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Study on genetic variations of kappa casein gene in local and exotic breeds of cattle

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Abstract
The kappa casein (k-CN) is genetically polymorphic and is controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance. The present research work was undertaken to analyze the genetic variation of kappa casein gene and their association with milk quality traits in Jersey, Hallikar and Malnad Gidda breeds of cattle by PCR-RFLP. A forward (JK5) and a reverse (JK3) primers were used to amplify 350 bp fragment of kappa casein gene and observed no variation in size of amplified product either within or between the breeds. The 350 bp PCR amplified product was digested using the enzyme *Hinf* I and resulted in three allelic patterns. The Bovine k-casein AA genotype has two restriction sites for *Hinf* I at 350 bp amplicon yielding two major fragments of 134 bp and 132 bp and a minor fragment of 84 bp. BB genotype had only one restriction site resulting in two fragments of sizes 266 bp and 84 bp. The AB genotype had fragments of 266, 134, 132 and 84 bp. Milk sample of the animals selected for blood sampling were also collected for milk analysis. The association between the kappa casein gene and milk composition was found to be non significant in Jersey. However due to almost monomorphic pattern in Hallikar and Malnad Gidda breeds, the association could not be ascertained. The present study gave an indication of association of kappa casein "B" allele with high milk fat and protein content.

Keywords: Kappa casein, Hallikar, Malnad Gidda, jersey, PCR, RFLP

Introduction
Milk protein is one of the important components of milk that determines its quality. Bovine milk protein are divided into four groups, the most important ones are Alpha-lactoglobulin, Beta-lactoglobulin and whole casein. Whole casein constitutes a mixture of four proteins *viz*, Alpha Casein S_1, Alpha Casein S_2, Beta casein and Kappa casein (Swaisgood, 1992) [12], which are genetically polymorphic and are controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance (Aschaffenburg and Drewy, 1957) [1]. Kappa casein gene was consider as genetic marker for altering the milk composition and its variants had significant effect on milk composition (Bovenhuis *et al*., 1992) [2].
Though extensive work has been done on milk protein gene in exotic breeds of cattle, such studies are very limited in indigenous cattle though they are traditionally believed to possess better milk quality. With this background, the present research work was undertaken to study and analyze the genetic variation of kappa casein gene and their association with milk quality traits in Jersey, Hallikar and Malnad Gidda breeds of cattle by PCR-RFLP.

Materials and Methods
Blood and milk samples were collected from 25 Malnad Gidda cows from village of Shivamogga district, 25 Hallikar cows from villages of Tumkur district and 10 Jersey cows from Semen station, Karnataka Milk Federation, Kakol, Bangalore rural district. Genomic DNA was isolated from venous blood by high salt method as described by Miller *et al.* (1988) [9]. A forward primer JK5 (5' ATCATTTATGGCCATTCCACCAAAG 3') and a reverse primer JK3(5'GCCCATTTCGCCTTCTCTGTAACAGA3') which were the flanking region of Kappa casein gene locus in exon IV and part of intron IV were used to amplify 350 bp fragment of kappa casein gene (Medrano and Aguilar-Cordova, 1990) [8].
All the reactions were carried out in 0.2 ml reaction tubes. Just before setting of the reaction, a master mix was prepared combining 1OX PCR buffer (500 mM KCl, 100 mM Tris. HCl, pH 8.3) (2 µl), 2.0 mM $MgCl_2$ (1 µl), 200 µM dNTP's (1.6 µl), 1.0 unit of Taq DNA polymeras

(0.33 µl), 25 picomole of each primer (1 µl) and Filtered Milli Quartz (FMQ) water (12.07 µl). Each reaction mix consisted of 19 µl of master mix and one µl (100 ng) of template DNA and placed in the thermal cycler block.
An initial denaturation was done at 94 °C for two minutes and subsequent denaturation primer annealing and primer extension were carried out at 94 °C, 58.2° and 72 °C each for one minute, respectively. The number of cycles was kept constant at 34. After the last cycle, a final extension was carried out at 72 °C for ten minutes and the samples were then cooled down to 15 °C until retrieved.

