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Genetic polymorphism in prolactin gene and its association with milk production traits of Sahiwal cattle in Assam

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Abstract

Background: The exploration of milk production traits and prolactin gene polymorphisms in Sahiwal cattle has illuminated the genetic basis for their exceptional milk production process. These findings contribute to tailored breeding approaches aimed at augmenting milk yield, fat and protein content and reproductive efficiency of Sahiwal cattle. Amid evolving agricultural landscapes, this research plays a pivotal role in sustainable livestock production, boosting food security, and supporting rural economies, particularly in challenging climate regions. The selection of genetically superior animals at an early stage of life, the molecular markers are used along with traditional selection. The study was carried out to identify the genetic polymorphism in the exon3 region of the Prolactin and enumerate its effect on milk production performance in Sahiwal cattle.

Methods: The study was conducted on various production traits and association of prolactin gene with the various traits under study on the herd of Sahiwal cattle maintained at the Sahiwal cattle farm, Livestock Farm Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22. Information from a total of 65 lactation records were utilized for the present study for Milk Production traits mainly Average daily milk yield, Lactation milk yield, Peak yield, Number of days to attain peak yield, Persistency of milk yield. A total of 382 milk samples were utilized to study the milk constituent traits. Milk samples were analysed using "Milk Quality Analyser Laktan 1-4" which includes Fat %, SNF (Solid Not Fat) %, Protein % and Density (kg/m^3). Genomic DNA were extracted from the collected whole blood samples by using Nucleo-pore (Genetix Brand, India) Genomic DNA Purification Kit #DNASure@Blood Mini Kit (NP-61105) 50 preps.

Results: The least-squares means with standard errors for milk production traits *viz*; average daily milk yield, lactation milk yield, peak yield, number of days to attain peak yield and persistency of milk yield were found to be 5.93 ± 0.08 kg, 1609.36 ± 31.21 kg, 13.20 ± 0.16 kg, 47.39 ± 0.88 days and 92.88 ± 0.43 days, whereas, the Least Square means with standard errors for milk constituent traits *viz*; fat%, SNF%, Protein%, Density were found to be $4.34 \pm 0.04\%$, $8.41 \pm 0.03\%$, $3.34 \pm 0.07\%$ and 29.28 ± 0.12 , respectively. PCR-RFLP was carried out using RsaI restriction endonuclease and revealed three genotypes AA, AB and BB, were 0.5, 0.312 and 0.188, the frequency of A and B allele of prolactin gene (exon 3) were 0.752 and 0.248, respectively. The result shows that cows with AA genotypes yielded most milk as compared to cows with genotype AB and BB.

Keywords: Sahiwal, milk production traits, prolactin gene, polymorphism

Introduction

Sahiwal cattle represent unique genetic resources that has evolved to adapt to specific local conditions. The breed's ability to thrive in diverse environments makes it a valuable asset for breeding programs aimed at improving other cattle breeds for various traits. Milk production is a multifaceted phenomenon regulation with diverse factors but among more conspicuous is genetic and non-genetic factors and there is always a coordinated between these factors. It is influenced by the interaction of a large number of genes of which, prolactin plays a pivotal role in the commencement of lactation in all animals. (Chrenek *et al.*, 1998) [5] noted silent mutation in prolactin (exon3) in 156 bp and they also revealed this gene could be used as a popular genetic marker in dairy animals. Therefore, Prolactin (PRL) is a potential candidate gene for linkage analysis of Quantitative Trait Loci (QTL) and genetic marker for production traits in dairy cattle (Brym *et al.*, 2005) [3].

