Research

Hepatic Gene Expression Reveals Functional Diversity in Indigenous Chicken Breeds

Meena Bagiyal[1,2], Sonika Ahlawat[1]*, Sunita Khatak[2], Pooja Chhabra[1], Upasna Sharma[1], Reena Arora[1], Rekha Sharma[1], Ram Parsad[1] and Ritika Gera[1,2]

[1]*ICAR-National Bureau of Animal Genetic Resources, Karnal-132001, Haryana, India*
[2]*UIET, Kurukshetra University, Kurukshetra-136119, Haryana, India*

ABSTRACT

Poultry meat ranks among the most widely consumed sources of animal protein across the globe, with chicken breeds varying significantly in their genetic makeup, physiological traits, and adaptability. Indigenous breeds contribute not only to rural livelihoods but also possess valuable traits like disease resistance, stress tolerance, and superior meat quality. The liver, being central to metabolism and lipid regulation, plays a crucial role in determining growth, reproduction, and stress response in poultry. This study employed high-throughput RNA sequencing to compare hepatic transcriptomes of three distinct chicken breeds - Aseel, Kadaknath, and Punjab Brown, to identify gene expression patterns in each. Notably, genes such as *APOV1* and *VTG2* showed higher expression in Aseel, *SPIA3, HMGCS1*, and *HSP90B1* in Kadaknath and *DIO2* and *EPAS1* in Punjab Brown. These differences reflect key roles in lipid metabolism, stress response, immunity and reproduction in these breeds, indicating distinct metabolic adaptations among the breeds.

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*Corresponding author: sonika.ahlawat@gmail.com

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INTRODUCTION

Indigenous chicken breeds play a vital role in both nutrition and rural livelihoods by offering high-quality protein and significantly contributing to household income (Luo *et al.,* 2021). Raised under free-range systems, indigenous breeds are essential part of livelihood to rural and tribal communities due to their inherent strengths, including resilience, disease resistance, adaptability, high survival rates, strong mothering instincts and efficient scavenging and nesting behaviours. Easily distinguishable by their varied physical characteristics, these breeds are well-known for specialized qualities such as superior meat and egg, as well as serving as game birds for cockfighting. The wide range of diversity among them offers a valuable resource for identifying and utilizing genetic factors that control economically important traits, including growth, reproduction, feather and skin colour, aggression and adaptation to local management and environmental conditions (Sharma *et al.,* 2023). India is rich in biodiversity, with a vast array of animal species and considerable genetic variability within them. Despite this diversity, more than 50% of the country's poultry population consists of nondescript or native (desi) birds (Arora *et al.,* 2024a). Although indigenous chickens often receive less attention than commercial breeds due to their comparatively lower production performance (Rathi *et al.,* 2024), the global spread of selectively bred commercial chicken breeds, favoured for their higher production efficiency, has increasingly threatened the survival of native breeds. This loss is concerning, as indigenous breeds possess valuable traits such as environmental adaptability and rich genetic diversity, which are vital for sustainable poultry farming (Luo *et al.,* 2021). Understanding the molecular mechanisms underlying these adaptive traits, in key metabolic organs like the liver, is crucial for unlocking the full genetic potential of indigenous chickens.

The main metabolic roles of the liver include breaking down red blood cells, controlling glycogen storage, and generating hormones (Xu *et al.,* 2019). In chickens, the liver is responsible for more than 90% *de novo* fatty acid synthesis (Li et al. 2016). Therefore, the liver is responsible for lipid metabolism and homeostasis. As a consequence, it plays a critical role in growth, laying performance and meat quality. It also enables birds to adapt to environmental changes by regulating lipid metabolism (Ning *et al.,* 2020). Advancements in next-generation sequencing technologies have enabled the exploration of a vast array of SNPs and genes linked to particular traits (Arora *et al.,* 2024b; Vijh *et al.,* 2024) and resulting regulatory mechanisms across various species (Ahlawat *et al.,* 2024). In the present study, high-throughput RNA sequencing (RNA-seq) were employed to profile the liver transcriptomes of three chicken breeds: Aseel, Kadaknath, and Punjab Brown. These breeds were selected to represent a spectrum of genetic diversity, production goals, and physiological characteristics. Kadaknath is the only Indian chicken breed with black meat, which is protein-rich (25.47%) with a unique flavour and reputed medicinal properties (Arora *et al.,* 2023). Punjab Brown is a dual-purpose breed raised for both meat and egg production, while Aseel is distinguished by its exceptional fighting abilities and majestic gait (Ahlawat *et al.,* 2023). Comparative transcriptomes of multiple breeds help in identifying both differentially expressed and highly expressed genes that reveal core functional signatures unique to each chicken breed. The present study therefore, attempted to investigate the top most highly expressed hepatic genes in each breed to explore the functional diversity in relation to metabolic, immune and physiological traits.

