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Study of milk production genes and their association with production traits in Rathi cattle

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ABSTRACT

The study aimed to identify polymorphism of LEP, and STAT5A milk-producing genes and their association with production traits in Rathi cattle. An overall 160 animals were selected from a population of Rathi cattle from Livestock Research Station, Rajasthan University of Veterinary and Animals Sciences, Bikaner (Rajasthan). The phenotypic information on total milk yield, peak yield and lactation length were recorded from the years 2012-2018. Each animal's milk sample (100 ml) was analysed for milk composition parameters. Genomic DNA was extracted from the whole blood sample through the spin column method and association analysis was done. The polymorphism have been observed in exon-3 (454 bp) of LEP gene, intron 9-10 (224 bp) and intron-15-exon-16 (379 bp) of STAT5A genes by PCR-SSCP and revealed two types of genotypic pattern. The STAT5A exon-7 (215 bp) also showed polymorphism with three genotypic patterns. The AB pattern of LEP exon-3 and AA pattern of intron 9-10 and intron-15-exon-16 of STAT5A genes showed a significant effect on total milk yield. The BB genotypic pattern of the STAT5A exon-7 gene showed a significant impact on peak yield. The AB pattern of LEP and STAT5A genes were associated with more milk fat % in the studied population of Rathi cattle. All these genes showed a significant effect on total solids. The association analysis of LEP and STAT5A genes with different milk production and milk composition traits illustrated the worth of these genes for marker-assisted selection of dairy cattle. Thus, the present study was designed to unmask the relevant genetic factors responsible for variation in milk performance traits.

Keywords: Genotype, Lactation length, PCR, Peak yield, Polymorphism, SSCP

In dairy animals, milk production and its composition are important economic traits. Quality and quantity of milk are controlled by SNP primarily linked to candidate genes and QTLs in bovines. For improvement in these products and economic traits, selection could be based on heritability, genetic variations and the genetic make-up of dairy animals. The selection for beneficial QTLs increases the frequency of that allele in the population and production and performance can be magnified (Kumar *et al.* 2020). The Rathi cattle breed is known for its hardiness to withstand the harsh agro-climatic conditions in the arid and semi-arid zone of Rajasthan. Dhaka *et al.* (2015) observed that Rathi cattle produce good lactation milk yield in the semi-arid region of Rajasthan. LEP and STAT5A genes are candidate genes for milk production (total milk yield, peak yield and lactation length) and milk composition traits (fat %, total solids %, protein content %, Lactose % and SNF %).

The bovine Leptin gene includes its promoter region, three exons and two introns and spanned about 18.9 kb. The LEP gene shows an association with milk production and milk fat %. It has role in appetite, metabolism, growth, nutritional status, fertility and reproductive function and milk production in cattle. Exon 2 and exon 3 parts of the LEP gene analyzed among three cattle breeds (Singh *et al.* 2014). STAT5A genes are located on bovine chromosome 19 and associated with some serum cytokines, mastitis and milk production traits (Zhou *et al.* 2020). STAT5A gene has been sequenced in bovine. The results of sequencing showed evolutionary divergence (Naveed *et al.* 2021). Overexpression of the STAT5A gene has a positive impact on lactation, mammary gland development and milk protein gene expression (Li *et al.* 2020). Signal transducers and activators of transcription 5 (STAT5) are involved in the proliferation, and differentiation of mammary gland epithelial cells (Tian *et al.* 2020). The role of LEP and STAT5A genes in determining milk production and milk constituents is very important. Therefore it is important to analyze these genes and their association in Rathi cattle in the arid zone of Rajasthan.

MATERIALS AND METHODS

Sample collection: The Rathi cattle (160) for the present trial were selected from Livestock Research Station, RAJUVAS, Bikaner (Rajasthan) having a minimum of 120

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days of lactation. The phenotypic information on various lactation traits such as total milk yield, peak yield and lactation length were recorded on the farm from the year 2012-2018. Information on outlier animals and aberrant lactation was excluded from the present study. The milk sample (100 ml) was collected and analyzed for fat, protein content, lactose, solid not fat and total solids by automated milkosan tester.

Blood samples, DNA extraction, purification and quantification: About 2 ml of blood samples were collected aseptically from jugular vein puncture into the EDTA containing vacutainers tube. Genomic DNA from the whole blood sample was extracted through the spin column method as per the standard method. The purity (OD ratio 260/280) and concentration (ng/µl) of extracted genomic DNA was determined by a Nanodrop spectrophotometer. The quality of genomic DNA was assessed through 0.8% agarose electrophoresis method.