Many researchers (Alipanah *et al.*, 2008; Ghasemi *et al.*, 2009) [2, 8] reported that the PRL gene is highly polymorphic and had an association with milk production traits. Particularly, PRL-RsaI locus had a significant effect on milk production and fat percentage in dairy cattle (Chung *et al.*, 1996; Mitra *et al.*, 1995; Udina *et al.*, 2001; Sacravarty *et al.*, 2008) [6, 15, 19, 17]. In comparison to the previous livestock census, the total population of the crossbred cattle in the country has increased by 26.9% in 2019 with a downfall of 6% in the indigenous zebu cattle population (20th Livestock census, 2019) [13]. Amongst the various registered breeds of humped zebu cattle (*Bos indicus*) in India, Sahiwal cattle of the tropic is considered as one of the best and most important milch breed in the country. The milk production capacity of Sahiwal cattle is a result of intricate interplays between genetic factors and the environment. Originating from the Indian subcontinent Punjab region. Sahiwal cattle stand out for their ability to yield substantial milk quantities even in challenging climatic conditions. Their adaptability, resilience to diseases, and tolerance to heat have made them favourites in tropical region. Due to the importance and high yielding nature the Sahiwal cattle has been extensively studied in various aspects like genomic selection and candidate gene approaches. For the prolactin gene in particular, studies has proven that there is faster genetic gain when genomic selection is done. Most of the candidate genes in cattle which are said to be associated with milk production are: Prolactin (*PRL*) gene, Pituitary transcription factor-1 (*PIT-1*) gene, Signal transducers and transcription-1 (*STAT-1*) gene, Beta-lactoglobulin (*BLG*) gene, etc. (Chauhan *et al.*, 2015; Gayari *et al.*, 2020). The prolactin gene has many functions mainly related to milk production and milk constituent traits. It also plays a very important role in the mammary gland development (mammogenesis), lactogenesis, and secretion of milk (galactopoiesis). It also regulates immunological functions and cell differentiation resulting in growth of the cells. Therefore, the bovine prolactin gene could be considered as an excellent candidate gene for milk production traits in Sahiwal cattle (Karuthadurai *et al.*, 2019) [11].

Materials and Methods

The study was conducted by using a total of 65 lactation records were utilized to study the milk production traits, whereas a total of 382 milk samples were utilized to study

the milk constituent traits. The blood samples were collected from the lactating Sahiwal cows maintained at the Sahiwal cattle farm, Livestock farm complex, College of Veterinary Science, Assam Agricultural University.

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The Least Squares Analysis of Variance Technique of Harvey (1975) [9] as suitable for non-orthogonal data was used to study the effects of various genetic and non-genetic factors on different milk production and milk constituent traits. To make a pair-wise comparison among the means, Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957) [12] was used wherever significant differences among different levels of factors were observed.

Genomic DNA were extracted from the collected whole blood samples by employing Nucleo-pore (Genetix Brand, India) Genomic DNA Purification Kit #DNASure@Blood Mini Kit (NP-61105) 50 preps. The quality of the DNA was checked by using Agarose gel electrophoresis and the concentration was assessed using Nanodrop Spectrophotometer. The ratio of OD values at 260 and 280 nm was used as criterion to judge the purity of the obtained DNA samples. The samples having OD ratio (260 nm/280 nm) ranging between 1.60 to 1.90 and a concentration of 40-60 ng/μl were used for experiments.

The concentration of DNA was estimated by using the following formula:

DNA concentration (μg/ml) = (OD 260nm x 50 × Dilution factor).

The targeted region of prolactin gene was exon 3 and the sequence of the primers and their nucleotide numbers are as follows:

F -5' CGA GTC CTT ATG AGC TTG ATT CTT 3'

R-5' GCC TTC CAG AAG TCG TTT GTT TTC 3'

The amplified product was checked using 2% agarose gel electrophoresis.

The amplified product was subjected to RFLP and the 156 amplified product was digested with RsaI restriction enzyme. The restricted PCR products were checked at 2% agarose gel. The agarose gels were photographed in the gel doc system under the UV light and then scored to their respective genotypes.

Results and Discussions

The Least square means along with the standard errors for various productive traits under study are highlighted in Table.1

Table 1: Least-square means with standard errors for different productive traits under study

Sl. No.	Traits under studies	N	Mean±SE
1.	Average daily milk yield	65	5.93±0.08
2.	Lactation milk yield	65	1609.36±31.21
3.	Peak yield	65	13.20±0.16
4.	Number of days to attain peak yield	65	47.39±0.88
5.	Persistency of lactation yield	65	92.88±0.43

N: Number of observations; SE: Standard error.

The Least squares mean and its standard error to determine different constituents in milk is presented in Table.2. In the present study, the least-squares mean for the fat %, SNF%,

protein % and density were reported as 4.34±0.04%, 8.41±0.0%, 3.34±0.07%, and 29.28±0.12, respectively.