Restriction enzyme digestion of the PCR product of kappa-Casein gene
A 20 µl of digestion mixture, in 0.5 ml micro centrifuge tube consisted of 15 µL of the PCR product, 2µl (1X) of recommended buffer, 2µl of FMQ and six units of (0.6µl) of restriction enzyme. The digestion mixture was mixed thoroughly in vortex mixture and incubated at optimum temperature of the enzyme *Hinf* I for three and half hour in dry bath.The digested product was run on two percent gel along with the standard DNA molecular marker of 100 bp at 50 volts for three hours and observed under UV transilluminator. The Agarose gel photographs were taken by using gel documentation instrument.

Results and Discussion
The high salt DNA extraction method of Miller *et al* (1988) [9] was followed for the isolation of DNA, which yielded good quality DNA. The OD ranged between 1.7 and 1.9 and the bright streaks on gel electrophoresis.

Optimization of PCR parameters: PCR conditions were standardized in the present study to obtain the optimal yield of the desired product. Except with some differences in PCR parameters like annealing temperature and primer concentration, rest of the PCR parameters were similar to the technique adopted by Medrano and Aguilar-Cordova (1990) [8] and Darshan Raj (2006) [4].
The amplification was optimum with the primer concentration of 25 pico mole. Gradient temperature between 58-59 °C was kept in thermal cycler programme and 58.2 °C was found to be the optimal annealing temperature for amplification K-casein gene. Medrano and Aguillar-Cordova (1990) [8] found 60 °C as the optimal annealing temperature in *Bos taurus* species for the amplification of exon IV of kappa casein gene with the primers JK3 and JK5, where as Darshan raj (2006) [4] had observed 58.4 °C as the optimal annealing temperature in the *Bubalus bubalis* species with the same set of primers. The variation observed in the optimal annealing temperature may possibly due to the varying laboratory conditions.

PCR amplification of Kappa casein gene
The amplified product was approximately of 350 bp in length with no variation in size either within or between the breed studied (Figure.1). The sizes of the amplification products were identical in the two indigenous cattle breeds studied suggesting that this region is conserved in all the cattle breeds.

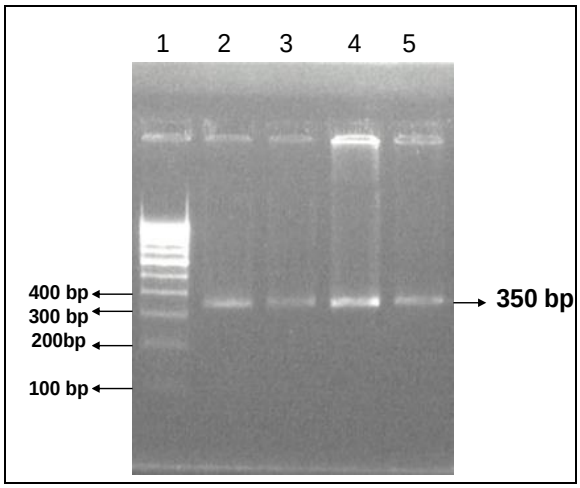

Fig 1: PCR amplified products of 350 bp, kappa casein gene

Lane 1 - molecular marker (100 bp).
Lane 2 - Malnad gidda
Lane 3 - Jersey
Lane 4 & 5 - Hallikar

Medrano and Aguillar-Cordova (1990) [8] and Darshan Raj (2006) [4] also had obtained amplified product of similar size in *Bos taurus* and *Bubalus bubalis* species respectively suggesting the conservation of kappa casein gene between *Bos taurus, Bubalus bubalis* and *Bos indicus* species.

