Vietnam Journal of Agricultural Sciences

ISSN 2588-1299

VJAS 2023; 6(1): 1711-1718
https://doi.org/10.31817/vjas.2023.6.1.02

Association between the *MUC4* g.243A>G Polymorphism and Production Performance of Landrace and Yorkshire Pigs in Vietnam

Do Duc Luc[1*#], Nguyen Hoang Thinh[1], Ha Xuan Bo[1#], Do Thi Phuong[2], Phan Thi Tuoi[3], Vu Dinh Ton[1] & Frederic Farnir[4]

[1]Faculty of Animal Science, Vietnam National University of Agriculture, Hanoi 131000, Vietnam
[2]Asia Livestock Innovation Center, Sunjin Vina Co., Ltd, Ha Nam Branch, Ha Nam 400000, Vietnam
[3]Faculty of Agriculture – Forestry - Fishery, Hong Duc University, Thanh Hoa 441539, Vietnam
[4]Unit of Biostatistics, Bioinformatics, Economics, Animal Selection, University of Liège, Liège 4000, Belgium

Abstract

Porcine mucin 4 (*MUC4*) is a candidate gene for controlling the adhesion of the enterotoxigenic *Escherichia coli* (ETEC) F4 receptor. Polymorphisms of the *MUC4* gene have been used as markers to identify the susceptibility of neonatal diarrhea in piglets for breeding selection. The objective of this study was to evaluate the effects of MUC4 g.243A>G polymorphisms on the production traits of Landrace and Yorkshire pigs in Vietnam. A total of 1,057 Landrace and 1,361 Yorkshire piglets were used to estimate the allelic and genotypic frequencies of the polymorphisms. Body weights at birth, at weaning, at initial fattening (IBW), and at the end of fattening period (FBW), backfat thickness (BFT), and depth of the *longissimus dorsi* muscle (DLD) were measured and lean meat percentage was estimated. Frequency of the susceptibility allele A to ETEC was higher than the resistance allele G for both breeds based on genotyping piglet tails collected at birth. The AA, AG, and GG genotypes were present in Yorkshire while GG was not found in Landrace. The production traits were not affected (P >0.05) by MUC4 polymorphisms except BFT and DLD (P <0.05). There were interactions between gender and MUC4 genotype (P <0.05) for IBW, FBW, average daily gain, and DLD. These traits of GG males were significantly higher than those of GG females (P <0.05). The results suggest that selecting pigs carrying the GG genotype of *MUC4*, known as providing resistance to ETEC, do not negatively affect productive performance in Landrace and Yorkshire pigs.

Keywords

Swine, growth, polymorphisms, diarrhea resistance

Received: April 25, 2022
Accepted: March 14, 2023

Correspondence to
ddluc@vnua.edu.vn

[#]Authors contributed equally to this study

ORCID
Do Duc Luc
https://orcid.org/0000-0003-3364-1296

Introduction

Pre-weaning and post-weaning diarrhea are common in the pig industry. This disease results in significant economic losses due to high mortality and morbidity. In addition, an outbreak of diarrhea leads to a low growth rate after recovery from the illness as a consequence of using antibiotics for treatment, which increases the risk of anti-microbial resistance (Fairbrother *et al.*, 2005). According to Luise *et al.* (2019), most types of diarrhea in piglets are caused by enterotoxigenic *Escherichia coli* (ETEC). There are two types of virulence factors that allow ETEC to cause diarrhea, namely enterotoxins and fimbrial adhesins. ETEC expressing F4 fimbriae causes more severe diarrhea in nursery, weaning, and weaned piglets (Vu-Khac *et al.*, 2007; Kim *et al.*, 2010). Nevertheless, not all pigs are susceptible to ETEC F4 infection due to the presence and number of F4 specific receptors in the brush border of the small intestine, which prevent ETEC from adhering and developing diarrhea (Sterndale *et al.*, 2019). The mucin 4 (*MUC4*) gene, which is located on chromosome 13 and physically mapped to SSC13q41 (Jacobsen *et al.*, 2011), encodes a membrane-bound O-glycoprotein (F4 receptor) in pigs (Fontanesi *et al.*, 2012).

