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Differential Expression of Transforming Growth Factor Beta Receptor 3 and *Ubiquitin-protein Ligase E3C* in LWY and Ankamali Pigs in Kerala

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ABSTRACT

Present study investigates the differential expression of *Transforming Growth Factor Beta Receptor 3 (TGFBR3)* and *Ubiquitin-Protein Ligase E3C (UBE3C)* genes in Large White Yorkshire (LWY) and Ankamali pigs, two distinct genetic groups in Kerala. *TGFBR3*, a key regulator of the TGF-β signalling 1 pathway, influences muscle growth, fibrosis, and fat deposition, impacting meat quality. *UBE3C*, a component of the ubiquitin-proteasome system (UPS), plays a crucial role in protein turnover and lipid metabolism, affecting muscle composition. Using quantitative real-time PCR (qRT-PCR), gene expression was analysed in skeletal muscle tissues from six pigs per genetic group. Results revealed a significant down regulation of *TGFBR3* and *UBE3C* in Ankamali pigs, suggesting reduced TGF-β pathway activity and proteasomal function. These differences may contribute to lower fat deposition and higher lean meat yield in Ankamali pigs, while LWY pigs exhibit higher intramuscular fat and superior growth rates. Present findings highlighted *TGFBR3* and *UBE3C* as potential genetic markers for optimising growth performance and meat quality.

HIGHLIGHTS

- *TGFBR3* and *UBE3C* are identified as potential biomarkers for selecting and breeding pigs with optimized growth performance and improved meat quality characteristics.
- Both genes showed significantly lower expression in Ankamali pigs compared to LWY pigs, suggesting decreased activity in the TGF-β signalling and proteasomal pathways.

Keywords: LWY pigs, Ankamali pigs, *TGFBR3*, *UBE3C*

Pig farming plays a crucial role in the livestock sector, serving as a significant source of high-quality animal protein and providing economic stability for farmers. In Kerala, Large White Yorkshire (LWY) pigs and Ankamali pigs represent two genetically distinct genetic groups with varying meat production, physiological and adaptive traits. The LWY pigs, a globally recognised commercial breed, are known for their rapid growth rate, high feed efficiency and superior meat yield, making them a preferred choice in intensive farming systems (Jaysree *et al*., 2019). In contrast, Ankamali pigs, a native domesticated variety, exhibit better adaptability to local conditions, disease resistance and lean meat composition, making them suitable for small-scale and sustainable pig farming (Gupta *et al*., 2007). Understanding the genetic factors influencing meat quality and muscle development in these

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breeds is essential for improving pig breeding strategies and enhancing pork production efficiency.

Transforming Growth Factor Beta Receptor 3 (TGFBR3) and *Ubiquitin-Protein Ligase E3C (UBE3C)* are two key genes implicated in muscle growth, development and meat quality regulation. The TGFBR3 plays a crucial role in muscle tissue remodelling, fat deposition and extracellular matrix organisation, influencing tenderness and texture of meat (Schabort *et al*., 2009). On the other hand, UBE3C, a component of the ubiquitin-proteasome system, is involved in protein turnover, fat deposition and lipid metabolism, expressed in muscle and are associated with meat quality in pigs (Ponsuksili *et al*., 2010; Huynh *et al*., 2013; Abe *et al*., 2014). Differences in the expression levels of these genes between LWY and Ankamali pigs may provide insights into breed-specific variations in muscle development, fat content and overall meat characteristics.

The present study aimed to analyse and compare the expression of *TGFBR3* and *UBE3C* in skeletal muscle tissues of LWY and Ankamali pigs in Kerala using quantitative real-time PCR (qRT-PCR). Investigating the differential gene expression patterns can help identify genetic markers associated with improved meat quality, muscle growth efficiency and adaptability to different farming conditions, thereby aiding in selective breeding programmes and sustainable pork production.

