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D Harish
MVSc Scholar, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Elizabeth Kurian
Associate Professor, Center for Advanced Studies in Animal Genetics and Breeding (CASAGB), Mannuthy, Thrissur, Kerala, India

LF Anand
Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

N Thomas
Associate Professor, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

KA Bindu
Professor and Head, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

B Chacko
Associate Professor and Head, Department of poultry science, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Corresponding Author:
D Harish
MVSc Scholar, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Differential expression of *Chac Glutathione Specific Gamma-Glutamylcyclotransferase 1, APOBEC1* complementation factor and fibrinogen beta chain in Tellicherry and Australorop chicken in Kerala

D Harish, Elizabeth Kurian, LF Anand, N Thomas, KA Bindu and B Chacko

Abstract
Poultry farming is a vital component of Kerala's livestock sector, contributing substantially to nutrition and rural livelihoods. The Tellicherry chicken, the state's only recognised indigenous breed, is valued for its adaptability and meat quality, while the Black Australorp is a dual-purpose exotic breed known for productivity. This study investigated the differential expression of three genes *CHAC1* (Glutathione Specific Gamma-Glutamylcyclotransferase), *A1CF* (APOBEC1 Complementation Factor) and *FGB* (Fibrinogen Beta Chain) in Tellicherry and Australorp chickens. Six male birds (three of each breed) were reared under uniform management and breast muscle tissues were collected at eight weeks of age. Total RNA was extracted, converted to cDNA, and analysed using quantitative real-time PCR (qRT-PCR) with *GAPDH* as the reference gene. Relative expression was determined using the 2^-ΔΔCT method. *CHAC1* was significantly upregulated in Tellicherry chickens compared to Australorps, indicating enhanced oxidative stress adaptation. In contrast, *A1CF* and *FGB* were significantly downregulated in Tellicherry chickens, suggesting disruptions in apoB mRNA editing, lipid metabolism, fibrinogen synthesis and extracellular matrix organisation. The upregulation of *CHAC1* may confer adaptive advantages in muscle development, while reduced expression of *A1CF* and *FGB* could affect tissue repair and stability. The differential expression of these genes highlights breed-specific molecular mechanisms influencing muscle growth and meat quality. These findings provide a basis for utilising *CHAC1, A1CF* and *FGB* as potential molecular markers in poultry breeding programmes aimed at improving productivity and adaptability.

Keywords: *CHAC1, A1CF,* Kerala, improving productivity and adaptability, fibrinogen, Tellicherry, Australorop

Introduction
Poultry farming is a key component of Kerala's livestock sector, contributing substantially to nutrition, rural livelihoods, and the state's economy. The poultry population has shown steady growth over the past decade, rising from 29.24 million in 2012 to 37.6 million in 2022, a 28.6 per cent increase. Intensive commercial production systems dominate, with 21.82 million birds reported in the 2019 Livestock Census, while backyard poultry, with 7.42 million birds, continues to play an important cultural and economic role in rural households. Among the breeds reared, the Tellicherry chicken is the only officially recognised indigenous variety in Kerala, valued for its adaptability and meat quality (Vij *et al*., 2007) [11]. The Black Australorp, a dual-purpose breed of Australian origin, is widely preferred for both meat and eggs and is well suited to intensive and semi-intensive rearing systems (Pawankar *et al*., 2022) [7]. In contrast, commercial broiler strains dominate large-scale intensive farms due to their rapid growth and feed efficiency (Prakash *et al*., 2020) [8]. Together, Tellicherry, Australorp and broiler strains reflect the dual structure of Kerala's poultry sector, which accounts for 17.5 per cent of the state's total livestock population and supplies 29.13 per cent of its meat, highlighting its central role in food security and the economy.

ChaC Glutathione Specific Gamma-Glutamylcyclotransferase 1 (*CHAC1*), *APOBEC1 Complementation Factor* (*A1CF*) and *Fibrinogen Beta Chain* (*FGB*) are three key genes associated with skeletal muscle physiology, metabolic regulation and meat quality traits. *CHAC1* plays a crucial role in skeletal muscle development by promoting myoblast proliferation, differentiation and muscle regeneration (Chen *et al*., 2024). *The A1CF* gene is important because it encodes the apobec-1 complementation factor, a crucial RNA-binding protein required for site-specific C-to-U editing of apoB mRNA. This makes *A1CF* indispensable for apoB regulation and suggests additional roles in RNA processing (Mehta *et al*., 2002; Fossat *et al*., 2014) [6, 4]. *FGB* encodes the beta subunit of fibrinogen, essential for coagulation, wound healing and tissue repair and is enriched in biological processes such as coagulation, hemostasis, platelet activation and hormone secretion while also contributing to immune defense and tissue remodeling (Simurda *et al*., 2020; Zheng *et al*., 2021) [10, 14]. Variations in the expression of these genes between Tellicherry and Australorp chickens may explain breed-specific differences in fat deposition, muscle growth and meat quality.

