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Research Article

Effects of *FUT1* and *MUC4* Polymorphisms on Intestinal Gene Expression in Yorkshire Pigs under Tropical Conditions

Luc Duc Do[1]*, Tuoi Thi Phan[2], Phuong Thi Do[3], Ton Dinh Vu[1], Thinh Hoang Nguyen[1]

[1]Department of Animal Breeding and Genetics, Faculty of Animal Science, Vietnam National University of Agriculture, Hanoi 10000, Vietnam; [2]Department of Animal Science, Faculty of Agriculture - Forestry - Fishery, Hong Duc University, Thanh Hoa 40132, Vietnam; [3]Sunjin Vina Co., Ltd – Ha Nam Branch, Ha Nam 400000, Vietnam.

Abstract | This study was conducted at a nucleus breeding pig farm in northern Vietnam from August to September 2018 to evaluate the effects of mutation c.307G>A in *alpha (1,2)-fucosyltransferase* (*FUT1*) and g.243A>G in mucin 4 (*MUC4*) on gene expression in intestinal tissues of Yorkshire pigs. The polymorphisms of *FUT1* and *MUC4* were identified from ear tissue samples of 28 21-day-old pigs using the PCR-RFLP method. From the genotype information, a total of 12 clinically healthy piglets (6 females and 6 uncastrated males) with average age 32.5 days were selected for analyzing gene expression. Colon, duodenum and jejunum tissues were collected and determined the expression levels of *FUT1* and *MUC4* gene using q-PCR technique. The genotypes of interested genes (*FUT1* and *MUC4*), tissue (colon, duodenum and jejunum) and gender (male and female) were included in the statistical model to estimate the effects of factors on the gene expression. The results showed that *FUT1* gene expression did not differ among genotypes in any examined tissues. In contrast, *MUC4* expression was significantly higher in pigs with the AG genotype than in those with the GG genotype in the colon and across the overall tissues. *FUT1* expression was similar among intestinal tissues, whereas *MUC4* expression was significantly higher in the colon than in the duodenum and jejunum. In addition, male pigs showed higher *FUT1* expression in the colon, jejunum, and overall tissues, while higher *MUC4* expression in male pigs was observed only in the colon.

Keywords | *FUT1*, Gene expression, *MUC4*, Pigs, Polymorphisms, Tissues

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***Correspondence** | Luc Duc Do, Department of Animal Breeding and Genetics, Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi 12406, **Email:** ddluc@vnua.edu.vn.
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INTRODUCTION

In the pig farms, enterotoxigenic *Escherichia coli* (ETEC) has been considered as one of the main causes of morbidity and mortality due to diarrhea in neonatal and just weaned piglets, and resulting in huge economic losses in swine husbandry (Kim *et al*., 2022; Luise *et al*., 2019; Shakuntala *et al*., 2018). Antibiotic administration, a popular strategy for controlling diarrhea for a long time, has been reconsidered due to the increased risk of antibiotic resistance in pathogens (Shakuntala *et al*., 2018). Another strategy is vaccination the sows, that could protect piglets but only during lactation, not for post-weaning diarrhea (Matías *et al*., 2017; Ruan *et al*., 2011). Oral vaccination has been suggested to reduce the incidence of post-weaning diarrhea and fecal shedding in pigs infected with ETEC from 18 days of age, however, its cost was 3-fold of antibiotic treatment (Fratto *et al*., 2024). On the other

hand, the effects of nutritional supplements on post-weaning diarrhea in pigs were high variables (Canibe *et al*., 2022). Recently, research has focused on preventing and controlling diseases with minimal veterinary intervention or non-chemotherapeutic approaches. The potential for discovering genetic resistibility through the selection of genotypes resistant to diseases has been interested (Emam *et al*., 2019).

There are two types of important factors that allow ETEC to cause diarrhea, namely surface adhesions and enterotoxins. For the former, in the gastrointestinal tract of the animals, ETEC attach with their fimbriae or pili to specific receptors on the epithelium of the host small intestine or in the mucus, which coat the small intestine. For the latter, the bacteria proliferate rapidly, colonize the small intestine, and release enterotoxins and lipopolysaccharide, that trigger diarrhea (Fairbrother *et al*., 2005; Schroyen *et al*., 2012). Of the five well described types of fimbriae in ETEC, F4 and F18 are the most prevalent. F4 fimbriae are related to diarrhea in both neonatal and post-weaning piglets, while F18 fimbriae are associated with post-weaning piglets (Schroyen *et al*., 2012). To cause diarrhea, F4 or F18 fimbriae of ETEC necessarily bind to the specific receptor in the host brush border, which do not express at the same level in all pigs. The literature studies have suggested that, *alpha (1,2)-fucosyltransferase (FUT1)* gene located on chromosome 6 was candidate gene for genetical modulation of F18 receptor expression (Meijerink *et al*., 1997; Vögeli *et al*., 1997). An association between a mutation at position c.307G>A in *FUT1* gene and the susceptibility to ETEC F18 was reported, in which genotype AA was considered as resistant, whereas GG or AG as sensitive genotypes (Meijerink *et al*., 1997; Vögeli *et al*., 1997). On the other hand, *MUC4* g.243A>G mutation in intron 17 was demonstrated the association with susceptibility to ETEC F4ab/ac infection, in which GG related to resistant genotype, while AA and AG were sensitive (Peng *et al*., 2007).