MATERIALS AND METHODS

Skeletal muscle tissues were collected from six adult LWY and Ankamali pigs during slaughter at the Meat Technology Unit, Mannuthy, Thrissur, Kerala. All samples were obtained during the pre-rigor mortis phase, before the onset of rigor mortis and were immediately preserved in RNAlater (Sigma-Aldrich) to maintain RNA integrity. The preserved samples were then transported to the laboratory under controlled conditions and stored at -80°C until RNA extraction. Total RNA was isolated using the RNeasy Fibrous Tissue Mini Kit (Qiagen), followed by first-strand cDNA synthesis using the Thermo Scientific Verso cDNA Synthesis Kit. The relative expression levels of *TGFBR3* and *UBE3C* were quantified using Aldolase, fructose-bisphosphate A (*ALDOA)* as the reference gene. Gene-specific primers were designed based on porcine *TGFBR3* and *UBE3C* sequences, selected using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and custom synthesised is given in Table 1.

The qRT-PCR was performed for each sample in triplicate, in 10 µl final volume containing five µL SYBR green master mix, primers 0.3 µL each (10pmol/µL of primers) and 0.5 µg of template cDNA. The thermal cycling profile for the reaction includes initial denaturation for 3 min at 95ºC, followed by 40 cycles of denaturation at 94ºC for 30 sec, annealing at 63.9ºC, 65ºC and 61ºC for 15 sec for *TGFBR3, UBE3C* and *ALDOA*, respectively, followed by extension at 72ºC for 30 sec. Dissociation curve analysis was done after each PCR. The control set for each run were non-template control (NTC) for each gene and a negative control with nuclease free water. The qRT-PCR was normalised to the reference gene, *ALDOA*. The $2^{-\Delta\Delta CT}$ method, was used for calculating relative expression of *TGFBR3* and *UBE3C* gene (Livak and Schmittgen, 2001). Statistical comparisons between the LWY and Ankamali pigs were conducted using independent sample t-tests and a significance level of $p< 0.05$ was used to determine statistical significance.

Table 1: Primer sequences of *TGFBR3* and *UBE3C* genes

Gene	Primer sequence	Product size
TGFBR3	**FP:** 5'- GCCATCCAAACGTGCTTCAT - 3' **RP**: 5'- GTGGACTCTCTTGGGATCGT - 3'	123
UBE3C	**FP:** 5'- ATCGCCTACATCCACCTTGT - 3' **RP:** 5'- GAGCCACTCCAGATTCAGCA - 3'	108
ALDOA	**FP:** 5'- CAACCTCAACGCCATCAACA - 3' **RP:** 5'- GGGCTCGCTTGACATATTCTT -3'	152

RESULTS AND DISCUSSION

The mean RNA concentrations obtained were 296.36 ± 22.45 ng/µL for LWY pigs and 150.03 ± 17.53 ng/µL for Ankamali pigs. The A260/A280 and A260/A230 ratios for LWY pigs were 2.08 ± 0.01 and 1.94 ± 0.01, respectively, while Ankamali pigs exhibited corresponding values of 2.09 ± 0.01 and 1.96 ± 0.01. These values confirm the high quality of RNA, making it suitable for downstream molecular analyses. Only high-quality RNA samples, characterised by distinct 28S and 18S rRNA bands along with mRNA smearing, were selected for further analysis.

The expression analysis of the reference gene, *TGFBR3* and *UBE3C* genes was conducted using qRT-PCR. The amplification plot and melt curve confirmed the specificity of the reaction, as a single peak was observed, indicating the absence of primer dimers and non-specific products (Ruiz-Villalba *et al*., 2017). The amplification and melt curve data are presented in Fig. 1 to 3. Cycle threshold (C_T) mean values were calculated from data generated by the Bio-Rad CFX Opus Dx Real-time PCR detection system for LWY and Ankamali pigs, providing a comparative measure of gene expression levels (Bustin, 2002). The relative expression profiles of *TGFBR3* and *UBE3C* in LWY and Ankamali pigs are shown in Fig. 4. The relative expression profiles of *TGFBR3* and *UBE3C* in both genetic groups revealed a significant down regulation of these genes in Ankamali pigs compared to LWY pigs (Table 2). These findings have important implications for meat production, as both genes are associated with muscle development, protein turnover and metabolic regulation, which directly impact growth performance, carcass traits and meat quality in pigs.

