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SHORT COMMUNICATION

Elucidating the associations of polymorphism of growth hormone gene with milk production traits in Jamunapari goats of India

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Abstract: Growth hormone (GH) gene can be utilized as a major gene because its polymorphisms have been associated to milk traits in various livestock species. The aim of this present study was to investigate the polymorphism in the exon 1, 3 and 5 of gGH (goat GH) gene and to evaluate the possible associations with milk traits in Jamunapari goat, maintained at the ICAR-Central Institute for Research on Goats, Makhdoom, Uttar Pradesh, India.In this study, 100 primiparous lactating goats were randomly chosen, and their productive performances like 90-days, 140-days and total milk yield as well as milk compositional traits like fat %, SNF%, casein%, protein%, lactose% and ash % of three consecutive lactations were recorded. The exon 1, 3 and 5 of the gGH gene was PCR amplified and the resulting products were analyzed by Single-strand conformation polymorphism (SSCP). Two conformational patterns (A and B) for each the GH exon 3 and 5 were detected but no polymorphism existed in exon 1 of growth hormone gene.The frequency of the patterns varied from 41.4 to 58.6% in these two fragments. Association studies of SSCPs patterns at GH gene with milk production and milk composition traits in Jamunapari breed showed that only polymorphic patterns at exon 3 were positively associated with milk production traits, i.e., with 90-days milk yield, 140-days milk yield and total milk yield of animals but there was no significant effect on milk composition traits. Animals with pattern A/A for exon 3 were significantly superior milk producers (P<0.01) than animals having B/B pattern for 90-days, 140-days and total milk yield of animals, whereas GH conformation patterns (A and B) for exon 3 didn't have any impact on milk compositions. Further, in case of exon 5, the milk yields and milk compositions of animals didn't vary significantly between animals having different GH conformation patterns (A and B).These findings may be used for preserving genetic diversity of the population and may be useful for marker assisted selection in order to improve milk production of this breed.

Keywords: Growth hormone gene, Milk traits, SSCP polymorphism, Jamunapari Goat

Milk production is a physiological function that is under the control of several genes. Genotyping animals for all the genes encoding a polygenic traits seems impractical and so it appears more realistic to focus on only a few genes having effects that account for a significant genetic variation in milk production traits. The growth hormone (GH), an anabolic hormone synthesized and secreted by the anterior pituitary somatotroph cells, plays an important role in regulation of postnatal growth and metabolism in mammals and is directly involved in animal processes such as lactation, protein, lipid and carbohydrate metabolism, tissue growth, and fertility in dairy animals (Seevagan et al. 2015; Agaoglu et al. 2019).The wide physiological activities of growth hormone gene make it an important candidate gene worth investigating for its role in growth and milk production traits of animals. Several authors demonstrated the associations of GH gene polymorphisms with growth (Singh et al. 2015; Pandya et al. 2021; Rashijane et al. 2022), milk yield (Moneva et al. 2020) and milk compositions (Dettori et al. 2009; Dettori et al. 2013) in different goat breeds.

Single-strand conformation polymorphism (SSCP) is a powerful method for identifying sequence variation in amplified DNA. The SSCP analysis of genes, whose product is associated with production traits, could be a valuable alternative approach for the establishment of allelic variants useful as markers to aid selection. Therefore, growth hormone gene is a potential target for studies of genetic sequence variation in connection with

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production performance of animals. This prompted us to investigate Single-strand conformation polymorphisms (SSCP) in each of the three exons of the goat GH gene and to establish the possible relationships of polymorphism patterns with milk production (lactation and milk compositional traits) traits in Jamunapari goats.

The present study was carried out on Jamunapari goats belonging to a farm located at ICAR- Central Institute for Research on Goats, Makhdoom, Uttar Pradesh, India. A total of 100 primiparous lactating goats were randomly chosen, and the lactation and milk compositional traits of these selected animals were recorded for three consecutive lactationsfor a period of 3 years. Milk yields at 90-days, 140-days and total lactational yield were recorded for each lactation and fresh milk samples for all animals under study were collected at 15 days intervals from kidding to 90 days of lactation. Milk samples were analyzed to estimate the fat, solid-not-fat (SNF), casein, protein, lactose and ash content of milk (Prajapati et al. 2017). Blood sample (8–10µl) was collected from each animal by jugular vein puncture in vacuum tubes treated with 15% ethylene di-amine tetra acetic acid (EDTA) as an anticoagulant and stored at 4°C till further processing. Genomic DNA was isolated from whole blood using phenol-chloroform method (Sambrook et al. 1989) with minor modifications.

