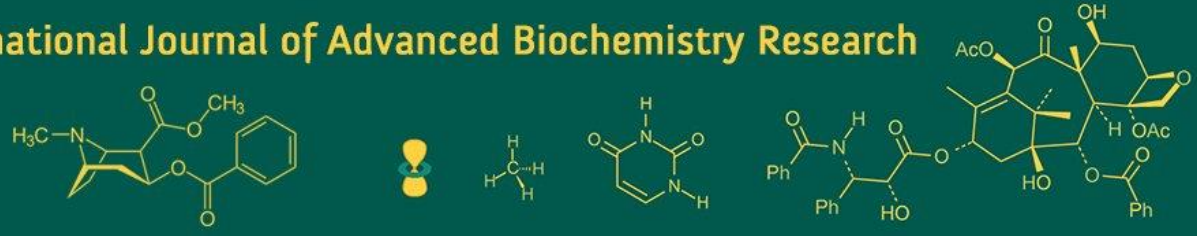
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 83-88
www.biochemjournal.com
Received: 12-10-2025
Accepted: 15-11-2025

Rohit Barwar
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

KA Saravanan
Scientist, AG division, ICAR-Central Sheep and Wool Research Institute, Avikanagar Rajasthan, India

Gyanendra Kumar Gaur
Principal Scientist, Animal Genetics, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Munish Gangwar
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Ashok Chaudhary
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Tapendra Saini
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Manoj Kumar Goud Pyatla
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Rohit Barwar
Ph.D. Scholar, AG Division, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Selection signature analysis for immunity and production traits in semi-arid Indian sheep breeds

Rohit Barwar, KA Saravanan, Gyanendra Kumar Gaur, Munish Gangwar, Ashok Chaudhary, Tapendra Saini and Manoj Kumar Goud Pyatla

DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1Sb.6886

Abstract
Understanding genomic regions under selection is crucial for improving productivity and resilience in indigenous livestock. In this study, whole-genome resequencing (LC-WGS) was performed on Malpura and Patanwadi sheep to identify nucleotide diversity patterns and selection signatures associated with immune and production traits. After stringent quality control, 8.37 million and 8.19 million SNPs were retained for Malpura and Patanwadi, respectively. Genome-wide nucleotide diversity (π) was estimated using sliding-window analysis, and the top 5% genomic regions were subjected to functional enrichment. Both breeds exhibited strong enrichment of immunity-related pathways, including Type I interferon receptor binding, natural killer cell activation, and antiviral dsRNA recognition. Malpura displayed higher enrichment intensity for antiviral and immune cell-mediated pathways, while Patanwadi showed elevated representation of haptoglobin-haemoglobin complex and acute-phase inflammatory responses. Production-related selection signatures highlighted distinct metabolic specialization: Malpura showed enrichment for fructokinase activity, keratin filament binding, and reproductive pathways, whereas Patanwadi exhibited enrichment for oxygen transport and haemoglobin-binding functions consistent with wool production efficiency. Several key genes, including β-defensins (*DEFB1*, *DEFB5*), interferon-stimulated genes (*MX1*, *ISG15*, *IRF3*, *IRF7*), and lipid-metabolism genes (*FASN*, *ACAA1*, *ACAA2*), were identified within the selected regions. These findings demonstrate strong directional selection for pathogen resistance and production performance in semi-arid Indian sheep breeds and provide genomic resources for future genetic improvement and conservation strategies.

Keywords: Malpura, Patanwadi, selection signatures, nucleotide diversity, whole-genome resequencing

Introduction
As domestication began in the Neolithic age, sheep and goats were among the first livestock species that humans domesticated to control their food, habitat, and behaviour. Over millennia of selective breeding, sheep populations have evolved considerable phenotypic variation, encompassing diverse physical morphologies, behavioural traits, and productive capacities, particularly relating to meat yield, fibre production, and lactation performance. This phenotypic diversity reflects the varied production objectives pursued by different human societies and agro-ecological regions (Chessa *et al.*, 2009; Lukic *et al.*, 2023) [3, 12]. Genomic selection has become a more effective choice for identifying suitable candidate genes in animal breeding, due to its potential benefits and better returns over traditional breeding methods (Mishra *et al.*, 2021) [15]. Thus, human-led selection increases the frequency of beneficial traits in the population. According to the neutral genetic premise, closely linked neutral loci in their locality show the same pattern of variation due to factors such as genetic drift, a phenomenon known as a selection signature or selective sweep (Nielsen *et al.*, 2007) [18]. Under sustained natural or artificial selection pressures, genomic regions harbouring advantageous alleles experience linkage disequilibrium patterns distinct from neutral loci, creating characteristic genomic signatures. These selection signatures represent the molecular legacy of long-term evolutionary and breeding pressures and constitute valuable markers for investigating how domestic species adapt to diverse environments and develop specialised production traits.

