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GENETIC PROFILING OF β-CASEIN IN GIR, HF, AND INDIGENOUS CATTLE: A STUDY ON MILK YIELD CORRELATION

Genetics

Shailja J. Vyas Research Scholar, P.G. Department Of Genetics, Ashok And Rita Patel Institute Of Integrated Study & Research In Biotechnology And Allied Science (ARIBAS), The Charutar Vidya Mandal University, Vallabh Vidyanagar, Anand, Gujarat- 388120.

Anjali Kulkarni* Assistant Professor, Natubhai V. Patel College Of Pure And Applied Sciences (NVPAS), The Charutar Vidhya Mandal University, Vallabh Vidhyanagar, Anand, Gujarat-388120. *Corresponding Author

ABSTRACT

Beta-casein, a key milk protein in cattle, exists in two genetic variants, A1 and A2, differing by a single amino acid. This study aimed to determine the allelic and genotypic frequencies of the β-casein gene in 200 unrelated cattle in 2 different farms of Savarkundla (Gir, Holstein Friesian (HF), Kankrej, and HF × Jersey crossbreeds) and analyze milk production traits based on their β-casein genotypes. A 121 bp fragment in exon 7 of the β-casein gene was amplified using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Three genotypes were identified: A1A1, A1A2, and A2A2, with respective frequencies of 8%, 0%, and 92% in Gir cattle; 59%, 35%, and 6% in HF cattle; 50%, 50%, and 0% in Kankrej cattle; and 56%, 28%, and 16% in HF × Jersey crossbreeds, with overall allele frequencies of A1 = 0.0746 and A2 = 0.92. Milk yield and fat content varied among genotypes. In Gir cattle, A2A2 cows had higher milk yield (11.59 ± 9.4) and fat content (4.48 ± 0.40%) compared to A1A1 cows (9.8 ± 1.16, 4.26 ± 0.29%), with similar trends observed across other breeds. Crossbreed cattle exhibited the highest milk yield, with A1A1, A1A2, and A2A2 cows producing 17.4 ± 2.8, 17.0 ± 1.24 and 17.6 ± 2.44, respectively. This study highlights significant differences in milk traits among β- casein genotypes, suggesting that A2-focused breeding may benefit both market demand and public health due to the growing preference for A2 milk's potential digestive benefits.

KEYWORDS

Cattle, Beta-gene, A2 milk, PCR – RFLP

INTRODUCTION:

Milk is considered a nutritionally complete food, comprising approximately 87% water and 13% essential nutrients, including lactose, protein, fat, vitamins, and minerals (Nagpal et al., 2012). Casein, the predominant milk protein, constitutes about 80% of the total protein content and plays a crucial role in milk's nutritional and functional properties (Sulimova et al., 2007). It is categorized into four types: alpha s1 (38%), alpha s2 (10%), beta (36%), and kappa-casein (13%) (Sulimova et al., 2007; Jaiswal et al., 2014). Beta-casein, encoded on chromosome 6q31–33 (Sulimova et al., 2007), exhibits significant genetic polymorphism, with multiple variants, including A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, and G, where A1 and A2 are the most common (Jaiswal et al., 2014; Sharma et al., 2013).The key difference between A1 and A2 variants is a cytosine-to-adenine mutation, replacing histidine in A1 with proline in A2 at position 67 (Jaiswal et al., 2014). This genetic variation affects beta-casein's structure and bioactivity, influencing human health. The presence of proline in A2 prevents cleavage, leading to the formation of beta-casomorphin-9 (BCM-9), while A1 releases beta-casomorphin-7 (BCM- 7), an opioid peptide linked to an increased risk of type 1 diabetes, coronary heart disease, and autism (Elliott, 1999; McLachlan, 2001). Conversely, A2 beta-casein is associated with health benefits like lower cholesterol and reduced cardiovascular risk (Elliott et al., 1988). Epidemiological studies have further linked A1 consumption to cardiovascular disease and type 1 diabetes (Panicke et al., 1997). A widely used technique to identify beta-casein polymorphisms is Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), which differentiates genotypes through enzymatic digestion and electrophoresis (Ratna Kumari et al., 2008; Panneerchelvam et al., 2003).

This study aims to investigate the Genotyping Of the Beta-casein gene and the ITS Association on milk yield in Gir and Kankrej, indigenous cattle breeds. The findings will contribute to improving dairy productivity and promoting Indigenous cattle breeds with superior milk traits.

