

Short Paper

Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF × Sahiwal) cross breed of Indian origin

Deb, R. *; Singh, U.; Kumar, S.; Singh, R.; Sengar, G. and Sharma, A.

Molecular Genetics Laboratory, Animal Genetics and Breeding Division, Indian Council of Agricultural Research-Central Institute for Research on Cattle, Meerut Cantt. Meerut-250001, Uttar Pradesh, India

*Correspondence: R. Deb, Molecular Genetics Laboratory, Animal Genetics and Breeding Division, Indian Council of Agricultural Research-Central Institute for Research on Cattle, Meerut Cantt. Meerut-250001, Uttar Pradesh, India. E-mail: drrajibdeb@gmail.com

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Summary

The aim of the present study was to screen the genotype profile of bovine kappa-casein gene among Frieswal (HF × Sahiwal) crossbred cattle developed in India. A total number of two hundred Frieswal cows were evaluated for *HinfI* RFLP based genotyping of kappa-casein gene. We observed that only two genotypes (AA and AB) exist among the studied population with the genotype frequency of 0.58 (n=117) and 0.42 (n=83), respectively. The calculated allele frequency for A and B was 0.79 and 0.21, respectively. Association of genotypes with certain milk production traits revealed that AB had significant ($P < 0.05$) effect on total milk yield, peak yield, yield at 300 days and SNF% as compared to AA.

Key words: Kappa-casein, Polymorphism, Frieswal, RFLP, Milk production traits

Introduction

Casein is a family of milk proteins that exists in several molecular forms and is the main protein present in the bovine milk (Alipanah *et al.*, 2005). The bovine milk specific proteins include casein fractions: α s1 casein (CSN1S1), α s2 casein (CSN1S2), β casein (CSN2) and κ -caseins (CSN3) as insoluble fractions, α lactalbumin (LALBA) and β lactoglobulin (LGB), which are classified as soluble fractions (Galila and Darwish, 2008). The κ -casein (CSN3) molecule is a single-chain polypeptide of 169 amino acids with a molecular weight of 19.2 kDa. CSN3 plays an important role in milk chemistry by providing colloidal to the casein micelle. In the micelle, κ -casein is mostly located at the periphery, with its hydrophilic C-terminal sequence protruding into the solvent (Rachagani and Gupta, 2008).

The κ -casein gene comprises a 13 kb sequence divided into 5 exons (Alexander *et al.*, 1988). Point mutations in exon IV of the bovine kappa-casein (CSN3) gene determine two allelic variants, A and B (Alipanah *et al.*, 2007). The A and B variants differ in the amino acids 136 and 148. At position 136, threonine is replaced by isoleucine, while at position 148, aspartic acid is replaced by alanine, for A and B, respectively (Alexander *et al.*, 1988). This variation, associated with processing properties like cheese production technology (Alipanah *et al.*, 2007) and physiological processes such as cytotoxic and antibacterial effects, enhances the

immunity (Hamza *et al.*, 2010). The B allele was found to be associated with thermal resistance, shorter coagulation time, better curdles and micelles of different sizes, which are preferable in cheese making (Azevedo *et al.*, 2008). The cheese yield from cows with genotype BB is 10% higher when compared with AA cows (Azevedo *et al.*, 2008). These variants were distinguished by polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP) analysis (Rachagani and Gupta, 2008). The aim of this study was to determine possible κ -casein gene polymorphism and their association with milk production traits among Frieswal cattle of Indian origin. Frieswal cows were developed in India using 62 percent exotic (HF) and 38 percent indigenous (Sahiwal) blood which exhibit high milk yielding and better fat percentage capability.

Materials and Methods

A total of 200 Frieswal heifers were included in the study. Genomic DNA was isolated from the venous blood using standard phenol chloroform extraction method (Sambrook *et al.*, 1989). For detection of kappa-casein genotypes PCR amplification was done using primer CSN3-F: 5'-TGTGCTGAGTAGGTATCCTAGT TATGG-3' and CSN3-R 5'-GCGTTGTCTTCTTTGAT GTCTCCT-3' (Barroso *et al.*, 1998). PCR was carried out from a starting template of approximately 50 ng of

genomic DNA in a final reaction volume of 25 μ l containing 1 X Taq DNA polymerase buffer (Sigma), 1.5 mM MgCl₂ (Sigma), 200 μ M dNTPs (Sigma), 0.5 μ M of each primer and 1 U Taq polymerase (Sigma). The PCR reaction included pre-denaturation for 5 min at 95°C followed by 35 cycles 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension of 10 min at 72°C. PCR products were visualized in 1.0% agarose gels. The amplicon was sequenced directly using automated DNA sequencer by Sanger's dideoxy chain termination method.