By mapping selection signatures across the genome, we can infer the genetic architecture underlying phenotypic differentiation and environmental adaptation.

Various methods are currently employed to assess genetic diversity and identify signatures of selection. Nucleotide diversity is a key metric for measuring the level of genetic variation within populations. Nei's foundational concept defined nucleotide diversity as the average number of base-pair differences at corresponding loci when comparing any two randomly chosen genomic sequences from a population (Nei *et al.*, 1979) [17]. The approach involves systematically counting nucleotide differences across homologous sites in paired DNA sequences from population samples, then aggregating these differences to compute a population-level nucleotide diversity metric. This value is typically denoted as "π" (pi). The pi coefficient remains a widely used tool for detecting signs of natural selection within populations. The higher the value, the higher the nucleotide diversity. It is commonly used to measure nucleotide diversity within a population and can also be used to infer evolutionary relationships. The π value is calculated based on the sliding window method and displayed using the Manhattan chart. It can be intuitively seen in which genome interval the π value of the population is significantly reduced (Shi *et al.*, 2023) [20]. Despite advances in more complex analytical methods, the pi metric remains a reliable indicator of genetic polymorphism and adaptive evolutionary pressures at the population level, especially in comparative genomic studies analysing selection processes within specific geographic or ecological contexts.

Sheep are a vital aspect of Indian culture, raised by farmers and stakeholders for their wool, meat, and milk since their domestication. Our study focuses on two native semi-arid sheep breeds: Malpura and Patanwadi. Malpura, a popular mutton breed, is found in Tonk, Ajmer, and Sawai-Madhopur districts, as well as across Rajasthan's semi-arid regions. Known for their resilience and adaptability, these sheep have low prolificacy, with ewes usually lambing once (Mishra *et al.*, 2007) [14]. The second breed, Patanwadi, originates from Gujarat's Patan region, including districts like Patan, Mehsana, Surendranagar, Rajkot, and Jamnagar. Characterised by a white coat, brown face, and legs, Patanwadi sheep are medium to large in size and excel at producing high-quality carpet wool, thriving under tough environmental and management conditions (Jyotsana *et al.*, 2010; Kadam *et al.*, 2025) [10, 11].

Technological advances in DNA sequencing, SNP genotyping platforms, and computational bioinformatics have substantially enhanced the resolution and interpretability of selection signature analyses. These methods permit investigation of the genetic mechanisms underlying complex economic traits without extensive phenotypic data collection or extended study periods. Given the widespread adoption of selective sweep methodology across diverse livestock species, contemporary research increasingly focuses on characterising genomic signatures associated with production efficiency, environmental adaptation, and immunological competence in indigenous livestock populations.

Materials and Methods

In this study, we collected 50 samples of ewes of both Malpura and Patanwadi breeds from their respective herds at ICAR-Central Sheep and Wool Research Institute. After quality control, the genomic DNA was sequenced by the LC-WGS method with the Illumina NovaSeq 6000 sequencing protocol. The sheep genome *ARS-UI_Ramb_v3.0* was used as a reference genome. Quality control was performed using *Plink v1.9* software (Chang *et al.*, 2015) with the criteria of (1) minimum allele frequency (MAF) > 0.01, and (2) Hardy Weinberg equilibrium P-value > 10-6 (Shi *et al.*, 2023) [20], resulting in a total of 8370560 and 8192064 SNPs for Malpura and Patanwadi subsequent analysis.