MATERIALS AND METHODS

1. Selection Of Cattle Breeds For The Study :

Cattle Breeds selected for the present study include Gir: Originating from the Gir forests of South Kathiawar in Gujarat, Gir cattle are renowned for their high milk yield and disease resistance. Holstein Friesian (HF): Originally from the Netherlands, HF cattle are characterised by their black-and-white markings and are globally recognised for their exceptional milk production. Kankrej: Native to the region of Rann of Kutch in Gujarat and neighbouring Rajasthan, Kankrej cattle are known for their hardiness and dual-purpose utility for milk production and draught work. HF×Jersey Crossbreeds: These crossbreeds combine traits from Holstein Friesian and Jersey breeds, aiming to enhance milk production while maintaining adaptability to various climatic conditions.

2. Samples Collection:

In the present study, blood samples were collected from 200 unrelated cattle viz 134 Gir, 34 Holstein Friesian (HF), 14 Kankrej, and 18 HF×Jersey crossbreeds, from two organized farms in Savarkundla, Gujarat. Milk yield and fat percent data given by farm's owners. Samples were drawn into vacutainers containing ethylenediaminetetraacetic acid (EDTA). Collected blood Samples were transported to the laboratory on ice maintained .

3. Genomic DNA isolation:

DNA was extracted using a standard protocol using the phenol: chloroform extraction procedure described by John et al., 1991. The quality of the isolated genomic DNA was checked by 0.8% agarose gel electrophoresis, and the quantity was measured using a Nanodrop spectrophotometer (Thermo scientific™ Nanodrop 2000).

4. Polymerase Chain Reaction (PCR):

In the present study, a 121base pair (bp) fragment of exon 7 from the bovine β-casein gene (CSN2) was amplified using Polymerase Chain Reaction (PCR). The PCR amplification was conducted in a 25 µL reaction volume using Takara PCR Mix, which includes 1X PCR buffer with 1.5 mM $MgCl_2$, 0.4 mM dNTPs, and 1 unit of DNA polymerase. The reaction mixture also contained 100 ng of genomic DNA as the template, 10 picomoles of each forward (5'-CCT TCT TTC CAG GAT GAA CTC CAG G-3') and reverse (5'-GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT-3') primers (McLachlan et al., 2006) and nuclease-free water to reach the final volume. The PCR cycling conditions were as follows: an initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 40 seconds, annealing at 58°C for 60 seconds, and extension at 72°C for 90 seconds, followed by a final extension at 72°C for 7 minutes. amplification of the target exon 7 fragment of the CSN2 gene was successfully achieved.

5. PCR-RFLP analysis:

For genotyping, 10 µL of the PCR product was digested with 2 units of the restriction enzyme DdeI in a 20 µL reaction mixture, incubated overnight at 37°C. The digested fragments were then separated on a 3% agarose gel, stained with ethidium bromide, and visualized under

UV light to identify specific polymorphisms in the CSN2 gene.

6. Calculation Of Gene And Genotype Frequency:

To assess the genotypic and allelic distribution of the CSN2 gene polymorphism, allele frequencies were calculated using the direct counting method, adhering to Hardy-Weinberg equilibrium principles. The Chi-square test was then applied to evaluate deviations between observed and expected genotype frequencies. Expected frequencies were derived using the Hardy-Weinberg equation, where p and q represent the frequencies of alleles health fundamental in population genetics to determine if a population's genetic structure remains constant over time or is influenced by evolutionary forces.

$$\textbf{Genotype Frequency} = \frac{\text{Total number of observed genotypes}}{\text{Total number of genotypes}} \times 100 \quad (1)$$

$$P = (2AA + AB)/2N;\ q = (2BB + AB)/2N \quad (2)$$

RESULTS:

The extracted DNA was assessed for quality using 1% agarose gel electrophoresis, revealing high molecular weight sharp bands indicative of good DNA integrity, suitable for subsequent PCR amplification. Purity was confirmed via NanoDrop spectrophotometry, measuring absorbance ratios at 260/280 nm. To investigate the polymorphism of the β-casein (CSN2) gene, a 121 bp PCR-amplified fragment was digested with the DdeI restriction enzyme. This enzyme cleaves the A2 allele, producing two fragments of 86 bp and 35 bp, while the A1 allele remains uncut at 121 bp. Thus, the A1A1 genotype yields a single 121 bp fragment, A2A2 results in 86 bp and 35 bp fragments, and the heterozygous A1A2 genotype presents all three fragments: 121 bp, 86 bp, and 35 bp. These distinct fragment patterns facilitate the differentiation of genotypes in Gir and other cattle breeds. Visual representations of these patterns are provided in Figure 1.

