

# Mutation c.307G>A in *FUT1* gene has no effect on production performance of Yorkshire pigs in the tropics: the case of Vietnam

Do Duc Luc, Nguyen Hoang Thinh, Ha Xuan Bo, Nguyen Thi Vinh, Tran Xuan Manh, Nguyen Van Hung, Vu Dinh Ton, and Frédéric Farnir

**Abstract:** The alpha (1) fucosyltransferase gene (*FUT1*) is a candidate gene for controlling the adhesion of *Escherichia coli* F18 receptor. Indeed, a single-nucleotide polymorphism, c.307G>A, located in the gene is such that pigs with AA genotype are resistant to entero-toxicogenic *E. coli* F18, whereas those with AG and GG genotypes are sensitive. An experiment was carried out in northern Vietnam from March 2016 to May 2017 to determine *FUT1* genotype frequencies and the effect of these genotypes on production performance of Yorkshire pigs. A total of 613 animals were genotyped using polymerase chain reaction – restriction fragment length polymorphism method. The body weights at birth, weaning, initial fattening period, and final fattening period were collected from 611, 516, 479, and 418 animals, respectively, whereas backfat thickness, depth of *longissimus dorsi*, and lean meat percentage were recorded from 328 animals. The frequencies of *FUT1* genotypes were found to be in Hardy–Weinberg equilibrium ( $P = 0.51$ ). Effect of *FUT1* genotype was not observed for all production traits ( $P > 0.05$ ), whereas final body weight and depth of *longissimus dorsi* were significantly different between females and males ( $P < 0.05$ ). These results suggest that selection of Yorkshire pigs resistant to entero-toxicogenic *E. coli* F18 could be effective without adversely affecting average daily gain and lean meat.

**Key words:** swine, productive performance, *FUT1*, diarrhea resistant.

**Résumé :** Le gène de l'alpha (1) fucosyltransférase (*FUT1*) est un gène candidat pour le contrôle de l'adhésion au récepteur F18 d'*Escherichia coli*. En effet, un polymorphisme à nucléotide simple, c.307G>A, localisé dans le gène est tel que les porcs ayant le génotype AA sont résistants à l'*E. coli* entérotogène F18 tandis que ceux ayant les génotypes AG et GG y sont sensibles. Une expérience a été effectuée au nord du Vietnam entre mars 2016 et mai 2017 afin de déterminer les fréquences génotypiques du gène *FUT1* ainsi que les effets de ces génotypes sur la performance de production des porcs Yorkshire. Un total de 613 animaux ont été génotypés au moyen de la méthode de la réaction en chaîne de la polymérase-polymorphisme de longueur des fragments de restriction. Les poids corporels à la naissance, au sevrage, et lors de la période initiale et finale de l'engraissement ont été collectés de 611, 516, 479, et 418 animaux respectivement, tandis que l'épaisseur du gras dorsal, la profondeur du muscle *longissimus dorsi* et le pourcentage de viande maigre ont été enregistrés chez 328 animaux. Les fréquences des génotypes *FUT1* se sont montrées comme un équilibre Hardy-Weinberg ( $P = 0,51$ ). L'effet du génotype *FUT1* n'a pas été observé pour toutes les caractéristiques de production ( $P > 0,05$ ) tandis que le poids corporel final et la profondeur du muscle *longissimus dorsi* étaient significativement différents entre les femelles et les mâles ( $P < 0,05$ ). Ces résultats

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**D.D. Luc, N.H. Thinh, and H.X. Bo.** Department of Animal Breeding and Genetics, Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi 12406, Vietnam.

**N.T. Vinh.** Department of Biology and Zoology, Faculty of Animal Science, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi 12406, Vietnam.

**T.X. Manh and N.V. Hung.** Dabaco Nucleus Breeding Pig Company, Tien Du District, Bac Ninh Province 16416, Vietnam.

**V.D. Ton.** Center of Multidiscipline Research for Rural Development, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi 12406, Vietnam.

**F. Farnir.** Department of Animal Production, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège, Belgium.

**Corresponding authors:** Do Duc Luc (email: [doducluc@yahoo.co.uk](mailto:doducluc@yahoo.co.uk)) and Frédéric Farnir (email: [F.Farnir@uliege.be](mailto:F.Farnir@uliege.be)).

