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Fertility, Growth Rate, Polymorphism of GH and FUT1 Genes of Hybrid between Wild Boars and Indigenous Breeds

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Industrial breeding has contributed to the erosion of genetic diversity in pig breeds. The wild pig subjugating and crossbreeding with indigenous breeds is a method of restoring genetic resources and improving the quality of pig breeds. This study was conducted to investigate fertility, neonatal mortality, growth rate, and single nucleotide polymorphism in FUT1 and GH genes of Vietnam native wild pigs (WP) and hybrid of them with two indigenous breeds Mong Cai (MC) and Soc (S). 34 native wild pigs, including 18 boars and 16 sows, were collected from the wild boar at a young age and subjugated and randomly paired. The wild boars were randomly paired with 10 indigenous sows in each breed. According to the need, pigs are bred based on sell grazing model that the livestock has large space and supplement food sources. The results: The number of piglets/parity in wild pigs, (WPxMC) and (WPxS) was about 7-8 piglets; The birth weight of F1 in two-hybrid combinations (WPxMC) and (WPxS) was lower than the birth weight of WPxWP, (533.75 g, 489.00 g, and 613.43 g); The weaning weight (at 60 d old) in all 3 groups of pigs were similar, 6.45 kg (WPxMC), 6.32 kg (WPxS), 6.45 kg (WPxWP); the neonatal mortality rate was similar in WPxWP group (7.67 %) and F1 (WPxMC and WPxS) hybrids (9.28 % and 8.61 %). The growth rate from birth into 60 d of the group (WPxMC) was the highest (98.60 g/d) ($P<0.05$). At the period from weaned to 6 months old, the growth rate of piglet in group WPxMC was lower than that of two groups WPxWP and WPxS (92.01 g/d and 88.67 g/d). The growth rate in period 6 months to 10 months of piglet in group WPxWP was highest (245.03 g/d). Among the samples analyzing polymorphism level of the nucleotide C in GH gene at +378 and +329 position and FUT1 gene at M307 C/G point mutation were appeared in all group. The hybrid formula showed that hybrid pigs have good reproductive power, vigour, and good weight gain.

1. Introduction

In Vietnam, there are 26 indigenous pig breeds, which are breeds that have acquired unique biological characteristics and conditions due to a long process of breeding and immobilization (Ishihara et al., 2020). These breeds have been shown to have many good traits, such as high fertility, excellent adaptability to the environment, and delicious meat quality (NIAS, 2015). Unlike raising other livestock, pigs do not provide any secondary products such as milk and wool, nor do they have other uses such as transportation or energy. The sole aim of the modern pig industry is to focus only on the efficiency of meat production, so the current selection of "good pigs" is those with high meat yields (Dang- Nguyen et al., 2010). Non-imported pig breeds are being raised industrially, accounting for an increasingly large proportion of livestock production, contributing to the erosion of genetic diversity in pig breeds (Ishihara et al., 2020). The domestication of wild pigs and crossbreeding with indigenous breeds is a method of restoring genetic resources and improving the quality of indigenous breeds, adding natural genetic resources to reared pig breeds, and contributing to the improvement of pig breeds yield and quality of the current pig breed.
For pig production, growth rate (Balatsky et al., 2016), and disease resistance are two essential traits in livestock production (Luc et al., 2020). These traits were affected by multi-factors, including the breed's characteristics, diet composition, genetic improvement (Franco et al., 2014), and environment (Ngilangil and Quinquito, 2020).

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The identification of genetic markers for the quantitative trait (QTL) related to the characteristics of weight gain and disease resistance in pigs has been published (Silva et al., 2019). The growth hormone gene (GH) plays a vital role in regulating tissue growth and metabolism in animals, and the GH gene influences growth characteristics, lean percentage, and milk production (Jing, 2006). The GH gene was one of the first genes used as a functional candidate gene related to growth and carcass traits (Thomas et al., 2007). The GH gene polymorphism was associated with growth traits between different generations (Ologbose et al., 2020). The microenvironment of the barn was determined related to the disease rate (Kaumbekova et al., 2021), there were many research screening genetic marker abilities resistant disease in pigs. The FUT1 gene plays an important role in regulating the resistance of pigs to Escherichia coli (ETEC), expressing of FUT1 associated with post-weaning diarrhea (PWD) in piglets (Dai et al., 2017). The variation of the FUT1 gene on the M307 locus is correlated with resistance to ETEC (Meijerink et al., 1997). System of plasma metabolism and some specific plasma metabolites involved in intestinal microbial metabolism (hippuric acid, oxindole, betaine) (Poulsen et al., 2018). The FUT1 gene at locus M229 has the potential to improve resistance to diarrhea and reduce backfat thickness (Zhang et al., 2020).