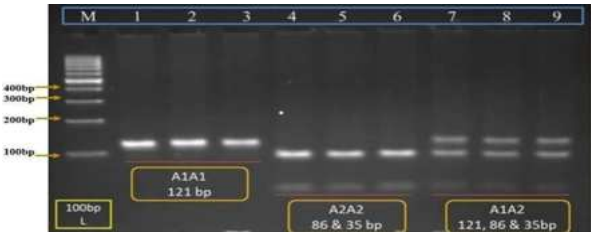

Figure 1: Electrophoresis pattern of PCR-RFLP of β-casein gene on 3% Agarose Gel. Lane M: 100 bp DNA ladder. Lanes 1to 3 sample Nos. S111 to S113 of HF cattle, respectively, Lanes 4 to 9 Sample Nos. S114 to S119 of Gir cattle, respectively. Lanes 1,2 & 3 show A1A1 Genotype (121bp), Lanes 4,5 & 6 show A2A2 Genotype (86 & 35 bp), Lanes 7,8 & 9 show A1A2 Genotype (121,86 & 35) respectively.

The study analyzed β-casein (CSN2) gene polymorphism in Gir, Kankrej, Holstein Friesian (HF), and HF × Jersey crossbreds using PCR-RFLP. Genotypic frequencies for A1A1, A1A2, and A2A2 were determined, along with A1 and A2 allele frequencies. Chi-square tests confirmed Hardy-Weinberg equilibrium (P = 0.01) across all breeds. Gir cattle showed 0.080 (A1A1), 0.0 (A1A2), and 0.92 (A2A2) genotype frequencies, with allele frequencies of 0.0746 (A1) and 0.9254 (A2). HF cattle had 0.589 (A1A1), 0.352 (A1A2), and 0.059 (A2A2), with allele frequencies of 0.60 (A1) and 0.40 (A2). Kankrej exhibited 0.5 (A1A1), 0.5 (A1A2), and no A2A2, leading to allele frequencies of 0.75 (A1) and 0.25 (A2). HF × Jersey crossbreds had 0.56 (A1A1), 0.28 (A1A2), and 0.16 (A2A2), with allele frequencies of 0.69 (A1) and 0.31 (A2). The results confirm a higher prevalence of the A2 allele in indigenous breeds (Gir, Kankrej) and a higher A1 allele frequency in exotic breeds (HF, crossbreds), highlighting the importance of A2-focused breeding programs for healthier milk production.

Table 1: Allele frequencies and result of Chi- square test for comparison of proportions to all different cattle breeds:

Breed Name	Total No.	A1A1	A1A2	A2A2	A1	A2	Chi square test	P value
Gir	124	O=10 E=0.080 (8%)	O=00 E=00	O=114 E=0.92 (92%)	0.0746	0.9254	134.00	P<0.05 (0.01) (DF=2)
HF	34	O=20 E=0.589 (59%)	O=12 E=0.352 (35%)	O=02 E=0.059 (6%)	0.60	0.40	29.95	P<0.05 (0.01) (DF=2)
Kankrej	14	O=7 E=0.5 (50%)	O=7 E=0.5 (50%)	O=0 E=0 (0%)	0.75	0.25	1.55	P<0.05 (0.01) (DF=2)
Hf× Jersey	18	O=10 E=0.56 (56%)	O=5 E=0.28 (28%)	O=3 E=0.16 (16%)	0.69	0.31	2.14	P<0.05 (0.01) (DF=2)

(O = Obeserved frequency , E = Expected frequency)

The study analyzed β-casein (CSN2) gene polymorphism's impact on milk yield and fat percentage in Gir, Holstein Friesian (HF), and crossbred cattle using ANOVA ($p < 0.05$). Results showed that A1A1 genotype cattle had a significantly higher total milk yield than A2A2 cattle. Conversely, A1A2 genotype cattle produced milk with a higher fat percentage than A2A2. These findings highlight a trade-off between milk volume and fat content linked to CSN2 genotypes. This knowledge is valuable for breeding programs aiming to balance milk yield and composition based on market and nutritional demands.