F4 receptors are present on the surface of gastrointestinal epithelial cells and act as a functional barrier to cover and protect mucosal surfaces. From literature studies, *MUC4* has been considered as the most promising candidate gene for F4 receptors (Jørgensen *et al.*, 2003; Joller *et al.*, 2009; Jacobsen *et al.*, 2011). The study by Joller *et al.* (2009) reported the strong association of the XbaI polymorphism in intron 7 of *MUC4* with the susceptibility of the animal, where allele C is associated with resistance while allele G is associated with susceptibility. In the study of Peng *et al.* (2007), the *MUC4* g.243A>G mutation in intron 17 was associated with susceptibility/resistance to ETEC F4ab/ac infection.

The effects of *MUC4* on animal resistance to ETEC have been shown repeatedly. Polymorphisms of the *MUC4* gene have been used as markers to identify the susceptibility of animals for breeding selection in many countries. However, the frequencies of alleles and genotypes of *MUC4* polymorphisms have been shown to vary in different breeds (Fontanesi *et al.*, 2012). In addition, *MUC4* polymorphisms may be associated with other production traits of pigs. Therefore, selection focused on candidate genes such as *MUC4* to improve disease resistance should consider the impact selection has on other important economic traits. A study of Balcells *et al.* (2011) showed that the mutation g.243A>G in intron 17 of *MUC4* did not affect the total number of piglets born or number born alive. Inversely, a study by Liu *et al.* (2015) confirmed that the GG genotype, which relates to the resistant genotype, showed a strong association with improvement of IL8, IL10, and age for pigs that reached a bodyweight of 100kg compared with the AA and AG genotypes.

The present study aimed to estimate the allelic and genotypic frequencies of the *MUC4* g.243A>G mutation polymorphisms and evaluate the effects of the polymorphisms on the production traits of Landrace and Yorkshire pigs in Northern Vietnam.

Materials and Methods

Experimental design

The experiment was carried out at the pig farm of the Dabaco Nucleus Breeding Pig Company, Tien Du district, Bac Ninh province, Vietnam from April 2015 to October 2018. At birth, the tails of 2,418 piglets were docked and used to estimate the allelic and genotypic frequencies of the *MUC4* g.243A>G polymorphisms. The production traits measured were body weights at birth (BWB, kg), at weaning (BWW, kg), at initial fattening (IBW, kg), and at the final fattening period (FBW, kg), backfat thickness (BFT, mm), depth of the *longissimus dorsi* muscle (DLD, mm), and lean meat percentage (LMP, %).

A total of 2,418 piglets were tattooed and their body weights were recorded individually at birth. At weaning (23.2 ± 3.22, mean ± SD days), 2,252 piglets including 1,057 Landrace (626 females and 431 intact males) and 1,361

Yorkshire (808 females and 553 intact males) were notched by an ear tag and individually weighted. The fattening period began at 80.8 ± 6.49 days. A total of 1,671 animals from the 2,252 weaning piglets were housed in the fattening units based on available pens after recording the body weights by ear tag number. Females (1,202 in 60 pens) and intact males (469 in 23 pens) were raised separately in groups of approximately 20 animals. The fattening period was ended at 155 ± 10.3 days, and 59 pens (44 and 15 pens for females and intact males, respectively) were randomly selected for measurements. The FBWs of 1,135 pigs (846 females and 289 intact males) were measured. At the same time, BFT and DLD were measured between the third and fourth last ribs at 6cm perpendicularly from the dorsal midline using the ultrasound device AgroScan AL with a linear probe ALAL350 (ECM, France) according to the methods of Youssao *et al.* (2002). LMP was predicted by the regression equation from BFT and DLD recommended by the Ministre des classes moyennes et de l'agriculture de Belgique (1999):

$$Y = 59.902386 - 1.060750X1 + 2.229324X2$$

where, Y is LMP; X1 is BFT (mm); and X2 is DLD (mm).