The nucleotide diversity pi was calculated for both breeds using *VCFtools v0.1.16* (Danecek *et al.*, 2011) [4] with a 100-kb sliding window (-window-pi 100, 000) and a step size of 50 kb (-window-pi-step 50, 000) across the genome (Rodrigues *et al.*, 2025) [19]. The pi values for each autosomal SNP were plotted against their chromosomal positions in Manhattan plots for each breed using the R package ggplot (Wickham, 2016) [24]. The top 5% (approximately 1700 and 1220) of genome-wide SNPs were selected as candidate loci for the selection signal in both breeds (Zhang *et al.*, 2023; Rodrigues *et al.*, 2025) [25, 19].

Gene annotation was performed on the candidate selection signatures and regions of 500 kb upstream and downstream of the significant SNPs identified in the GWAS using the GALLO R package (Fonseca *et al.*, 2020) [5]. To further understand the biological functions of the candidate genes, we performed Gene Ontology (GO) analysis of Genes and functional pathway enrichment analysis using the *DAVID v6.8* tool (Huang *et al.*, 2009) [7]

Results and Discussion:

The pi values for each autosomal SNP were plotted against their chromosomal position in terms of a Manhattan plot for individual breeds using the R package: ggplot (Wickham, 2016) [24] (Figures 1 & 2)

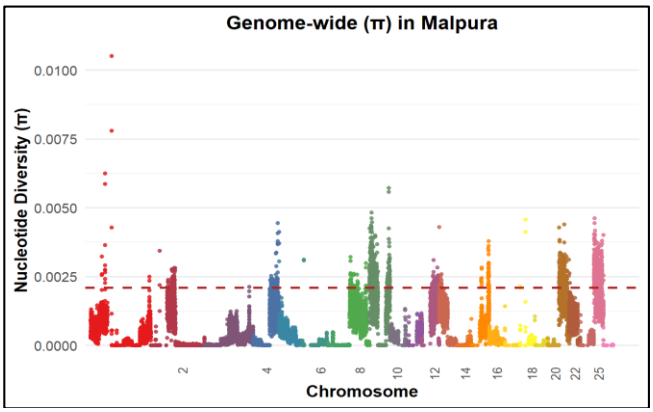


Fig 1: Genome-wide nucleotide diversity distribution of the Malpura breed

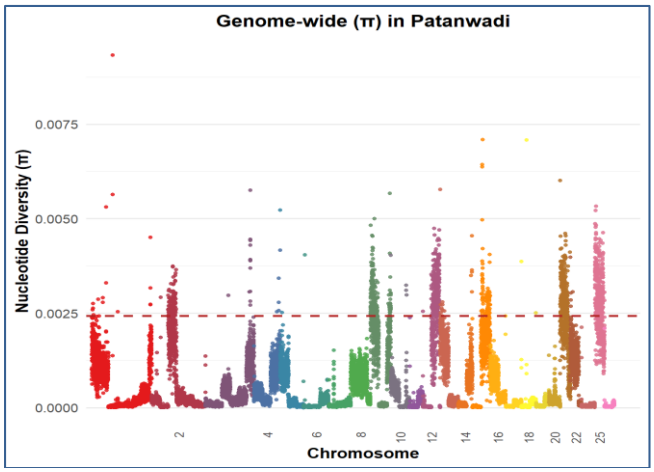


Fig 2: Genome-wide nucleotide diversity distribution of the Patanwadi breed

Functional Enrichment Analysis across Selection Signature Pathways in Malpura and Patanwadi Sheep

Gene Ontology (GO) enrichment analysis revealed distinct functional pathway signatures across immunity and production categories in both Malpura and Patanwadi semi-arid sheep breeds (p<0.05).

Immunity Pathways: Both breeds exhibited substantial enrichment of immune function genes, though with notable differences in magnitude. Malpura sheep demonstrated exceptional enrichment in type I interferon receptor binding (fold enrichment ~12×) and natural killer cell activation (fold enrichment ~12×), with comprehensive B and T cell-mediated immune responses (fold enrichment 9-11×). Patanwadi sheep showed comparable immune pathway enrichment with pronounced T cell activation (fold enrichment ~12×) and natural killer cell activation (fold enrichment ~10×), alongside elevated haptoglobin-haemoglobin complex representation (fold enrichment ~8×), indicating enhanced acute-phase inflammatory capacity. Both breeds displayed enrichment of antiviral response mechanisms, including dsRNA recognition pathways, suggesting strong selective pressure for pathogen resistance in semi-arid environments (Figures 3 & 4).

