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The use of PCR-RFLP techniques to investigate POU1F1 gene polymorphism in Sirohi goats

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Abstract
Background: Sirohi goats are raised for their meat and milk, thus rendering them multipurpose animals even in poor quality rearing conditions, the animals are well-known for their lactation and weight gain. Thus, the purpose of the current study was to investigate the association between the growth trait in Sirohi goats and the polymorphism of the POU1F1 gene.
Methods: Fifty Sirohi goats in total, grouped into four age groups: 3-6 months (n = 12), 6-9 months (n = 12), 9-12 months (n = 12), and above 12 months (n = 14). Estimates were made for body weight and body measurements such as body length (inches), wither height (inches), heart girth (inches), and paunch girth (inches). Genomic DNA was extracted from whole blood using the method described by John *et al.* (1991) followed by PCR amplification. *PstI* restriction enzymes were used to perform RFLP on the PCR amplicon in order to detect polymorphism.
Results: The studied samples were monomorphic with TT genotype an observed genotypic frequency of TT genotype was 1, while no genotypic frequency was found for the TC and CC genotypes. T allele allelic frequency was 1, whereas C allele allelic frequency was 0. The existence of monomorphism in the populations of Sirohi goats indicates that the T allele has been fixed in the population, which has led to HW disequilibrium. The fact that the POU1F1 gene is monomorphic in our study suggests that there hasn't been a mutation in the gene; hence we were unable to conduct any association study.

Keywords: Sirohi goat, PCR-RFLP, POU1F1 gene

Introduction
The goat, commonly referred to as the "poor man's cow," is a significant livestock species in India because of its high prolificacy, ability to adapt to the climate in the area, and widespread acceptance of its products by all communities. India stands 2nd in the world with 148.88 million goat population (Livestock Census, 2019) [9]. India has 37 recognized breeds of goat distributed across different agro-ecological regions (NBAGR, 2023) [11]. One of the well-known breed that originated in Rajasthan is the Sirohi goat. Sirohi goats are dual-purpose animals, being reared for both milk and meat. The animals are popular for their weight gain and lactation even under poor quality rearing conditions. According to Supakon (2009) [15], the pituitary specific transcription factor 1 (POU1F1) is one of the candidate genes that affects the animal's growth and body mass.
The POU1F1 gene, commonly referred to as growth hormone factor-1, GHF1, or pituitary specific transcription factor-1, PIT-1, is found on chromosome 1q21–22 in Caprine, Ovine, and Bovine species (Woollard *et al.*, 2000) [17]. The anterior pituitary gland is the primary site of expression for PIT-1, the POU1F1 gene's product. It regulates itself and the expression of the GH, PRL, and TSH-β genes. It is essential for differentiation, reproduction and survival of somatotropes lactotropes and thyrotropes. The inhibition of POU1F1 synthesis leads to a marked decrease in proliferation of cell lines producing PRL and GH (Sun *et al.*, 2002) [14]. In cattle and goats, the POU1F1 polymorphisms have associations to birth weight, body weight, milk yield, milk proteins, fat yield, and litter size. Considering the potential effect of POU1F1 gene on growth traits in various livestock, present research was undertaken to investigate caprine POU1F1 gene polymorphism and its association with the body weights of Sirohi goat.

Materials and Methods

A total of 50 Sirohi goats maintained at Livestock Farm, Amanala, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (MP). Further breed was grouped according to their age i.e., 3-6 month (n=12), 6-9 month (n=12), 9-12 month (n=12) and above 12 month (n=14). Body weight and body measurements like body length (inch), wither height (inch), heart girth (inch) and paunch girth (inch) were calculated for the age groups 3-6 month ((n=12), fortnightly interval); 6-9 month ((n=12), monthly interval); 9-12 month ((n=12), monthly interval) and above 12 month. ((n=14), quarterly). Three millilitre bloods sample was collected from each animal from the jugular vein in sterile blood collection tubes containing Ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The samples were brought to laboratory at 4^0C, temperature being maintained with the aid of ice packs. In the laboratory the blood was stored at -20ºC until DNA extraction. Genomic DNA was extracted from whole blood using the method described by John *et al.* (1991) [7].

The purity of the DNA was verified by measuring the ratio between the optical density reading at 260 nm and 280 nm wavelengths. DNA samples with an OD 260/280 ratio of 1.6 to 1.9 were used for further analysis. The quality of DNA was checked by using 0.8% agarose gel electrophoresis. The PCR-RFLP method was performed for polymorphism of POU1F1 gene using *Pst1*restriction enzyme.