This study focused on analysing the expression of *CHAC1*, *A1CF* and *FGB* in the breast muscle tissues of Tellicherry and Australorp chickens reared under Kerala conditions using quantitative real-time PCR (qRT-PCR), and the findings are expected to provide a framework that could be utilised in breeding programmes to enhance adaptability and productivity.

Materials and Methods

The chickens used in this study were obtained from the University Poultry and Duck Farm, Mannuthy, and were reared under uniform housing and management conditions. Breast muscle tissues were collected from six male birds (three Tellicherry and three Australorp) at eight weeks of age during slaughter. All experimental procedures and animal handling protocols were approved by the Institutional Animal Ethics Committee (IAEC). Tissue samples were collected from each bird and immediately submerged in RNA later (Sigma-Aldrich) and stored at-80 °C until RNA isolation. Total RNA was extracted using the RNeasy Plus Universal Mini Kit (Qiagen), and cDNA was synthesised with the Bio-Rad cDNA kit. The relative expression levels of *CHAC1, A1CF* and *FGB* were quantified using *GAPDH* as the reference gene. Gene specific primers for the four genes were designed using Primer-BLAST, (Table 1), (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

Table 1: Oligonucleotide primers

Sl. No.	Gene	Primer sequence (5'-3')	Product size (bp)
1	*CHAC1-F*	5'-AGCACCTGTTCTCCATCGAA-3'	118
	CHAC1-R	5'-AAGAAGTTGTCCCTTTCGGC-3'	
2	*A1CF-F*	5'-AAACCCGTTGACAAGGACAG-3'	119
	A1CF-R	5'-TAGGTAGCAGCAGGGTCGTA-3'	
3	*FGB-F*	5'-TAACTACAAAGGCAATGCAG-3'	100
	FGB-R	5'-ACTGAAGTACATGCCATTGTGA-3'	
4	*GAPDH-F*	5'-ACAGCCACACAGAAGACGGT-3	126
	GAPDH-R	5'-CTCAGGGATGACTTTCCCCAC-3'	

QRT-PCR analysis was carried out in triplicates for each sample using a 10 µL reaction mixture consisting of 5 µL SYBR Green Master Mix, 0.3 µL of each primer (10 pmol/µL), 0.5 µg of cDNA template, and the remaining volume adjusted with nuclease free water. The amplification program included an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing for 15 s at 59.8 °C, 63.4 °C, 57.0 °C and 62.9 °C for *CHAC1*, *A1CF*, *FGB* and *GAPDH*, respectively and extension at 72 °C for 30 s. Melt curve analysis was performed at the end of each run to verify amplification specificity. Each run included non-template controls (NTC) and negative controls containing nuclease-free water. *GAPDH* was used as the reference gene for data normalisation, and relative expression levels of *CHAC1*, *A1CF* and *FGB* were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) [12]. Statistical differences between Tellicherry and Australorp chickens were analysed using independent sample t-tests, with significance considered at $p<0.05$.

Results and Discussion

The mean RNA concentration was 4154±73.53 ng/µL in Australorp chickens and 2387±106.6 ng/µL in Tellicherry chickens. The purity of RNA, assessed by spectrophotometric ratios, showed A260/A280 values of 2.11±0.01 and 2.09±0.00 for Australorp and Tellicherry chickens, respectively, while A260/A230 values of 2.16±0.01 and 2.05±0.01 Australorp and Tellicherry chickens, respectively. These values indicate high RNA quality, suitable for downstream molecular applications. Furthermore, only RNA samples exhibiting distinct 28S and 18S rRNA bands along with clear mRNA smearing were selected for subsequent analyses.