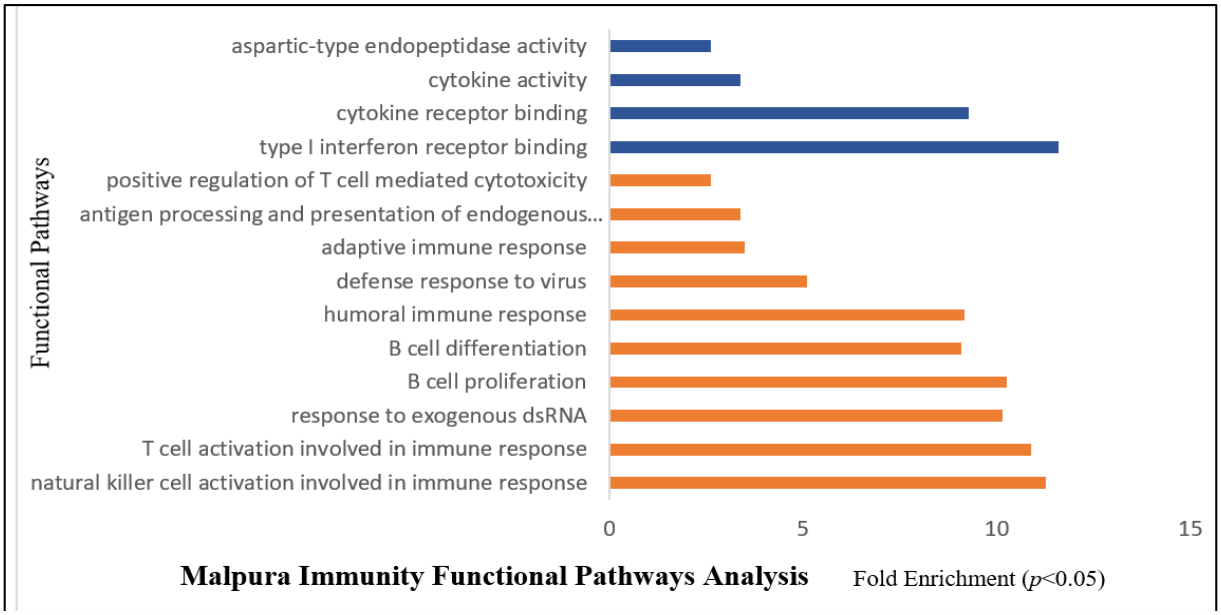


Fig 3: Analysis of immunity functional pathways of the Malpura breed

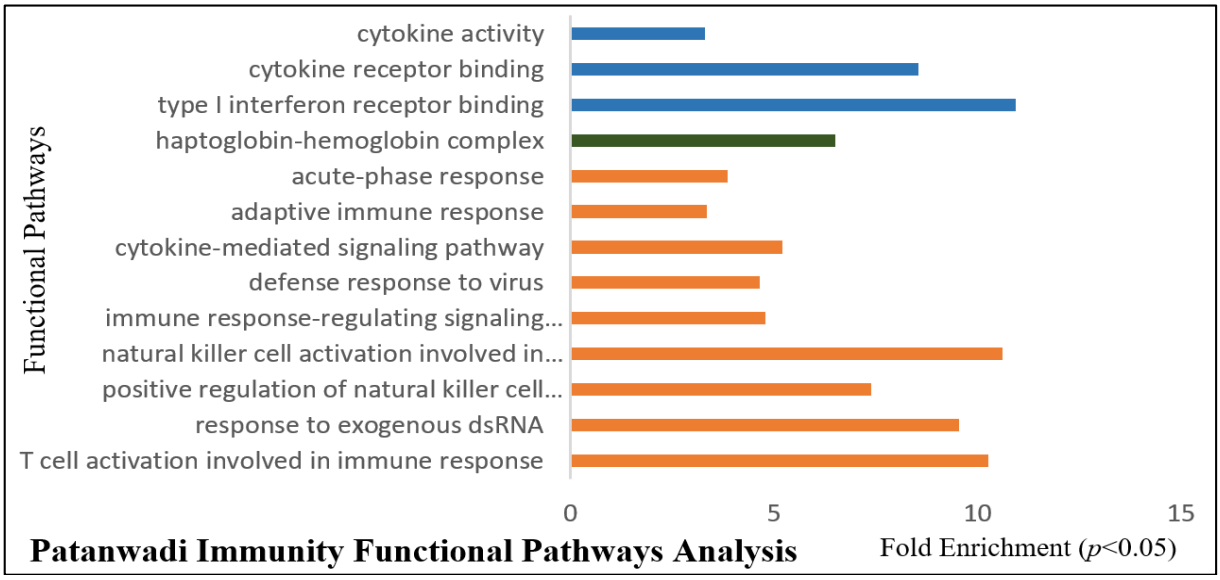


Fig 4: Analysis of immunity functional pathways of the Patanwadi breed

Production Pathways: Production trait enrichment revealed metabolic and structural specialisations. Malpura sheep showed enrichment in fructokinase activity (~8×), keratin filament binding (~8×), and acetylcholine receptor inhibitor activity (~8×), with reproductive pathway enrichment (female pregnancy, fold enrichment ~5×). Patanwadi sheep displayed elevated haemoglobin alpha binding (~10×) and haptoglobin-haemoglobin complex (~7×) alongside acetylcholine receptor inhibitor activity (~5×), suggesting enhanced oxygen transport capacity and metabolic efficiency for sustained wool production under harsh conditions (Figures 5 & 6).