Although the information on GH and FUT gene polymorphisms in different pig breeds has been published, there is no information on single nucleotide polymorphisms (SNPs) of these genes in Vietnamese indigenous pig breeds. In this study, fertility, growth rate and polymorphism of GH, and FUT1 gene of Vietnamese native wild pigs, Mong Cai pigs, Soc pigs, and hybrid pigs between these breeds were investigated.

2. Material and methods

2.1 Experimental animal

The native wild pigs were collected in the mountainous areas in the Central Highlands of Vietnam, domesticated and raised at the Institute of Tropical Biology in Ho Chi Minh City, the Vietnam Institute of Science and Technology. These wild pigs are classified by morphology and molecular biology, which their characteristics compared to the wild breeds of pigs of Thailand and Europe (Long et al., 2014). The Soc breed is collected in villages, raised by the Ede and M'nong ethnic people in the Central Highlands of Vietnam. The Mong Cai breed was collected at the Mong Cai pig breeding and conservation facility (Quang Ninh province, Vietnam). These two indigenous pig breeds were transferred to acclimatization at the Institute of Tropical Biology in Ho Chi Minh City, Vietnam Institute of Science and Technology before conducting the research. According to the need, pigs are bred based on sell grazing model that the livestock has ample space and supplement natural food sources.

2.2 Experimental design

Three experimental groups consisted of 5 healthy wild boars randomly paired with 10 wild sows (not the same bloodline, acquired in different geographical locations) (WPxWP), 10 sows of Soc breeds (WPxS), and Mong Cai breeds (WPxMC).

The number of offspring per litter; the number of newborns per nest; the neonatal mortality rate; the birth weight/piglet; the number of animals weaned per nest; the weaning volume/head; the survival rate to weaning, mass growth up to 10 m of age, and calculate the average weight gain during the rearing period (g/head/d) in wild pig, wild boar crossed with Mong Cai and Soc pigs were monitored and recorded. The weight gain of pigs was calculated according to the formula:

$$A = \frac{V_2 - V_1}{T_2 - T_1} \quad (1)$$

where A is increase in absolute weight (g/head/d), V1 is the mass corresponding to the time T_1 (g), and V_2 is the mass corresponding to time T_2 (g).

2.3 Determination of the FUT and GH genotypes

The ear tissues were collected from random individuals from five groups: native wild pig (WP), Soc pig (S), Mong Cai pig (MC), WPxS, and WPxMC. The 20 mg of pig ear tissue samples were obtained using specialized pliers, then keep in keeping sample boxes, transported to the laboratory, stored at -20 °C until genomic DNA was extracted. The DNA extraction was used the GeneJET Genomic DNA Purification Kit (Thermo Scientific, UK), and the extraction procedure consists of steps following the manufacturer's instructions. The DNA concentration of samples was measured by the NanoDrop machine (Eppendorf, Germany) to evaluate the content and purity, and then they were stored at -20 °C until gene analysis. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on SNP the +C at 329 and 378 of GH gene, and c.307 G>A mutation of the FUT1 gene, the following primers were used to amplify the target DNA sequences: primer GH (Thuy et al., 2004), forward primer 5'-TTATCCATTAGCAACTGCCTGCCAG-3' and reverse primer 5'-

CTGGGGAGCTTACAAACTCCTT-3'; primer FUT1 (Cuong et al., 2012), forward primer 5'-CCAACGCCT-CCGATTCCTGT-3' and reverse primer 5'-GTGCATGGC AGGCTGGATGA-3'.
The PCR reaction with a volume of 25 µLconsisted of Buffer 2X MyTaq™ Mix (Bioline, UK), forward primer (10 pmol), reverse primer (10 pmol), DNA sample (final concentration 50 ng/µL), and distilled water up to enough volume. PCR cycle was performed under the following conditions: one cycle of DNA denaturation at 94 °C in 5 min; 40 cycles at 94 °C in 30 s; annealing at 55 °C in 45 s; extension at 72 °C in 45 s; final extension at 72 °C in 10 min. A single band was visualized following electrophoresis of the reaction product in a 1 % agarose gel. Which samples were correct band will be used for restriction fragment analysis. The amplified DNA (10µl) was digested at 37 °C with 1 unit of restriction enzyme *HinP*I (New England Biolabs, America) in 2 h for GH gene and 1 unit of restriction enzyme *HaeIII* (New England Biolabs, America) in 2 h for the FUT gene. The DNA fragments were separated by electrophoresis on 2 % agarose gel, where genotypes could be extracted.