MATERIALS AND METHODS

Ethics Statement

All procedures were followed in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals). The study was approved by the Institutional Research Committee, ICAR-National Bureau of Animal Genetic Resources, Karnal, India (Code-7.72).

Sample Collection

Approximately 10-20 g of liver samples were collected in RNALater, at the marketing age from four birds of each Aseel, Kadaknath, and Punjab Brown breeds, in collaboration with local butchers.

RNA Extraction, RNA Sequencing and Data Analysis

Total RNA was extracted from liver tissue samples using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA was then further purified with the RNeasy Kit (Qiagen, Hilden, Germany), and its quality and concentration were evaluated using an Agilent 2100 Bioanalyzer. Only RNA samples with a RIN value ≥ 7 were selected for cDNA library preparation. The sequencing libraries were prepared and sequenced on the Illumina NovaSeq 6000 platform, producing 150-base-pair paired-end reads. The quality of the raw sequencing data was checked using FastQC (version 0.11.5) (Andrews, 2010). Clean and high-quality reads were aligned to the *Gallus gallus* reference genome (GCF_016699485.2) using the 'Map Reads to Reference' tool in CLC Genomics Workbench 6.5.1 (CLC Bio, Aarhus, Denmark). Normalization of mapped reads was performed in terms of Reads Per Kilobase Million (RPKM), and transcripts with RPKM values below 0.25 were excluded from downstream analyses. The Venn diagram was generated using InteractiVenn (Heberle *et al.,* 2015). The schematic diagram depicted in Figure 1 illustrates the core workflow used for the analysis of transcriptomic data.

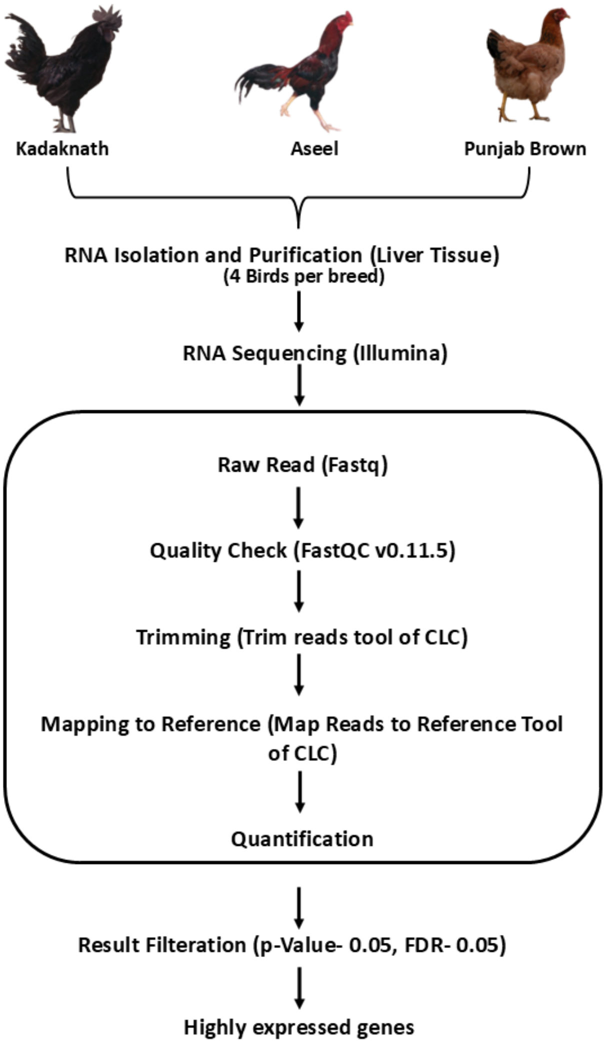

Fig. 1: *Schematic representation of the transcriptomic analysis pipeline*

RESULTS AND DISCUSSION

The raw transcriptomic sequencing data have been deposited in the NCBI with accession numbers SAMN46784021-SAMN46784024 for Kadaknath, SAMN46784025- SAMN46784028 for Aseel and SAMN46784029-SAMN46784032 for Punjab Brown. After processing, the average number of high-quality reads per sample exceeded 126 million for Kadaknath, 100 million for Aseel, and 119 million for Punjab Brown. A total of 11,986 genes were commonly expressed across all three breeds, while several transcripts were uniquely expressed or highly enriched in individual breeds (Fig. 2).