Table 2: Least-Squares Means (LSM) And Standard Errors (SE) With Least Square Constants (LSC) for different Subclasses along with the results of DMRT for Fat, SNF, Protein Percentages and Density in Milk

Effect	N	FAT%		SNF%		PROTEIN%		DENSITY	
		LSM±SE	LSC	LSM±SE	LSC	LSM±SE	LSC	LSM±SE	LSC
μ	382	4.34±0.04	-	8.41±0.03	-	3.34±0.07	-	29.28±0.12	-
Lactation Order									
1	89	4.33±0.08	-0.01	8.49±0.06	0.07	3.18±0.15	-0.15	28.92±0.25	-0.36
2	145	4.39±0.06	0.04	8.34±0.05	-0.06	3.37±0.11	0.03	29.41±0.19	0.12
3	148	4.31±0.07	-0.03	8.40±0.05	-0.01	3.45±0.11	0.11	29.52±0.19	0.23
Season of calving									
1	113	4.20±0.07 ^b	-0.13	8.50±0.06 ^b	0.08	3.22±0.13	-0.11	29.83±0.22 ^a	0.54
2	105	4.17±0.08 ^c	-0.17	8.25±0.06 ^c	-0.16	3.24±0.13	-0.09	29.05±0.22 ^b	0.22
3	66	4.20±0.10 ^b	0.01	8.15±0.08 ^d	-0.26	3.23±0.17	-0.10	28.36±0.29 ^c	-0.92
4	98	4.64±0.08 ^a	0.29	8.75±0.06 ^a	0.33	3.65±0.14	0.31	29.89±0.24 ^a	0.60
Milking Time									
1	194	4.13±0.06 ^b	-0.21	8.16±0.04 ^b	-0.25	3.12±0.10 ^b	-0.21	28.65±0.17 ^b	-0.63
2	188	4.56±0.06 ^a	0.21	8.66±0.04 ^a	0.25	3.55±0.10 ^a	0.21	29.91±0.17 ^a	0.63

N.B. N = Number of observation, LSM: Least squares mean; LSC: Least squares constant; SE: Standard error.

Tona *et al.* (2013) [18] noted that the average morning, evening, and total daily milk ranged from 9.37 to 12.67% in the case of protein and 4.83 to 6.07% in the case of milk fat. The effect of feeding time on milk composition during the summer season was such that the percentage of fat was 4.17% in the morning period and 4.21% in the evening period; again the protein percentage was found as 3.42% and 3.44% in the morning and evening period respectively (Niu and Harvatine, 2018) [16].

The PCR-RFLP technique was carried out to identify the variants in prolactin gene based on the variations produced by digestion of 156 bp (Fig 1) amplified product with restriction enzymes *RsaI* (Fig 2). The restriction fragments were then dissolved by agarose gel electrophoresis (2%). The PCR-RFLP studies on *PRL* gene in Sahiwal cattle, using *RsaI* revealed three alleles, arbitrarily designated as AA, BB and AB with a genotype frequency of 0.5, 0.188 and 0.312, respectively, The allele frequency detected for A and B were 0.752 and 0.248



Fig 1: The 156 bp (100 bp MM) fragment of *prl* gene amplified from genomic DNA by PCR in sahiwal cattle

PCR-RFLP analysis in *PRL* gene using *RsaI*

The PCR-RFLP technique was carried out to identify the variants in *PRL* gene based on the variations produced by digestion of 156bp amplified product with restriction enzymes *RsaI*. The restriction fragments were then dissolved by agarose gel electrophoresis (2%). The PCR-RFLP studies on *PRL* gene in Sahiwal cattle, using *RsaI* revealed three alleles, (Fig.2) arbitrarily designated as AA, BB and AB with a genotype frequencies of 0.5, 0.188 and 0.312, respectively.

In Sahiwal cattle, PCR-RFLP investigations of the *PRL* gene, utilizing *RsaI*, identified three alleles labelled as AA, BB, and AB, with respective genotype frequencies of 0.5, 0.188, and 0.312. Similar genotype frequencies were also reported by Ishaq *et al.* (2012) [10] with proportions of 72%,

18%, and 10% in Sahiwal cattle, and 44%, 34%, and 22% in Achai cattle, respectively.

The A and B allele frequencies were determined to be 0.752 and 0.248, respectively, with a Shanon Index of 0.378 (Table 3).

The findings indicated that cows with AA genotypes produced more milk compared to those with AB and BB genotypes (Table 4). Similar results were reported by Karuthadurai *et al.* (2019) [11] and Mahajan *et al.* (2012), who observed that animals with BB genotypes exhibited longer lactation lengths, while those with AB genotypes had higher lactation yields. They concluded that the prolactin gene could serve as a marker for genetically selecting dairy traits in Frieswal cattle.

