A STUDY OF KAPPA AND BETA CASEIN ALLELES IN CROSS-BRED AND ZEBU CATTLE FROM INDIA USING POLYMERASE CHAIN REACTION AND SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBES (PCR-SSOP).

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SUMMARY

Cross-bred cattle are produced in India to increase the frequencies of desired k-casein -B (CASK-B) allele as well as β-casein A_2 (CASB-A2) and B (CASB-B) alleles in them because of economic importance of these alleles in industrial milk production. To ascertain that the required goals were achieved, frequencies of the alleles of CASK and CASB in Cross-breds and Sahiwal breed of cattle were studied using Polymerase Chain Reaction (PCR) and Sequence Specific Oligonucleotide Probes (SSOP). The results showed predominance of CASK-A allele in Sahiwal while CASK-B allele was predominant in Cross-breds. At CASB level CASB-A_2 allele showed predominance in both Sahiwal and Cross-breds and an increased frequency of CASB-B allele in Crossbreds.

Key words: Cross-breds, Polymerase chain reaction, Sequence specific oligonucleotide probes.

INTRODUCTION

Caseins constitute major part of bovine milk proteins. The genes for four major types of caseins i.e. αs1(CASAS1), αs2 (CASA2), β (CASB) and k (CASK) caseins reside on less than 220 Kb of DNA on bovine chromosome 6 (Threadgill and Womack, 1990). Four alleles of CASK gene have so far been identified A,B,C and E. The B allele of CASK has been shown to correlate with higher cheese production. Cheese produced from the CASK-BB genotype has been reported to have a higher protein content, higher yield and better quality than produced from CASK-AA milk (Mariani *et al.* ,1976, Medrano and Anguilar cordova,1990). CASB has seven alleles i.e. A_1, A_2, A_3, B, C, D and E (Eigel *et al.*, 1984). The A_2 allele of CASB gene has been associated with higher milk production (Matyukov,1983, Bech and Kristiansen,1990) and B variant has been associated with increased protein content and better cheese making properties (Marziali and Ng KwaiHang, 1986 and Pecorari and Mariani,1990).

MATERIALS AND METHODS

Genomic DNA's were extracted from 24 unrelated animals of Sahiwal breed randomly selected from a herd of zebu cattle (*Bos indicus)* maintained at Livestock Research Centre, G.B.Pant University of Agriculture and technology, Pant nagar, India and 15 Crossbred cattle (1/2HolsteinFriesian(HF).1/4Jersey.1/4Hariana) randomly selected from a

herd maintained at Live stock Production Research (Cattle and Buffalo). Indian Veterinary Research Institute (IVRI) Izatnagar, India.

PCR-SSOP

Primers designed for amplification of 202 bp variable fragment of k-casein gene are forward primer 5'-ATCATTTATGGCCATTCCACCAAAG-3'(nucleotide positions 324-348) and reverse primer 5'-TTAGACTGCAGTTGAAGTAAC-3'(nucleotide positions 503-523) and The sequences of the probes are 5' CACCTACCACCGAAGCAGT 3' and 5' CACCTACCATCGAAGCAGT 3'(411-429 nucleotide positions) for A and B alleles, respectively. The sequences of these oligonucleotide primers and probes were based on the nucleotide sequences of K-casein published by Kang and Richardson (1988). Amplification of 328 bp fragment of β-casein gene was done using primers and oligonucleotide probes as described by Pinder *et al.* (1991). For amplification of beta and kappa casein gene fragment 200 ng genomic DNA in the presence of 10x PCR buffer, 10mM$MgCl_2$, 10 µl of 1X dNTP's, 25 pmoles of each of the two primers, 0.25µl of Taq DNA polymerase (5 units/µl, Promega Madison,WI) in MJR(PTC-100) thermocycler for 30 cycles. Each cycle consisted of denaturation at 94°C for 1 minute, annealing at 55°C for 50 seconds and extension at 72°C for 1.5 minutes and final extension at 72°C for 5 minutes. Dot blots were prepared from 2 µl of each of the amplified DNA sample. Hybridization was done with oligonucleotide probes for the identification of different CASK and CASB alleles under stringent conditions.

RESULTS AND DISCUSSIONS

Crossbreds showed a higher frequency of CASK-B allele(0.542), while Sahiwal showsed predominance of CASK-A allele (0.625) (table-1) The genotype frequency of CASK-AA was found 8.3% in Cross-bred and 33.33% in Sahiwal cattle. The heterozygous AB genotype was found in 58% in both Sahiwal as well as crossbred cows. An increased frequency of CASK-BB genotype was observed in Crossbreds (25%) as compared to Sahiwal (4.2%). The data show an increase of CASK-A in Sahiwal, while CASK-B allele and its homozygosity in Crossbreds. The relative increase in the homozygosity of B allele is due to the introduction of exotic genes from Jersey in Crossbreds. Jersey breed has been reported to have a higher frequency of B allele (Bech and Kristiansen, 1990). In earlier studies of K-Casein on Indian cattle (Jairam and Nair, 1983 and Majumdar and Ganguli, 1970) a complete absence of homozygous BB genotype was observed in sahiwal breed using starch gel electrophoresis, which is difficult to explain considering mendelian inheritance. Since the frequency of homozygous BB is very low, it may have been just a chance that they did not find any animal with CASK-BB in Sahiwal. There seems to be a change in the frequencies of the two alleles CASK-A and CASK-B in Sahiwal breed since earlier reports, where A allele was found with a frequency of 0.78 to 0.84 and B allele was found with a frequency of 0.16 to 0.22 (Jairam and Nair, 1983, Majumdar and Ganguli,1970) as compared to 0.625 and 0.33 for CASK-A and CASK-B alleles, respectively in the present study, which seems to be nearing the Jersey's gene frequencies in terms of increase in the frequency of B allele and a decrease in that of A allele. The

Cross-breds studied in the present report, have a higher frequency of genotype BB (25%) as compared to Sahiwals where the present genotype frequency of homozygous BB is 4% only confirming the addition of desired alleles in the Cross-bred population.