qRT-PCR was used to evaluate the expression of *CHAC1, A1CF* and *FGB.* The amplification and melt curve analyses showed a single sharp peak, confirming reaction specificity and the absence of primer-dimer formation or non-specific amplification (Ruiz-Villalba *et al*., 2017) [9]. Amplification and melt curve profiles are shown in Figure 1-4. Mean cycle threshold (Ct) values were obtained from the Bio-Rad CFX Opus Dx real-time PCR detection system for Tellicherry and Australorp chickens, providing a reliable basis for comparative gene expression analysis (Bustin *et al*., 2025) [1]. The relative expression profiles of *CHAC1, A1CF* and *FGB* in Tellicherry and Australorp chicken in Figure 5 and 6. *CHAC1* expression was significantly upregulated in the Tellicherry chickens compared Australorp chickens (Figure 5), whereas *A1CF* and *FGB* in a significantly downregulated in the Tellicherry chickens compared Australorp chickens (Figure 6). In the present study, *CHAC1* was significantly upregulated in the breast muscle tissue of Tellicherry chickens compared to Australorp chickens. *CHAC1* encodes a glutathione-degrading enzyme that regulates intracellular redox balance and promotes calcium mobilisation, both of which are vital for skeletal muscle fibre development (Yadav *et al*., 2019) [13]. Recent studies have also demonstrated that *CHAC1* supports muscle regeneration by enhancing myoblast proliferation and differentiation through the lncMDP1/miR-301a-5p axis (Chen *et al*., 2024) [2]. The increased expression of *CHAC1* in

Tellicherry chickens may therefore reflect an adaptive advantage in muscle growth, repair and oxidative stress management. This suggests that *CHAC1* could contribute to improved muscle development and meat quality traits in Tellicherry chickens. *FGB* gene was down regulated in the breast muscle of Tellicherry chickens. The *FGB* gene encodes the β-subunit of fibrinogen, a critical protein involved in blood coagulation, wound healing and tissue repair. Reduced expression of *FGB* may impair fibrinogen synthesis and secretion, leading to disruption of extracellular matrix organisation and weakening of muscle fibre stability (Glavey *et al*., 2017) [5]. Such alterations can compromise muscle regeneration and repair, ultimately influencing growth performance and meat quality. Flood *et al*. (2006) [3] demonstrated that structural changes in fibrinogen delay fibrinolysis by conferring proteolytic resistance, despite normal plasminogen activation. In addition, Gene Ontology annotations highlight the broad functional roles of *FGB* in coagulation, platelet activation, immune defence, tissue remodelling and stress adaptation (Simurda *et al*., 2020; Zheng *et al*., 2021) [10, 14]. Thus, down regulation of *FGB* in breast muscle may adversely affect hemostatic balance and tissue physiology, contributing to breed-specific differences in muscle function and adaptability. The *A1CF* gene encodes the apobec-1 complementation factor, an RNA-binding protein essential for site-specific C-to-U editing of apolipoprotein B (apoB) mRNA. It binds to the mooring sequence and recruits apobec-1, enabling precise cytidine deamination. In the absence of *A1CF*, this editing process cannot occur, highlighting its regulatory role (Mehta *et al*., 2002) [6]. In this study, *A1CF* was downregulated in the breast muscle, indicating potential impairment of apoB editing and RNA processing, which may affect lipid metabolism, intramuscular fat deposition and ultimately muscle growth and meat quality traits.