The average daily gain (ADG) was the quotient between the weight gain and the duration of the fattening period. All animals were kept indoors and given free access to water. The pigs were fed *ad libitum* with rations according to their different ages (**Table 1**).

Determination of the *MUC4* genotypes

Piglet tails were collected immediately at birth and stored at -20°C until DNA extraction. DNA was extracted following the procedures of Sambrook *et al.* (1989). The mutation g.243A>G (referred to as rs698037138 on position 13:134237729 in the last version of Sscrofa11.1 of the pig genome) located in *MUC4* was identified according to Peng *et al.* (2007) using the PCR-RFLP technique. The DNA fragment of 538bp was amplified using the forward and reverse primers: F5′-CAGGATGCCCAATGGCTCTAC-3′ and R5′-CCCCGAAGTTGTGAAAGGAAG-3′. The PCR reaction included 2μL (50 ng/μL) of template DNA, 0.5μL (10μM) of each primer, 2.5μL DreamTaq^{TM} buffer (including $MgCl_2$), 0.5μL dNTP, 0.2μL DreamTaq^{TM}, and 18.8μL ddH_2O. The thermal cycling conditions were: (i) 5min at 95°C, (ii) 30 cycles of 45 sec at 95°C, 45 sec at 62.5°C, and 45 sec at 72°C, and (iii) 10min at 72°C. The *MUC4* genotypes were identified using electrophoresis on 3% agarose gel after digesting the DNA fragments overnight with the restriction enzyme *HhaI*, which recognizes GCG^C sites at 37°C. Two alleles (A and G) and three genotypes (AA, AG, and GG) were identified. Alleles G (295 and 243bp) and A (538bp) denote the resistance and susceptibility alleles, respectively. All genetic procedures were conducted at the Genetic Laboratory, Faculty of Animal Science, Vietnam National University of Agriculture.

Statistical analysis

A general linear model was used to evaluate the fixed effects on the study traits, namely *MUC4* genotype, breed, and sex, as follows:

$$Y_{ijkl} = \mu + MUC4_i + Breed_j + Sex_k + Breed_j*Sex_k + \varepsilon_{ijkl}$$

where: Y_{ijk} is BWB, BWW, IBW, FBW, ADG, BFT, DLD, or LMP; μ is the overall mean; $MUC4_i$ is the effect of *MUC4* genotype i (AA, AG, or GG); $Breed_j$ is the effect of breed j (Landrace or Yorkshire); Sex_k is the effect of sex k (female or intact male); $Breed_j*Sex_k$ is the interaction between breed j and sex k; and ε_{ijkl} is the residual error.

The following ages of individuals (in days) were adjusted in the statistical model as covariates: (i) age at weaning for BWW, (ii) age at initial fattening for IBW, and (iii) age at final fattening for FBW, ADG, BFT, DLD, and LMP. The pairwise comparisons between the least-square means (LSM) were conducted using Tukey's test. Hardy-Weinberg equilibrium was tested using a chi-square test with 1 degree of freedom. A significant difference was considered when the P-value was below 0.05. The data analyses were performed by SAS software (SAS, 1989). The values presented in the tables are least square means (LSM) and root mean square errors (RMSE).

Results

Genotypic and allelic frequencies of *MUC4*

The allelic and genotypic frequencies of the *MUC4* gene of Landrace and Yorkshire pigs at birth are shown in **Table 2**. At *MUC4* g.243A>G, three genotypes (AA, AG, and GG) were observed in Yorkshire whereas there were no pigs with the GG genotype found in Landrace. Allele G, which has been identified as conferring resistance to ETEC, was present at an extremely low frequency (0.002) in the Landrace breed but had a higher frequency (0.457) in Yorkshire pigs. The frequency of the susceptibility allele A to enterotoxigenic *Escherichia coli* (ETEC) was higher than the resistance allele G for both breeds, especially in Landrace in which allele A was found at a frequency of 0.998. The most frequent genotype in Landrace was AA (0.996) whereas it was the heterozygote AG in Yorkshire pigs (0.516). The polymorphisms of *MUC4* g.243A>G were in Hardy Weinberg equilibrium for both Landrace (*P* = 0.9509) and Yorkshire pigs (*P* = 0.1474).

