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IMPACT OF KAPPA CASEIN GENE POLYMORPHISM ON MILK COMPONENTS OF GAOLAO CATTLE

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Abstract: Kappa Casein gene has significant role in stabilizing casein micelle and thus govern the protein content in bovine milk. The present study was undertaken to investigate the genotypes of kappa casein in 83 Gaolao cattle using PCR-RFLP technique. A 379 bp K-casein fragment was amplified using forward and reverse primers viz. 5'- CACGTCACCCACACCCACATTTATC- 3' and 5'- TAATTAGCCCATTTCGCCTTC - - TCTGT- 3', respectively. The amplified PCR products were digested by restriction enzyme *HindIII*, followed by gel electrophoresis to observe genetic variants (A and B) of K-casein. The digested products of 379 bp k-casein showed two variants A and B with gene frequencies of 0.93 and 0.07 respectively. Three genotypes viz. AA (379), AB (379,225,154) and BB (225,154) were observed in 88.89, 8.89 and 2.22 percent cows respectively. The least squares mean values for protein%, fat%, Lactose%, SNF% and Total milk yield as 4.15±0.12, 3.21±0.09, 4.99±0.15, 9.53±0.27 and 312.47±9.38, respectively. The location, season and genotype had no significant effect (P>0.05) on milk components (Fat %, protein%, Lactose% and SNF %) and Total Milk Yield (kg). However, parity had significant effect on Protein, Lactose, SNF content as well as on total milk yield, but it did not affect Fat percent. The study suggested the need to screen larger population of Gaolao cattle to reveal the impact of polymorphic nature of K-casein gene with milk composition traits and thereby suggests the feasibility of inclusion of k-casein genotype in bovine breeding plans.
Keywords: Kappa-casein, polymorphism, PCR-RFLP, Gaolao, Milk traits.

INTRODUCTION

Gaolao is the only cattle breed from the Vidarbha region of Maharashtra state. It is dual type, medium sized, fair milk yielder breed with quick draft capacity. Nagpur and Wardha districts cover the prime breeding tract of Gaolao, where it is well known as Arvi or Gaulani. Milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals, produced in mammals. Indian cattle (*Bos indicus*) are poor yielder of milk but it has a high fat (3.9 %) and protein percentage (3.2 – 4.5 %) compared to taurine cattle (3.6 - 5.2%). The milk protein and fat content are important constituents for dairy industries with respect to industrial competition for import and export of dairy products. Trend in consumer market for value added processed products lead to

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scientific approach for genetic improvement by identifying candidate milk genes and thereby enhancing quality milk production.

Casein genes are located on Chromosome 6 in Cattle (Locus – 6q31), Size – 250 kb and arranged in order as αS1, β- casein, αS2 and k-casein, respectively. Amongst these, k-casein is the largest one i.e. 95-120 kb. Milk components being quantitative traits, are influenced by environmental and genetic factors. Genetic variants of milk proteins differ from each other by one or more amino acid residues in the polypeptide chains, which is due to various types of mutations in the genes encoding them (Hristov et al. 2012). The allelic variation in kappa casein gene has been extensively studied due to its vital role in stabilizing casein micelle, which has wide application cheese making industries (Zukiewicz et al. 2012). Several workers elucidated significant role of kappa casein genetic polymorphism with composition of milk and milk production traits. However, some studies have shown contradictory results and confirmed no significant association between kappa casein genotypes and milk traits. The present study was undertaken with objective to detect the allelic variants of Kappa casein gene in Gaolao breed of cattle in Maharashtra using PCR-RFLP method.