PCR amplification and PCR-SSCP: The sequences of primers, the accession number of the reference sequence and expected fragment length of the different selected regions are represented in Table 1. The allele-specific PCR was carried out as standard protocol. The quality and size of the PCR amplicons were assessed on a 1.5% agarose gel containing ethidium bromide (1% solution). To detect mutations, SSCP analysis was performed according to guidelines described with slight modifications. Denatured PCR products were subjected to 8% polyacrylamide gel electrophoresis in Tris-Borate-EDTA buffer at constant voltage (120 V) for 15 h at a constant temperature of 4°C, and then gels were stained (1% EtBr) and documented by gel documentation system.

Statistical analysis and association study: The gene and genotypic frequencies of these genes were analyzed through the POPGENE program (ver. 3.1). The significance of association of traits with genetic marker information was done by a univariate analysis of variance carried out through a general linear model (GLM) procedure of SPSS ver.25.0 for Windows (SPSS Inc., Chicago, IL, USA).

$$X_{ijk} = \mu + g_i + h_j + e_{ijk}$$

Where, X_{ijk}, mean observed value of milk performance parameter; µ, general mean; g_i, fixed effect of the i^{th} genotype; h_j, fixed effect of the j^{th} location; e_{ijk}, random error, e_{ijk} ~NID (0, σ_e^2).

RESULTS AND DISCUSSION

Polymorphism analysis and Gene and genotype frequencies: The *LEP* exon-3 (454 bp), *STAT5A* intron 9-10 (224-bp) and *STAT5A* intron-15 exon-16 (379 bp) segments revealed the presence of two unique patterns reflecting their respective SSCP genotypic pattern (AA and AB) (Fig.1, 3 and 4). In these genes, the AA genotypic pattern was more prevalent than the AB genotype in the studied population The *STAT5A* exon-7 (215-bp) region revealed the presence of three unique patterns reflecting their respective SSCP genotypic patterns, viz. AA, AB and BB (Fig. 2). The AA pattern was more common than other genotypic patterns (Table 2). Our results show similarities with Cobanoglu *et al*. (2020), Kiyici *et al*. (2022) and Putra *et al*. (2020)

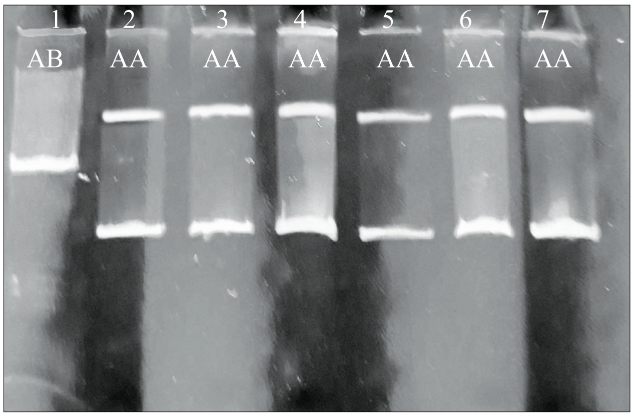

Fig 1. SSCP patterns of *LEP* exon-3 gene.

Table 1. Primer sequences and expected fragment sizes of PCR products of selected genomic regions

Selected region	Primer sequences	GenBank accession No.	Expected fragment length	References
LEP Exon-3	Forward 5'-GGGAAGGGCAGAAAGATAG-3' Reverse 5'-CCAAGCTCTCTCCAAGCTCTC-3'	JQ711179.1	454	Kumar *et al*. (2018)
STAT5A Exon-7 region	Forward 5'-CTG CAG GGC TGT TCT GAGAG-3' Reverse 5'-TGG TAC CAG GACTGTGCACAT-3'	AJ237937	215	Selvaggi *et al*. (2009)
STAT5A Intron 9-10	Forward 5'CCAGGGTGCATACAGGACAG3' Reverse 5'GCAGGTTACGAGGACTCAGG3'	AJ237937.1	224	He *et al*. (2012)
STAT5A Intron-15 Exon-16	Forward 5'CTTGGGAGAACCTAACATCACT3' Reverse 5'AGACCTCATCCTTGGGCC3'	AJ237937.1	379	Flisikowski *et al*. (2004)

Table 2. Gene and genotypic frequencies of *LEP* and *STAT5A* gene detected through SSCP analysis

Gene	Genotypic pattern			Gene frequency	
	AA	AB	BB	A	B
Exon-3 region of *LEP*	0.832 (133)	0.168 (27)	-	0.915	0.085
Exon-7 of *STAT5A*	0.594 (95)	0.288 (46)	0.118 (19)	0.738	0.262
Intron 9–10 of *STAT5A*	0.843 (135)	0.156 (25)	-	0.921	0.069
Intron-15 exon-16 of *STAT5A*	0.875 (140)	0.125 (20)	-	0.937	0.062

Note: Number in parenthesis is number of observations, - = nil observation.

