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Comparative study of kappa casein gene sequence in Hallikar and Malnad Gidda breeds of cattle

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Abstract
The milk proteins in cattle are controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance. The present research work was undertaken to analyze the Kappa Casein gene of Malnad Gidda and Hallikar breeds of cattle. A forward (JK5) and a reverse (JK3) primer were used to amplify 350 bp fragment of kappa casein gene. Denaturation, primer extension and annealing were carried out at 94 °C, 72 °C & 58.2 °C, respectively. The number of cycles was kept constant at 34. The amplified product was approximately of 350 bp in length with no variation in size either within or between the breeds studied. The PCR products were sequenced and were subjected to pairwise alignment on online EBI (Europian Bioinformatics Institute) tool. It revealed 83.2% homology between kappa casein gene sequence of Hallikar and Malnad Gidda breed. There were 27 base deletions and 13 base insertions involving all the four bases in Malnad Gidda when compared with Hallikar. Transversion of bases C/G was also observed at positions 9 and 180. Study revealed significant difference in the nucleotide sequence of kappa casein gene between Hallikar and Malnad Gidda breeds.

Keywords: Kappa Casein, Hallikar, Malnad Gidda, PCR, DNA sequence

Introduction
Milk protein is one of the important components of milk that determines its quality. In cattle there are five major milk proteins *viz*, α-Casein, ***β***-Casein, k-Casein, α-lacto globulin and ***β***-lacto globulin which are genetically polymorphic and are controlled by co-dominant autosomal genes in accordance with Mendelian law of inheritance (Aschaffenburg and Drewy, 1957) [2]. The kappa casein (k-CN) is one of the major milk protein and it determines the size of the micelles and stabilizes their structure (Schaar, 1985) [6] and also influences the manufacturing properties of milk products (Rahali and Menard, 1991) [5].
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 Kappa Casein gene of Malnad Gidda and Hallikar breeds of cattle by PCR amplification followed by sequencing.

Materials and Methods
Blood and milk samples were collected from 25 Malnad Gidda cows from villages of Shivamogga district, 25 Hallikar cows from villages of Tumkur district. Genomic DNA was isolated from venous blood by high salt method as described by Miller *et al.* (1988) [8].
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) [7].
All the reactions were carried out in 0.2ml 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 polymerase (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.

Sequencing of kappa casein gene and Sequence data analysis

PCR products of 350bp fragment of the region exon IV and a part of intron IV of each breed were selected randomly and sent for sequencing (Biochromous Pvt. Ltd., Bangalore). The data base search of sequences for possible match to the DNA sequence of kappa casein gene was conducted using the BLAST algorithm available at the National Center for Biotechnology Information (NCBI).(www.ncbi.com). Multiple sequences were aligned by EBI (Europian Bioinformatic Institute) pairwise alignment tool, online application.

Results and Discussion

The high salt DNA extraction method of Miller *et al*. (1988) [8] was followed for the isolation of DNA, which yielded good quality DNA. The OD ranged between 1.7 and 1.9. The amplification was optimum with the primer concentration of 25 pico mole each, while increasing the primer concentration above 50 pico mole and more resulted in formation of primer-dimer. 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) [7] 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) [3] had observed 58.4^{0}C as the optimal annealing temperature in the *Bubalus bubalis* species with the same set of primers.

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.

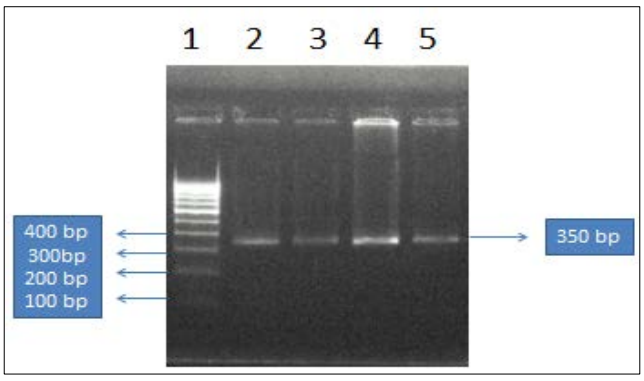

Fig 1: PCR amplified products of 350 bp, kappa casein gene.

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

Medrano and Aguillar-Cordova (1990) [7] and Darshan Raj (2006) [3] also 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.