For genotyping, PCR product was digested with *HinfI* restriction enzyme which was used for the determination of kappa-casein alleles. Gene fragments were subjected to digestion by restriction enzymes in a total volume of 20 μ l (8 μ l PCR product, 1 X enzyme buffers, 4 U enzymes and distilled water) and placed in the incubator at 37°C for 5 h. The restriction products were analyzed by electrophoresis on a 2% agarose gel. Gene (allele) and genotype frequencies were calculated as per Falconer and Mackay (1996). Data were analyzed by using SPSS statistical program (SPSS 10.0 for Windows; SPSS, Inc., Chicago, IL, USA). Significant differences were determined by one-way analysis of variance (ANOVA) using the SPSS program according to the following statistical model:

$$Y_{ij} = \mu + G_i + M_j + e_{ij}$$

Where,

Y_{ij} : The analyzed trait of each cow

M: The overall mean

G_i : The fixed effect of the i^{th} genotype

M_j : The fixed effect of j^{th} season of calving

e_{ij} : The random error

Results

The PCR product of the kappa-casein gene using specific set of primers (CSN3-F and CSN3-R) was a fragment of 453 bp DNA. Digestion of 453 bp fragment of kappa-casein gene by *HinfI* restriction endonuclease generated four fragments, i.e. 453, 326, 100 and 27 bp (Fig. 1). Three fragments of 326, 100 and 27 bp represent homozygotes A allele wherein four fragments viz. 453, 326, 100 and 27 bp represent heterozygotes AB for kappa-casein gene. No BB genotypes were observed among the studied population. Estimated genotypic frequencies were 0.58 and 0.42 for AA and AB, respectively (Table 1).

The least-squares means of total milk yield, peak yield, yield at 300 days, fat%, protein%, lactose% and SNF% for different genotypes are presented in Table 1. The animals with genotype AB had a significantly ($P < 0.05$) higher total milk yield, peak yield and yield at

300 days than those with genotype AA. Table 1 also showed that AB genotypes have significantly ($P < 0.05$) higher SNF percentage than AA genotypic animals. However, the statistical analysis revealed that, there was non-significant ($P > 0.05$) difference between the genotypes with fat, protein and lactose percentage.

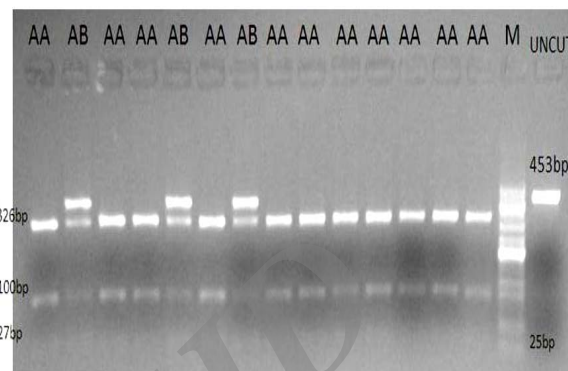


Fig. 1: PCR-RFLP product of kappa-casein gene. After the PCR product was digested by *HinfI* and visualized on 1% agarose gel, the results were the 453 bp fragment of uncut PCR product representing homozygotes B allele (not shown), three fragments of 326, 100 and 27 bp representing homozygotes A allele, and four fragments 453, 326, 100 and 27 bp representing heterozygotes (A/B) for kappa-casein gene