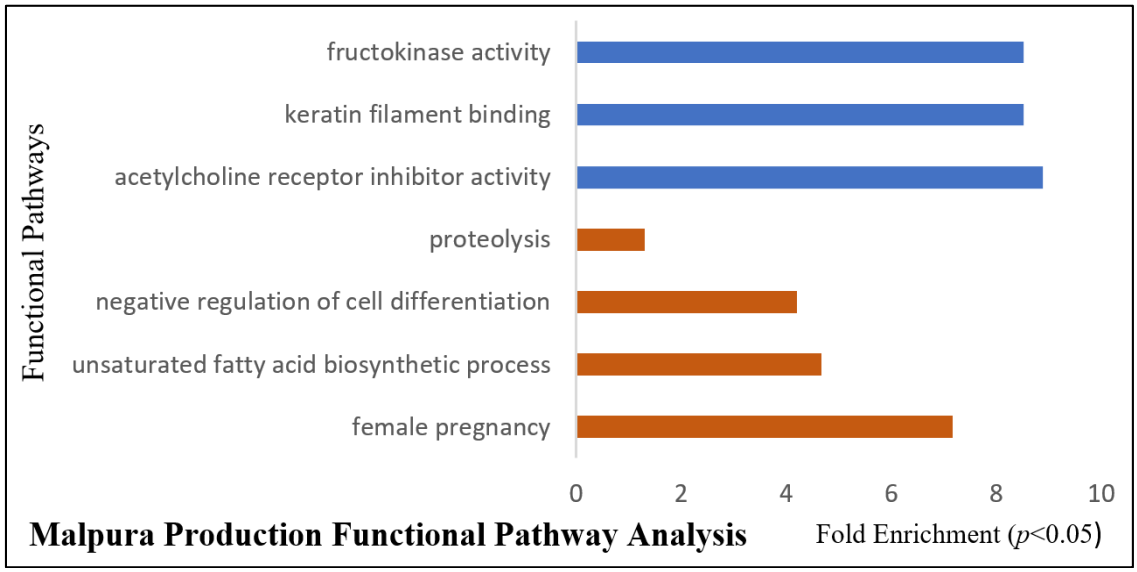


Fig 5: Analysis of production functional pathways of the Malpura breed

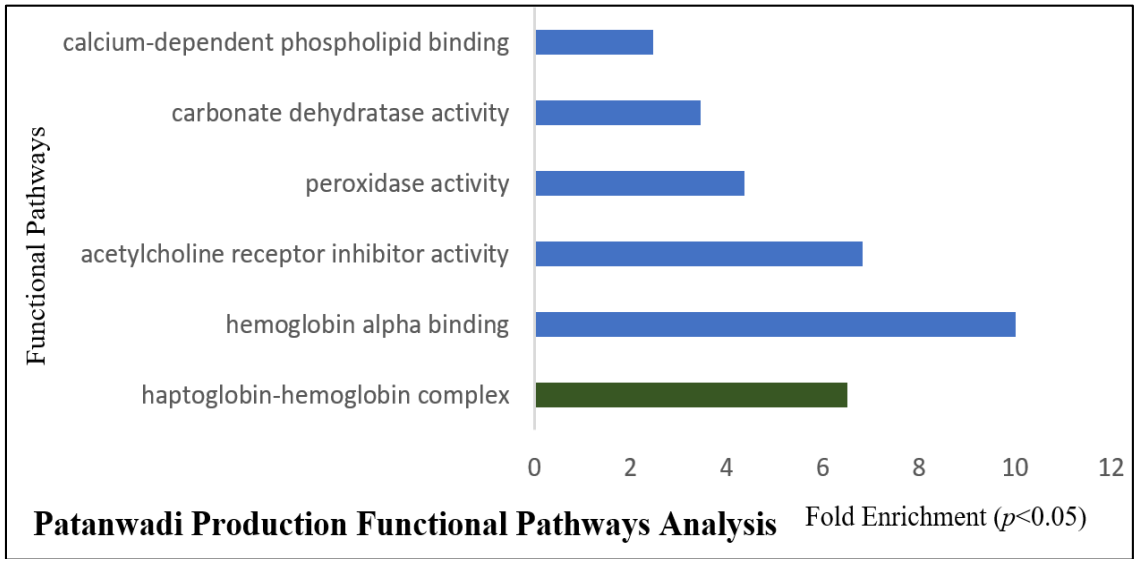


Fig 6: Analysis of production functional pathways of the Patanwadi breed

Breed-Specific Differentiation: While both breeds share core immune and sensory pathway enrichment, Malpura exhibits amplified immune signature intensity, whereas Patanwadi demonstrates enhanced oxygen transport and haematological pathway representation, reflecting their respective breeding objectives, mutton production versus carpet wool specialisation.

Several genes were identified through nucleotide diversity for production and immune-related genes in both Malpura and Patanwadi genes, like β-defensin genes (*DEFB1* and *DEFB5*) located within copy number variable (CNV) regions of North Chinese and Hu sheep breeds, which function as primary components of innate immune defence (GO:0006952) (Wang *et al.*, 2019) [22]. These antimicrobial peptides directly kill pathogens while simultaneously modulating adaptive immunity through chemokine signalling and immune cell recruitment. Interferon-stimulated genes (*MX1*, *ISG15*, *IRF3*, *IRF7*, *RNASEL*, *CXCL10*) identified in CNV regions represent the antiviral effector machinery (GO:0002504) (Wang *et al.*, 2017; Luo *et al.*, 2024) [23, 13]. These genes coordinate type I interferon responses through transcriptional regulation (*IRF3/IRF7*), viral replication inhibition (*MX1*), and protein ubiquitination (*ISG15*), creating a multilayered antiviral defence critical for pastoral environments with endemic viral pathogens. *TMC6* (transmembrane