MATERIALS AND METHODS

Samples collection and DNA extraction

A total of 83 unrelated lactating Gaolao cows were considered for study, comprised of 60 cows from organized farm (Bull Mother Farm, Pohara Dist. Amravati) and 23 cows from the breeding tract (various villages of Arvi block of Wardha district), during 2015-2016. The blood samples were collected aseptically from jugular vein in 10 ml vacuum tube (BD Plymouth) containing EDTA and stored in deep freezer at – 20^{0}C pending to DNA extraction. The genomic DNA was extracted using DNA isolation kit (Make MoBio, 100 prep) and Phenol-chloroform extraction protocol (Sambrook *et al.,*1989). The quality and quantity of DNA were measured by spectrophotometer at 260/280 nm and the genomic DNA was diluted to 50 ng/µL.

The data of milk records (season, parity, lactation length) was collected from lactating Gaolao cows under study from organized farm and field by regular visits. The milk components (protein%, fat%, Lactose% and SNF) were estimated from fresh milk samples using milk analyzer.

PCR amplification and RE digestion

A 379 bp fragment of Exon IV of kappa casein gene was amplified by PCR using forward 5'-CACGTCACCCACACCCACATTTATC- 3' and reverse 5'-

TAATTAGCCCATTTCGCCTTCTCTGT- 3' primers as reported by Doosti *et al.* (2011). The PCR was carried out with reaction mixture of 25 μl final volume containing forward and reverse primer 1 μL each, 2 μL DNA sample, 12.5 μL PCR Mastermix and 8.5 μL nucleus free water, subjected to Master Pro S Thermal Cycler (Eppendorf). The PCR amplification reactions were used as follows: 95°C for 5min (initial denaturation), followed by 35 cycles of (denaturation) 94°C for 4min then (annealing) 56°C for 1min and (extension) at 72°C for 1min, and (final extension) at 72°C for 10 min. DNA implication was verified by electrophoresis of the PCR product with loading dye on 1.5% (W/V) agarose gel in 1X TAE buffer, using DNA marker to confirm the desired PCR products length.

The PCR products were digested with restriction endonucleases *HindIII* at 37^0C for 3 hr followed by electrophoresis in 2% agarose gel stained with ethidium bromide. Restriction digestion fragments were loaded on 2 % agarose gel containing 1 × TAE buffer at 120 V for 10 minutes and 100 V for 35 minutes. The gels were analyzed in the UV rays (Transilluminator) to record the results.

Statistical Analysis

The estimates of daily milk yield and daily fat yield based on measured yield and milking frequencies were computed using method of Delorenzo and Wiggans (1986) for adjustment of milking interval and lactation stage, as well as Test day milk yield was computed following Test Interval method (Sargent, 1968 and Liu *et al.* 2000) as per the International Council for Animal Recording (2017).

Data pertaining to milk components of different genotypes were subjected to analysis software package of LSML (Harvey, 1990) using general linear model as:

$$\mathbf{Y_{ijklm} = \mu + G_i + L_j + P_k + S_l + e_{ijklm}}$$

Where, Y_{ijklm} is the observation on each trait of ijklmth animal, μ is the general mean of each trait, G_i is the fixed effect of ith kappa casein genotype, L_j is the fixed effect of jth location, P_k is the fixed effect of kth parity, S_l is the fixed effect of lth season and e_{ijklm} is the random error effect associated with the ijklmth observation.

RESULTS AND DISCUSSIONS

The digested products of 379 bp K-casein indicated two variants A and B with gene frequencies of 0.93 and 0.07 respectively. Three genotypes viz. AA (379), AB (379,225,154) and BB (225,154) were observed in 88.89, 8.89 and 2.22 percent cows respectively (Figure 1). The results are in agreement with the earlier studies in Sahiwal cattle (Mitra *et al.,*1998), Holstein cows (Galila *et al.,* 2008) and Holstein and Iranian native cattle (Doosti *et al.,* 2011) while investigating K-casein gene polymorphism by PCR-RFLP technique using *HindIII* restriction enzymes. Mitra *et al.,* (1998) observed the frequency of B allele in Sahiwal cattle as 0.16 with no homozygous BB animal. Pipaliya (1999) investigated monomorphic pattern (BB genotype) for K casein gene by PCR RFLP technique in Jaffarabadi, Surti, Mehsani and Pandharpuri buffaloes. Similarly, Otaviano *et al.,* (2005) reported monomorphic pattern (BB genotype) in exotic buffaloes and Othman (2005) in Egyptian buffaloes.

