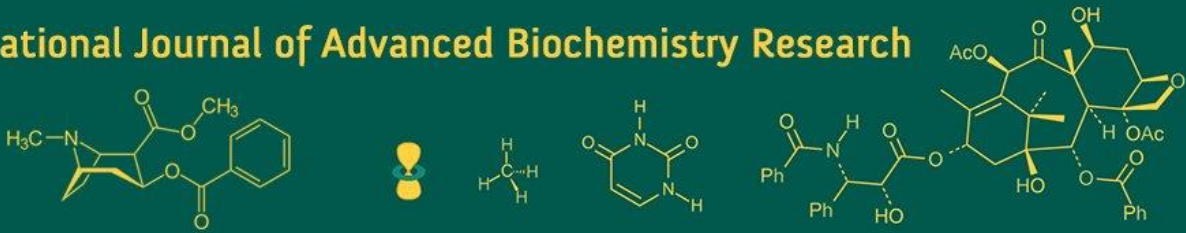


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## Uncovering genomic diversity and trait annotations in Garole sheep for improvement

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**Abstract**

The study conducted in Garole sheep aimed to identify genome-wide single nucleotide polymorphisms (SNPs) and annotate them to various production traits. Using Illumina OvineSNP50v1 genotyping data from 26 Garole sheep samples, a total of 46,820 high-quality SNPs were identified after rigorous quality control measures. Annotation of these SNPs revealed 760 SNPs associated with 175 candidate genes implicated in milk production traits, including genes regulating fatty acid oxidation, milk yield, and energy metabolism. Notable candidate genes identified included Acetyl-CoA carboxylase Beta (ACACB), Growth hormone receptor (GHR), and Leptin (LEP). This analysis provides valuable insights into the genetic architecture of Garole sheep, highlighting the potential for targeted breeding programs aimed at improving economically important traits such as milk yield and composition. Understanding the genetic basis of these traits is crucial for designing effective breed improvement strategies and enhancing overall productivity in Garole sheep.

**Keywords:** Garole, microarray, production traits, SNPs, sheep

**Introduction**

Sheep and goats, among the earliest domesticated animals, have been integral to human civilization for millennia, serving as sources of meat, wool, milk, and other essential products. Their domestication around 11,000 years ago marked a pivotal moment in agricultural history, facilitating the transition from nomadic hunting and gathering to settled farming societies (Chessa *et al.*, 2009) [7]. In India, this relationship with sheep is deeply entrenched in the socio-economic fabric, particularly among small and marginal farmers, who rely on these animals for livelihoods and sustenance.

India's vast and diverse landscape, ranging from the frigid Himalayan regions to the tropical climates of the south, has given rise to a rich array of sheep breeds adapted to various agro-climatic zones. Currently, the country boasts 45 registered sheep breeds, each uniquely suited to its local environment (NBAGR, 2020). The significant contribution of sheep to India's livestock sector is evidenced by their presence in the 19th Livestock Census, where they comprise approximately 13.87% of the total livestock population, with 74.26 million individuals (DAHDF, 2019). However, despite their historical and economic importance, many of these breeds face challenges related to productivity, adaptability, and sustainability. Traditionally, selective breeding practices have played a crucial role in enhancing desirable traits in sheep breeds. However, the genetic potential of these animals remains largely untapped. With the advent of modern genomic tools, there exists a tremendous opportunity to delve deeper into the genetic makeup of sheep populations and unravel the underlying genetic mechanisms governing various traits. Single nucleotide polymorphisms (SNPs), in particular, have emerged as valuable markers for understanding genetic variation within and among populations.

The Garole sheep, a breed indigenous to India, represents an important genetic resource with unique characteristics suited to its native habitat. Yet, comprehensive genomic studies on Garole sheep remain limited. In this context, the present study aims to address this gap by conducting a genome-wide analysis of SNPs in Garole sheep and annotating them to production traits.

By identifying candidate genes associated with traits such as milk production, composition, and other economically important factors, this research seeks to enhance our understanding of the genetic architecture underlying key phenotypic traits in Garole sheep.

Through a combination of genotyping and bioinformatics analyses, this study aims to shed light on the genetic diversity and structure of Garole sheep populations. Such insights are vital for the development of targeted breeding strategies aimed at improving productivity, resilience, and adaptability in this indigenous breed. Furthermore, understanding the genetic basis of traits related to milk production and composition holds immense promise for the development of sustainable livestock management practices and the promotion of genetic conservation efforts in Garole sheep.