The performance results of the Landrace and Yorkshire pigs of each genotype at different measurement periods (at birth, weaning, initial fattening, and final fattening) and by gender (female and intact male) are presented in **Tables 4 and 5**, respectively. At initial fattening, the frequencies were 0.631, 0.262, and 0.107 for AA, AG, and GG, respectively. While these values at the end of fattening were 0.561, 0.309, and 0.130 in AA, AG, and GG genotypes, respectively, which indicated that the G allele provided resistance to ETEC. The frequencies varied according to the measurement period due to the decrease in the number of measurement pens from birth to fattening and the random selection of pigs.

Association between the *MUC4* g.243A>G polymorphisms and production performance

The levels of significance of *MUC4*, Breed, Gender, and the interaction Breed*Gender on production performance of Landrace and Yorkshire pigs are presented in **Table 3**. The

Table 1. Feed ratio according to age (week) and body weight (kg) for Landrace and Yorkshire pigs

Age (week)	Body weight (kg)	Feed	Protein (%)	Kcal/kg
<5	<10	Starter	20.0	3,350
5-8	10-30	Grower 1	18.0	3,100
8-12	30-50	Grower 2	16.0	3,100
>12	>50	Finisher	13.5	2,850

Table 2. Frequency of the genotypes and alleles at the porcine *MUC4* g.243A>G locus of Landrace and Yorkshire breeds

Item	Genotype			Allele		P-value for HWE
	AA	AG	GG	A	G	
Landrace						
Observed count	1053	4	0			
Expected count	1053.004	3.992	0.004			
Observed frequency	0.996216	0.003784	0	0.998	0.002	0.9509
Expected frequency	0.996219	0.003777	0.000004			
Yorkshire						
Observed count	388	702	271			
Expected count	401.26	675.47	284.26			
Observed frequency	0.285085	0.515797	0.199118	0.543	0.457	0.1474
Expected frequency	0.294831	0.496305	0.208864			

production performance of Landrace and Yorkshire pigs according to the polymorphisms of the *MUC4* gene are presented in **Table 4**.

There were significant associations of the *MUC4* polymorphisms with the BFT and DLD of pigs at the end of the fattening period ($P < 0.05$). The pigs that carried the GG genotype were shown to have higher BFT and DLD values than those with the AA genotype ($P < 0.05$). The pigs that carried the AG genotype resulted in higher DLD ($P < 0.05$) values while having similar levels of BFT compared to those with AA ($P > 0.05$). No significant differences in body weight at all the measured times, ADG, or LMP were detected among the *MUC4* genotypes in this study ($P > 0.05$).

The interactions between gender and the *MUC4* genotypes for the production traits are presented in **Table 5**. There were gender x *MUC4* interactions for IBW, FBW, ADG, and DLD ($P < 0.05$). For females, these traits in AG pigs had a trend higher than in AA and GG pigs. Inversely, the IBW, FBW, ADG, and DLD values of GG intact males were higher than those of AG and AA. The BWB, BWW, BFT, and LMP were not affected by the interaction between gender and the *MUC4* genotypes.

The production traits were significantly different between the Landrace and Yorkshire breeds ($P < 0.05$) except ADG ($P = 0.2642$). The BWs of Landrace were higher than those of Yorkshire while the LMP values were lower ($P < 0.05$).