Table 2: Means and Standard Deviations of Milk Production Traits in different Cattle Breeds:

Breed	Genotypes	N (Total No.)	Milk Production	Fat Percent
			Mean ± SD	Mean ± SD
Gir	A1A1	10	9.8 ±1.16	4.26 ± 0.29
	A2A2	124	**11.59± 9.4**	**4.48 ± 0.40**
HF	A1A1	20	14.6±1.8	3.63 ± 0.72
	A1A2	01	**5.5±5.5**	1.95±1.95
	A2A2	13	12.69±1.20	3.56 ± 0.59
Kankrej	A1A1	07	11.50±4.98	3.91±0.37
	A2A2	07	11.52±1.67	3.95±0.31
Cross breed	A1A1	10	17.4±2.8	4.45 ±0.18
	A1A2	03	17 ±1.24	4.6±0.57
	A2A2	05	**17.6±2.44**	4.14±0.66

DISCUSSION:

The genotyping of β-casein (CSN2) variants across various cattle breeds has revealed distinct patterns in allele and genotype frequencies, particularly between indigenous and exotic breeds. Indigenous breeds, such as Gir cattle, exhibit a high prevalence of the A2A2 genotype, with frequencies reported at 92% in some studies. In contrast, exotic breeds like Holstein Friesian (HF) show a higher frequency of the A1A1 genotype, observed at 59%, with A2A2 at 6% and A1A2 heterozygotes at 35%. Crossbreeds, such as HF× Jersey, display intermediate frequencies, with 56% for A1A1, 16% for A2A2, and 28% for A1A2 genotypes. Notably, in the Kankrej breed, both A1A1 and A1A2 genotypes are present at 50%, while the A2A2 genotype is absent.

These findings are in accordance with Pandey et. al., (2019) examined β-casein gene polymorphism in Sahiwal and HF crossbred cattle, reporting a high A2 allele frequency (0.85) in Sahiwal and a higher A1 allele frequency (0.32) in HF crossbreeds. These findings are also in accordance with Shende et al. (2017) analyzed HF crossbred cattle and found genotype frequencies of 28% for A1A1 and 72% for A1A2, with no A2A2 genotypes detected and Martina et al. (2014) reported allele frequencies for Holstein breed was higher frequency of allele A2 (0.7072 and 0.6322) & population of Pinzgau breed was present higher frequency of the allele A1 (0.5618).

Recent studies further support these observations Mukesh et al. (2022) found that the A2 allele is predominant in native Indian cattle breeds, reporting an A2 allele frequency of 0.95. They also noted a higher percentage of A1A2 heterozygous genotypes in breeding bulls, emphasizing the need to screen sire lines to shift herds toward the A2 allele. Similarly, a study on indigenous cattle breeds reported a mean A2A2 genotype frequency of 80%, with the A1A1 genotype absent, and the highest A2A2 frequency observed in the Gir breed (93%). In contrast, exotic breeds exhibited higher frequencies of A1A1 and A1A2 genotypes, with a mean A2A2 frequency of only 3%.

These patterns suggest a breed-dependent distribution of β-casein alleles, with Indigenous breeds favouring the A2 allele and exotic breeds showing a higher prevalence of the A1 allele. This information is crucial for breeding programs aiming to produce milk with specific β-casein compositions, as the A2 variant has been associated with potential health benefits. Therefore, strategic selection and breeding practices could increase the frequency of the A2 allele in dairy herds,

aligning with consumer preferences and potential nutritional advantages.

CONCLUSION:

This study investigated β-casein gene polymorphism in a large population of 200 cattle (Gir, Holstein Friesian, Kankrej, and HF × Jersey crossbreeds) using the PCR-RFLP method. Three genotypes—A1A1, A1A2, and A2A2were identified with allele frequencies of A1 = 0.0746 and A2 = 0.92. The results showed that β-casein genotypes significantly influenced milk production traits ($P < 0.05$), with A1A1 cattle producing higher milk yield and A2A2 cattle yielding milk with higher fat content but slightly lower yield, supporting the known negative correlation between these traits. The A2 allele was identified as a potential genetic marker for improved milk production. Given the increasing demand for A2 milk due to its potential health benefits, this study emphasizes the importance of genetic selection in breeding programs and highlights the need for further large-scale research across different regions to validate the role of β- casein/Dde1 markers in milk production.

Conflict of interest:
All authors no conflict of interest.

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Ethical Statement:
All procedures involving animals were conducted in accordance with the guidelines set forth by the Canadian Association for Laboratory Animal Science (CALAS) and the Canadian Council on Animal Care (CCAC). Blood samples were collected exclusively by licensed veterinary doctors to ensure ethical and professional standards of animal care and use." Information regarding milk yield and fat content was obtained from records maintained by the farm owners.