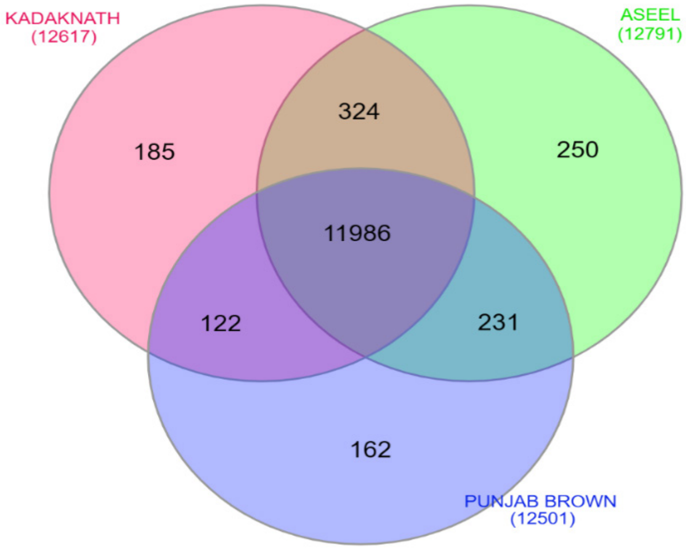

Fig.2: *Venn diagram of expressed genes across three indigenous chicken breeds.*

Transcriptomic profiling revealed distinct hepatic gene expression patterns across the three indigenous chicken breeds, indicating diverse transcriptional landscapes (Fig.3). While similar genes were highly expressed across all breeds, their expression levels differed significantly between breeds.

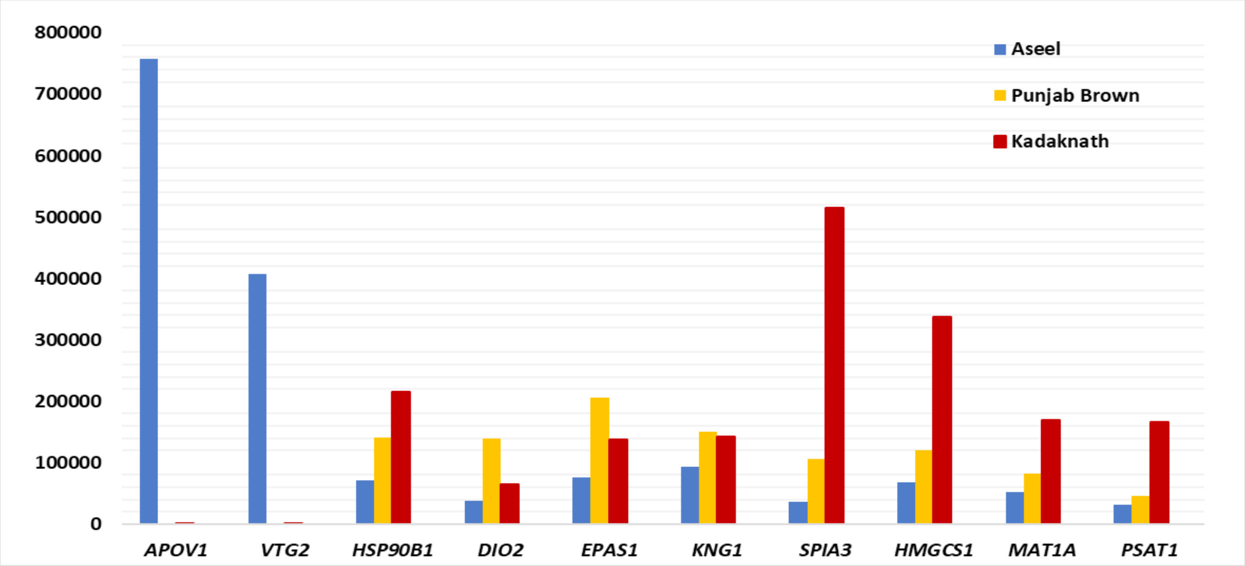

Fig. 3: *Diverse hepatic expression profiles of selected highly expressed genes in three chicken breeds.*