Discussion

The present study was aimed to screen the *HinfI* RFLP pattern of bovine kappa-casein among Frieswal cattle and, further, to identify its association with certain lactogenic traits. The present finding indicated that the A allele was more frequent than the B allele among the Frieswal population studied here. Interestingly no BB homozygous was observed among the studied population. Results from the present study was in agreement with the earlier observations reported by various authors. Kučerová *et al.* (2006) reported that A allele (0.60) are more frequently present than B (0.38) among Czech Simmental cattle. Similar findings of the allele and genotype frequencies among Czech Fleckvieh cattle population was reported by Bartoňová *et al.* (2012). The observation made by Keating *et al.* (2007) showed that, A allele (0.80) was most frequently present among various dairy cattle population. Curi *et al.* (2005) identified a higher frequency of A allele among Simmental and Aberdeen Angus cattle. Trakovická *et al.* (2012) also showed confirmed that existence of A allele among the crosses of Simmental and Holstein cattle breeds was more frequent than B allele. In their study, the predominant allele was A with the observed frequency of 0.76.

Table 1: Determination of the relation between genotypes and its association with milk production traits among Frieswal cattle

Genotype	First lactation milk yield (kg)	Peak yield (kg)	Yield 300 days (kg)	Fat%	Protein%	Lactose%	SNF%
AA (n=117)	2492.75±146.01 ^a	11.30±0.64	2820.90±163.91 ^c	4.0319±0.03	3.005±0.01	4.570±0.01	8.56±0.05 ^c
AB (n=83)	2778.79±215.67 ^b	13.68±0.65 ^d	3012.78±189.99 ^f	3.990±0.04	2.690±0.02	4.430±0.01	8.77±0.03 ^d

Number in parenthesis is the number of animals. Mean values with the different superscript lower case letters (^{a, b, c, d, e} and ^f) in the same column denote significant difference ($P < 0.05$)

The present study showed that animals with AB genotype had a significantly ($P < 0.05$) higher total milk yield, peak yield and yield at 300 days than those with genotype AA. However, Lin *et al.* (1986) found that the BB genotype had a higher average milk yield than AA and AB. In contrast, Curi *et al.* (2005) reported that the κ -casein genotype AA was associated with higher milk production than BB, with the heterozygous AB being intermediate. In the present study, we could not achieve any significant difference between the two identified genotypes with respect to fat, protein and lactose percentage so far. However, Ng-Kwai-Hang *et al.* (1986) and Strzalkowska *et al.* (2002) reported that the κ -casein variant BB genotypes were associated with increased milk fat percentage. However, Bovenhuis *et al.* (1992), Strzalkowska *et al.* (2002) and Rachagani *et al.* (2008) found no significant difference in milk protein content between the two κ -casein variants among Ayrshire, Jersey, brown Swiss, Canadianne, Guernsey and Polish Black-and-White cattle. Interestingly, our study indicated that, AB genotypes had significantly ($P < 0.05$) higher SNF percentage than AA among the Frieswal cows. Though, Rachagani *et al.* (2008) earlier reported that BB animals of Sahiwal and Tharparker cattle had higher monthly SNF percentage and yield than those with genotypes AA and AB.

In conclusion, AB genotypes may have greater influence on certain lactogenic traits among Frieswal crossbred cattle which require further study with a higher number of studied population.