Discussion

Genotypic and allelic frequencies of the *MUC4* gene

The observed results of our study were in agreement with the report by Liu *et al.* (2015) that allele A was the dominant allele (frequency

Table 3. Level of significance of *MUC4*, Breed, Gender, and the interaction Breed*Gender on production performance of Landrace and Yorkshire pigs

Variable	*MUC4*	Breed	Sex	*MUC4**Sex	R² (%)
Weight at birth (BWB, kg)	0.1437	<.0001	0.0035	0.1283	6.67
Weight at weaning (BWW, kg)	0.8495	<.0001	0.0019	0.118	8.78
Weight at initial fattening (IBW, kg)	0.0476	<.0001	0.0066	0.0158	34.02
Weight at final fattening (FBW, kg)	0.2009	0.0454	<.0001	0.0035	14.08
Average daily gain (ADG, g/day)	0.8751	0.2642	<.0001	0.0084	15.16
Backfat thickness (BFT, mm)	0.0075	0.0013	0.0002	0.3703	3.88
Depth of *longissimus dorsi* (DLD, mm)	<.0001	<.0001	0.0548	0.0174	15.49
Lean meat percentages (LMP, %)	0.2721	<.0001	0.0017	0.768	10.38

Table 4. Production performance of Landrace and Yorkshire pigs with various *MUC4* g.243A>G genotypes

Variable	AA		AG		GG		RMSE
	n	LSM	n	LSM	n	LSM	
Weight at birth (BWB, kg)	1441	1.42	706	1.45	271	1.47	0.27
Weight at weaning (BWW, kg)	1366	6.67	646	6.72	240	6.69	1.37
Weight at initial fattening (IBW, kg)	1054	33.4	438	34.2	179	34.6	5.27
Weight at final fattening (FBW, kg)	637	95.2	351	96.6	147	97.4	10.9
Average daily gain (ADG, g/day)	637	819	351	824	147	822	110
Backfat thicknesses (BFT, mm)	491	12.3^b	285	12.6^b	131	13.4^a	2.60
Depth of *longissimus dorsi* (DLD, mm)	491	60.1^b	285	62.3^a	131	63.8^a	6.46
Lean meat percentages (LMP, %)	491	60.6	285	60.8	131	60.4	2.36

Note: Within rows, LSM followed by different letters are significantly different (P <0.05).

Table 5. Interaction between gender and *MUC4* g.243A>G genotypes for production performance in Landrace and Yorkshire pigs

Variable	Female						Male						RMSE
	AA		AG		GG		AA		AG		GG		
	n	LSM	n	LSM	n	LSM	n	LSM	n	LSM	n	LSM	
Weight at birth (BWB, kg)	878	1.40	400	1.45	156	1.43	563	1.45	306	1.45	115	1.50	0.27
Weight at weaning (BWW, kg)	843	6.60	369	6.69	137	6.45	523	6.74	277	6.76	103	6.94	1.37
Weight at initial fattening (IBW, kg)	764	33.5^{b}	314	33.9^{ab}	124	34.4^{b}	290	33.3^{b}	124	34.6^{ab}	55	35.8^{a}	5.27
Weight at final fattening (FBW, kg)	482	92.6^{c}	256	94.7^{bc}	108	91.5^{c}	155	97.7^{ab}	95	98.5^{a}	39	103^{a}	10.9
Average daily gain (ADG, g/day)	482	779^{bc}	256	802^{b}	108	763^{c}	155	859^{a}	95	847^{a}	39	880^{a}	110
Backfat thicknesses (BFT, mm)	371	12.0	214	12.3	96	12.7	120	12.6	71	12.9	35	14.0	2.60
Depth of *longissimus dorsi* (DLD, mm)	371	60.5^{b}	214	61.9^{b}	96	62.2^{ab}	120	59.7^{b}	71	62.7^{ab}	35	65.4^{a}	6.46
Lean meat percentages (%)	371	61.0	214	61.1	96	60.7	120	60.2	71	60.6	35	60.0	2.36

Note: Within rows, LSM followed by different letters are significantly different (P <0.05).

of 0.65) in Large White pigs. According to the study of Fontanesi *et al.* (2012), genotypic and allelic frequencies of the *MUC4* gene differed among various pig breeds. In another report, the frequencies of alleles A and G in Meishan pigs were 0 and 1, respectively, while they were 0.5 for both two alleles in Iberian pigs (Balcells *et al.*, 2011). For Landrace pigs, the frequency of the allele G polymorphism of *MUC4* g.243A>G in the present study was extremely low, and consequently, it was difficult to improve the frequency of the resistant allele in the given pig population by phenotypic breeding selection.