In addition, the associations between *FUT1*, *MUC4* polymorphisms and the economically important traits in pigs were investigated. A study by Liu *et al*. (2015b) demonstrated the positive effect of $MUC4^{GG}$ genotype on age reached 100kg body weight of pigs compared with the genotypes AA and AG. Inversely, Geraci *et al*. (2019) reported no association between the polymorphisms of *MUC4* g.243A>G with average daily gain and backfat thickness of Italian Large White pigs. For the reproductive traits, the effect of *MUC4* genotypes on total number of piglets born or number born alive were not detected (Balcells *et al*., 2011; Liu *et al*., 2015b). Similarly, polymorphism of *MUC4* did not have negative effects on the growth performance (Luc *et al*., 2023) and sperm quality traits (Luc *et al*., 2022) of Landrace and Yorkshire pigs. Previous studies have confirmed the positive effect of genotype $FUT1^{AA}$ on the productive traits of pigs, including body length, body height, back width (Bao *et al*., 2011), survival rate of piglets (Kim *et al*., 2013). Whereas, no significant effects of *FUT1* polymorphisms on productive traits of pigs were observed (Luc *et al*., 2020; Sukhno *et al*., 2022).

Despite extensive investigations on the associations between *FUT1, MUC4* polymorphisms with ETEC susceptibility, the biological mechanisms underlying these associations remain poorly understood. One possible mechanism is that disease-resistant genotypes may influence gene expression patterns in intestinal tissues during the critical post-weaning period, thereby affecting host resistance to ETEC infection (Wenhua Dong *et al*., 2015a). Previous studies have demonstrated that the expression of *MUC4* and *FUT1* genes can be influenced by several factors, including age, breed, and dietary components such as zinc oxide, laminarin, resistant starch, and inulin (Bao *et al*., 2012b; Wenhua Dong *et al*., 2015; Liu Y *et al*., 2015; Sargeant *et al*., 2010; Smith *et al*., 2010; Xia *et al*., 2016; Zhou *et al*., 2017; Święch *et al*., 2025). While previous studies have examined these relationships in other breeds or under temperate conditions (Bao *et al*., 2012a; Dong WenHua *et al*., 2016), investigations specifically focusing on post-weaned Yorkshire pigs raised under tropical conditions in Vietnam remain limited. The objective of this study was to investigate the effects of mutations *c.307G>A* in *FUT1* and *g.243A>G* in *MUC4* on their expression in different intestinal tissues of post-weaning Yorkshire pigs.

MATERIALS AND METHODS

The study protocol was approved by the Faculty Council, Faculty of Animal Science, Vietnam National University of Agriculture under approval number FAS-VNUA-2018/602.

Time and location

The study was conducted at Dabaco nucleus breeding pig farm in northern Vietnam from August to September 2018. *FUT1* and *MUC4* genotyping, and gene expression were conducted at the Genetic Laboratory, Faculty of Animal Science, Vietnam National University of Agriculture.

Animals and sample collection

The ear tissue samples of 28 Yorkshire piglets at 21 days of age were collected to identify *FUT1* and *MUC4* genotypes. Among the 28 individuals, the numbers of animals with *FUT1* genotypes AA, AG, and GG were 3, 10, and 15, respectively, corresponding to genotype frequencies of 0.11, 0.36, and 0.54. The numbers of individuals carrying *MUC4* genotypes AA, AG, and GG were 11, 15, and 2, respectively, corresponding to genotype frequencies of 0.39,

0.54, and 0.07. Based on this genotype information, 12 clinically healthy piglets at 32-33 days old of age (average 32.5 days, 6 females and 6 uncastrated males) were chosen from above 28 piglets for evaluating gene expression. The animals were slaughtered at Dabaco food processing company according to TCVN 8402:2010 (Ministry of Science and Technology, 2010). The tissue samples were collected from 3 regions of the intestine, including colon, duodenum, and jejunum. At each region, all tissues were taken in duplicates and stored individually in nuclease-free collection tubes with NucleoProtect® RNA for stabilization, protection and storage tissues (followed the User manual - NucleoProtect® RNA (MACHEREY-NAGEL GmbH & Co. KG). After complete permeation of samples with NucleoProtect® RNA solution (≥ 12 h at 4 °C – 25 °C), the samples were frozen at -30 °C and stored until RNA extraction.