REFERENCES:

1. Elliott, R.B., Reddy, S.N., Bibby, N.J., and Kida, K.,(1988) Dietary prevention of diabetes in the non-obese diabetic mouse, Diabetologia 31, 62–64.17.
2. Hosking, L., Lumsden, S., Lewis, K., Yeo, A., McCarthy, L., Bansal, A., ... & Xu, C. F. (2004). Detection of genotyping errors by Hardy–Weinberg equilibrium testing. European Journal of Human Genetics, 12(5), 395-399.
3. John, S. W., Weitzner, G., Rozen, R., & Scriver, C. R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. Nucleic acids research, 19(2), 408.
4. John, S. W., Weitzner, G., Rozen, R., & Scriver, C. R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. Nucleic acids research, 19(2), 408.
5. Kamiński, S., Cieślińska, A., & Kostyra, E. (2007). Polymorphism of bovine beta-casein and its potential effect on human health. Journal of applied genetics, 48, 189-198.
6. Machugh, D. E., Shriver, M. D., Loftus, R. T., Cunningham, P., & Bradley, D. G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (Bos taurus and Bos indicus). Genetics, 146(3), 1071-1086.
7. McLachlan, C.N., Breeding and milking cows for milk free of β-casein A1, United States Patent 7094949, 2006.
8. Mukesh, M., Swami, S., Bhakhri, G., Chaudhary, V., Sharma, V., Goyal, N., Vivek, P., Dalal, V., Mohanty, A. K., Kataria, R. S., Kumari, P., Niranjan, S. K., & Sodhi, M. (2022). Demographic pattern of A1/A2 beta casein variants indicates conservation of A2 type haplotype across native cattle breeds (Bos indicus) of India. 3 Biotech, 12(8), 167.
9. Nagpal, R., Behare, P. V., Kumar, M., Mohania, D., Yadav, M., Jain, S. & Yadav, H. (2012). Milk, milk products, and disease free health: an updated overview. Critical reviews in food science and nutrition, 52(4), 321-333.
10. Oleński, Kamil & Cieślińska, Anna & Suchocki, Tomasz & Szyda, Joanna & Kaminski, Stanislaw. (2012). Polymorphism in coding and regulatory sequences of beta-casein gene is associated with milk production traits in Holstein-Friesian cattle. Animal science papers and reports. 30. 5-12.
11. Panicke, L., Freyer, G., Erhardt, G., (1997) Effects of milk protein genotypes on milk production traits, 48th Annual Meetin of the European Association for Animal Produktion am 25.-28.08.19 in Vienna/Austria.
12. Panneerchelvam, S., & Norazmi, M. N. (2003). Forensic DNA profiling and database. The Malaysian journal of medical sciences: MJMS, 10(2), 20.
13. Ratna Kumari, A., Singh, K. M., Soni, K. J., Patel, R. K., Chauhan, J. B., & Sambasiva Rao, K.R. S. (2008). Genotyping of the polymorphism within exon 3 of prolactin gene in various dairy breeds by PCR RFLP (Brief report). Archives Animal Breeding, 51(3), 298-299.
14. Safa, M. A., Mou, M. A., Hridoy, M. F. A., Siddiqua, S. A., Bhuiyan, A. K. F. H., & Bhuiyan, M. S. A(2024) Analysis of β-casein gene for A1 and A2 allelic variants in Holstein-Local crossbred cattle of Bangladesh using allele-specific PCR, Journal of Advanced Biotechnology and Experimental Therapeutics; 7(1): 74-82.
15. Shende, T. C., Kulkarni, P. S., & Pawar, P. C. (2017). Genotyping of HF crossbred cattle for ß-casein genes using PCR-RFLP. Indian Research Journal of Extension Education, 105-107.
16. Sulimova, G. E., Azari, M. A., Rostamzadeh, J., Mohammad Abadi, M. R., & Lazebny, O. E. (2007). κ-casein gene (CSN3) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. Russian Journal of Genetics, 43, 73-79.
17. Wan, X., Wang, W., Liu, J., & Tong, T. (2014). Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC medical research methodology, 14, 1-13.
18. Yadav, A. K., Pandey, A., & Pandey, Y. (2023). Status of A1 / A2 beta casein Gene and its association with Lactose, SNF% and density% of milk in Gir and HF crossbred, reared in Madhya Pradesh, India. The Pharma Innovation Journal, 12(6): 268-271.