Genotyping genetic variants of kappa casein gene using RFLP technique
The 350 bp kappa casein gene amplified product was digested with enzyme *Hinf* I. This resulted in three different allelic patterns. The first allelic pattern with two major fragments of 134 bp and 132 bp and a minor fragment of 84 bp, was classified as AA genotype. Second allelic pattern with a two fragments of sizes 266 bp and 84 bp was classified as BB type and third pattern with four fragments of 266, 134, 132 and 84 bp was classified as AB genotype (Figure 2). The present findings were in agreement with earlier reports (Medrano and Aguillar-Cordova, 1990; Darshan raj, 2006) [8, 4].

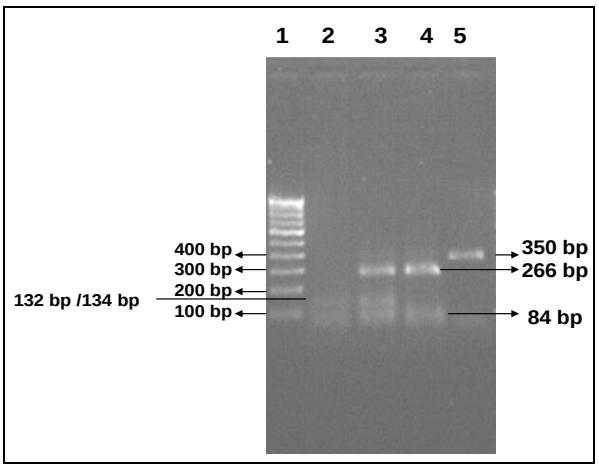

Fig 2: Genotypic variants of kappa kasein gene variants

Lane 1 Molecular marker
Lane 2 Genotype AA
Lane 3 Genotype AB
Lane 4 Genotype BB
Lane 5 Uncut 350 bp amplified product

The observed gene frequency of 0.98 and 0.96 for kappa casein variant A in Malnad Gidda and Hallikar observed in the present study was in agreement with the reports of Kemenes *et al* (1999) [5] who observed almost similar frequencies of A allele in few other *Bos indicus* breeds, viz; Gyr (0.93), Guzera (0.92) and Nelore (0.90). Almost similar A allele frequency was also reported by Murphy and Downey (1969) [11] in Irish Kerry breed.

Complete absence of BB genotype was observed in the present study in Malnad Gidda and Hallikar cattle. Similar result of absence of BB genotype was also reported by Mitra *et al* (1998) [10] in Sahiwal cattle and Nili ravi, Murrah and Egyptian buffalo breeds.

In contrast to the present study, Burzynska and Topczewski, (1995) [3] genotyped exon IV of the k-casein gene of *Bison bonoses* (European bison) and found only BB variants with allele A being totally absent. They concluded that homozygosity could be due to the loss of genetic variability in the small and isolated population studied and same results were also observed by Darshan Raj, (2006) [4] in *Bubalus bubalis* species, who recorded only BB variants with the complete absence of allele A.

In the present study B allele was predominantly found in Jersey (*Bos taurus*) animals and was in very small frequency in Malnad Gidda and Hallikar (*Bos indicus* breeds) where allele A was predominant. Similar result was also reported by Malik *et al* (2000) [6] who observed the predominance of A allele in Sahiwal cattle and B allele in cross breds. The predominance of B alleles in Jersey was also reported by MacLean *et al.* (1984) [7]. The above results indicate that there is significant difference in the genotype of *Bos taurus* and *Bos indicus* cattle.

Association of kappa casein gene and milk composition

In Malnad Gidda and Hallikar cattle the AA genotype was found in almost all the animals, except in one and two animals respectively. Due to this monomorphic pattern it was not possible to determine the association between milk traits with these genotypes in these breeds. Jersey animals exhibited all the three genotypes, however no association could be established which could be due to the fact that only a small number of Jersey animals were studied.

Conclusion

The PCR amplified product obtained in the present study was approximately of 350 bp in length with no variation in size either within or between the breeds studied indicating that this region is conserved in all the cattle breeds. The presence of B allele predominantly in high milk yielding cattle breed such as Jersey and higher frequency of A allele in low producing *Bos indicus* cattle gives some indication of association of B allele with high milk production. However further, extensive study is needed to confirm this by involving larger samples and more breeds.

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