## Materials and Methods

### SNPs Identification

A total of 26 samples of Garole sheep genotyped using Illumina OvineSNP50v1 were accessed from WIDDE online repository (<http://widde.toulouse.inra.fr/widde/widde/main.do?module=sheep>). In quality control, individuals whose genotype is known for less than 95% of markers were ignored. Markers whose genotype is known for less than 75% of individuals were not considered. Markers for which  $p\text{-Value} < 0.001$  were neglected. Markers for which  $\text{MAF} < 1\%$  within the selected dataset were also ignored.

### Annotation

Retained SNPs obtained of Garole were annotated using SnpEff v4.4 (Cingolani *et al.*, 2012) [15]. Then, the annotated SNPs presented within the genes were screened on literature and also in animal QTL (Quantitative Trait Loci) database v 2019 ([www.animalgenome.org](http://www.animalgenome.org)) for identifying candidate genes associated with production traits. As a result of that, candidate genes associated with production traits were identified and annotated gene-wise separately using Vcftools v0.1.15 and SnpSift v4.4.

## Results and Discussion

Genetic variation underlies the diverse phenotypic traits observed in livestock species. In this study, we aimed to identify genome-wide single nucleotide polymorphisms (SNPs) in the Garole sheep breed and annotate them to production traits, shedding light on the genetic architecture of this important livestock resource.

The domestication of sheep marked a crucial milestone in human civilization, with sheep serving as a vital source of meat, wool, milk, and other products for thousands of years (Chessa *et al.*, 2009) [7]. In India, the sheep population plays a significant role in the socio-economic landscape, particularly among small and marginal farmers. With 45 registered sheep breeds in India and a diverse range of agro-climatic conditions, sheep breeding and management practices have evolved to adapt to various environmental challenges. Among these breeds, the Garole sheep, indigenous to India, holds particular importance due to its unique genetic makeup and adaptation to local conditions.

Utilizing data from 26 Garole sheep genotyped using Illumina OvineSNP50v1, we identified a total of 46,819 high-quality SNPs in the Garole genome following stringent

quality control measures. Annotation of these SNPs revealed their distribution across various genomic regions, with a notable proportion located within transcript regions. The observed transition-to-transversion (Ts/Tv) ratio of 2.43 per sample on average indicated a higher frequency of G>A and C>T substitutions, consistent with previous findings in related species (Patel *et al.*, 2017) [15].

Among the annotated SNPs, 760 were associated with 175 candidate genes implicated in production traits, particularly milk composition and yield. Noteworthy genes included Acetyl-CoA carboxylase Beta (ACACB), Growth hormone receptor (GHR), and Leptin (LEP), which have known roles in regulating fatty acid oxidation, milk yield, and energy metabolism, respectively (Han *et al.*, 2019; Stasio *et al.*, 2005; Buchanan *et al.*, 2002) [9, 19, 2]. These findings align with previous studies highlighting the importance of these genes in shaping milk production traits in various livestock species.

The identification of SNPs linked to candidate genes associated with production traits in Garole sheep provides valuable insights into the genetic basis of economically important traits in this breed. The presence of considerable genetic variation offers opportunities for targeted breeding programs aimed at enhancing traits such as milk yield, composition, and overall productivity. Furthermore, the annotated SNPs serve as valuable genetic markers for future genomic selection and breeding efforts in Garole sheep.

Comparisons with studies in other livestock species, such as cattle and buffalo, underscore the unique genetic characteristics of Garole sheep and highlight species-specific variations in SNP distribution and candidate gene associations. While this study provides a comprehensive analysis of SNPs and candidate genes in Garole sheep, validation of these findings using whole-genome sequencing data and functional studies will further enhance our understanding of the genetic mechanisms underlying production traits in this breed.

In conclusion, the identification and annotation of genome-wide SNPs in Garole sheep, particularly those associated with production traits, offer valuable insights into the genetic architecture of this important livestock breed. These findings lay the foundation for targeted breeding programs aimed at improving economically important traits in Garole sheep and contribute to the overall genetic resource conservation and enhancement efforts in Indian livestock farming.

## Conclusions

This study presents SNPs which are polymorphic in Garole sheep genome and their annotation. The presence of considerable existing variation in candidate genes related to various traits provides the opportunity for further improvement of these traits. The identified variants in candidate genes will also serve as useful genetic tools, in the quest for nucleotide changes resulting in a phenotype modification and also to design appropriate genetic improvement programs. However the SNPs can be validated in the whole genome sequence data.

## Statements and Declarations

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**Conflict of interest**

No potential conflict of interest was reported by the author(s).

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