FUT1 and *MUC4* genotypes identification

DNA extraction was followed method of (Sambrook *et al*., 1989) with slight modifications for suitable of laboratory condition. The determination of concentration, quality and purity of DNA was tested by agarose gel electrophoresis and measured using NanoDrop 2000 *Spectrophotometer* device *(*Thermo Fisher Scientific, USA*)*. Then DNA working solution (concentration of 50 ng/µl) was prepared by dilution of DNA stock solution with nuclease-free water for PCR reaction. The genotypes of *FUT1 G307A* and *MUC4 G243A* were identified by identified using the PCR-RFLP technique based on the methods described in study of Meijerink *et al*. (1997) and Liu *et al*. (2015a), respectively. The information on primer sequences, PCR product size, restriction enzymes and allele sizes are shown in Table 1.

The components of a PCR reaction were a total 25 µL mixture containing 2 µL (50 ng/ µL) of template DNA, 0.5 µL (10 µM) of each primer, 2.5 µL Dream Taq™ buffer (including $MgCl_2$), 0.5 µL dNTP, 0.2 µL Dream Taq™ and 18.8 µL ddH_2O. The PCR thermal cycling condition for *FUT1* and *MUC4* were presented in Table 2.

FUT1 and *MUC4* genes expression

The tissue samples (50-100 mg) from each intestinal region (colon, duodenum, or jejunum) were homogenized and a total RNA extraction was conducted by using NucleoSpin® RNA Set for NucleoZOL kit (Mecherey Nagel, Germany) according to the manufacturer instructions. The RNA quality and integrity were controlled by NanoDrop 2000 *Spectrophotometer* device (Thermo Fisher Scientific, USA).

Reaction of q-PCR was performed on an ABI Step one Plus system (Applied Biosystems, USA) using the Universal One-Step RT-qPCR kit with SYBR Green dye. *FUT1* and *MUC4* primers for q-PCR were followed to study of Xia *et al*. (2016) and Dong WenHua *et al*. (2015), respectively. Three commonly reference genes including *GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase), transferrin-binding protein1 (*TBP1*) and Actin beta *(ACTB)* was evaluated under laboratory conditions across the examined tissues. Based on these assessments, *TBP1* was selected as housekeeping gene in this study. The information on primer sequences, PCR product size of *FUT1*, *MUC4* and housekeeping genes are presented in Table 3. A q-PCR reaction conditions were initial step (15s at 95°C) and denaturation step (5s at 95°C and 34s at 62°C) for 40 cycles. Analysis of the melt curve was performed after the amplification. The peak values Tm (82.63 for *FUT1* and 79.41 for *MUC4*) of the melt curve were used to evaluate the specificity of the amplification reactions. The Tm value of each sample at each region was an average of 3 replicates from the q-PCR reaction. The $2^{-\Delta\Delta Ct}$ method was used to quantification for gene expression.

Statistical analysis

The effects of genotype (*FUT1* or *MUC4*), tissue and gender on gene expression were assessed using the statistical mixed model using REML method as below:

$$yijklm = \mu + Gi + Oj + Sk + IDl + \varepsilon ijklm$$

Where: y_{ijkl} = values of gene expression, µ = overall mean, Gi = fixed effect of genotype i (AA, AG and GG), O_j = fixed effect of tissue j (colon, duodenum and jejunum), S_k = fixed effect of gender k (male and female) ID_l = random effect of animal l, and + ε_{ijklm} = residual error. Additionally, the effect of genotype and gender on gene expression at each tissue was also estimated using the above statistical model without random (animal) and tissue effects.

Table 1: Primer sequences, PCR products, restriction enzymes, allele sizes of *FUT1* and *MUC4* and referenced methods.

Gene	SNP	Primer sequence	PCR product size (bp)	Restriction enzyme	Allele size (bp)	Reference
FUT1	G307A	F - CTTCAGCCAGGGCTCCTTTAAG R - CTGCCTGAACGTCTATCAAGACC	421	Hin6I	A: 328, 93 G: 241, 93, 87	Meijerink *et al.* (1997)
MUC4	G243A	F – CAGGATGCCCAATGGCTCTAC R - CCCCGAAGTTGTGAAAGGAAG	538	HhaI	A: 538 G: 295, 243	Peng *et al.* (2007)

Note: R: Reverse, F: Forward.

Table 2: Thermo cycling conditions of PCR reactions according to temperature (T, °C) and time (Time, sec).

Steps	FUT1			MUC4		
	T (°C)	Time (sec)	Number of cycles	T (°C)	Time (sec)	Number of cycles
Initial	94	180	1	95	180	1
Denaturation	94	45	35	95	45	30
Annealing	58	30		62.5	45	
Extension	72	45		72	45	
Final Extension	72	300	1	72	300	1

Table 3: Primer sequences, PCR product size of *FUT1*, *MUC4* and housekeeping gene *TBP1*.