protein 6), identified in North Chinese sheep CNVs, regulates innate immune signalling and zinc homeostasis (GO:0002323, GO:0002286, GO:0051607). This gene modulates epithelial barrier immunity and viral surveillance, suggesting selection for enhanced mucosal defence against respiratory and enteric infections.

Production and Metabolic Traits

Genes like *FASN* (fatty acid synthase), identified in CNV regions, drive de novo lipogenesis (GO:0006636, GO:0006090), directly determining intramuscular fat content and carcass quality in meat-type breeds (Moradi *et al.*, 2022) [16]. Variation in *FASN* copy number alters lipid deposition patterns and meat palatability. Genes *CA1*, *EEF2*, and *ASPA* identified in Tibetan sheep CNVs regulate growth and reproductive processes (GO:0007565) (Shi *et al.*, 2023) [20]. These genes control protein synthesis, pH homeostasis, and developmental signalling, affecting body growth and sexual maturation under highland environmental constraints. Genes like *ACAA1* (acetyl-CoA acylase 1) inhibit adipogenic differentiation (GO:0007565), partitioning energy toward skeletal muscle development and reproductive investment (Hu *et al.*, 2019) [22], thereby influencing meat composition and breeding efficiency. A multi-gene lipid metabolism module, including *ACSL*, *ACCSL*, *ACAD8*, *DAGLA*, *FMO5*, and *LIPF/LIPK* (GO:0009058) coordinates complex lipid biosynthesis (Cao *et al.*, 2022). In sheep, *ACAA2* associates with milk fatty acid profile and lactation yield (GO:0009058) (Jawasreh *et al.*, 2025) [1], reflecting artificial selection for enhanced milk production and nutritional composition.