Association between the *MUC4* g.243A>G polymorphisms and production performance

Our results showed an association of the resistant genotype GG with higher BFT and DLD values compared to the susceptible genotype AA. The increase of both of the above traits in pigs with the GG genotype might explain why the LMP values were not different among pigs with different genotypes. Other production traits were unbeneficial effects of the resistant genotype of the *MUC4* gene on production performance in pigs. A report by Liu *et al.* (2015) indicated that Large White pigs with the GG genotype grew slower in the fattening period than pigs with AA or AG. Similarly during the fattening period, White Duroc × Erhualian pigs carrying F4ab or F4ac receptors grew faster than those without these receptors (Yan *et al.*, 2009). In agreement, in Italian Large White pigs, having the susceptible allele was associated with higher ADG and BFT values (Fontanesi *et al.*, 2012). On the other hand, Geraci *et al.* (2019) reported that in Italian Large White pigs, the polymorphisms of *MUC4* g.243A>G did not affect ADG and BFT. For reproductive traits, the MUC4 g.243A>G polymorphisms have been shown to have no effect on the number of piglets born or the number of piglets born alive (Balcells *et al.*, 2011; Liu *et al.*, 2015). The inconsistent results from previous studies are probably caused by different factors such as pig breed, farm conditions, and the selection program. In the present study, because the frequency of the G allele was very low and the GG genotype was absent in the Landrace breed, the effects of the resistant allele were not significantly detected in several production traits.

From our review, the interactions between gender and *MUC4* genotype for production traits have not been well investigated. The results of this study revealed that intact male pigs with the GG genotype are associated with the highest IBW, FBW, ADG, and DLD values compared to other animals with different genotypes. Males presented greater FBW and ADG values than females. Therefore, the outcomes as well as the literature review indicated that selecting males with the GG genotype as a disease resistance marker for breeding not only did not have negative effects on production performance but also improved carcass traits for Landrace and Yorkshire pigs. Due to the low numbers of boars with the *MUC4* g.243 GG genotype in our study, further experimentation should be done to confirm the genetic effects of the GG *MUC4* polymorphism on disease resistance, production traits, and reproductive performance in boars.

Conclusions

Frequency of the susceptibility allele A of *MUC4* to enterotoxigenic *Escherichia coli* (ETEC) was higher than the resistant allele G for the Landrace and Yorkshire breeds. The AA, AG, and GG genotypes were observed in Yorkshire while GG was absent in Landrace. The production traits were not affected by the *MUC4* polymorphisms except backfat thickness and depth of *longissimus dorsi*. These results suggest that selection for pigs carrying the GG genotype of *MUC4*, known as providing resistance to ETEC, did not negatively affect productive performance in Landrace and Yorkshire pigs.

Acknowledgments

This study was funded by FIRST (Fostering Innovation through Research, Science and Technology, Vietnam) project No: 11/FIRST/1.a/VNUA3. The authors thank the technical staff and directorate of the Dabaco Nucleus Breeding Pig Company for their contributions.

References

Balcells I., Castelló A., Mercadé A., Noguera J. L., Fernández-Rodríguez A., Sànchez A. & Tomàs A. (2011). Analysis of porcine MUC4 gene as a candidate gene for prolificacy QTL on SSC13 in an Iberian× Meishan F 2 population. BMC Genetics. 12(1): 93.

Fairbrother J. M., Nadeau E. & Gyles C. L. (2005). Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Animal Health Research Reviews. 6(1): 17-39.

Fontanesi L., Bertolini F., Dall'Olio S., Buttazzoni L., Gallo M. & Russo V. (2012). Analysis of association between the MUC4 g. 8227C> G polymorphism and production traits in Italian heavy pigs using a selective genotyping approach. Animal Biotechnology. 23(3): 147-155.

Geraci C., Varzandi A. R., Schiavo G., Bovo S., Ribani A., Utzeri V. J., Galimberti G., Buttazzoni L., Ovilo C., Gallo M., Dall'Olio S. & Fontanesi L. (2019). Genetic markers associated with resistance to infectious diseases have no effects on production traits and haematological parameters in Italian Large White pigs. Livestock Science. 223: 32-38.