Based on the published nucleotide sequence of exon 1, 3 and 5 of goat growth hormone (Marques et al. 2003 and Malveiro et al. 2001a,b), the three exons of gGH gene were amplified by PCR using the following primer pairs shown in Table 1.

PCR reactions were performed using advanced primus 96 thermocycler using 200 mM each of dATP, dTTP, dGTP and dCTP; 50mM KCl, 10mM Tris-HCl (pH 9.0, 0.1% Triton X -100, 1.5 mM magnesium chloride; 0.75 unit of Taq DNA polymerase; 0.5μM of each primers and 50–100 ng of genomic DNA in the final volume of 25µl). The amplification began with denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56–61°C for 30s extension at 72°C for 30 s and final extension at 72°C for 5 min. The amplified product of each fragment was analyzed by electrophoresis on 2% agarose gel (5V/cm) using ethidium bromide staining.

PCR-Single strand conformation polymorphism (PCR-SSCP), a method for mutation detection, relies on the fact that denatured DNA molecules migrate across non-denaturing polyacrylamide gel according to their size and their sequence. For SSCP analysis, 6 µl of the each PCR product is mixed with 8 µl of denaturing loading buffer (0.05% xylene cyanol and 0.05% bromophenol blue, 5.5mM EDTA, pH 8.0, in formamide), denatured at 95°C for 5 min, and snap-chilled on ice for 2 min. Samples were then loaded onto a polyacrylamide gel containing 0.5x TBE (0.045M Tris-borate, 0.001M EDTA, pH 8.0).Acrylamide concentration was 9.2%, glycerol concentration was 1%, *bis* ratio (29.1), running temperature 10°C, and TBE concentration was 0.5x in using Bio-rad Seq GT Electrophoresis Systems. After the electrophoresis run, gels were silver stained after fixing for 5 min and stained for 20 min.The PCR-SSCP gels were scored based on differential conformation and movement patterns of each of the single strand of amplified DNA (Orita et al. 1989).

To study the associations of SSCP patterns of growth hormone gene with milk production and milk composition traits, least-squares analysis of fitting constant (Harvey, 1990) was conducted with the following model:

$$Y_{jklmn} = \mu + Y_j + F_k + P_l + S_m + e_{jklmn}$$

Table 1: Primer sequences along with fragment length of exon regions of growth hormone gene

Exons	Primer sequences	Fragment length and localization(bp)	References
1	5′-CAGAGACCAATTCCAGGATC-3′ 5′-TAATGGAGGGGATTTTTGTG-3′	112 (360-471)	Marques et al. 2003
3	5′-GTGTGTTCTCCCCCCAGGAG-3′ 5′-CTCGGTCCTAGGTGGCCACT-3′	157 (1063-1219)	Marques et al. 2003
5	5′-AAAGGACAGTGGGCACTGGA-3′ 5′-CCCTTGGCAGGAGCTGGAAG-3′	289 (1854-2142)	Malveiro et al. 2001a,b

Table 2: The band patterns and their frequencies at different fragments of growth hormone gene of Jamunapari goat

Exons	No. of pattern	Band Patterns	Pattern Frequencies (%)
gGH-1	1	A	100
gGH-3	2	A	41.4
		B	58.6
gGH-5	2	A	45.5
		B	54.5

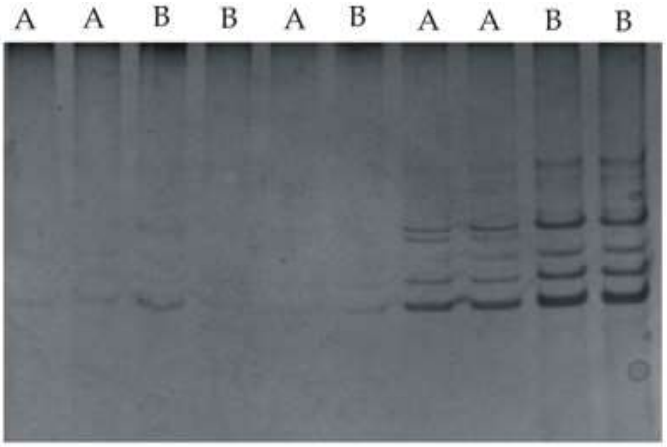

Fig. 1 PCR-SSCP of DNA region spanning from exon 3 of gGH gene in Jamunapari goat