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suggèrent que la sélection des porcs Yorkshire résistants à l'*E. coli* entérotoxigène F18 pourrait être efficace sans avoir d'effet négatif sur le gain moyen quotidien et la viande maigre. [Traduit par la Rédaction]

**Mots-clés :** porcs, performance de production, *FUT1*, résistance à la diarrhée.

## Introduction

Porcine post-weaning diarrhea (PWD) is an enteric disease of piglets after weaning. PWD has an important impact on economic losses due to a reduction of the growth rate and an increase in the mortality rate as well as the high costs of veterinary medication (Fairbrother et al. 2005). According to Coddens et al. (2008), no satisfactory method for controlling PWD is available. The alpha (1) fucosyltransferase gene (*FUT1*) is located on pig chromosome 6q11, and the existence of a c.307G>A mutation in *FUT1* (referred to as rs335979375 on position 6:54079650 in the last version Sscrofa11.1 of the pig genome) was found in a study of Meijerink et al. (1997). *FUT1* has been proposed as being a marker for selection of *Escherichia coli* F18 adhesion resistant pigs (Bao et al. 2011). The animals with AA genotype have been shown to be resistant to enterotoxigenic *E. coli* F18, whereas those with AG and GG are sensitive (Meijerink et al. 1997). Therefore, marker-assisted selection might help to control the disease in the targeted population by using preferably animals with genotype AA (Wang et al. 2012). The effect of *FUT1* genotypes on vital economic traits has been reported (Jiang et al. 2005; Bao et al. 2011; Zhu et al. 2014; Geraci et al. 2019), but varying results on the relationship between these genotypes and reproduction and production performances still remain. The gilts with AA genotype grow faster than the AG and GG ones, whereas sows with AA genotype have more piglets born alive than those with AG and GG genotypes (Zhu et al. 2014). In contrast, no effect of *FUT1* gene on growth performance traits of Duroc and Landrace boars was detected in Huang et al. (2008).

All of the above studies were conducted separately for males and females; and no mention was made of sex effect in the statistical model. Additionally, to our knowledge, there are no published studies on the relationship between *FUT1*, growth rate, and lean meat percentage for males and females. The objectives of this study were to determine the frequency of the *FUT1* genotypes and the effect of these single-nucleotide polymorphism (SNP) genotypes on the production traits of Yorkshire fattening pigs for both sexes under industrial condition in Vietnam.

## Materials and Methods

### Animals and data collection

The experiment was carried out at Dabaco Nucleus Breeding Pig Company, Bac Ninh Province, Vietnam (40 km north from Hanoi) from March 2016 to May 2017. Four piglets (two males and two females) from the

same litter were randomly selected from 160 sows, leading to a total of 613 Yorkshire piglets (320 females and 293 intact males) used to determine allelic and genotypic frequencies for *FUT1*. For the production performance, the body weights at birth (BWB), body weights at weaning ( $23.01 \pm 2.38$ , mean  $\pm$  standard deviation days), initial body weights (IBW) ( $79.39 \pm 5.42$ ), and body weights at final fattening period (FBW) ( $144.31 \pm 4.91$ ) were collected from 611, 516, 479, and 418 piglets, respectively, whereas backfat thickness (BFT), depth of *longissimus dorsi* (DLD), and lean meat percentage (LMP) were recorded from 328 animals.

Piglets were tattooed on the ear, and body weight was recorded individually at birth. At weaning, the piglets were notched using a 28 mm diameter ear tag, and individual weights were noted. The pigs were housed in the fattening unit after recording the individual weights using an electronic scale. Intact males and females were kept separately in groups of 20 animals. At the end of the fattening period, BFT and DLD were measured on living animals using an ultrasound device (AgroScan AL with a linear probe ALAL350; ECM, France) according to the method described in Youssao et al. (2002). Before ultrasound measurement, body weights of the individual pigs were recorded using an electronic scale. The LMP was predicted from BFT and DLD using the regression equation recommended by the Ministère des classes moyennes et de l'agriculture de Belgique (1999):

$$Y = 59.902386 - 1.060750X_1 + 2.229324X_2$$

where  $Y$  is the estimated carcass LMP;  $X_1$  is the BFT (including skin, mm); and  $X_2$  is the DLD (mm) measured between the third and fourth last ribs at 6 cm perpendicularly from the dorsal mid line.