The CASB-A_2 allele was observed (Table-2) in 93.34% of crossbreds as compared to 75% samples of the Sahiwal breed with gene frequencies of 0.57 and 0.50, respectively. CASB-B allele was observed in 73.34% samples in Cross-breds (gene feequency =0.37) as compared to 50% samples from Sahiwals (gene frequency = 0.25). An increased frequency of genotype A_2B was observed in crossbreds (73%) as compared to Sahiwal (29%) and this difference was statistically significant ($P< 0.008$). Crossbreds did not show A_2A_3 and A_3B genotypes, since A_3 allele was absent in them. Crossbreds showed higher frequencies of CASB-A_2 and CASB-B, since crossbreds are produced from the crossing of Holstein, Jersey and Hariana genome, which have been reported to have a high frequency of CASB-A_2 allele (Bech and Kristiansen, 1990, Singh and Bhat 1981). Crossbreds showed higher genotype frequency of desired heterozygote CASB-A_2B, which is required for both the better yield as well as higher protein content and better cheese making quality. In crossbreds A_3 allele was not observed. It was expected, since A_3 has not been reported in Jersey and Holstein cattle and reported very low frequency (0.0039) in Hariana breed in earlier studies (Bech and Kristiansen, 1990, Singh and Bhat, 1981). However, Ng Kwai-hang *et.al.* (1984) have shown A_3 as a rare allele in Holstein population. The higher gene frequency of CASB-B allele (0.37) in crossbreds may be due to the fact that Jerseys have been reported to have a higher frequency of CASB-B allele (Bech and Kristiansen, 1990). Thus the basic purpose of producing crossbred cattle i.e. to increase the frequencies of CASK-B, CASB-A_2 and CASB-B simultaneously has been fulfilled.

Table1. Gene and Genotype frequencies of the alleles of k-casein gene in local and cross-bred cattle

Alleles	Cross-breds			Sahiwal			Genotypes	Crossbreds		Sahiwal	
	No.	%	GF	No.	%	GF		No.	%GF	No.	%GF
A	8	66.66	0.375	22	91.66	0.625	AA	1	8.3	8	33.3
B	10	83.33	0.542	15	62.50	0.333	AB	7	58.3	14	58.3
Blank	1	8.33	0.083	1	4.16	0.040	BB	3	25.0	1	4.2
							Blank	1	8.3	1	4.2

Table-2. Gene and Genotype frequencies of the alleles of CASB gene in local and cross-bred cattle.

CASB Alleles	Crossbreds			Sahiwal			p<	Genotypes	Crossbreds		Sahiwal		p<
	No.	%	GF	No.	%	GF			No.	%	No.	%	
A_2	14	93.34	0.57	18	75.0	0.50	0.59	A_2A_2	3	0.20	6	0.25	0.52
A	0	0	0	10	41.0	0.21	0.003	A_2A_3	0	0	5	0.21	0.07
B	11	73.34	0.37	12	50.0	0.25	0.14	A_2B	11	0.73	7	0.29	0.008
Blank	1	6.67	0.07	1	4.16	0.04		A_3B	0	0	5	0.21	0.07
								Blank	1	0.07	1	0.04	

REFERENCES

Bech, A.M.and Kristiansen, K.R.(1990) *J. Dairy Res.* **57**:53-62.

Eigel, W.N., Butler, J.E., Ernstorm,C.A., Farrel,H.M.,Jr. Harwalkar, V.R., Jenness,R. and Whitney, R.M. (1984) *J. Dairy Sci.* **67**:1599-1631.

Jairam, B.T.and Nair, P.G.(1983) *Indian J. Dairy Sci.* **36**:5-11.

Kang ,Y.C.and Richardson, T.(1988) *J. Dairy Sci.* **71**:29-40.

Majumdar, G.C.and Ganguli, N.C.(1970) *Indian J. Dairy Sci.* **23**:201-204.

Mariani, P., Lossi, G., Russo, V., Castagnetti, G.B., Grazia, L., Morini, D.and Fossa, A.(1976) *Scienza e Tecnica Lattiero-Casearia,* **27**:208-227.

Marziali, A.S. and Ng-Kwai-Hang,K.F.(1986) *J. Dairy Sci.* **69**:2533-2542.

Matyukov,V.S.(1983) *Dairy Sci. Abs.***46**:374.

Medrano, J.F.and Anguilar-Cordova, E. (1990) *Biotechnology*. **8**:144-146.

Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley,J.E. and Monardes,H.G. (1984) *J. Dairy Sci.* **67**:835-840.

Pecorari, M. and Mariani,P.(1990) *Scienza e Technica Lattiero-Casearia.* **41**:225-244.

Pinder, S.J., Perry, B.N., Skidmore,C.J. and Savva, D. (1991) *Animal Genetics*. **22**:11-20.

Singh, H.P. and Bhat, P.N. (1981) *Indian J. Animal Sci.* **51**:11-16.

Threadgill,D.W. and Womack,J.E. (1990) *Nucleic Acids Res.* **18**:6935-6942.