BETA CASEIN A1A2 POLYMORPHISM AND MILK YIELD IN VECHUR, KASARGODE DWARF AND CROSSBRED CATTLE*

E. M. Muhammed[1] and M. Stephen[2]
College of Veterinary and Animal Sciences, Mannuthy, Thrissur
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ABSTRACT

β-Casein (β-CN) is the second most abundant protein in cow's milk that contains 209 amino acids. Among the twelve β-CN variants identified A1 and A2 are the common types and others are very rare. A1 variant has histidine at position 67 of the amino acid sequence while A2 possess proline at this position- this polymorphism has attracted much public health attention, since consumption of A1 milk is reported to cause various illness. The high producing Bos taurus cattle are found to possess more A1 allele. The present study was undertaken to assess the relationship between β-CN A1/A2 polymorphism with milk yield in Vechur, Kasargode and Crossbred cattle. The average daily milk yield was 1.27±0.05 kg in Vechur cattle and the peak yield of crossbred cattle was 10.71±1.046 kg. The average daily milk yield for A1A2 genotype in Vechur cattle was 1.52±0.08 kg and 1.14±0.04 kg for A2A2 genotype and the difference in the milk yield of the two genotypes was significantly different (p=0.05). In crossbred cattle the peak yield (kg) of A1A1 genotype was 14.64±3.181, 8.54±0.194 for A1A2 genotype and 9.09±0.125 for A2A2 genotype. The A1A1 genotype in crossbred cattle showed significantly higher average peak yield compared to other genotypes and difference between A1A2 and A2A2 genotypes were not significantly different (p=0.05). Thus it can be concluded that selection for enhancing milk production may increase the frequency of harmful A1 allele in bovine population.

Key words: Beta casein gene A1and A2, Vechur cattle, AS-PCR, Milk yield.

[1*]PhD scholar, Email:dremmuhammed@gmail.com
[2]Professor at Department of Animal Breeding, Genetics and Biostatistics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur

INTRODUCTION

India has vast resources of livestock. It plays a vital role in the Indian economy and in improving the socio economic conditions of rural masses. Agriculture and livestock sector share 16.74 percent and 4.36 percent in the economy of India. As per Livestock census 2003, India has total livestock population of 485 million with 185.2 million cattle and 97.2 million buffalo which are 65 percent and 34 percent respectively of total bovine population (283.1million). In Kerala 96 percent of livestock population is cattle producing 98.50 percent of total milk (Anon, 2009). More

than 82 percent of the cattle in Kerala are crossbreds except indigenous breeds like Vechur. Vechur cattle once thought to be extinct due to extensive crossbreeding are the first native cattle of Kerala to be approved as a distinct breed and the smallest breed in the world. Vechur cattle are maintained by a few farmers and at Kerala Veterinary and Animal Sciences University.

Milk is a common source of animal protein and associated micro elements for vegetarians. Cow's milk contains two major protein groups: caseins and whey proteins and out of which caseins account for 80 percent of milk proteins. Bovine milk contains four caseins: alpha S1 (CSN1S1 39-46 percent of total casein), alpha S2 (CSN1 S2 8-11 percent), beta (CSN2, 25-35 percent), and kappa (CSN3 8-15 percent). β-Casein (β-CN) is the second most abundant protein in cow's milk that contains 209 amino acids. Bovine β-CN gene belongs to the cluster of four casein genes located on chromosome 6. There are 12 genetic variants of β-CN: A1, A2, A3, B, C, D, F, H1, H2, I and G out of which A1 and A2 are the most common. So the present study was undertaken, realising the importance of β-CN gene polymorphism in discovery of markers linked to economically important traits such as milk production.

MATERIALS AND METHODS

Blood samples were collected from 72 Vechur cattle (60 from KVASU farm and 10 from Vechur Conservation Trust, Vechur), 14 Kasargode dwarf cattle (KVASU farm) and 100 crossbreds from different parts of Kerala *viz*, Kozhikode (22), Kannur (15), Malappuram (14), Wayanad (15), Thrissur (24) and Kottayam (10).

Collection of Samples

About 5 ml of blood was collected from each animal in a sterile 15 ml polypropylene centrifuge tube (vacutainer) containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant (1 mg/ml of blood). The samples were brought to the laboratory at 4^0C and stored at -20^0C until DNA extraction.

AS-PCR amplification

There are 12 alleles (A1, A2, A3, B, C, D, F, G, H1, H2, I and G) of β-CN in bovines. Of all the 12 β-CN variants all except A2 produce BCM-7 upon enzymatic digestion in the gut. Among BCM-7 yielding variants A1 is the predominant type and other alleles are very rare. Therefore in this study alleles are classified as A1 and A2 only. Based on this, two allele specific reverse primers each one matched to one of two alleles at 3' end (G for β-CN A2 and T for β-CN A1) along with a common forward primer were used (Keating *et. al.*, 2008). The ASPCR products were checked electrophoretically using 2 percent agarose.

Statistical analysis

The allelic and genotype frequencies at A1/A2 locus were calculated by direct counting method and the variations of the allelic frequencies among the three groups were analyzed by the *Chi*-square test of significance as described by Snedecor and Cochran (1994) considering the allelic frequencies in a 2×2 table.

Collection of data

The average daily milk yield of Vechur cattle was collected from the records maintained

in the farm. For crossbred animals, the peak yield was obtained from the farmers. The milk yield of Kasargode dwarf cattle were not recorded as the number of observations was very less. There were only two genotypes (A1A2 and A2A2) in Vechur cattle and three genotypes- A1A1, A1A2 and A2A2 in crossbred cattle. The difference in milk yield of the genotypes was compared by t-test and ANOVA.