The average daily gain (ADG) was calculated as the weight gain between FBW and IBW divided by the duration of the fattening period.

All pigs were housed indoor with fans and dripping water system to control the housing climate. Animals had free access to water through nipple drinkers and were fed ad libitum. The feed rations were presented in Supplementary Table S1<sup>1</sup>.

### Determination of the *FUT1* genotypes

The piglet tails were docked at birth, then stored in keeping sample boxes and transported to the laboratory the Faculty of Animal Science, Vietnam National University of Agriculture, where the samples were stored

<sup>1</sup>Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjas-2019-0084>.

**Table 1.** Frequency of genotypes and genes at *FUT1* locus of Yorkshire pigs.

Item	Genotype			Allele		P for HWE
	AA	AG	GG	A	G	
Observed count	6	137	470	—	—	—
Expected count	9.07	130.88	473.05	—	—	—
Observed frequency (%)	0.98	22.35	76.67	12.15	87.85	0.51
Expected frequency (%)	1.48	21.35	77.17	—	—	—

**Note:** *FUT1*, alpha (1) fucosyltransferase; HWE, Hardy–Weinberg equilibrium.

at  $-20^{\circ}\text{C}$  until genomic DNA was extracted. Genomic DNA was isolated from the porcine tail sample following standard procedures (Sambrook et al. 1989). Polymerase chain reaction (PCR) – restriction fragment length polymorphism based on the c.307G>A mutation of the *FUT1* gene was used to genotype the pigs (Meijerink et al. 1997). Forward and reverse primer sequences to amplify the *FUT1* polymorphism described above were 5'-CTTCAGCCAGGGC TCCTTTAAG-3' and 5'-CTGCCTGAACGTCTATCAAGACC-3'. The PCR reaction was performed on a 25  $\mu\text{L}$  volume, including 20 ng genomic DNA, 0.25  $\mu\text{mol L}^{-1}$  of each primer, 2.5  $\mu\text{L}$  10 $\times$  PCR buffer (containing 1.5  $\text{mmol L}^{-1}$   $\text{Mg}^{2+}$ ), 0.2  $\text{mmol L}^{-1}$  dNTPs, 1.25 U *Taq* DNA polymerase, and 18.5  $\mu\text{L}$  double-distilled water. PCR was carried out on a PCR system PTC-100 (MJ Research Inc., Watertown, MA, USA) with the following procedures: an initial denaturation of 3 min at  $94^{\circ}\text{C}$ , followed by 35 cycles of 45 s at  $94^{\circ}\text{C}$ , 30 s at  $58^{\circ}\text{C}$ , 45 s at  $72^{\circ}\text{C}$ , and then a 5 min final extension at  $72^{\circ}\text{C}$ . The amplified DNA (8  $\mu\text{L}$ ) was digested at  $37^{\circ}\text{C}$  with 1 unit of *Hin6I* for 8 h. The digestions were separated by electrophoresis on 3% agarose gel, where genotypes could be extracted.

#### Statistical analysis

A linear model including the fixed effects of the *FUT1* genotypes, the sex, and the IBW as a covariate was adjusted to the data.

$$Y_{ijk} = \mu + \text{FUT1}_i + \text{Sex}_j + \text{IBW}_{ijk} + e_{ijk}$$

where  $Y_{ijk}$  is the IBW, FBW, ADG, BFT, DLD, or LMP of pig  $k$ , sex  $j$ , and *FUT1* genotype  $i$ ;  $\mu$  is the overall mean;  $\text{FUT1}_i$  is the fixed effect of *FUT1* genotype  $i$  (AA, AG, and GG);  $\text{Sex}_j$  is the fixed effect of sex  $j$  (female and intact male);  $\text{IBW}_{ijk}$  is the covariate for FBW, ADG, BFT, DLD, and LMP; and  $e_{ijk}$  is the residual error. The least-square means of the various genotypes and of both sexes were compared using Tukey's test. Hardy–Weinberg equilibrium (HWE) was tested using a  $\chi^2$  test.  $P$  values  $< 0.05$  were considered as significant. The data were analyzed using the general linear model procedure of SAS software version 9.3 (SAS Institute Inc. 1989) to identify significant sources of variation.