2.4 Data analysis

The analysed data were performed by analysing the variation of error (ANOVA), mean values, comparison a difference between groups by t-test. The values of a, b and c, were used to indicate the difference between the groups of values is statistically significant ($p < 0.05$). The results are expressed as mean X (means) and standard error (SE). Genotypic analysis results will be calculated according to the genotype percentage, the allele ratio by Excel software.

3. Results and discussion

3.1 Fertility efficiency in three groups

Reproductive efficiency in three groups is presented in Table 1. The results show that the number of piglets per parity is approximately 7-8 in all three groups. The neonatal mortality rate in the hybrid group of wild boar and Mong Cai pig (WP x MC) accounted for a relatively high rate (9.28 %), and the lowest rate was 7.67 % at WPxWP group. Although the parameters are different between the experimental groups, there were no differences significantly ($p <0.05$). The number of piglets/parity in WPxWP group was higher than that in Muong Khuong (5 piglets/parity), it was similar of them in Meo and Co pig (6–7 piglets/parity), and lower than that in I pig (8.8–11.3 piglets/parity), Soc pig (6–10 piglets/parity) and Mong Cai pig (10–14 piglets/parity) (Dang-Nguyen et al., 2010). The number of piglets/parity in two hybrid groups (WPxMC and WPxS) was similar in WPxWP group (7.44 ± 1.16, 7.67 ± 1.00 and 7.44 ± 1.16), but it was lower than that in Mong Cai and Soc pig (Dang-Nguyen et al., 2010).

Table 1: Reproductive efficiency of Vietnamese native wild pig and crossbreeds

Parameters	WP x WP $(\bar{X} \pm SE)$	WP x MC $(\bar{X} \pm SE)$	WP x S $(\bar{X} \pm SE)$
Number of sows (n)	10	10	10
Number of piglets/parities	7.44 ± 1.16[a]	7.67 ± 1.00[a]	7.22 ± 1.73[a]
Number of live piglets/parities	6.78 ± 0.74[a]	7.11 ± 0.86[a]	6.56 ± 1.56[a]
Neonatal mortality rate (%)	7.67 ± 8.52[a]	9.28 ± 6.11[a]	8.61 ± 6.70[a]
Number of weaned/parity	6.78 ± 0.57[a]	6.67 ± 0.89[a]	6.22 ± 1.67[a]

[a] superscripts within a row indicate that there was no significant difference ($P<0.05$), the WP x WP was the group of wild pig x wild pig, the WP x MC was the F1 hybrid group of wild boar with Mong Cai pig, the WP x S was the F1 hybrid group of wild boar with Soc pig.

3.2 Growth performance in three groups

The growth performance in three studied groups of pigs was shown in Table 2. The mean of birth weight of piglets in the WPxWP group was higher than that in WPxMC and WPxS groups (613.43 g, 533.75 g, and 489.00 g) According to Dang-Nguyen et al. (2010) the mean of birth weight of Mong Cai pig is 0.45 kg, and the Soc pig is 0.4 - 0.45 kg. Although, at the weaned, the weight of the piglet seems to the same in three groups, but it was higher than that in Soc pig (5–6 kg) (Dang-Nguyen et al., 2010). The growth rate of piglets from birth to weaning in WPxMC groups was 98.42 g/d, higher than that in two groups WPxWP and WPxS (97.37 g/d and 97.18 g/d, $P<0.05$). Although, the growth rate of piglets from weaning to 6 months was highest in WPxWP group, 92.01 g/d ($P<0.05$). There is a slight decrease in weight gain from weaning to 6 months of age because after weaning, piglets have to go through a process of adapting to feed-based feeding. Because of human supply, the process of absorbing nutrients is still weak, so the growth rate is lower than that of the breastfed new born period. In the period of pigs from 6 m to 10 m of age, the weight gains of pigs increased sharply in all 3 groups of pigs studied, specifically in the WPxMC group with a weight gain of 165.03 g/d, in the group of pigs. The WPxS group reached

178.00 g/d, especially in the purebred wild boar group, the weight gain rate was 245.03 g/d higher than the two groups of WPxMC and WPxS. From the results of the study on the growth rate at 3 stages, the piglet stage from birth to weaning, the weaning stage to 6 months old, and the pig period from 6 months old to 10 months old, it is concluded that using purebred wild boars for crossbreeding with Mong Cai and Soc pig breeds will promote weight gain and higher breeding efficiency. At present, very little is known about the growth traits in wild pigs in breeding conditions. The results of this research are in agreement with other reports indicating a slower growth rate of wild pig (average is only about 150 - 300 g/d) (Thom et al., 2021). The growth rate of WPXMC was also lower than that in Mongcai pig in previous reports, which was from 273 g/d (Nguyen and Ly, 2002) to 328 g/d (Pham et al., 2010), or average 367.27 - 381.00 g/d (Duyen, 2010).