Gene	SNP	Primer sequence	PCR product size (bp)	Reference
FUT1	G307A	F - TTTTAAGCCCCCAAACTGCC R - TAAATCGACCCCATCAGCCTC	126	(Xia *et al.*, 2016)
MUC4	G243A	F - GGCCCACCTTAAGATTCCCA R - GCTTCTCCTTAGCATGCCCAG	131	(Wenhua Dong *et al.*, 2015)
TBP1		F: AACAGTTCAGTAGTTATGAGC R: AGATGTTCTCAAACGCTTCG	153	

Note: R: Reverse, F: Forward.

Normality and homogeneity of variances were checked. These assumptions were met for *FUT1* data but not with *MUC4*. Therefore, data transformation was applied for *MUC4* using log10 of the initial value plus 9. All values for *MUC4* are displayed in the log10 scale.

The statistical parameters were observation (n), Least Square Mean (LSM) and standard error (SE). The Tukey test was used for pairwise comparison between LSM. All data were performed using SAS® OnDemand for Academics.

RESULTS AND DISCUSSION

This study was conducted at a pig farm in northern Vietnam, a region climatically characterized by high ambient temperatures (approximately 28–35 °C), high relative humidity (often exceeding 80%), and frequent rainfall, reflecting a hot and humid monsoon season. These environmental factors may influence physiological status and gene expression in livestock. For example, heat and humidity can induce thermal stress, which may alter metabolic activity, immune responses, and intestinal function. Such changes could, in turn, affect the expression of genes such as *FUT1* and *MUC4*, particularly in tissues associated with digestion and host–microbe interactions.

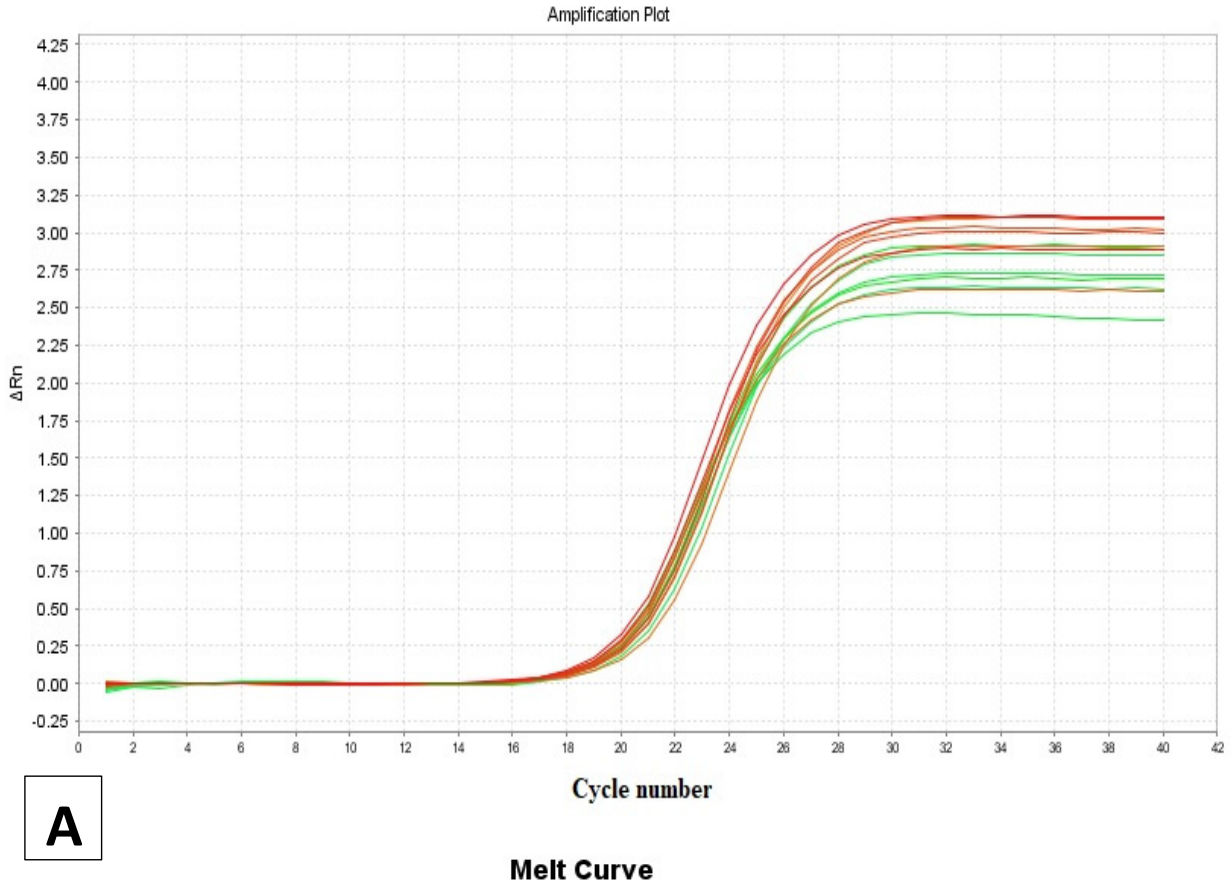
Figures 1 and 2 show the melting curves for the *FUT1* and *MUC4* qPCR products. We performed simultaneous amplification of multiple samples, which resulted in multiple peaks. However, the critical point is that these peaks cluster around nearly the same melting temperature (Tm), thereby confirming the specificity of the qPCR assay and suggesting the accurate quantification of the target genes.