Jacobsen M., Cirera S., Joller D., Esteso G., Kracht S. S., Edfors I., Bendixen C., Archibald A. L., Vogeli P. & Neuenschwander S. (2011). Characterisation of five candidate genes within the ETEC F4ab/ac candidate region in pigs. BMC Research Notes. 4(1): 225.

Joller D., Jørgensen C. B., Bertschinger H., Python P., Edfors I., Cirera S., Archibald A., Bürgi E., Karlskov‐Mortensen P. & Andersson L. (2009). Refined localization of the Escherichia coli F4ab/F4ac receptor locus on pig chromosome 13. Animal Genetics. 40(5): 749-752.

Jørgensen C., Cirera S., Anderson S., Archibald A., Raudsepp T., Chowdhary B., Edfors-Lilja I., Andersson L. & Fredholm M. (2003). Linkage and comparative mapping of the locus controlling susceptibility towards E. coli F4ab/ac diarrhoea in pigs. Cytogenetic and Genome Research. 102(1-4): 157-162.

Kim Y. J., Kim J. H., Hur J. & Lee J. H. (2010). Isolation of *Escherichia coli* from piglets in South Korea with diarrhea and characteristics of the virulence genes. Canadian Journal of Veterinary Research - Revue canadienne de recherche veterinaire. 74(1): 59-64.

Liu Y., Yin X. M., Xia R. W., Huo Y. J., Zhu G. Q., Wu S. L. & Bao W. B. (2015). Association between the MUC4 g. 243A> G polymorphism and immune and production traits in Large White pigs. Turkish Journal of Veterinary and Animal Sciences. 39(2): 141-146.

Luise D., Lauridsen C., Bosi P. & Trevisi P. (2019). Methodology and application of Escherichia coli F4 and F18 encoding infection models in post-weaning pigs. Journal of Animal Science and Biotechnology. 10(1): 53.

Ministère des classes moyennes et de l’agriculture de Belgique (1999). Arrêté ministériel relatif au classement des carcasses de porcs, 03 mai 1999 [Online]. Bruxelles: Ministère des Classes Moyennes et de l’Agriculture. Retrieved from http://www.ejustice.just.fgov.be/doc/rech_f.htm on May 12, 2020.

Peng Q. L., Ren J., Yan X. M., Huang X., Tang H., Wang Y. Z., Zhang B. & Huang L. S. (2007). The g.243A>G mutation in intron 17 of MUC4 is significantly associated with susceptibility/resistance to ETEC F4ab/ac infection in pigs. Animal Genetics. 38(4): 397-400.

Sambrook J., Fritsch E. & Maniatis T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). New York: Cold Spring Harbor Press.

SAS (1989). SAS/STAT. User’s Guide, Version 6, 4th Edition. SAS Institute. Cary, NC.

Sterndale S. O., Evans D. J., Mansfield J. P., Clarke J., Sahibzada S., Abraham S., O’Dea M., Miller D. W., Kim J. C. & Pluske J. R. (2019). Effect of mucin 4 allele on susceptibility to experimental infection with enterotoxigenic F4 *Escherichia coli* in pigs fed experimental diets. Journal of Animal Science and Biotechnology. 10(1): 56.

Vu-Khac H., Holoda E., Pilipcinec E., Blanco M., Blanco J. E., Dahbi G., Mora A., López C., González E. A. & Blanco J. (2007). Serotypes, virulence genes, intimin types and PFGE profiles of Escherichia coli isolated from piglets with diarrhoea in Slovakia. The Veterinary Journal. 174(1): 176-187.

Yan X., Ren J., Huang X., Zhang Z., Ouyang J., Zeng W., Zou Z., Yang S., Yang B. & Huang L.-S. (2009). Comparison of production traits between pigs with and without the *Escherichia coli* F4 receptors in a White Duroc× Erhualian intercross F2 population. Journal of Animal Science. 87(1): 334-339.