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# Comparative expression of growth-associated genes in white Leghorn and Australorp chickens

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**Abstract**
Poultry industry plays a significant role in India's food and nutritional security, contributing nearly half of the nation's total meat output. Differences in energy metabolism among chicken breeds reflect their selection history with White Leghorn specialised for egg production and Australorp serving as a dual-purpose breed. To explore the molecular basis of these differences, the expression of three genes involved in hepatic metabolism were investigated. Male White Leghorn and Australorp chickens at 8 weeks of age were sampled and total RNA was extracted from liver tissues. Following cDNA synthesis, RT-qPCR was performed using gene-specific primers, with *GAPDH* as the reference gene. This study highlights the differences in the expression of *LDHB*, *UPP2* and *SQLE* between White Leghorn and Australorp breeds. In the liver, *LDHB* and *UPP2* were significantly upregulated in Australorp compared to White Leghorn whereas *SQLE* was downregulated suggesting variation in lactate utilisation, pyrimidine metabolism and cholesterol biosynthesis. These results indicated that White Leghorn metabolism favours oxidative energy pathways, supporting lean physiology and egg-laying specialisation, whereas Australorp exhibits lipid and cholesterol biosynthesis, consistent with its dual-purpose role. Based on the result of the study these genes can be served as bio marker for marker assisted selection to improve growth related traits in future breeding programmes.

**Keywords:** White Leghorn, Australorp, Liver, *LDHB*, *UPP2*, *SQLE*

## Introduction
Poultry production in India plays a significant role in providing the rural livelihood by ensuring food security, generating employment opportunities and it is one of the fastest growing sectors of agriculture today (BAHS, 2020), as more than 71 per cent of the adult population consumes poultry meat (RGI, 2016). It is widely recognised as low investment, high return livelihood strategy providing income to millions of farmers including smallholder and landless labourers. Poultry sector offers an affordable and high-quality protein through egg and meat. India ranks fifth in the poultry meat production of world with an annual production of 4.47 MT (PIB, 2023) [18]. The total poultry meat production in India is 4.06 million tonnes, which accounts for 48.96 per cent of total meat production in the country (DAHD, 2023-24). Though, the per capita availability remains suboptimal which is 103 eggs/year and 3.0 to 3.5 kg of poultry meat/year which are significantly below the Indian Council of Medical Research's recommendation of 182 eggs and 11 kg poultry meat per person per annum.
In chickens, the liver is the principal site of 90 per cent de novo lipogenesis which is responsible for more than 70 per cent of fatty acid synthesis. The analysis of difference in expression of lipid and energy pathways related genes in chicken liver tissues holds significant importance in understanding mechanism of growth-related traits. Breed differences in energy metabolism reflect their selection history. White Leghorn chickens, bred primarily for egg production are characterised by leaner physiology and efficient nutrient utilisation. Australorp, a dual-purpose breed, requires a metabolic balance that supports both growth and reproduction. These differences are expected to be reflected in the expression of genes regulating lipid metabolism, energy pathways and muscle growth. The present study investigated the expression profiles of genes involved in hepatic and breast muscle metabolism in White Leghorn (egg-type) and Australorp (dual-purpose) chickens.

In this study we conducted an expression analysis of three genes such as *LDHB, UPP2* and *SQLE* in the liver to assess breed-specific differences, thereby providing insight into the molecular basis of growth-related traits in poultry.

## Materials and Methods

White Leghorn (egg-type) and Australorp (dual-purpose) male chickens were sampled at 8 weeks of age. The liver tissues were collected immediately post-euthanasia, rinsed in PBS, snap-frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Total RNA was isolated from those tissues and treated with DNase I. The cDNA was synthesized from 1 µg of RNA using Verso cDNA Synthesis Kit (Thermo Scientific, USA). The RT-qPCR was performed in a Bio-Rad CFX Opus Dx Real-Time PCR System using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) in a 10 µL reaction volume. The components of reaction mixture are shown in Tables 1 and thermal cycling parameters given in Table 2.

### Primer design and verification

For analysing the differences in gene expression between White Leghorn and Australorp liver, three genes such as *Lactate Dehydrogenase B* (*LDHB), Uridine Phosphorylase 2* (*UPP2)* and *Squalene Epoxidase* (*SQLE)* which were previously reported in the literature were selected for quantitative real-time PCR (RT-qPCR) analysis. The *Glyceraldehyde 3-phosphate dehydrogenase* (GAPDH) was selected as the internal reference gene for the study.

Gene-specific primers for RT-qPCR were designed using the PrimerQuest™ Tool (Integrated DNA Technologies, IDT). The primer sets were optimised to amplify 100-200 bp products and were checked to avoid secondary structures and primer-dimer formation. The quality and specificity of each primer pair were evaluated using Oligo Analyzer Tool (IDT), Sequence Manipulation Suite and Multiple Primer Analyzer (Thermo Fisher Scientific). Both forward and reverse primer details and their annealing temperature was mentioned in Table 3.