In this finding the observed homozygosity and heterozygosity of the prolactin gene in the population deviated from the Hardy-Weinberg expectation, as evidenced by the Chi-Square test value of 4.3022. Similar findings were reported by Alfonso *et al.* (2012) [1], who observed that the Chi-square values for genotype

distributions did not conform to the Hardy-Weinberg equilibrium ($p < 0.05$). The results indicated that animals with the AA genotype exhibited significantly higher milk production ($p < 0.05$), while the BB genotype was associated with the lowest production ($p < 0.05$).

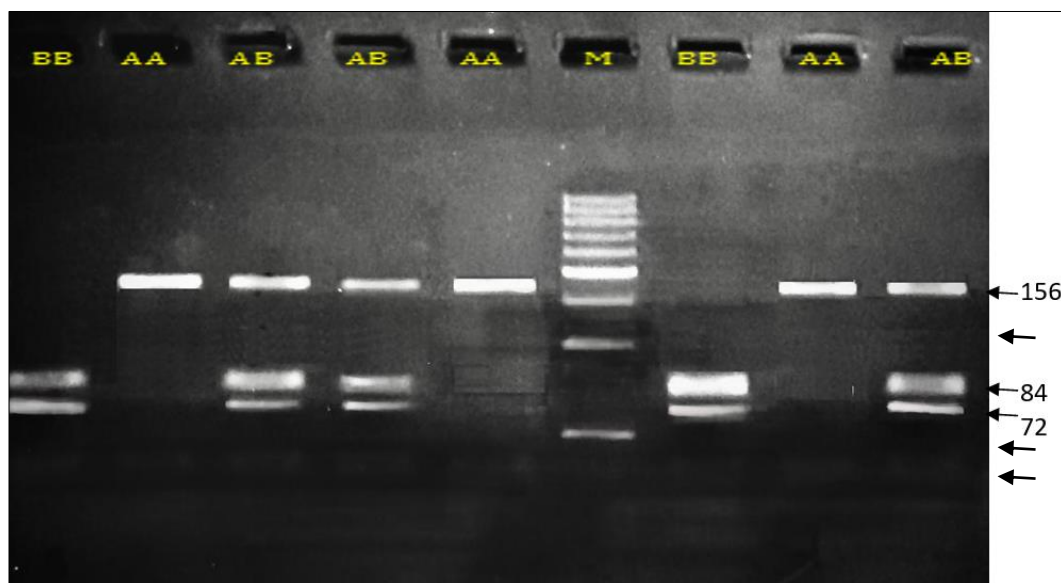


Fig 2: PRL/RsaI Genotypes resolved on gel electrophoresis. A/A GENOTYPES-156bp, B/B GENOTYPES 84 AND 72 bp, A/B GENOTYPES 156, 84 AND 72bp. M-100 bp DNA ladder

Table 3: Calculated Allelic and genotypic frequencies of PRL gene in Sahiwal cattle:

Locus	Genotype frequency			Allele frequency		Shanon Index	Chi Square test HW test	Heterozygosity (Ho)
	AA (8)	AB (5)	BB (3)	A	B			
PRL-RFLP-RSai	0.5	0.312	0.188	0.752	0.248	0.378	4.30216 Degree of freedom:1 Probability:0.038602	0.1898

Table 4: Associations between the prolactin (PRL) genotypes with milk yield traits in Sahiwal Cattle

Traits	Genotypes		
	AA (8)	AB (5)	BB (3)
Lactation milk yield	1609.36±31.28 ^a	1362.02±25.08 ^b	1525.25±29.25 ^a
Peak Yield	13.20±13.12	12.95±8.23	11.98±11.02

Data presented in parentheses are the total number of animals. Least square (LS) means bearing different superscripts in a row indicate statistically significant differences. * Significant at 5% level ($p < 0.05$).

Conclusion

In this study the association of prolactin gene with milk production and milk constituent traits were analysed and calculated. Amplification of DNA samples revealed 156bp products and digestion with RsaI showed three genotypes viz; (AA, AB and BB) The frequency of genotypes AA, AB and BB were 0.5, 0.312 and 0.188, respectively. The frequency of allele A and B were 0.752 and 0.248 with Shanon Index of 0.378. The result shows that cows with AA genotypes yielded most milk as compared to cows with genotype AB and BB. The observed homozygosity and heterozygosity of prolactin gene in the population were not within the Hardy- Weinberg expectation as revealed by the Chi-Square test value (4.30216)

Above all, the information obtained from this study is expected to help underscore the durability and performance

of Sahiwal cattle outside of its native hometract and in the agro-climatic condition of Northeast region, pertaining particularly to state of Assam.