Genome-wide nucleotide diversity analysis identified selection signature regions in both Malpura and Patanwadi sheep, with enriched immunity pathways indicating strong adaptive selection for pathogen resistance. Malpura demonstrated exceptional enrichment in type I interferon receptor binding (fold enrichment ~12×) and natural killer cell activation (fold enrichment ~12×), reflecting amplified antiviral competence. Patanwadi showed comparable immune enrichment with elevated haptoglobin-haemoglobin complex (fold enrichment ~8×), suggesting enhanced acute-phase inflammatory capacity for harsh pastoral conditions.

β-defensin genes (*DEFB1*, *DEFB5*) and interferon-stimulated genes (*MX1*, *ISG15*, *IRF3*, *IRF7*, *RNASEL*, *CXCL10*) identified within selection signature regions function in innate immune defence (GO:0006952) and antiviral effector machinery (GO:0002504). *TMC6* SNPs regulate epithelial barrier immunity (GO:0002323, GO:0002286, GO:0051607), collectively demonstrating genomic adaptation to endemic pathogen burdens in semi-arid pastoral systems.

Production Specialization

Breed-specific metabolic differentiation reflected distinct artificial selection objectives. Malpura enrichment in fructokinase activity (~8×) and reproductive pathways (fold enrichment ~5×) indicates selection for muscle development and reproductive efficiency. Patanwadi enrichment in haemoglobin alpha binding (~10×) and oxygen transport pathways reflects selection for sustained wool production under resource-limited conditions. *FASN* SNPs drive de novo lipogenesis (GO:0006636, GO:0006090), directly determining intramuscular fat content in Malpura. *ACAA1* SNPs inhibit adipogenesis (GO:0007565), partitioning energy toward muscle development. *ACAA2* SNPs associate with milk fatty acid profile in dairy sheep (GO:0009058). These metabolic SNPs show directional allele frequency changes reflecting artificial selection for production traits.

Conclusion

This study revealed distinct genomic signatures of selection in two important semi-arid Indian sheep breeds, reflecting both natural adaptation and targeted artificial selection. Strong enrichment of immune-related pathways in both Malpura and Patanwadi indicates long-term selection for pathogen resistance under harsh environmental and disease-challenged pastoral systems. Malpura exhibited particularly elevated enrichment in interferon-mediated antiviral responses and NK-cell activation, while Patanwadi showed stronger haemoglobin-related and acute-phase response pathways, supporting their resilience and wool-production characteristics. Production-related pathways also showed clear breed-specific patterns, with Malpura demonstrating signatures linked to reproductive efficiency and metabolic regulation, and Patanwadi exhibiting genomic regions associated with oxygen transport and wool-related physiology. Key genes such as DEFB1, DEFB5, MX1, IRF3, IRF7, FASN, ACAA1, and ACAA2 highlight the molecular basis of immunity and production traits under selection. Overall, the findings emphasise the complex evolutionary forces shaping these indigenous breeds and underscore their value as genetically diverse resources for breeding programs and long-term conservation.