RESULTS AND DISCUSSION

Genotype and Allele Frequencies of β-CN

The allele and genotype frequencies of β-CN A1A2 in Vechur, crossbred and Kasargode dwarf cattle are presented in Table 4.1. In Vechur cattle the genotype frequencies of A1A1, A1A2 and A2A2 were observed as 0, 0.34 and 0.66 whereas in crossbred cattle the corresponding frequencies were 0.32, 0.28 and 0.40 respectively. Of the 72 Vechur cattle typed, none of the animals were of A1A1 genotype. No A1A1 genotypes were found in Kasargode dwarf animals also and the corresponding frequencies in Kasargode dwarf cattle was recorded as 0.79 (A1A2) and 0.21 (A2A2). The frequencies for A1 and A2 alleles in Vechur cattle were noted as 0.2 and 0.80 respectively and for crossbreds the frequencies were recorded as 0.46 and 0.54, respectively. In the Kasargode dwarf cattle A1 and A2 allele frequencies were obtained as 0.39 and 0.61 respectively.

β- CN Polymorphism and Milk Yield

The average daily milk yield of 50 Vechur cows were found to be 1.27±0.07 kg and the average peak yield of 100 crossbred cattle were found to be 10.71±1.05 kg. β-CN A1/A2 polymorphism had significant effect on average daily milk yield in the Vechur cattle and peak yield in crossbred cattle (Table 4.2). In t test, Vechur cattle carrying A1A2 genotype showed a significantly higher average daily milk yield (1.52±0.08 kg) compared to A2A2 genotype (1.14±0.04 kg). ANOVA showed significant difference between two genotypes in crossbreds. Crossbred cattle carrying A1A1 genotype showed significantly higher average peak yield (14.64±3.181 kg) whereas A1A2 and A2A2 genotypes had lower average peak yield (8.54±0.019 kg and 9.09±0.125 kg respectively). The peak yields of A1A2 and A2A2 were not significantly different while the peak yield of A1A1 genotype was significantly higher than the other two genotypes.

Table 4.1 Genotype and allele frequencies of β-CN A1/A2 polymorphism in Vechur, crossbred and Kasargode dwarf cattle of Kerala

Population	Genotype frequency			Allele frequency	
	A1A1	A1A2	A2A2	A1	A2
Vechur cattle (72)	0.00	0.34 (29)	0.66 (43)	0.20 (29)	0.80 (115)
Crossbred cattle (100)	0.32 (32)	0.28 (28)	0.40 (40)	0.46 (92)	0.54 (108)
Kasargode cattle (14)	0.00	0.79 (11)	0.21 (3)	0.39 (11)	0.61 (17)

Figures in parenthesis denotes number of observations

Table 4.2 β-CN A1/A2 polymorphism and milk yield in Vechur and Crossbred cattle

Population	Milk production traits	Genotypes (Mean± SE)		
		A1A1	A1A2	A2A2
Vechur	Average daily yield(Kg)		1.52 ± 0.08^{a} (29)	1.14 ± 0.04^{b} (43)
Crossbred	Peak yield (kg)	14.64 ± 3.181^{a} (32)	8.54 ± 0.194^{b} (28)	9.09 ± 0.125^{b} (40)

Where a, b, c are allelic frequencies in different groups

The recorded average daily milk yield of Vechur cattle was 1.27±0.07 kg in the present study against 2.17 kg reported by Girija (1994), 2.2 kg reported by Iype and Venketachelapathy (2001). In crossbred cattle the peak yield was recorded as 10.71±1.046 kg. The 305 day milk yield of crossbred cattle of Kerala is reported as 2406 kg by Chandran and Stephen (2007) and 2106 kg by Anand (2009).

Significantly increased level of milk yield obtained in A1A1 crossbred cattle is in line with findings of Ron *et.al*. (1994) in Israeli Holstein cows and Velmala *et.al*. (1995) in Finnish Ayrshire cattle. Higher milk production from A2 allele is reported by Ng-Kwai-Hang *et.al*.(1990) in Holstein Friesian, Ikonen *et.al*. (1999) in Finnish Ayrshire, Freyer *et.al*. (1999) in Holstein Friesian, Comin *et.al*.

Means with different superscript in a row differ significantly (p=0.05) Figures in parenthesis denotes number of observations (2008) in Italian Holstein Friesian and Nilsen *et.al*. *(*2009) in Norwegian Red cattle.A higher milk yield was reported for heterozygous A1A2 animals by Ng-Kwai-Hang *et.al*. (1986) and Ojala *et.al*. (1997) in Holstein Friesian cattle. In contrast, milk yield was not associated with β-CN A1/A2 polymorphism as reported by Sarbour *et.al*. (1996) in Canadian Holstein and Boettcher *et.al*. (2004) in Holstein Friesian cattle.

As observed in the present study, selection for increasing milk yield may contribute for the higher proportion of undesirable A1 alleles in the population (Ron *et.al*. 1994 and Velmala *et.al*. 1995). Considering the public health implication, adequate weightage should be given to select bulls with A2A2 genotype while making selection for increasing milk yield of crossbreds. The proportion of A2 allele is high in Vechur cattle. Based on the present results, careful breeding strategies can be adopted to develop a Vechur herd/breed with A2A2 genotype and its milk can be marketed at a premium price.

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