Conflict of interest

Authors declare no Conflict of Interests for this article.

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References

- Alfonso E, Rojas R, Herrera JG, Ortega ME, Lemus C, Ruiz J, *et al.* Polymorphism of the prolactin gene (Prl) and its relationship with milk production in American Swiss cattle. *Afr J Biotechnol.* 2012;11:7338-7343.
- Alipanah M, Kalashnikova A, Rodionov GV. Kappa-casein and PRL-RsaI genotypic frequencies in two

- Russian cattle breeds. Arch Zootec. 2008;57(218):131-138.
3. Brym P, Kamiński S, Wójcik E. Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. J Appl Genet. 2005;46(2):179-185.
 4. Chauhan A, Tiwari M, Singh SP, Sharma D, Kumar S, Goel R, *et al.* Association of PIT-1 gene polymorphism with milk production traits in Sahiwal cattle. Indian J Anim Sci. 2015;85(6):610-612.
 5. Chrenok P, Huba J, Oravcova M, Hetenyi L, Peskovicova D, Bulla J. Genotypes of bGH and bPRL genes in relationships to milk production. Proceedings of the 50th Annual Meeting of the EAAP; 1998 Aug 24-27; Zürich, Switzerland. Book of Abstracts. p. 40.
 6. Chung ER, Kim WT, Lee CS. DNA polymorphism of kappa casein, B-lactoglobulin, growth hormone and prolactin genes in Korean cattle. Aust J Anim Sci. 1996;11:422-427.
 7. Gayari I, Ferdoci AM, Aziz A, Zaman GU, Akhtar F, Deka R. Prolactin gene polymorphism in crossbred cattle of Assam and its association with productive and reproductive traits. Int J Chem Stud. 2020;8(2):717-22.
 8. Ghasemi N, Zadehrahmani M, Rahimi G, Hafezian SH. Associations between prolactin gene polymorphism and milk production in Montbeliard cows. Int J Genet Mol Biol. 2009;1(3):48-51.
 9. Harvey WR. Least-squares analysis of data with unequal subclass numbers. Technical Report ARS H-4. Washington, DC: U.S. Department of Agriculture, Agricultural Research Service; c1975.
 10. Ishaq R, Suleman M, Naeem RM, Yousaf M, Shah A, Ghafoor A. Prolactin gene polymorphism in Nili-Ravi buffaloes in relation to Sahiwal and Achai cattle. Int J Dairy Technol. 2012. doi:10.1111/j.1471-0307.2012.00875.x.
 11. Karuthadurai T, Chakravarthy AK, Kumaresan A, Kour A, Nag BS, Rana E, *et al.* Identification of genetic marker for prolactin gene related to milk yield in pedigreed Sahiwal population. Indian J Anim Res. 2019;53(5):566-571.
 12. Kramer CV. Extension of multiple range test to group correlated adjusted mean. Biometrics. 1957;13:13-20.
 13. Livestock Census (2019). The 20th Livestock Census-2019. Key Results. Available from: <http://www.drishtias.com>. Accessed 3 Feb 2022.
 14. Mahajan V, Parmar SNS, Thakur MS, Sharma G, Vaishali PM. Prolactin gene polymorphism and its association with milk production in Malvi, Nimari and Frieswal cattle. Indian J Anim Sci. 2012;82(4):388-391.
 15. Mitra A, Schlee R, Balakrishnan CR, Pirchner F. Polymorphisms at growth hormone and prolactin loci in Indian cattle and buffalo. J Anim Breed Genet. 1995;112:71-74.
 16. Niu M, Harvatine KJ. Short communication: The effects of morning compared with evening feed delivery in lactating dairy cows during the summer. J Dairy Sci. 2018;101:396-400.
 17. Sacravarty G, Vadodaria VP, Brahmkshtri BP, Shah RR, Solanki JV. Prolactin gene polymorphism and its association with economic traits in Kankrej cattle. Indian J Dairy Sci. 2008;61(4):273-276.
 18. Tona GO, Oseni SO, Adeyemo BY. Morning, evening and entire lactation milk yield and composition of Jersey cows under hot and humid tropical environment of Nigeria. Int J Sci Res. 2013;5(6):2324-2328.
 19. Udina IG, Turkova SO, Kostuchenko MV, Lebedeva LA, Sulimova GE. Polymorphism of bovine prolactin gene, microsatellites, PCR-RFLP. Russ J Genet. 2001;4:407-411.