The gene was amplified using following primers

(F):5'- CCATCATCTCCCTTCTT - 3'

(R):5' - AATGTACAATGTGCCTTCT - 3'

The PCR reaction contained 25.0 µl reaction mixture including 12.5 µl 2X PCR master mix (Gotaq® Green Master mix), 2 µl forward primer (10pmol /µl), 2 µl reverse primer (10pmol /µl), 1 µl Bovine serum albumin and 2.5 µl nuclease free water. To each reaction mix, 5 µl genomic DNA (30ng /µl) was added as template. The PCR was carried out in PCR machine. The cycling protocol was initial denaturation for 05 min at 94 ºC followed by 35 cycles (94 ºC for 45 sec, 54 ºC annealing for 1 min and 72 ºC for 1 min), with a final extension at 72 ºC for 05 min. PCR products were subjected to 1.5-2% agarose gel electrophoresis with 100 bp molecular weight marker (Himedia).

Amplified products were digested with *PstI* enzyme according to the manufacturer's instructions. The reaction mixture was incubated at 37 ºC in water bath for 5 hr. Then the digestion products were subjected to 2% submarine horizontal agarose gel electrophoresis with 50 bp molecular weight marker (Himedia). The allelic and genotypic frequencies of POU1F1 gene were estimated by standard procedure (Falconer and Mackay, 1996) [2]. The Chi-Square test (Snedecor and Cochran, 1994) [13] was used to test the populations of different breeds for Hardy-Weinberg equilibrium. To find out the association of trait with polymorphic variants of POU1F1 gene linear model was used (Harvey, 1990) [4].

Results and Discussion

In Sirohi breed of goats, significant difference (p<0.05) was observed for body weight and body measurements at different age groups. The results obtained in the current study are in accordance with Bharathidhasan *et al.* (2009) [1]. The PCR RFLP assay of 450 bp (Plate 01) PCR product showed the presence of TT genotype with a genotypic frequency of 1.0. TC and CC genotype were not observed in screened samples and had zero genotypic frequency. The result was comparable with the results of Lan *et al.* (2009) [8] as they found high genotypic frequency of TT genotype (0.917), very low genotypic frequency of TC genotype (0.083) and zero genotypic frequency of CC genotype in Inner Mongolian Cashmere goats. T allele allelic frequency was 1, whereas C allele allelic frequency was 0. The result was comparable with the results of Lan *et al.* (2009) [8] as they obtained very high allelic frequency of POU1F1-T allele (0.959) and almost negligible allelic frequency of POU1F1-C allele (0.041) in a large population size of 847 Inner Mongolian Cashmere goats. The existence of monomorphism in the populations of Sirohi goats indicates that the T allele has been fixed in the population, which has led to HW disequilibrium. The results were also in accordance with the reports of Sharma *et al.* (2013) [12]. The fact that the POU1F1 gene is monomorphic in our study suggests that there hasn't been a mutation in the gene; hence we were unable to conduct any association study contrary to this finding positive associations between POU1F1gene and growth traits in sheep and goats were reported by Jiangzuo (2010) [6], Wang *et al.* (2013) [16], Ma *et al.* (2017) [10] and Han *et al.* (2019) [3].

Although the gene and variation within is hypothesized to be associated with growth trait, but we were unable to detect any polymorphism at the proposed SNP site. Hence, further analysis could not be done. Also it should be noted that monomorphic pattern may have been observed due to either limited sample size or due to effect of breed. Hence looking into the vast goat genetic resources of India, the SNP should be tested on various breeds with larger sample size to establish its association with growth trait and further proposition of applicability as genetic marker for selection.

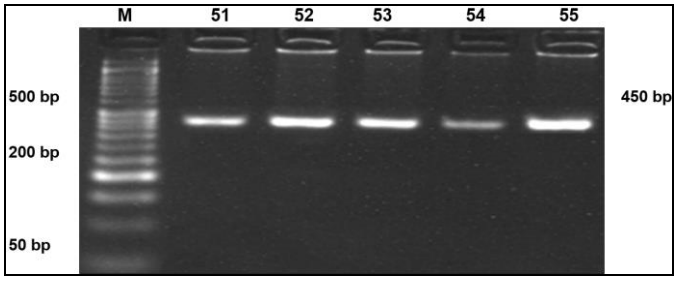

Plate 1: Agarose gel electrophoresis showing PCR-RFLP pattern of POU1F1 gene in Sirohi breeds of goat. M = 50 bp DNA ladder.

Conclusion

In this study, genetic analysis of the POU1F1 gene in Sirohi goats revealed a monomorphic pattern, with only the TT genotype observed and no presence of the TC or CC genotypes. This finding suggests that the T allele has been fixed in the population, leading to Hardy-Weinberg disequilibrium. The allelic frequency of the T allele was 1, while the C allele was absent. This monomorphism aligns with previous research findings in Inner Mongolian Cashmere goats and indicates a lack of mutation in the gene within the studied population. Although the POU1F1 gene is hypothesized to be associated with growth traits, the absence of polymorphism at the proposed SNP site in this study precluded further association analysis. However, positive associations between the POU1F1 gene and growth traits have been reported in other studies. Therefore, further analysis on larger sample sizes and different goat breeds is warranted to establish the gene's association with growth traits and its potential as a genetic marker for selection in goat breeding programs.

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