## Results

### Genotypic and allelic distribution of *FUT1* gene

For the SNP corresponding to the mutation c.307G>A of *FUT1*, a 421 bp DNA fragment was amplified, digested

with the restriction enzyme *Hin6I*, and identified genotyping by electrophoresis on 3% agarose gel. For the enzyme *Hin6I*, the fragment had two restriction sites. Two allele (A and G) and three genotypes (AA, AG, and GG) were identified in Yorkshire. Allele A is characterized by digestion of the 421 bp PCR product to fragments of 328 and 93 bp, whereas allele G with a polymorphic restriction site is represented by fragments of 241, 93, and 87 bp.

A total of 640 piglet tail samples were collected at birth. Due to losses between birth and phenotyping, only 613 animals (320 females and 293 intact males) were used to identify the *FUT1* genotypes. The genotype and allele frequencies of *FUT1* gene are presented in Table 1. The three genotypes (AA, AG, and GG) were observed in the studied population. From 613 piglets, AA genotype was found on six individuals (0.98%), GG on 470 (76.67%), and AG on 137 (22.35%). Consequently, the gene frequency for disease resistant allele A was 12.15%, whereas the frequency for sensitive allele G was 87.85%. The result of  $\chi^2$  test indicated that the genotype frequencies of *FUT1* in our Yorkshire pigs were found to be in HWE ( $P = 0.51$ ).

### Effects of *FUT1* genotype and sex on the growth performance of Yorkshire pigs

In this study, the interactions between the *FUT1* genotype and the sex were not significant for any trait, and we, therefore, ignored them in the final models. The least-square means for the genotypic effects of *FUT1* (AA, AG, and GG) and of sex (female and intact male) associated to the production traits are presented in Table 2 and Supplementary Table S2<sup>1</sup>, respectively.

Sex significantly affected BWB, FBW, and DLD (Supplementary Table S2<sup>1</sup>). The males had higher BWB, FBW, and lower muscle thickness when compared with the females ( $P < 0.05$ ). However, ADG and LMP were not significantly different between females and intact males.

## Discussion

### Genotypic and allelic distribution of *FUT1* gene

A low frequency of the resistant genotype AA in Yorkshire breed was found in this study. Based on past researches, low frequencies of resistant animals were also found in pigs from western countries. Meijerink et al. (1997) reported that no more than 5%–10% of Swiss Landrace, Large White, and Duroc pigs are resistant to F18.

**Table 2.** Growth performance traits of Yorkshire pigs according to *FUT1* genotype.

Variable	AA			AG			GG		
	n	LSM	SE	n	LSM	SE	n	LSM	SE
Body weight at birth (kg)	6	1.61	0.11	137	1.55	0.02	468	1.57	0.01
Body weight at weaning (kg)	6	6.51	0.72	115	6.48	0.16	395	6.54	0.09
Initial weight (kg)	6	32.55	2.56	102	34.48	0.62	371	34.39	0.33
Final weight (kg)	6	85.27	3.18	89	85.60	0.83	323	86.60	0.44
Average daily gain (g)	6	820.46	47.44	84	787.65	12.34	300	803.13	6.48
Backfat thickness (mm)	6	10.88	0.91	67	11.14	0.27	255	10.81	0.14
Muscle thickness (mm)	6	48.28	1.91	67	49.42	0.57	255	49.33	0.29
Lean meat percentage (%)	6	59.43	1.00	67	59.42	0.30	255	59.75	0.15