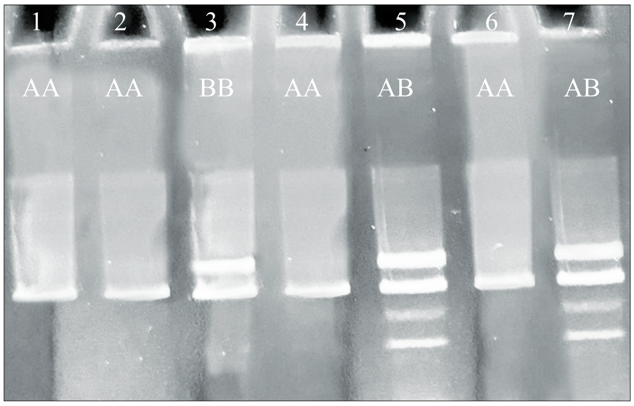

Fig. 2. SSCP patterns of STAT5A exon-7.

who observed polymorphism in *LEP* and *STAT5A* genes in different breeds of cattle. Results of Yadav *et al*. (2020) show dissimilarities with our results in *LEP* gene with three genotypic patterns- AA, AG and GG in Hardhenu cattle population.

Association studies: In the present study, genotypic patterns of exon-3 of LEP and intron 9-10 of STAT5A gene had a significant ($P<0.05$) effect on TMY, while a non-significant effect on PY, LL had been observed (Table 3). Rambachan *et al*. (2019) found an association of *LEP* gene with these traits in Hariana cattle. Dar *et al*. (2021) also found an association of the *LEP* gene with milk production traits in different cattle breeds. The genotypic patterns of exon-7 of STAT5A gene had a significant ($P\leq0.05$) effect on PY and a non-significant ($P>0.05$) effect on TMY, and

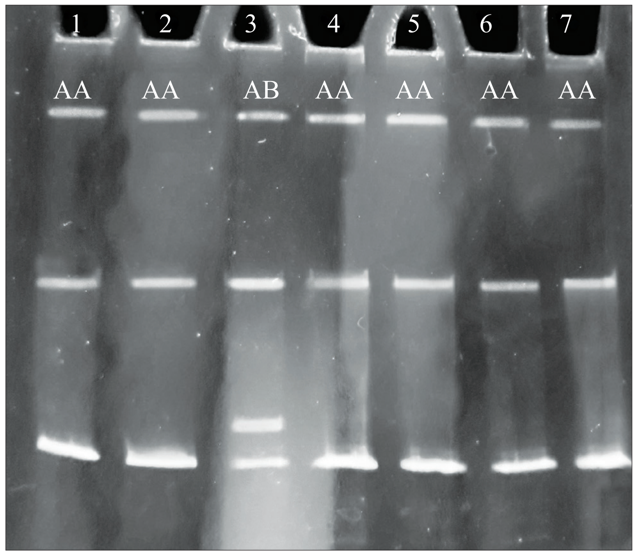

Fig. 3. SSCP patterns of *STAT5A* intron 9–10.

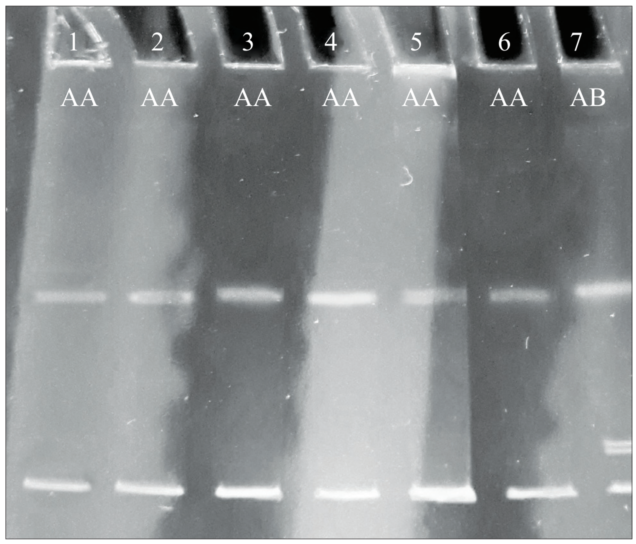

Fig. 4. SSCP patterns of *STAT5A* intron-15 exon-16.