Table 4: Gene expression of *FUT1* and *MUC4* according to genotype and tissue.

Tissue	Genotype	*FUT1*			*MUC4*		
		n	LSM	SE	n	LSM	SE
Overall	AA	9	0.785	0.305	12	0.999^{ab}	0.009
	AG	12	0.990	0.261	18	1.020^{a}	0.008
	GG	15	1.363	0.235	6	0.975^{b}	0.014
	P-value		0.314			0.0352	
Colon	AA	3	0.688	0.337	4	1.021^{ab}	0.019
	AG	4	0.972	0.288	6	1.078^{a}	0.016
	GG	5	1.514	0.259	2	0.976^{b}	0.029
	P-value		0.189			0.0334	
Duodenum	AA	3	0.899	0.416	4	0.999	0.015
	AG	4	1.064	0.355	6	0.992	0.013
	GG	5	1.236	0.321	2	0.977	0.023
	P-value		0.818			0.7286	
Jejunum	AA	3	0.768	0.284	4	0.976	0.006
	AG	4	0.934	0.242	6	0.989	0.005
	GG	5	1.339	0.219	2	0.973	0.010
	P-value		0.290			0.2694	

Within each gene, means with different superscript letters are significantly different (P<0.05).

The gene expression according to the genotypes of *FUT1* and *MUC4* genes is presented in Table 4. Three expected genotypes (AA, AG and GG) of each gene and their expressions were detected at all the selected tissues. The expression levels of *FUT1* did not differ significantly among pigs carrying the AA, AG, and GG genotypes, either across all examined tissues or within each of the three intestinal tissues individually. These findings were