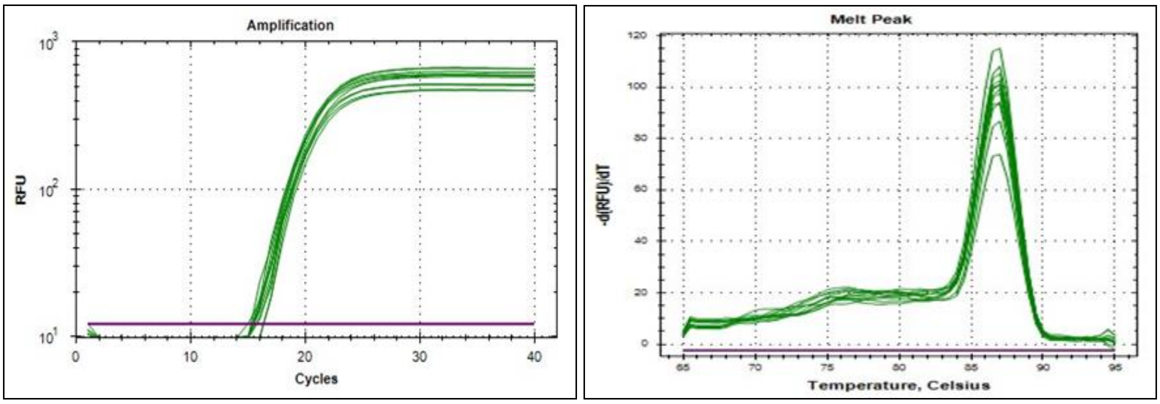

Fig 1: Amplification plot and melt curve of GAPDH gene

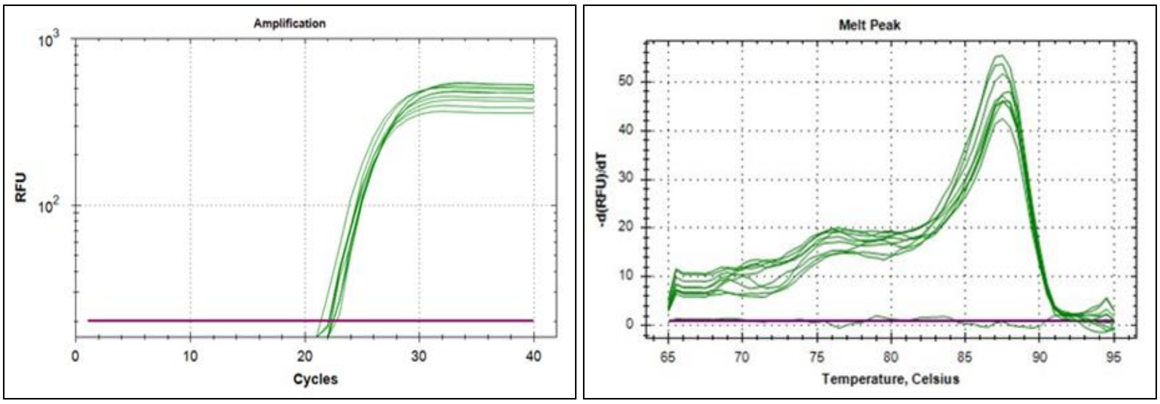

Fig 2: Amplification plot and melt curve of CHAC1 gene

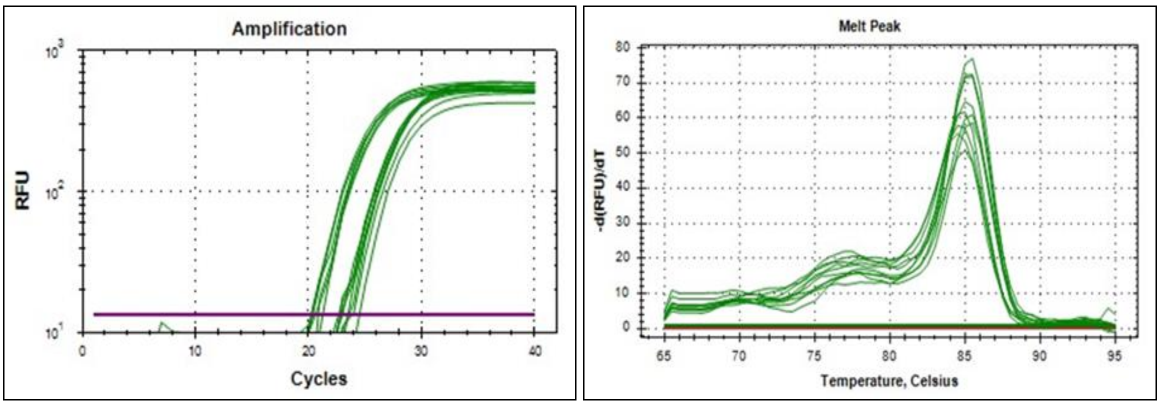

Fig 3: Amplification plot and melt curve of A1CF gene

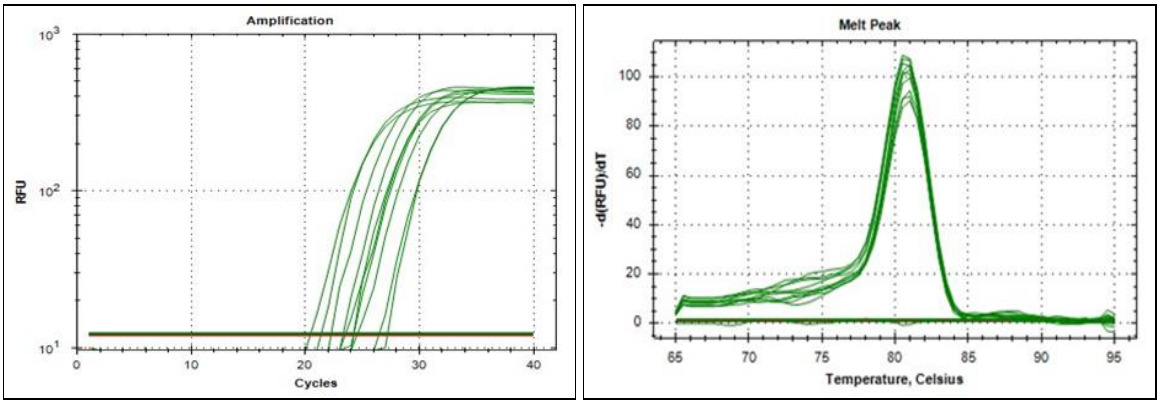

Fig 4: Amplification plot and melt curve of FGB gene

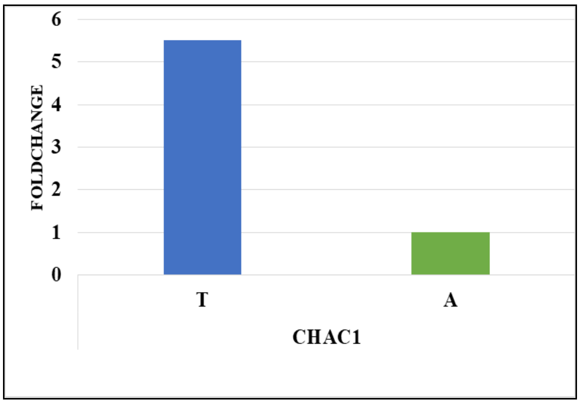

Fig 5: Relative expression profile of *CHAC1*

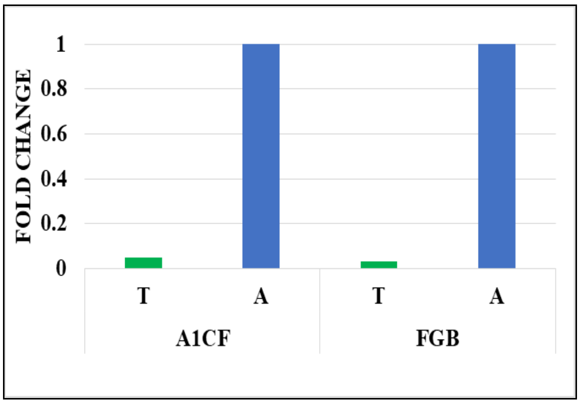

Fig 6: Relative expression profile of *A1CF* and *FGB*

Table 1: Relative expression profile of genes CT values, fold change and p values of *CHAC1, A1CF* and *FGB.*

Sample	Gene	Mean CT ± SE		ΔCT± SE	ΔΔCT± SE	Fold change from control (2-ΔΔCT)	P-Value
		Target gene	*GAPDH*				
T	*CHAC1*	21.16±0.20	18.53±0.02	2.63±0.20	-2.46±0.20	5.51[a] (4.78-6.36)	0.03
A		24.51±0.35	19.42±0.01	5.09±0.35	0.00±0.35	1[b] (0.78-1.27)	
T	*A1CF*	27.16±0.47	18.53±0.02	8.63±0.47	5.22±0.47	0.02[b] (0.02-0.03)	0.04
A		22.83±0.89	19.42±0.01	3.41±0.89	0.00±0.89	1[a] 0.54-1.85	
T	*FGB*	31.7±0.51	18.53±0.02	13.17±0.51	6.12±0.51	0.01[b] (0.01-0.02)	0.00
A		26.47±0.41	19.42±0.01	7.05±0.41	0.00±0.41	1[a] 0.75-1.33	

Figures in parenthesis represent RQ lower value-RQ upper value
Values with different superscript differ significantly ($p<0.01$ and $p<0.05$)

Conclusion

CHAC1 upregulation in Tellicherry chickens indicates enhanced muscle fibre development, regeneration and oxidative stress adaptation. Conversely, downregulation of *FGB* may impair fibrinogen synthesis, extracellular matrix organisation and muscle stability, while downregulation of *A1CF* suggests disrupted apoB editing and lipid metabolism. Together, these gene expression changes highlighted breed-specific differences influencing muscle growth, repair and meat quality traits.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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