LL. Similar results were found by Al-Azzawi *et al.* (2020) in Holstein cows while, in Agerolese cows observed a non-significant association ($P>0.05$) of this region with TMY, PY and LL. Intron-15 and exon-16 of STAT5A gene had a significant ($P\leq0.05$) effect on peak yield, while a non-significant ($P>0.05$) effect on total milk yield, and lactation

Table 3. Effect of genotypic patterns of genes on milk production traits

Gene	Genotypes	Sign	Total milk yield (kg)	Sign	Peak yield (kg)	Sign	Lactation length (days)
Exon-3 region of *LEP*	AA	*	1932.90^{b}±80.25	NS	11.50±0.29	NS	283.19±7.45
	AB		1883.13^{a}±90.58		11.91±0.36		272.80±10.08
Exon-7 of *STAT5A*	AA	NS	1982.87±90.25	*	11.27^{c}±0.29	NS	285.95±9.88
	AB		1877.75±96.43		11.54^{b}±0.32		275.84±9.80
	BB		1794.90±93.35		12.90^{a}±0.41		269.05±12.17
Intron 9–10 of *STAT5A*	AA	*	1967.72^{b}±88.67	NS	11.56±0.28	NS	280.59±9.60
	AB		1676.75^{a}±96.37		11.95±0.45		280.20±10.33
Intron-15 exon-16 of *STAT5A*	AA	NS	1940.2±92.23	NS	11.47±0.21	NS	281.64±9.60
	AB		1875±91.34		12.45±0.42		272.80±9.89

Note: Sign, Significance; *, Significant; NS, Non-significant.

Table 4. Effect of genes on milk composition parameters

Parameter	*LEP* exon-3			*STAT5A* exon-7				*STAT5A* intron 9–10			*STAT5A* intron-15 exon-16		
	Sig	AA	AB	Sig	AA	AB	BB	Sig	AA	AB	Sig	AA	AB
Fat (%)	*	$4.49^{b}\pm$ 1.30	$4.63^{a}\pm$ 1.70	*	$4.27^{b}\pm$ 0.40	$4.77^{a}\pm$ 0.1	$3.56^{c}\pm$ 0.23	*	$4.4^{b}\pm$ 1.36	$4.83^{a}\pm$ 1.40	*	$4.4^{b}\pm$ 1.38	$5.26^{a}\pm$ 1.32
PC (%)	NS	3.8± 0.65	3.76± 0.48	NS	3.9± 0.70	3.8± 0.57	3.9± 0.62	NS	3.8± 0.64	3.79± 0.52	NS	3.86± 0.61	3.95± 0.64
Lac (%)	NS	4.52± 0.51	4.58± 0.32	NS	4.5± 0.48	4.5± 0.43	4.5± 0.50	NS	4.5± 0.56	4.5± 0.30	NS	4.5± 0.56	4.52± 0.39
SNF (%)	NS	8.93± 0.69	8.92± 0.51	NS	8.92± 0.69	8.98± 0.58	9.03± 0.56	NS	8.93± 0.69	8.97± 0.45	NS	8.9± 0.68	9.05± 0.45
TS (%)	*	$7.03^{b}\pm$ 1.30	$13.3^{a}\pm$ 1.42	*	$12.8^{b}\pm$ 1.02	$13.5^{a}\pm$ 0.4	$12.2^{c}\pm$ 0.30	*	$12.4^{b}\pm$ 1.50	$13.4^{a}\pm$ 1.35	*	$12.0^{b}\pm$ 1.55	$13.2^{a}\pm$ 1.28

Note: PC, Protein content; Lac, Lactose; SNF, Solid not fat; TS, Total solids; Sign. , Significance; *, Significant; NS, Non-significant.

length. In Holstein cows, He *et al.* (2012) also observed a non-significant (P>0.05) effect of intron-15 exon-16 of STAT5A gene on milk yield. Similar work was reported in Jersey cows, Selvaggi *et al.* (2013) who had observed no significant difference in genotypes of intron-15 exon-16 of STAT5A gene with TMY, PY and LL.

In present study, the genotypic patterns of the LEP and STAT5A genes had a significant (P≤0.05) effect on milk fat (%) and total solids (%) while a non-significant (P>0.05) effect on lactose (%), SNF (%) and protein contents (%) (Table 4). A similar association of these genes with milk yield traits and milk composition traits was found by Glantz *et al.* (2012) in different cattle breeds. Some researchers showed dissimilarities with the present work. In Holstein cows, Michel-Regalado *et al.* (2020) observed a non-significant association of intron-9 of the STAT5A gene with milk constitution traits. Rathi cattle were polymorphic in both studied genes and AA genotypic pattern was found more frequently than other patterns. In this study, it can be concluded that LEP and STAT5A genes were highly associated with milk production and milk composition in Rathi cattle of Rajasthan.

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