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References

- Alexander, LJ; Stewart, AF; Mackinlay, AG and Kapelinskaya, TV (1988). Isolation and characterization of the bovine kappa-casein gene. *Eur. J. Biochem.*, 178: 395-401.
- Alipanah, M; Kalashnikova, L and Rodionov, G (2005). Kappa-casein genotypic frequencies in Russian breeds Black and Red Pied cattle. *Iran. J. Biotech.*, 3: 191-194.
- Azevedo, ALS; Nascimento, CS; Steinberg, RS; Carvalho, MRS; Peixoto, MGCD; Teodoro, RL; Verneque, RS; Guimarães, SEF and Machado, MA (2008). Genetic polymorphism of the kappa-casein gene in Brazilian cattle. *Gen. Mol. Res.*, 7: 623-630.
- Bartoňová, P; Vrtková, I; Kaplanová, K and Urban, T (2012). Association between CSN3 and BCO2 gene polymorphisms and milk performance traits in the Czech Fleckvieh cattle breed. *Gen. Mol. Res.*, 11: 1058-1063.
- Bovenhuis, H; Van Arendonk, JAM and Korver, S (1992). Associations between milk protein polymorphism and milk production traits. *J. Dairy Sci.*, 72: 2549-2559.
- Curi, RA; Oliveira, HND; Gimenes, MA; Silveira, AC and Lopes, CR (2005). Effects of CSN3 and LGB gene polymorphisms on production traits in beef cattle. *Gen. Mol. Biol.*, 28: 262-266.
- Falconer, DS and Mackay, TFC (1996). *Introduction to quantitative genetics*. 4th Edn., Essex, UK, Longman Group Ltd., P: 464.
- Galila, ASE and Darwish, SF (2008). A PCR-RFLP assay to detect genetic variants of kappa-casein in cattle and buffalo. *Arab. J. Biotech.*, 11: 11-18.
- Hamza, AE; Wang, XL and Yang, ZP (2010). Kappa casein gene polymorphism in Holstein Chinese cattle. *Pak. Vet. J.*, 30: 203-206.
- Kučerová, J; Metějčková, A; Jandurová, OM; Sørensen, P; Němcová, E; Stípková, M; Kott, T; Bouška, J and Frelich, J (2006). Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. *Czech J. Anim. Sci.*, 51: 241-247.
- Lin, CY; McAllister, AJ; Ng-Kwai-Hang, KF and Hayes, JF (1986). Effects of milk protein loci on first lactation production in dairy cattle. *J. Dairy Sci.*, 69: 704-712.
- Ng-Kwai-Hang, KF; Hayes, JE; Moxley, JD and Monardes, HG (1984). Association of genetic variants of casein and milk serum protein with milk fat and protein production in dairy cattle. *J. Dairy Sci.*, 67: 835-840.
- Rachagani, S and Gupta, ID (2008). Bovine kappa-casein gene polymorphism and its association with milk production traits. *Gen. Mol. Biol.*, 31: 893-897.
- Sambrook, J; Fritsch, EF and Maniatis, T (1989). *Molecular cloning: a laboratory manual*. 2nd Edn., N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press. P: 1659.
- Strzalkowska, N; Krzyzewski, J and Ryniewicz, Z (2002). Effects of κ -casein and β -lactoglobulin loci polymorphism, cow's age, stage of lactation and somatic cell count on daily milk yield and milk composition in Polish Black-and-White cattle. *Anim. Sci. Paper Rep.*, 20: 21-35.
- Trakovická, A; Moravčíková, N and Navrátilová, A (2012). Kappa-casein gene polymorphism (CSN3) and its effect on milk production traits. *Acta fytotechnica et zootechnica*. Nitra, Slovaca Universitas Agriculturae Nitriae. (Suppl. 3/2012): 61-64.

با صفات تولید شیر میان نژاد آمیخته فریسوال (سایوال × HF) با منشا هندی

راجیب دب، یومش سینگ، سوشیل کومار، رانی سینگ،

گیانندرا سنگار و آرجاوا شارما

آزمایشگاه ژنتیک مولکولی، بخش پرورش و ژنتیک دام، معاونت تحقیقات کشاورزی هند، انستیتو مرکزی تحقیقات گاو، میرته کانت. میرته-۱-۲۵۰۰۰، یوتر پردش، هند

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هدف از مطالعه حاضر غربال‌سازی پروفایل ژنتیکی ژن کاپا-کازئین میان گاوهای دو رگه فریسوال (سایوال × HF) پرورش یافته در هند بود. تعداد ۲۰۰ گاو فریسوال برای Hinfl RFLP بر اساس تعیین ژنوتیپ ژن کاپا-کازئین مورد ارزیابی قرار گرفتند. ما مشاهده کردیم که تنها دو ژنوتیپ (AA و AB) در میان جمعیت مورد مطالعه به ترتیب با فراوانی ژنوتیپ ۰/۵۸ (تعداد = ۱۱۷) و ۰/۲۴ (تعداد = ۸۳) وجود دارند. فراوانی الل مورد محاسبه برای A و B، به ترتیب ۰/۷۹ و ۰/۲۱ بود. رابطه ژنوتیپ‌ها با صفات تولید شیر مشخص تأیید نمود که AB اثر معنی‌داری ($P < 0.05$) روی تولید کل شیر، تولید حداکثر، تولید در ۳۰۰ روز و درصد SNF در مقایسه با AA دارد.

واژه‌های کلیدی: کاپا کازئین، پلی مورفیسم، فریسوال، RFLP، صفات تولید شیر

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