References

1. Cao Y, Yu Y, Zhang L, Liu Y, Zheng K, Wang S, *et al.* Transcript variants of long-chain acyl-CoA synthase 1 have distinct roles in sheep lipid metabolism. Frontiers in Genetics. 2022;13:1021103.
2. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. 2015;4(1):1-16.
3. Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, *et al.* Revealing the history of sheep domestication using retrovirus integrations. Science. 2009;324(5926):532-536.
4. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, *et al.* The variant call format and VCFtools. Bioinformatics. 2011;27(15):2156-2158.
5. Fonseca PA, Suarez-Vega A, Marras G, Canovas A. GALLO: An R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci. GigaScience. 2020;9(12):giaa149.
6. Hu R, Jiang X, Yang H, Liu G. Selection signature analysis reveals RDH5 performed key function in vision during sheep domestication process. Archives of Animal Breeding. 2023;66(1):81-91.
7. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols. 2009;4(1):44-57.
8. Jawasreh KI, Khrais D, Alu'datt M, Haddad NJ, Awabdeh S, AlMadani MI, *et al.* Fatty acids profile in Awassi sheep milk affected by some genes' single and combined effects. Journal of Advanced Veterinary and Animal Research. 2025;12(1):238-247.
9. Jyotsana B, Jakhesara S, Prakash V, Rank DN, Vataliya PH. Genetic variability and relationship among Patanwadi, Marwari and Dumba sheep breeds. Indian Veterinary Journal. 2011;88(3):39-42.
10. Jyotsana B, Jakhesara S, Prakash V, Rank DN, Vataliya PH. Genetic features of Patanwadi, Marwari and Dumba sheep breeds (India) inferred by microsatellite markers. Small Ruminant Research. 2010;93(1):57-60.

11. Kadam V, Singh A, Kumar A, Kumar A. Patanwadi sheep wool profile: yield, morphology and physico-mechanical properties. Tropical Animal Health and Production. 2025;57(2):1-9.
12. Lukic B, Curik I, Drzaic I, Galić V, Shihabi M, Vostry L, *et al.* Genomic signatures of selection, local adaptation and production type characterisation of East Adriatic sheep breeds. Journal of Animal Science and Biotechnology. 2023;14(1):142-156.
13. Luo J, Min Q, Sun X, Guo X, Song M, Zeng X, *et al.* Comparative whole-genome analysis of production traits and genetic structure in Baiyu and Chuanzhong Black goats. Animals. 2024;14(24):3616-3629.
14. Mishra AK, Arora AL, Kumar S, Kumar S, Singh VK. Improving productivity of Malpura breed by crossbreeding with prolific Garole sheep in India. Small Ruminant Research. 2007;70(2-3):159-164.
15. Mishra DC, Budhlakoti N, Majumdar SG, Rai A. Innovations in genomic selection: statistical perspective. Special Proceedings. 2021;101-111.
16. Moradi MH, Mahmodi R, Farahani AHK, Karimi MO. Genome-wide evaluation of copy gain and loss variations in three Afghan sheep breeds. Scientific Reports. 2022;12(1):14286-14298.
17. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences USA. 1979;76(10):5269-5273.
18. Nielsen R, Hellmann I, Hubisz M, Bustamante C, Clark AG. Recent and ongoing selection in the human genome. Nature Reviews Genetics. 2007;8(11):857-868.
19. Rodrigues JL, Braga LG, Watanabe RN, Schenkel FS, Berry DP, Buzanskas ME, *et al.* Genetic diversity and selection signatures in sheep breeds. Journal of Applied Genetics. 2025;66:1-13.
20. Shi H, Li T, Su M, Wang H, Li Q, Lang X, *et al.* Whole genome sequencing revealed genetic diversity, population structure, and selective signature of Panou Tibetan sheep. BMC Genomics. 2023;24(1):50-64.
21. Sun G, Zhao H, Mu X, Li X, Wang J, Zhao M, *et al.* Whole-genome re-sequencing association study on body size traits at 10-weeks of age in Chinese indigenous geese. Frontiers in Veterinary Science. 2024;11:1506471.
22. Wang S, Hu D, Wang C, Tang X, Du M, Gu X, *et al.* Transcriptional profiling of innate immune responses in sheep PBMCs induced by Haemonchus contortus soluble extracts. Parasites & Vectors. 2019;12:182-196.
23. Wang W, Xu L, Su J, Peppelenbosch MP, Pan Q. Transcriptional regulation of antiviral interferon-stimulated genes. Trends in Microbiology. 2017;25(7):573-584.
24. Wickham H. Programming with ggplot2. In: ggplot2: Elegant Graphics for Data Analysis. Cham: Springer International Publishing; 2016. p. 241-253.
25. Zhang W, Jin M, Li T, Lu Z, Wang H, Yuan Z, *et al.* Whole-genome resequencing reveals selection signal related to sheep wool fineness. Animals. 2023;13(18):2944-2958.