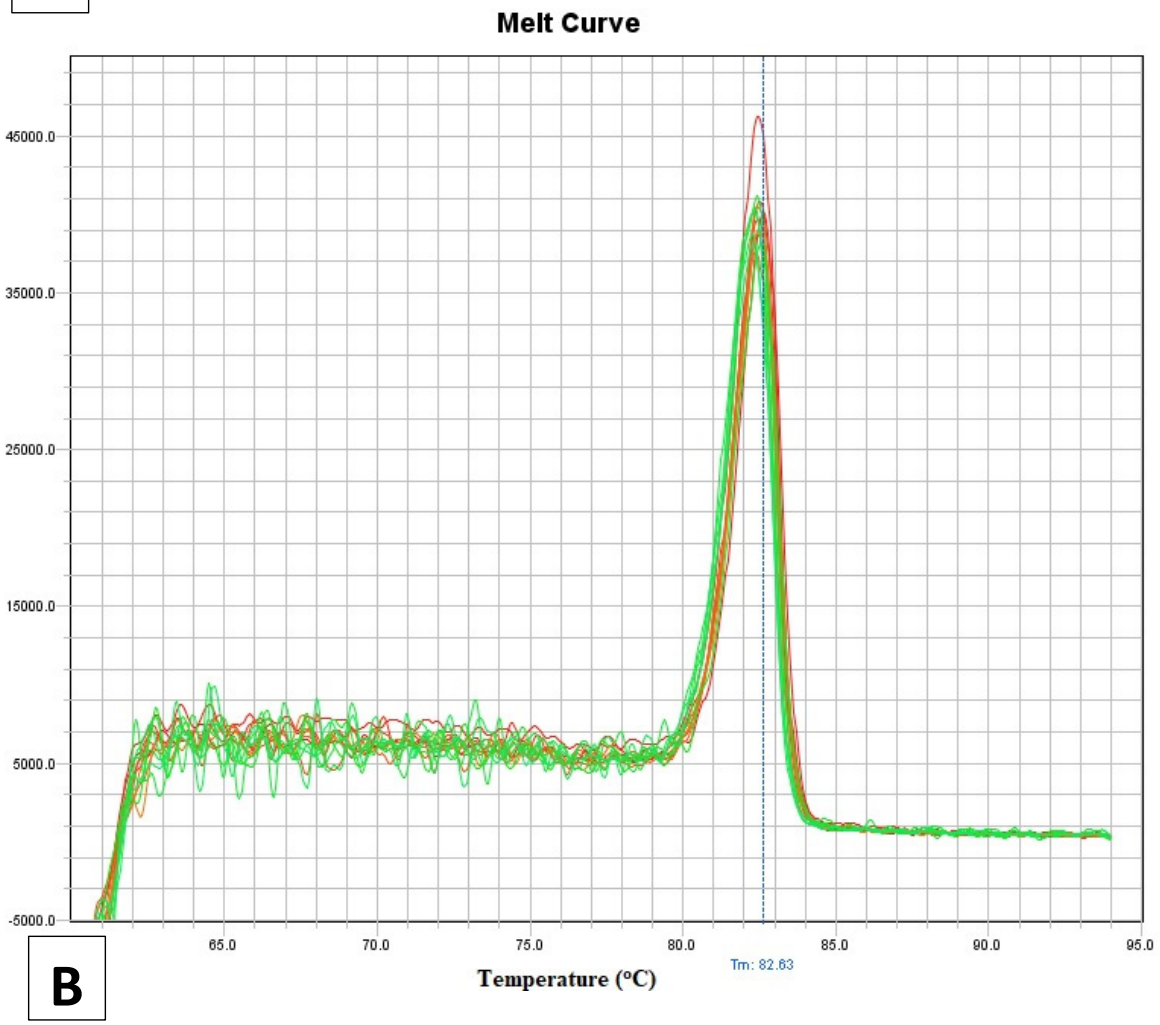


Figure 1: Amplification curve (A) and dissociation curve (B) for the *FUT1* gene.

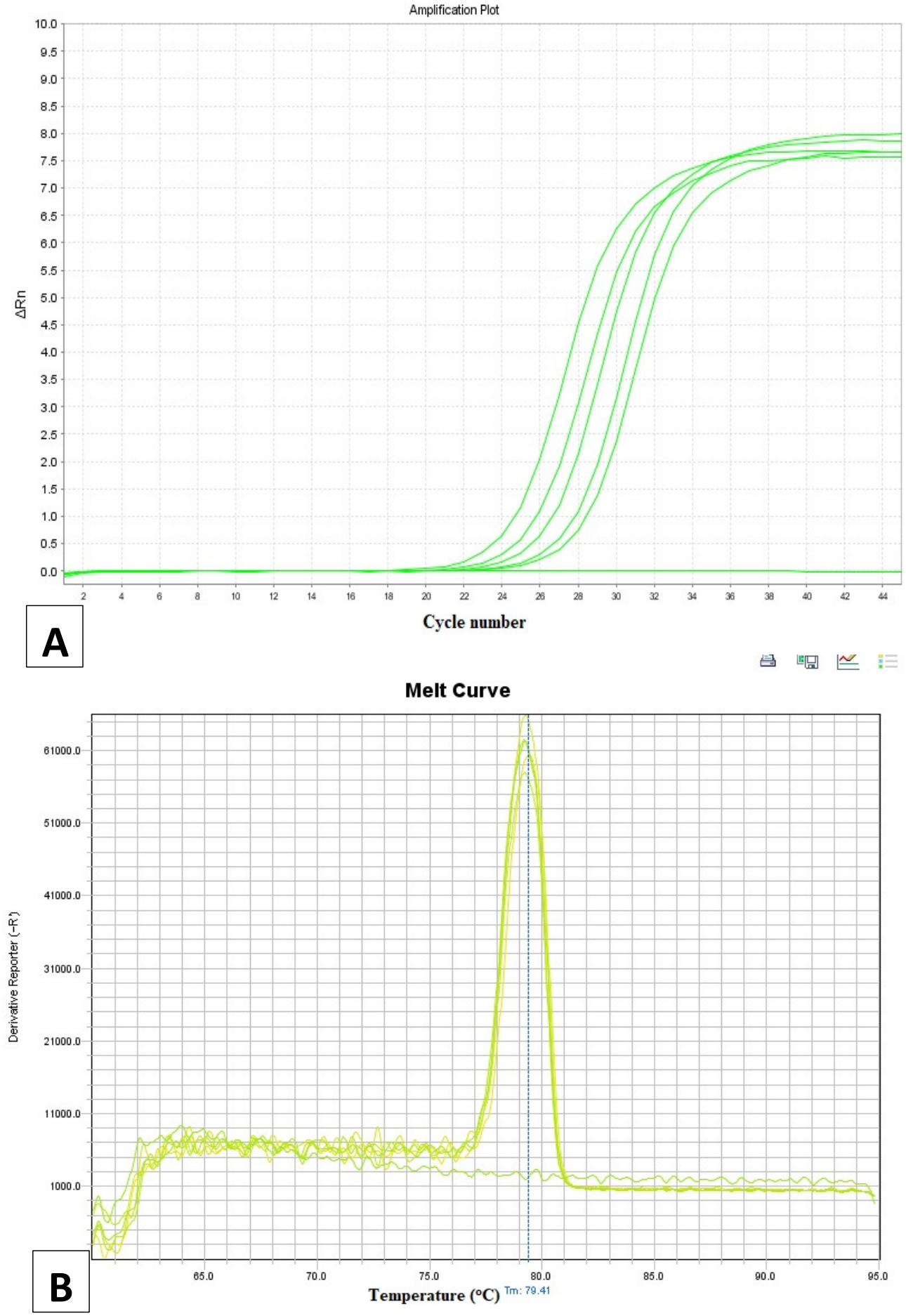


Figure 2: Amplification curve (A) and dissociation curve (B) for the *MUC4* gene.

consistent with the previous study in Sutai piglets, which reported *FUT1* expressions at 11 different tissues of 35-day-old pigs with the highly expression levels at lung, liver, stomach and duodenum (Bao *et al*., 2012b). Similarly, the effect of *FUT1* polymorphisms (AA, AG and GG) on gene expressions in each of four highly expressing tissues were not significant (Bao *et al*., 2012a). The non-significant effect of *FUT1* polymorphisms on gene expression in various tissues of pigs suggest that the expression of this gene may be regulated by other loci or factors.

