve the value of the native breed, Vechur, and the crossbred cattle population of Kerala.

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SAŽETAK

Vechursko govedo (*Bos indicus*) jedina je autohtona pasmina goveda u Kerali. Istraživanje je poduzeto s ciljem da se odredi povezanost polimorfizma gena za leptin s obilježjima proizvodnje mlijeka u 74 goveda vechurske pasmine i 108 križanih goveda (*Bos taurus* x mješano govedo) u Kerali. Mikrosatelitska analiza markera BM1500 smještenoga 3,6 kb nizvodno od gena za leptin provedena je lančanom reakcijom polimerazom i poliakrilamid gel elektroforezom (6%). Aleli za BM1500 u vechurskoga goveda bili su veličine 126 bp, 132 bp, 136 bp, 144 bp i 150 bp, dok su u križanih aleli bili 126 bp, 132 bp, 136 bp, 138 i 144 bp. Najčešće je bio dokazan alel od 132 bp s učestalošću 0,40 u vechurskoga i 0,46 u križanoga goveda. Alel od 138 bp nije bio ustanovljen u vechurskih goveda, a alel od 150 bp u križanih goveda. U objema skupinama najčešći je bio alel od 132 bp: za vechursko govedo 0,40, a križano 0,46. Informacijski paket za polimorfizam markera BM1500 u vechurskoga goveda bio je 0,6595, a u križanoga 0,656. Najduže prosječno vrijeme laktacije od 305 dana s prosječno najvećom dnevnom količinom mlijeka ustanovljeno je u goveda koja su imala alel od 136 bp. Najveći postotak mliječne masti dokazan je u vechurskih goveda s alelom od 136 bp, a križanih goveda s alelom od 138 bp.

Ključne riječi: Vechur, leptin, gen, polimorfizam, križano govedo, marker BM1500, proizvodnja mlijeka, Kerala