The present study was aimed to screen the *HindIII* RFLP pattern of bovine kappa-casein (379 bp) among Gaolao cattle. The present finding indicated that the A allele was more frequent than the B allele among the Gaolao population studied here.

Result revealed the least squares mean values for protein%, fat%, Lactose%, SNF% and Total milk yield as 4.15±0.12, 3.21±0.09, 4.99±0.15, 9.53±0.27 and 312.47±9.38, respectively.

Table 1: Effect of kappa casein genotypes, location, parity, seasons of calving on milk yield and milk composition yields (N=83)

Parameter	N	Protein (%)	Fat (%)	Lactose (%)	SNF (%)	TMY(kg)
Overall Mean	83	2.97 ± 0.28	3.93 ± 0.36	4.59 ± 0.44	8.74 ± 0.81	279.69 ± 27.59
Location		NS	NS	NS	NS	NS
Farm	60	2.85 ± 0.25	3.68 ± 0.32	4.39 ± 0.39	8.35 ± 0.72	274.63 ± 24.74
Field	23	3.09 ± 0.36	4.19 ± 0.47	4.78 ± 0.57	9.14 ± 1.04	284.75 ± 35.68
Parity		*	NS	*	*	*
1	14	2.47 [a] ± 0.32	3.79 ± 0.41	3.86 [a] ± 0.49	7.39 [a] ± 0.91	242.07 [a] ±31.21
2	18	3.46 [b] ±0.31	4.71 ± 0.39	5.33 [b] ± 0.48	10.21 [b] ±0.89	342.06 [b] ±30.33
3	26	3.31 [b] ±0.29	4.25 ± 0.39	5.14 [b] ± 0.47	9.81 [b] ±0.86	315.26 [b] ±29.37
4	13	3.32 [b] ±0.37	4.17 ± 0.48	5.15 [b] ± 0.59	9.79 [b] ±1.08	303.37 [b] ±36.78
5	09	2.97 [a] ± 0.42	3.68 ± 0.55	4.51 [a] ± 0.67	8.60 [a] ± 1.22	273.98 [a] ± 41.81
6	03	2.31 [a] ± 0.06	3.00 ± 0.75	3.54 [a] ± 0.91	6.66 [a] ± 1.67	201.41 [a] ± 57.08
Season		NS	NS	NS	NS	NS
1	22	2.84 ± 0.30	4.06 ± 0.39	4.35 ± 0.47	8.37 ± 0.87	272.72 ± 29.66
2	52	2.89 ± 0.25	3.81 ± 0.32	4.52 ± 0.39	8.62 ± 0.72	281.87 ± 24.72
3	09	3.18 ± 0.44	3.95 ± 0.57	4.89 ± 0.69	9.23 ± 1.26	284.48 ± 43.18
K-CSN genotype		NS	NS	NS	NS	NS
AA	73	3.19 ± 0.20	4.09 ± 0.26	4.94 ± 0.32	9.41 ± 0.58	297.22 ± 20.00
AB	08	3.08 ± 0.32	4.25 ± 0.42	4.78 ± 0.51	9.07 ± 0.93	299.25 ± 31.80
BB	02	2.63 ± 0.63	3.47 ± 0.81	4.04 ± 0.99	7.74 ± 1.81	242.61 ± 61.97

Figures bearing different superscripts in a column for each parameter differ significantly (P<0.05)

The results shown in Table 1 elucidated that, location, season and genotype had no effect (P>0.05) on milk components (Fat %, protein%, Lactose% and SNF %) and Total Milk Yield (kg). These results coincide with the findings of non significant effect of season on fat and the other milk solids by Teixeira et al. (2003), Kappa casein genotype on lactose yield and content by Lunden et al. (1997) and Tsiaras et al. (2005) in Holstein cattle; on milk components by Strzalkowska *et al.* (2002) in Polish black and white cattle; on milk, fat and protein yield by Trakovicka et al. (2012) in Simmental and Holstein cattle breeds. Zhang et al. (2007) reported no association between kappa casein gene and total milk yield and average milk components (Protein and Fat %) in Chinese Holstein cattle.