On the other hand, *MUC4* expression in the colon was significantly higher in pigs with the AG genotype than associated with differences in the physiological status of the pigs, particularly during the post-weaning period. In Vietnam, piglets are typically weaned at 25–28 days of age; therefore, the period from 30 to 35 days is considered the post-weaning stage. This is a period during which piglets are highly susceptible to diarrhea; therefore, we hypothesize that genes associated with resistance to diarrhea may exhibit elevated expression during this stage. In the current study, the pigs were approximately 32.5 days old, corresponding to the early post-weaning stage, which is characterized by substantial intestinal stress, abrupt dietary transition, and marked alterations in gut microbiota composition. These physiological changes may strongly influence the transcriptional regulation of the *MUC4* gene and consequently modify the relationship between genotype and gene expression. Supporting this hypothesis, Comelli *et al.* (2008) demonstrated that intestinal microbiota can regulate the mucus layer at the transcriptional level and that variations in bacterial composition significantly affect this process. Therefore, the higher *MUC4* expression observed in pigs carrying the AG genotype compared with the GG genotype in the present study may reflect genotype-specific responses to weaning-associated intestinal stress and microbiota alterations.

In addition, previous studies have shown that *MUC4* expression is influenced by age and developmental stage. Wenhua Dong *et al.* (2015) reported significantly higher *MUC4* expression levels in pigs at 30 and 35 days of age compared with those at 8 and 18 days of age. This supports the view that the post-weaning period is a critical stage for *MUC4* transcriptional activity. The discrepancy between studies may therefore be explained by differences in intestinal physiological conditions at the time of sampling, especially factors related to weaning stress and microbiota composition, rather than by genotype alone. Nevertheless, other factors such as genetic background, environmental conditions, diet, and methodological differences in tissue sampling, RNA extraction, normalization procedures, or statistical analyses may also contribute to the variation among studies. Moreover, only two individuals carrying the $MUC4^{GG}$ genotype were identified in the present study. Therefore, the limited sample size may have affected the statistical power of the analysis. Further studies with larger sample sizes are needed to draw more reliable conclusions regarding the effects of *MUC4* genotypes on gene expression in tissues.

The gene expression of *FUT1* and *MUC4* in different tissues is presented in Table 5. *FUT1* expression level was similar in all tested tissues (P=0.887) while the expression level of *MUC4* in colon was significantly higher than which in duodenum and jejunum (P<0.001). The result of *FUT1* expression in this study was consistent with previous articles, which reported that the expression of *FUT1* gene was relatively high in lung, stomach, liver and duodenum, jejunum among 11 tissues (Bao *et al*., 2012b; Liu *et al*., 2015a; Xia *et al*., 2016). In addition, there was not any significant difference at *FUT1* expression level in duodenal and jejunal tissues of experimental pigs in mentioned studies. According to previous study, post-weaning diarrhea in piglets normally associated with ETEC F18, which attach their F18 fimbriae to the specific receptors in the intestinal of the piglets (Schroyen *et al*., 2012). The piglets in this study were 32.5 day-old, in the period of post-weaning. Therefore, the relatively high expression of *FUT1* in the intestinal tissues such as colon, duodenum and jejunum, where suggests a role for *FUT1* in regulating the expression or structure of the ETEC F18 receptor.

Table 5: Gene expression of *FUT1* and *MUC4* according to tissues

Tissue	FUT1			MUC4		
	n	LSM	SE	n	LSM	SE
Colon	12	1.079	0.172	12	1.035[a]	0.009
Duodenum	12	1.046	0.172	12	0.984[b]	0.009
Jejunum	12	1.013	0.172	12	0.975[b]	0.009
P-value		0.887			0.0002	

Within each gene, the LSMs with different superscript letters are significantly different (P<0.05).

In this study, *MUC4* expressed in colon with significantly higher level (1.035) than those in duodenum (0.984) and jejunum (0.975). The result was consistent with the finding by Jacobsen *et al.* (2011), which observed the *MUC4* expression in the colon was markedly higher than those in the jejunum and duodenum of crossbred pigs, respectively. A study on Sutai pigs reported highly expression of *MUC4* gene in liver, spleen, stomach and intestinal tissues and the expression level were similar between duodenum and jejunum (Wenhua Dong *et al*., 2015). It is widely known that mucin was generally expressed in the epithelial line of secretory systems including the digestive system, respiratory system and reproductive system and played an important role in lubrication and protection of the mucosal epithelium