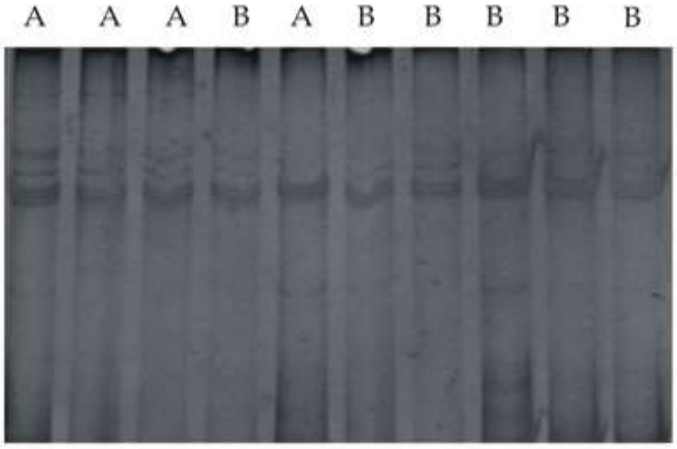

Fig. 2 PCR-SSCP of DNA region spanning from exon 5 of gGH gene in Jamunapari goat

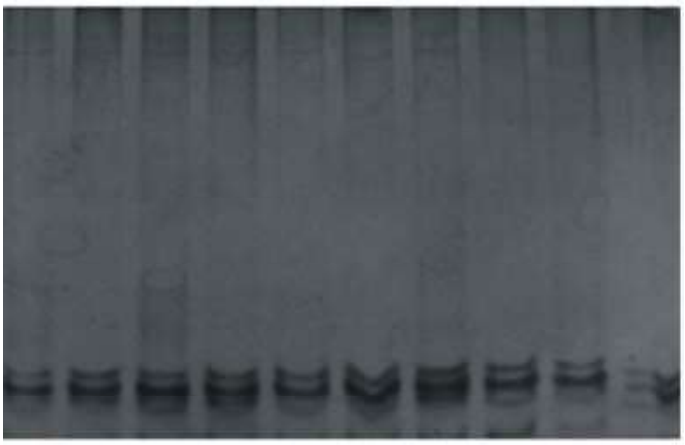

Fig. 3 PCR-SSCP of DNA region spanning from exon 1 of gGH gene in Jamunapari goat

Where, Y_{jklmn} is the record for the n^{th} animal, μ is overall mean, Y is the effect of j^{th} year of kidding (j =1,2,3), F_k is the effect of the k^{th} season of kidding (k=1, 2), P_l is the effect of the l^{th} parity of dam(l=1, 2, 3, 4,5, 6 or more), S_m is the effect of the m^{th} SSCP patterns (m= 1, 2) and e_{jklmn} is the residual error element with standard assumptions.

In this study, SSCP analysis of exons 1, 3 and 5 of the gGH gene of Jamunapari goats was performed on the fragments amplified by PCR using the primers described in Table 1, which showed the expected lengths. Polymorphism of gGH gene in Jamunapari goats showed two conformation patterns (A and B) for each of the GH exon 3 and 5 (Fig. 1 and 2), whereas no polymorphism was found in exon 1 (Fig. 3). The frequencies of each pattern are depicted in the Table 2. The SSCP pattern of growth hormone gene of Jamunapari goat in this study revealed polymorphism only in exon 3 and 5 regions. SSCP patterns analysis of exon 1, 3 and 5 of growth hormone gene of Algarvia goat was also observed by Malveiro et al. (2001a, b) and they reported that two conformational patterns existed in exon 1, four in exon 3 and five in exon 5 as compared to the present study. Marques et al. (2003) reported the existence of 6, 10, 5 SSCP pattern in exons 3, 4 and 5, respectively in Serrana goat. Vyas et al. (2008) also observed that high level of polymorphism existed at exons 3, 4 and 5 of the growth hormone gene of Jamunapari goats by SSCP analysis. In their study, they reported the presence of 7, 5 and 4 conformational patterns in exons 3, 5 and 4 of gGH gene of Jamunapari goats. Singh et al. (2018) observed the genetic polymorphism for growth hormone gene at 1 exon in Jamunapari kids by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), which was contrary to the present study. PCR-SSCP patterns of exons 4 and 5 of growth hormone gene of Jakhrana goats of India revealed the presence of 3 conformation patterns (viz., A, B and C) as reported by Gupta et al. (2009). In another study on polymorphism at the goat GH (gGH) gene, Mousavizadeh et al. (2009) reported the presence of nine conformational patterns in exon 4 of the gGH gene in SSCP-PCR analysis in Iranian Talli goats. The exon 3 of the gGH gene in Sarda goats was analysed after SSCP-PCR analysis by Dettori et al. (2009) and they detected six conformational patterns at the exon3 region of goat hormone gene. Wickramaratne et al. (2010) observed 3 conformational patterns (A, B and C) at each of exon 3 and 5 regions of growth hormone gene in Osmanabadi and Sangamneri goats. Further, genetic variations in the goat growth hormone (gGH gene) were investigated by single strand conformation polymorphism (SSCP) analysis in Sirohi goats by Kumar et al. (2011) and the fragments consisting of exon 1, exon 4 and exon 5 revealed 6 variants.