The liver, as a central metabolic organ, plays essential roles in lipid synthesis, degradation, and transport of triglycerides (Li *et al.,* 2015). Lipids, being the most energy-dense nutrients, are routinely included in poultry diets to meet energy requirements and enhance productivity (Ge *et al.,* 2019). Interestingly, the majority of the genes identified in analysis are associated with lipid metabolism across all three breeds underscoring pivotal role of the liver in regulating lipid metabolic processes. In Aseel chickens, *APOV1* and *VTG2* are among the most abundantly expressed hepatic genes, which are regulated by estrogen and are crucial for lipid metabolism in the liver (Ren *et al.,* 2021). *VTG2* is involved in lipid and fatty acid transport to the ovary and is associated with reproductive development under estrogen stimulation (Zhang *et al.,* 2022). It has also been linked to muscle growth, reduced muscle degradation, delayed skeletal muscle aging, and obesity prevention (Li *et al.,* 2021). *APOV1*, also known as *APOVLDLII*, encodes a liver-specific apolipoprotein responsible for the synthesis and transport of triglycerides and cholesterol to peripheral tissues, supporting energy storage and utilization of energy (Chen *et al.,* 2024; Gunawan *et al.,* 2019). These functions are critical for energy homeostasis, particularly in breeds with high activity levels. Consistent with these functional attributes, *APOV1* was significantly upregulated in Aseel, possibly reflecting the breed's elevated energy demands due to its traditionally aggressive behaviour and physical activity associated with their traditional role in cockfighting (Arora *et al.,* 2024b). This elevated expression of *APOV1* and *VTG2* suggests a physiological adaptation to enhance lipid mobilization and energy utilization in Aseel.

In contrast, Kadaknath chickens exhibited distinctly higher expression levels of *SPIA3, HMGCS1, HSP90B1* and *MAT1A*. Indigenous breeds like Kadaknath are known for their superior adaptability and enhanced disease resistance compared to exotic breeds (Yadav *et al.,* 2022). *SPIA3* (also known as *SERPINA3*), a serine protease inhibitor, is involved in maintaining cellular homeostasis and regulating lipid metabolism via modulation of *PPARγ* and *NF-κB*, key players in immune and inflammatory pathways (Soman and Nair, 2022; Lim *et al.,* 2012). *HMGCS1*, a critical enzyme in cholesterol biosynthesis and has been linked to hepatic cholesterol levels and skeletal muscle development in livestock (Shi *et al.,* 2021; Zhou *et al.,* 2025). Its high expression in Kadaknath may support cholesterol regulation and contribute to superior meat quality and metabolic resilience. Additionally, elevated expression of *HSP90B1*, a gene associated with thermotolerance and innate immunity, suggests potential genetic markers for heat resilience (An *et al.,* 2019; Wan *et al.,* 2017). Similarly, *MAT1A*, which plays a role in methionine metabolism and antioxidant defence via S-adenosylmethionine (SAM) synthesis, may offer protection against oxidative stress. Reduced *MAT1A* expression has been associated with increased oxidative stress and poor meat quality (Aggrey *et al.,* 2018; Nawaz and Zhang, 2021). Its high expression in Kadaknath chickens suggests a strengthened antioxidant capacity, potentially supporting better meat quality. Collectively, these gene expression patterns highlight unique adaptations of Kadaknath for stress tolerance, immune competence, and meat quality.

Punjab Brown, a hardy dual-purpose breed, showed high hepatic expression of *DIO2* and *EPAS1*. *DIO2* converts inactive thyroxine (T4) into the biologically active triiodothyronine (T3), which regulates metabolism, oxygen consumption, and thermoregulation (He *et al.,* 2016; Katarzyńska-Banasik *et al.,* 2024). T3 also enhances reproductive hormone synthesis, including follicle-stimulating hormone (FSH) gonadotropin-releasing hormone (GnRH), progesterone and estrogen, which are essential for egg production (Fatima *et al.,* 2025; Du *et al.,* 2022). *EPAS1* (also known as *HIF-2α*) is crucial for adaptation to hypoxic conditions and regulates iron metabolism, erythropoiesis and vascular development (Pan *et al.,* 2019). The upregulation of these genes suggests a robust physiological framework for reproduction, metabolic efficiency and environmental adaptability in Punjab Brown chickens. The distinct hepatic gene expression profiles identified among these indigenous breeds underscore their breed-specific metabolic and physiological adaptations, especially concerning lipid metabolism, stress resilience and immune regulation.

The results highlight the adaptive strengths of indigenous breeds and offer a valuable foundation for the genetic improvement, conservation, and sustainable utilization of native chicken resources, ensuring both productivity and biodiversity in poultry systems.

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