### Statistical analysis

The quantification of relative changes in gene expression using qPCR requires certain equations, assumptions and testing of these assumptions. The results were expressed as fold changes indicating an increase or decrease in the expression of the test one (White Leghorn) in relation to the control one (Australorp). The RT-qPCR was normalised to a reference gene (*GAPDH)* for liver based on the following equations

$\Delta CT$ = CT (Target gene)-CT (Reference gene)
$\Delta\Delta CT$= $\Delta CT$ (test)-$\Delta CT$ (control)
Fold difference = $2^{-\Delta\Delta CT}$

Statistical comparison between samples was performed for relative quantification (RQ) value of different samples using Analysis of variance (ANOVA) and independent sample t test (SPSS V.24).

**Table 1:** Reaction mixture used for RT-qPCR

| SL. No. | Components | Volume (µL) |
|---|---|---|
| 1 | Template (cDNA) | 0.5 |
| 2 | Maxima SYBR Green qPCR Master Mix (2X) | 5 |
| 3 | Forward Primer (10 picomol /µL)) | 0.3 |
| 4 | Reverse primer (10 picomol/µL) | 0.3 |
| 5 | Nuclease free water | 3.9 |
| | Total reaction volume | 10.0 |

**Table 2:** PCR conditions used for RT-qPCR

| SL. No | Step | Temperature | Duration |
|---|---|---|---|
| 1 | Initial denaturation | 95 °C | 10 min |
| 2 | Denaturation | 95 °C | 15 sec |
| 3 | Annealing | Respective Ta | 20 sec |
| 4 | Extension | 72 °C | 30 sec |

Steps 2 to 4 were repeated for 40 cycles, Data acquisition was performed during the extension step

**Table 3:** Primers for selected genes and their annealing temperatures

| Gene | Primer | Sequence (5'→3') | Annealing temperature (°C) |
|---|---|---|---|
| *LDHB* | Forward | ACCTATGTCACATGGAAGTT | 52.5 |
| | Reverse | TAGAATCACCATGTTCTCCT | |
| *SQLE* | Forward | ATGTGGACTTTCCTGGGCATCG | 61.5 |
| | Reverse | ACGGAAGTGGTAGCGATAGG | |
| *UPP2* | Forward | ATGCCTTCCACAAATCGGT | 56.7 |
| | Reverse | TCTGATGCTGCTTGACAAGTC | |
| *GAPDH* | Forward | ACAGCCACACAGAAGACGGT | 62.9 |
| | Reverse | CTCAGGGATGACTTTCCCCAC | |

**Table 4:** Relative expression profile of genes in liver sample of Australorp and White Leghorn

| Group | Gene | Mean CT ± SE | | ΔCT± SE | ΔΔCT± SE | Fold change from control (2-ΔΔCT) | P-Value |
|---|---|---|---|---|---|---|---|
| | | Target Gene | GAPDH | | | | |
| A | *LDHB* | 33.36±0.09 | 17.46±0.04 | 15.90 ±0.09 | -1.01±0.36 | 2.014±0.507 | < 0.05 |
| W | | 30.96±0.35 | 16.07±0.08 | 14.89±0.35 | 0.00 | 1 | |
| A | *UPP2* | 29.97±0.19 | 17.46±0.04 | 12.51±0.19 | -0.52±0.53 | 1.431 ±0.53 | < 0.05 |
| W | | 28.07±0.50 | 16.07±0.08 | 11.99±0.50 | 0.00 | 1 | |
| A | *SQLE* | 25.56±0.31 | 17.46±0.04 | 8.10±0.31 | 7.43±0.41 | 0.006±0.00 | < 0.05 |
| W | | 31.59±0.27 | 16.07±0.08 | 15.52±0.27 | 0.00 | 1 | |