The *TGFBR3* plays a pivotal role in the TGF-β signalling pathway, participates in many cellular processes like cell differentiation, proliferation and apoptosis. The TGF-β pathway is known to inhibit muscle growth by promoting

Table 2: C_T values, fold change and p values of relative expression of *TGFBR3* and *UBE3C* between LWY and Ankamali pigs

Genetic group	Gene	Mean C_T ± SE		ΔC_T ± SE	$\Delta\Delta C_T$ ± SE	Fold change from control ($2^{-\Delta\Delta CT}$)	p-value
		Target Gene	Reference Gene				
Ankamali pigs	*TGFBR3*	29.28±0.49	14.98±0.06	14.30±0.49	2.84±0.49	0.14[a] (0.09-0.19)	
LWY pigs		26.82±0.40	15.36±0.20	11.46±0.45	0.00±0.45	1[b] (0.73-1.37)	0.02
Ankamali pigs	*UBE3C*	24.87±0.09	14.98±0.06	9.89±0.11	1.94±0.11	0.26[a] (0.24-0.28)	
LWY pigs		23.31±0.14	15.36±0.20	7.95±0.25	0.00±0.25	1[b] (0.84-1.19)	0.00

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

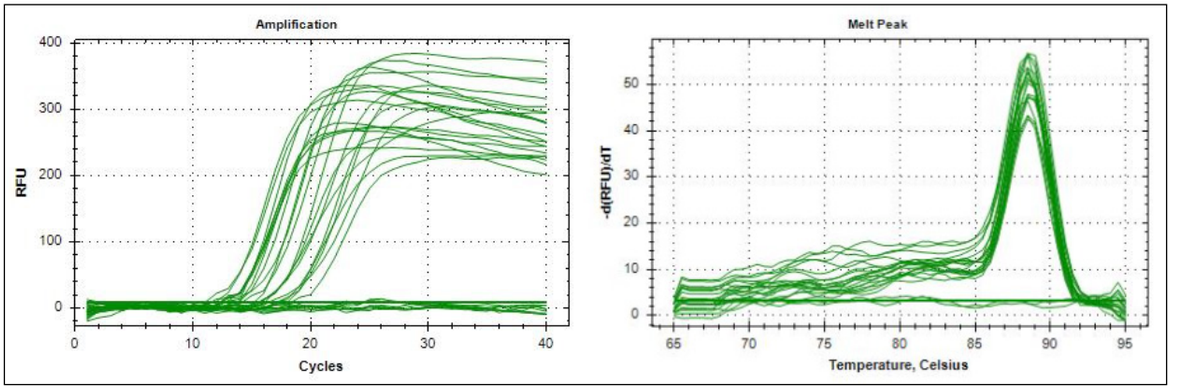

Fig. 1: Amplification plot and melt curve of *ALDOA* gene

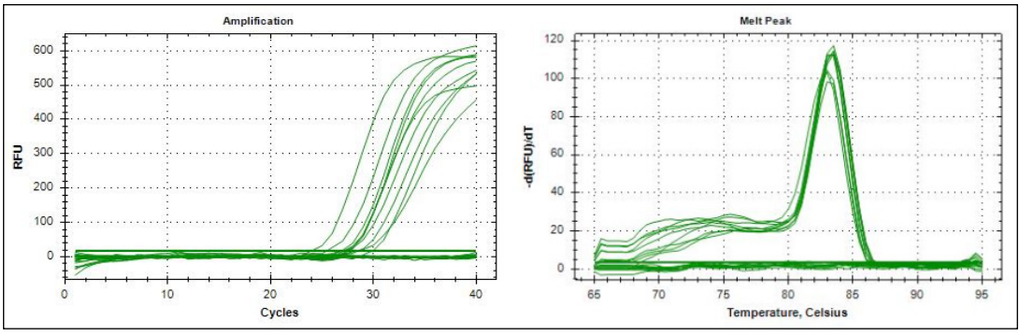

Fig. 2: Amplification plot and melt curve of *TGFBR3* gene

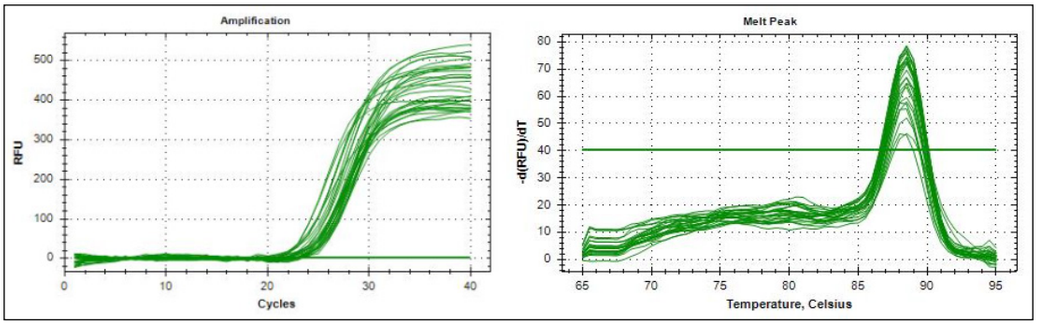

Fig. 3: Amplification plot and melt curve of *UBE3C* gene

fibrosis and regulating myogenic differentiation through the modulation of myostatin, a key inhibitor of muscle development (Delaney *et al*., 2017; Ismaeel *et al*., 2019). Since *TGFBR3* enhances TGF-β signalling, its reduced expression may lead to altered muscular proliferation and regeneration from satellite cells (Schabort *et al*., 2009). Additionally, Jeong *et al*. (2015) demonstrated through *in vitro* experiments that knockdown of *TGFBR3* inhibited both preadipocyte proliferation and differentiation, indicating its critical role in adipogenesis. The observed downregulation of *TGFBR3* in Ankamali pigs suggested a potential reduction in TGF-β pathway activity, which could influence muscle fibre characteristics, composition and meat quality traits.