**Note:** *FUT1*, alpha (1) fucosyltransferase; LSM, least-square means; SE, standard error.

In Belgian hybrid pigs, only a small fraction (4.6%) of the animals is resistant to F18 infection (Coddens et al. 2008). The low frequency of A allele was also observed in Landrace and Yorkshire pigs in People's Republic of China (Ruan et al. 2013). The AA genotype was absent in Asian local pigs breeds (Yan et al. 2003; Bao et al. 2008, 2011; Cuong et al. 2012). In their work on exotic Yorkshire pigs under industrial condition in Vietnam, Cuong et al. (2012) found the three genotypes and a frequency of AA estimated at 0.13. The relative low frequency of the A allele, which is the favorable allele for PWD resistance, should be questioned: selection on weight gain and (or) on number of piglets might lead to reduce the frequency of the allele A in the population, possibly indicating a deleterious effect of this allele on pig growth or reproduction traits, which would of course raise questions on the use of this allele in the selection process. Note, however, that in the study of Luise et al. (2019), the frequency of the A allele (0.29) was higher than in our study. The favorable allele frequency was deviation from close to 0 to 0.74 according to different breeds (Muñoz et al. 2018).

#### Effects of *FUT1* genotype and sex on the growth performance of Yorkshire pigs

Investigating the effect of the *FUT1* gene on the growth performance traits of Yorkshire pigs, our result show that no significant effect of *FUT1* genotypes on all studied traits can be detected: the AA individuals have growth performances (body weight at all ages, BFT, DLD, LMP at the end of fattening period) similar to AG and GG individuals.

Geraci et al. (2019) reported that there was no effect of *FUT1* gene on production traits of Italian Large White pigs. There are currently several different observations on the effect of *FUT1* genotypes on growth performance traits of pigs. Riis Poulsen et al. (2018) found that *FUT1* genotype did not affect the growth performance of piglets until 34 d old. The age at 100 kg of AA genotype pigs was shorter than that of AG, whereas BFT and DLD were similar in Zhu et al. (2014). Bao et al. (2011) indicated that ADG of animals with AA was higher than those of AG and

GG types. Jiang et al. (2005) proposed that AA genotype is a beneficial genotype for meat quality and carcass traits in hybrids Large White × Meishan pigs. If confirmed, increasing the frequency of the A allele not only would enhance the F18 infections resistance but also would improve the meat quality and carcass traits. Additionally, among surviving pigs, the resistant AA pigs demonstrated a 30% improvement of ADG compared with susceptible ones (Mellencamp et al. 2008). In contrast, Huang et al. (2008) reported that there was no effect of *FUT1* gene on the growth performance of males in Duroc and Landrace breeds, and Cuong et al. (2012) inferred that PWD has a negative relationship with growth rate. The slower average growth of pigs with diarrhea might either be due to the direct effect of the disease on the growth (i.e., piglets don't grow well at the beginning of the fattening period because they are sick) or to the effect of the resistant genotype which would also be favorable for the growth. Our experimental data also show that there is no significant difference in growth performance traits among the different genotypes of *FUT1* gene. Consequently, selective breeding in Yorkshire pigs resistant to *E. coli* F18 should globally improve the resistance to *E. coli* infections without adversely affecting the growth performance traits and meat percentage. Although we did not observe a significant effect of *FUT1* genotype on ADG and LMP, the number of individuals with favorable genotype AA was very low, making these comparisons hardly reliable. The low frequency of AA animals might be due to the way the selection is carried on in Vietnam, based on the animal conformation rather than on genetic basis. This issue should be considered in future research.

As mentioned above, we did not detect interactions between *FUT1* genotypes and the sex. ADG and LMP were not significantly different between females and intact males, although the values of the females tended to be lower than those of intact males. This result is consistent with the findings of Gispert et al. (2010), Quiniou et al. (2010), and Grella et al. (2013). In another study, Puls et al. (2014) confirmed that the intact males grew faster than females.

## Conclusions

Three genotypes (AA, AG, and GG) of the *FUT1* gene were recorded and the favorable genotype AA frequency was low in the studied Yorkshire pigs sample in Vietnam. There was no effect of the *FUT1* genotypes on ADG and LMP. This result suggests that a marker-assisted selection program using *FUT1* alleles could not only improve PWD resistance but also should not affect growth performance traits and meat percentage in male and female Yorkshire pigs. The current low frequency of AA should be questioned in future studies.

## Conflict of interest

The authors declare that they have no conflict of interest.

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