Sequencing of kappa casein gene: Pairwise alignment of Kappa casein gene between Hallikar and Malnad Gidda Breed are as follows,

```
# Aligned_sequences: 2
# 1: EMBOSS_001 Hallikar Breed
# 2: EMBOSS_002 Malnad Gidda Breed
# Identity: 317/381 (83.2%)
# Similarity: 317/381 (83.2%)
# Gaps: 62/381 (16.3%)

EMBOSS_001 1 TAGATGTTACGA--TTTTATTAATAAG-TCCATGAATCTTGG-C 40
||.|||||| | |||||||||| || |||||||||||| | |

EMBOSS_002 1 CCAATTTACATGTTA--ATATTTTATTAAT-AGATCCATGAATCTT-GCC 46
EMBOSS_001 41 TGTTATTCATTTTGCTTATTTACCTGCGTTTGTCTTCTTTGATGTCTCCT 90
||||||||||||||||||||||||||||||||||||||||||||||||||||||||

EMBOSS_002 47 TGTTATTCATTTTGCTTATTTACCTGCGTTTGTCTTCTTTGATGTCTCCT 96
EMBOSS_001 91 TAGAGT-TTTTAGACTG--CAGTTGAAGT-AACTTGGACTGTGTTGATCT 136
|||| | |||||||||| | ||||| | ||||||||||||||||||

EMBOSS_002 97 TAGA-TTTTTTAGACTGCCC--TTGAA-TTAACTTGGACTGTGTTGATCT 142
EMBOSS_001 137 CA-GGTGGGCTCTCAATAACTTCTGGA-AGAATCTTCTAGAGTAGC-TAC 183
|| | |||||||||||||||||||| | |||||||||.|| | |||

EMBOSS_002 143 CACG-TGGGCTCTCAATAACTTCTGGACA--ATCTTCTAGACTA-CCTAC 188
EMBOSS_001 184 AGTGCTCTCTACTGCT--TCGGTGGTA-GGTGTA-CTTGTAG--GCTCAC 227
|||||||||||||| | |||||| | || | |||| | ||||||

EMBOSS_002 189 AGTGCTCTCTACTGCTACT--GTGGTACG-TG-AACTTG--GGAGCTCAC 232
EMBOSS_001 228 CACTAGCAA--TGGTATTGATGGTAGGG-ATTTTCTGTTTTATCCTGA--272
||||| || |||||||||||||| | |||||||||||| | |

EMBOSS_002 233 CACTA--AATCTGGTATTGATGGTAGGGAA-TTTCTGTTTTAT--T-AAA 276
EMBOSS_001 273 T-TTTTCTTT-GGTGGAATGGCCATAAATGATAAATG-GGTGTGGGTGAC 319
| ||||||| |||||||||||||||||||||| ||||||||||

EMBOSS_002 277 TCTTTTCTTTAGGTGGAATGGCCATAAATGATAAATGTGGTGTGGGTGAC 326
EMBOSS_001 320 GTGCCATGGTAGTTGGCTGGGCTTGGCAGGA 350
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EMBOSS_002 327 GTGCCATGGTAGTTGGCTGGGCTT 350

The sequence data and pairwise alignment on online EBI revealed 83.2% homology between kappa casein gene sequence of Hallikar and Malnad Gidda breeds. The sequence data of Hallikar and Malnad Gidda breeds in the present study matched with another *Bos indicus* breed (Vechur) reported by Aravindakshan and James (2003) [1]. The alignment of 350 nucleotide showed almost 98 percent identity between these breeds.

Comparison of the sequence Between Malnad Gidda and Hallikar: There were 27 base deletions involving all the four bases in Malnad Gidda when compared with Hallikar. Further, there were 13 base insertions also involving all the four bases at various positions (Table 1). Transversion of bases C/G was also observed at positions 9 and 180. There was significant difference in the nucleotide sequence kappa casein gene between hallikar and malnad gidda breeds. Further study is needed in this regard to sequence and analyze the kappa casein gene of different Indian breeds.

Table 1: Comparison of kappa casein gene sequence of Malnad Gidda and Hallikar Cattle

Variant	Position	With Hallikar
1	20, 27,117,169	Del A
2	17, 43, 101, 118, 124, 147, 168, 183, 211, 202, 238, 273	Del G
3	16, 114, 115, 201, 239, 243, 270, 271	Del C
4	214, 222, 257	Del T
5	30, 226, 198, 255, 275, 276, 287	Inser A
6	225	Inser G
7	119,243	Inser C
8	19,103,242	Inser T
9	9,180	C/G

There are no other reports on the gene sequence analysis of the kappa casein gene in the breeds Hallikar and the Malnad Gidda.

BLAST (Basic Local alignment and search tool)

Kappa casein gene sequences when subjected to BLAST to identify the possible match to the DNA sequence yieldeded around 105 hits on the query sequence for *Bos indicus*. The *Bos indicus* (Malnad Gidda & Hallikar breed) kappa casein gene sequence had highest match with kappa casein gene of *Bos taurus* (Le Thi *et al*., 2004) [4] and *Bison bonasus* (Ward *et al.*, 1997) [9]. The score for the match was 617 ad 601 bits and the alignment of 350 base pair nucleotide showed 98 and 97 percent identity, respectively.

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. However, the DNA sequence of 350 bp kappa casein gene of the two breeds showed significant amount of deletion, insertion and transversion at different positions of kappa casein gene of Halliker and the Malnad Gidda breeds of cattle. Further study is needed to analyze the kappa casein gene of all the Indian breeds.

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