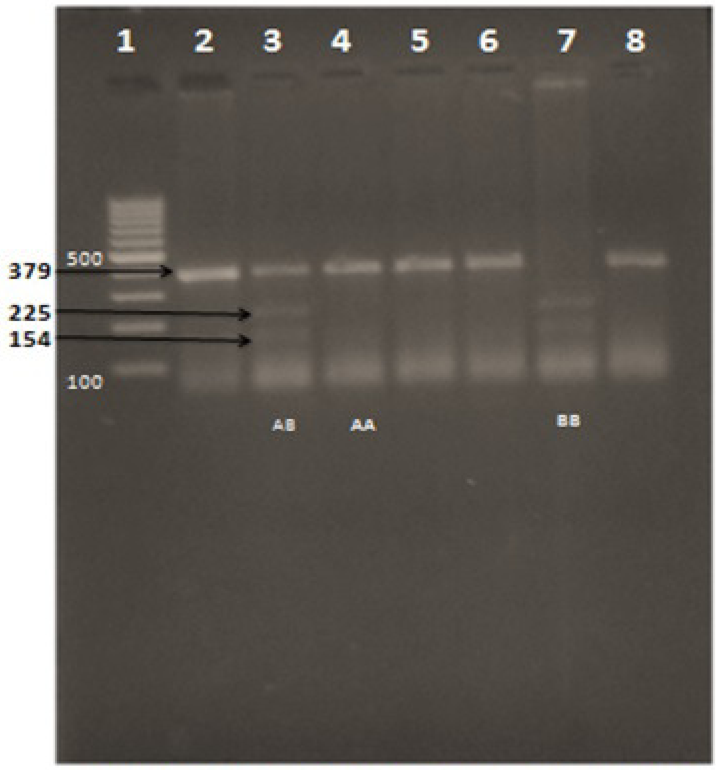
On the contrary, our results are different from those reported by NG Kwai- Hang *et al.* (1990), who claimed that kappa casein genotype had significant influence on milk protein and fat content but not milk yield. Similar results were given by Alipanah et al., (2007) in Russian breed Black and Red pied cattle; Sitkowska et al., (2008) in hiefers; Botaro et al., (2009); Nilsen et al., (2009) in Norwegian Red cattle; Morkuniene et al. (2016) in cows reared in Lithuania, elucidating the significant effect of genotype on milk components and milk yield.

Parity had significant effect on Protein, Lactose, SNF content as well as on total milk yield, but it did not affect Fat percent. Similar findings were reported by Tyrisevä (2008) revealing significant effect of parity number on protein content but not on fat content. The contradictory results may be due to differences in population size, breed and statistical approaches applied.

Conclusion

The polymorphic nature of K-casein gene in Gaolao cattle revealed by PCR-RFLP test indicated that K Casein genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the quality of production. The association of genotypes with milk yield and milk composition traits suggested the need to screen larger population of Gaolao cattle to reveal the impact of polymorphic nature of K-casein gene with milk composition traits and thereby suggests the feasibility of inclusion of k-casein genotype in bovine breeding plans.

Figure 1: Electrophoretogram of HindIII digested PCR product generated by amplification of genomic DNA using K-casein specific primers.



Lane #1- 100 bp DNA Marker, Lane# 2,5,6,8 – AA genotype, Lane # 3 – AB genotype and Lane #7- BB genotype

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