The *UBE3C* is a key component of the ubiquitin-proteasome system (UPS), which regulates fat deposition and lipid metabolism (Loix, 2024). The UPS plays a crucial role in maintaining muscle homeostasis by removing damaged or misfolded proteins, ensuring efficient protein synthesis and degradation balance, which is essential for muscle growth and meat yield (Bilodeau *et al*., 2016). Genetic variations in *UBE3C* have been significantly linked to intramuscular fat content and saturated fatty acid composition, reinforcing its role as a potential candidate gene for fat deposition in pigs (Uemoto *et al*., 2012; Supakankul and Mekchay, 2016). Additionally, research by Nitipongsuwan *et al*. (2016) indicated that *UBE3C* polymorphisms affected drip loss, highlighting its potential as a genetic marker for selective breeding programmes aimed at enhancing pork quality. The significant downregulation of *UBE3C* in Ankamali pigs suggested reduced proteasomal activity, which might affect protein turnover rates, fat deposition and muscle growth. This could result in lower fat deposition and higher lean meat yield in Ankamali pigs, a characteristic often observed in indigenous pig compared to commercial lines.

In conclusion, present findings underscore the genetic basis of muscle development and fat metabolism differences between LWY and Ankamali pigs. Understanding these

molecular mechanisms provides valuable insights for breeding strategies aimed at improving meat yield and quality in Ankamali pigs while maintaining their unique adaptations and desirable traits.