(Wenhua Dong *et al*., 2015). Therefore, the relatively high expression of *MUC4* observed in gastrointestinal tissues in the present study is likely related to its physiological role in maintaining mucosal integrity and epithelial protection. In this study, in colon, the expression levels of *MUC4* gene in the pigs with the GG genotype (known as resistant genotype) were lower than in those with the AG genotype. It is hypothesized that, if the disease-resistant genotype exhibits higher gene expression during critical susceptible periods, it may contribute to enhancing resistance to pathogenic microorganisms in weaned piglets and reducing the incidence of post-weaning diarrhea. (Wenhua Dong *et al*., 2015). Neverthless, resistance phenotypes were not evaluated, as no pathogen challenge experiment was performed. Therefore, the current data do not support a direct link between *MUC4* expression and disease resistance in pigs. Any potential role of *MUC4* in resistance to pathogens should be considered hypothetical and requires further investigation through studies integrating gene expression with phenotypic resistance data.

FUT1 gene expression levels in the colon, jejunum, and overall tissues were significantly higher in male pigs than in female pigs (P<0.05). However, no significant difference between sexes was observed in the duodenum (Table 6). For the *MUC4* gene, expression levels in the colon were significantly higher in male pigs than in female pigs (P = 0.048). Inversely, no significant sex-related differences were observed in the duodenum, jejunum, or across the overall tissues. The physiological reason for this sex difference in pre-pubertal piglets is unclear. While hormonal influences are possible, they are less likely at this young age. Further research is needed to explore potential sex-linked genetic or epigenetic regulatory mechanisms.

Table 6: Gene expression of *FUT1* and *MUC4* according to gender and tissue

Tissue	Gender	FUT1			MUC4		
		n	LSM	SE	n	LSM	SE
Overall	Female	18	0.673^{b}	0.213	18	0.987	0.009
	Male	18	1.149^{a}	0.213	18	1.009	0.007
	P-value		0.024			0.0763	
Colon	Female	6	0.610^{b}	0.245	6	0.995^{b}	0.019
	Male	6	1.506^{a}	0.235	6	1.055^{a}	0.015
	P-value		0.030			0.0480	
Duodenum	Female	6	0.765	0.303	6	0.984	0.015
	Male	6	1.367	0.290	6	0.994	0.012
	P-value		0.189			0.6253	
Jejunum	Female	6	0.645^{b}	0.207	6	0.981	0.006
	Male	6	1.382^{a}	0.198	6	0.978	0.005
	P-value		0.033			0.7020	

Within the gene and each tissue, the LSMs with different superscript letters are significantly different (P<0.05).

In this study, all pigs were of the same age and maintained under identical dietary conditions; therefore, the effects of age and diet were not evaluated. Although previous studies have reported influences of these factors on gene expression and the expression of *MUC4* in the intestinal tissues and of pigs were reported (Sargeant *et al*., 2010; Smith *et al*., 2010; Zhou *et al*., 2017; Święch *et al*., 2025). On the other hand, the effects of age (Bao *et al*., 2012b) and breed (Liu *et al*., 2015a; Xia *et al*., 2016), they are beyond the scope of the present data. Further studies are needed to clarify the potential role factors in regulating *FUT1* and *MUC4* expression in the intestinal tissues.

In presented study, given the limited sample size, the findings should be considered preliminary. Further studies with larger sample sizes and more robust experimental designs are required to validate these observations and clarify their biological and practical significance.

CONCLUSIONS

The results of this study show that *FUT1* gene expression did not differ among genotypes in any examined tissues. In contrast, *MUC4* expression was significantly higher in pigs with the AG genotype than in those with the GG genotype in the colon. However, given the very small number (n=2) of pigs with the GG genotype, this finding should be considered preliminary and requires validation in a larger cohort. *FUT1* expression was similar among intestinal tissues, whereas *MUC4* expression was significantly higher in the colon than in the duodenum and jejunum. In addition, male pigs showed higher *FUT1* expression in the colon, jejunum, and overall tissues, while higher *MUC4* expression in male pigs was observed only in the colon.

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NOVELTY STATEMENT

This study provides novel evidence on the relationship between polymorphisms of the *FUT1* (c.307G>A) and *MUC4* (g.243A>G) genes and their expression levels in intestinal tissues of Yorkshire pigs under Vietnamese production conditions. Unlike previous studies focusing mainly on genotypephenotype associations, this work is among the first to investigate how these specific mutations influence gene expression in different intestinal tissues (colon, duodenum, and jejunum) in young pigs. In addition, the

study highlights tissue-specific and sex-dependent differences in *MUC4* expression, contributing new insights into the biological regulation of genes associated with disease resistance in pigs.

AUTHORS CONTRIBUTIONS

Do Duc Luc, Vu Dinh Ton, Nguyen Hoang Thinh; Conceptualization and design the experiment, supervision, editing and finalization; Do Thi Phuong; data collection, laboratory manipulation, data analysis, manuscript preparation; Phan Thi Tuoi; data analysis, manuscript preparation.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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