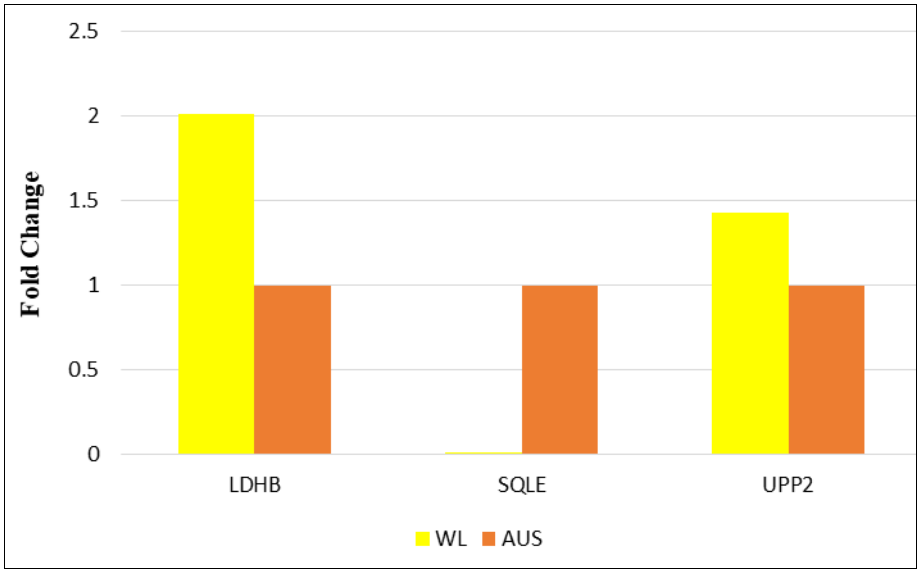


**Fig 1:** Relative expression profile of upregulated and downregulated genes in liver

## Results and Discussion

The liver serves as a central hub for coordinating carbohydrate and lipid metabolism, functioning as both a storage site and a source of metabolic energy. Through glycogen synthesis and de novo lipogenesis, it stores energy, while glycogenolysis and gluconeogenesis allow glucose mobilisation. In addition, the liver exports lipid fuels in the form of lipoproteins, linking it directly to systemic energy homeostasis (Kotani *et al.*, 2004) [12]. The comparative RT-qPCR analysis between Australorp (A) and White Leghorn (W) revealed differential expression of the studied genes such as *Lactate Dehydrogenase B* (*LDHB)*, *Uridine Phosphorylase 2* (*UPP2)* and *Squalene Epoxidase* (*SQLE).* The *LDHB* expression was significantly upregulated in White Leghorn compared to Australorp (fold change=2.01, $p<0.05$). Similarly, *UPP2* showed an upregulation in White Leghorn relative to Australorp (fold change=1.43, $p<0.05$). In contrast, *SQLE* was found to be markedly downregulated in White Leghorn, with a strong reduction in expression compared to Australorp (fold change=0.006, $p<0.05$).

White Leghorn liver showed higher expression of *LDHB* (fold changes 2.01) and *UPP2* (fold changes 1.43) genes which were associated with energy and nucleotide metabolism, respectively. The *LDHB*, favours oxidative energy metabolism by converting lactate into pyruvate, thereby fuelling the tricarboxylic acid (TCA) cycle for aerobic respiration (Cahn *et al.*, 1962; Dawson *et al.*, 1964; Pesce *et al.*, 1964) [4, 9, 17]. The higher expression of *LDHB* in White Leghorn suggested the enhancement of lactate clearance and energy-efficient oxidative phosphorylation, consistent with its high-output physiology for egg production.

The *UPP2* encodes *uridine phosphorylase 2*, an enzyme in the pyrimidine salvage pathway that converts uridine into uracil. The uridine confers hepatoprotective effects by suppressing lipogenesis and thereby reducing the obesity. The upregulation of *UPP2* leads to accelerated breakdown of uridine into uracil, ultimately promoting the fat accumulation in the body (Huixia *et al.*, 2024) [11]. In 8-week-old White Leghorn males, this could mean the body is prioritising faster RNA turnover and recycling of nucleotides to keep up with high cellular activity. However, this may come at the cost of proper fat regulation, suggesting a metabolic trade-off that reflects how these birds have been selectively bred for increased productivity.

Supporting this lipid-oriented metabolic profile, *SQLE*, a key rate-limiting enzyme in cholesterol biosynthesis, was significantly more expressed in Australorp liver. The *SQLE* catalyses the conversion of squalene to 2, 3-oxidosqualene, a critical step in cholesterol synthesis (D'Andre *et al.*, 2013) [8]. Its elevated expression in Australorp (fold change = 0.006, $p<0.05$) suggests a favouring of cholesterol production and lipid storage, which may provide a metabolic advantage for sustaining growth and reproduction.

The reference gene was used to ensure normalisation reliability: *GAPDH* for liver which is widely utilised as housekeeping genes in vertebrate gene expression studies (Chapman and Waldenström, 2015) [5].

## Conclusion

These findings highlight clear breed-specific differences in the expression pattern of hepatic tissues. White Leghorn exhibited a metabolic orientation toward efficient oxidative energy metabolism and nucleotide turnover, aligning with its lean phenotype and egg-laying specialisation. The expression pattern in Australorp revealed adaptations which favour lipid and cholesterol biosynthesis, consistent with its dual-purpose role requiring greater energy reserves to sustain both growth and reproduction. The genes used in this study could be serve as a basis for further investigation in improving the growth traits to achieve greater genetic improvement in poultry.

## Conflict of Interest

Not available

## Financial Support

Not available

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