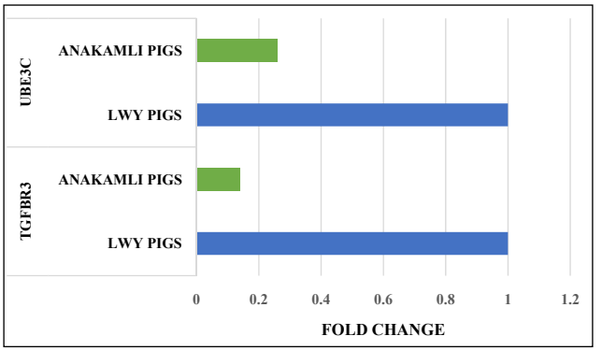

Fig. 4: Relative expression profile of *TGFBR3* and *UBE3C*

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REFERENCES

Abe, T., Hasebe, H., Fukuwaka, H. and Saburi, J. 2014. Genetic analysis of meat quality traits in pigs using genome-wide association study. *Anim. Sci. J.*, **85**(6): 617-623.

Bilodeau, P., Dufresne, F. and Guillemette, C. 2016. Ubiquitin-proteasome system and its role in muscle development. *J. Mol. Biol*., **428**(21): 4351-4363.

Bustin, S.A. 2002. Quantification of mRNA using real-time reverse transcription PCR (qRT-PCR): trends and problems. *J. Mol. Endocrinol*., **29**(1): 23-39.

Delaney, K., Kasprzycka, P., Ciemerych, M.A. and Zimowska, M. 2017. The role of TGF-β signaling in muscle fibrosis. *Cell Signal*., **40**: 1-11.

Gupta, R., Sharma, A. and Kumar, A. 2007. Genetic diversity and adaptability of indigenous pig breeds in India. *Indian J. Anim. Sci*., **77**(10): 1043-1050.

Huynh, T.T., Ponsuksili, S., Tholen, E. and Wimmers, K. 2013. Gene expression profiles of meat quality traits in pigs. *Anim. Genet*., **44**(5): 506-516.

Ismaeel, A., Kim, J.S., Kirk, J.S. and Smith, R.S. 2019. The TGF-β pathway and its role in muscle fibrosis. *Physiol. Rep*., **7**(2): e13968.

Jaysree, W., Kumar, R. and Sharma, P. 2019. Large White Yorkshire pigs: Growth, efficiency, and adaptability. *Indian Vet. J*., **96**(8): 12-18.

Jeong, Y.S., Lee, K.S. and Kim, J.B. 2015. Knockdown of TGFBR3 inhibits preadipocyte proliferation and differentiation. *Mol. Cell Biochem*., **407**(1-2): 183-193.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, **25**(4): 402-408.

Loix, S. 2024. The ubiquitin-proteasome system and its role in adipose tissue regulation. *J. Biochem. Res*., **58**(3): 145-158.

Nitipongsuwan, K., Surakhunthod, J. and Chaiwong, S. 2016. UBE3C polymorphisms and their association with drip loss in pigs. Asian-Australas. *J. Anim. Sci*., **29**(12): 1687-1694.

Ponsuksili, S., Murani, E., Trakooljul, N. and Wimmers, K. 2010. Expression profiling of candidate genes for meat quality in pigs. *Meat Sci.*, **84**(3): 465-470.

Ruiz-Villalba, A., Romero, J.P. and Hernández, S.C. 2017. qRT-PCR analysis: Melt curve validation and quality control. *Methods Mol. Biol*., **1663**: 29-45.

Schabort, E.J., van der Merwe, M. and Wannenburg, T. 2009. The role of *TGFBR3* in extracellular matrix organization and muscle regeneration. *J. Cell Physiol*., **218**(1): 52-60.

Supakankul, P. and Mekchay, S. 2016. Association of UBE3C gene variants with meat quality traits in pigs. *Genet. Mol. Res*., **15**(2): gmr.15027923.

Uemoto, Y., Ohnishi, C. and Sasaki, Y. 2012. Genetic variations in UBE3C and their effects on intramuscular fat content in pigs. *J. Anim. Sci*., **90**(12): 4211-4219.