The least-squares means along with standard errors associated with milk production and milk composition parameters for SSCP patterns at exon 3 and 5 of GH gene in Jamunapari goats have been depicted in Table 3. Different environmental factors associated with milk production and milk constituents traits of Jamunapari goats were found non-significant (P>0.05) for this set of data. The results also showed that SSCP patterns at exon

Table 3: Least-squares means (±SE) for lactation traits and milk compositional traits of Jamunapari goat associated with patterns of exon3 and exon 5

Traits	Patterns			
	Exon 3		Exon 5	
	A/A	B/B	A/A	B/B
Milk Production traits				
90-days MY	74.03[a]±3.03 (41)	65.05[b]±2.98 (58)	68.18±3.28 (45)	69.73±3.07 (54)
140-days MY	102.87[a]±4.52 (36)	89.72[b]±4.15 (52)	95.52±4.72 (39)	95.76±4.19 (49)
Total MY	109.46±5.24 (41)	96.75±4.73 (58)	101.53±5.18 (45)	103.08±4.84 (54)
Milk compositional traits				
Fat %	2.81±0.07 (41)	2.89±0.06 (58)	2.82±0.07 (45)	2.88±0.06 (54)
SNF %	9.26±0.07 (41)	9.21±0.06 (58)	9.31±0.07 (45)	9.16±0.06 (54)
Casein %	2.93±0.03 (41)	2.91±0.02 (58)	2.93±0.02 (45)	2.92±0.02 (54)
Protein %	3.28±0.03 (41)	3.23±0.03 (58)	3.26±0.03 (45)	3.24±0.03 (54)
Lactose %	5.00±0.07 (41)	4.90±0.06 (58)	5.01±0.06 (45)	4.89±0.06 (54)
Ash %	0.79±0.01 (41)	0.78±0.01 (58)	0.79±0.01 (45)	0.78±0.01 (54)

3 had only significant effect (P<0.01) on all milk yield traits. Animals with pattern A/A for exons 3 were significantly superior milk producers (P<0.01) for 90-days, 140-days and total milk yield of animals than animals having B/B pattern, whereas the milk yields and milk compositions of animals of different GH conformation patterns (A and B) for exon 5 didn't varied significantly between each other. Associations were established in the Portuguese Algarvia goat breed between gGH SSCP polymorphic patterns in exon 4 and 5 and milk production (Malveiro et al. 2001), later confirmed by Marques et al. (2003) for the Sarda goat. SSCP polymorphic patterns in exon 3 were also associated (P<0.01) with milk yield, fat and protein percentages, and with lactose content (P<0.05) in Sarda goats (Dettori et al. 2009). Further, Dettori et al. (2013) also reported that polymorphic patterns at exon 1 and exon 4 were positively associated with milk production, and with both fat and protein content in Sarda goat. The findings of the present study are preliminary based on small samples, and it should be confirmed on a larger sample size. The gGH gene polymorphisms may be used for marker assisted selection in Jamunapari goat, also taking into account short and long-term effects on population structure and rates of inbreeding, in order to improve dairy production along with preserving genetic diversity of the population.

In this study, Single-strand conformation polymorphism (SSCP) analysis of exons 1, 3 and 5 of the growth hormone gene of Jamunapari goats revealed that two conformation patterns (A and B) for each of the GH exon 3 and 5 but no polymorphism existed in exon 1 of growth hormone gene. Association studies between SSCPs patterns at GH gene with milk production and milk composition traits in this breed showed that only polymorphic patterns at exon 3 were positively associated with milk production traits of animals. As SSCP polymorphism at the exon 3 of the gGH exert a positive influence in milk production of Jamunapari goats in this study, so there is a possibility of exploring this approach for the search of genetic markers located at this region.The SSCP polymorphic variation makes it a potential candidate for the establishment of the association with quantitative traits. If specific haplotypes can be defined at this candidate gene that could be associated with milk production traits as well as milk composition traits, it would be a valuable genetic resource for improvement of this caprine breed.

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