Table 2: Weight growth of three groups

Parameters	WP x WP ($\bar{X} \pm SE$)	WP x MC) ($\bar{X} \pm SE$)	WP x S) ($\bar{X} \pm SE$)
Number piglets (n)	61	65	56
Birth weight (g)	613.43 ± 44.16[a]	533.75 ± 68.31[b]	489.00 ± 53.54[b]
Weaning weight (60 d old) (kg)	6.45 ± 2.26[a]	6.45 ± 0.85[a]	6.32 ± 1.39[a]
Growth rate of piglets from birth into weaned pig (60 d old) (g/d)	97.37 ± 1.11[b]	98.42 ± 0.39[a]	97.43 ± 0.67[c]
Growth rate of piglets from weaned pig into 6 months old (g/d)	92.01 ± 0.95[a]	86.80 ± 4.11[b]	87.90 ± 19.01[b]
Growth rate of piglets from 6 months old into 10 months old (g/d)	245.03 ± 0.31[a]	165.03 ± 9.37[b]	178.00 ± 15.54[c]

a, b, c: values within a row of table with different superscript differ significantly ($p < 0.05$)

3.3 Genotypes and gene frequencies of FUT1 gene in five pig Breeds

The results of determining the FUT1 genotype polymorphism are shown in Table 3. The results show that both A, and G alleles appeared in all studied groups of pigs. In which allele A is most common in the native wild boar population with a frequency of 65.12 %; followed by the population of WPxMC group with allele frequency A (53.66 %); WPxS, Mong Cai, and Soc pig groups have similar allele frequencies (47.06, 46.08 and 43.75 %). For the G allele, the frequency of occurrence was highest in the purebred Soc pig population (66.25 %) and the lowest in the native wild boar group (34.88 %); The groups of Mong Cai, WPxS and WPxMC had the frequency of G allele appearing (53.92 %, 52.49 %, and 46.36 %).

Table 3: Frequency of genotypes and alleles of FUT1 gene in five groups

Groups	N	Genotypes			Frequency of alleles (%)	
		AA $(\%, n)$	AG $(\%, n)$	GG $(\%, n)$	A (%)	G (%)
WPxWP	43	16 (37.21)	24 (55.81)	3 (6.98)	65.12	34.88
WP x S	34	5 (14.71)	22 (64.71)	7 (20.59)	47.06	52.94
WP x MC	41	8 (19.51)	28 (68.29)	5 (12.20)	53.66	46.36
MC	51	7 (13.73)	33 (64.71)	11 (21.57)	46.08	53.92
S	32	3 (9.38)	22 (68.75)	7 (21.88)	43.75	66.25

Genotype FUT1 AG appeared at a very high rate in all 5 groups of pigs studied, the most common among Soc pigs (68.75 %) and WPxMC group (68.29 %). This genotype appeared a lot in the Mong Cai group and the WPxS group with equal rates and equal to 64.71 %. Genotype FUT1 AG appeared in the native wild boar group with a rate of 55.81 %. Genotype FUT1 AA is most common in the wild boar population with a rate of 37.21 %, and occurs least in Soc pig group (9.38 %). In contrast, the FUT1 GG genotype had the highest rate in the Soc group (21.88 %) and the lowest in the wild boar group (6.98 %).

According to Zhang et al. (2013), when studying the FUT1 gene on 174 Pudong White pigs, the results showed that their respective genotype frequencies AA, AG, and GG were 0.052, 0.448, and 0.500. The frequency of the A allele is 0.276 and the frequency of the G allele is 0.724 (Zhang et al., 2013). Research by Meijerink et al. (1997) showed a G/A mutation at nucleotide 307 of the FUT1 open reading frame (ORF). Pigs with genotype AA are resistant to ETEC F18 infection, while pigs carrying the GG gene, or heterozygous for AG, genotype are susceptible to ETEC F18 infection (Meijerink et al., 1997). Kim et al. (2013) found that the piglet survival rate of individuals with genotype AA was almost two times greater than that of GG individuals. The FUT1 polymorphism can be used as a marker for selection programs to improve post-weaning piglet survival.

3.4 Genotypes and gene frequencies of GH gene in five pig Breeds

The results of identifying GH genotype polymorphisms are shown in Table 4. Through the results in Table 4, genotype C4/C4 appeared in all studied groups of pigs with a high percentage, the highest was the group of WPxMC, and MC groups (63.41 % and 63.16 %), in Soc group accounted for 50 %, and native wild boar group accounted for 30.23 %, the lowest in WPxS group (11.76 %). In addition, genotypes C1/C4 and C1/C2 also appeared in all groups of pigs but with less frequency. In the wild boar group, the genotype that appeared most was C1/C4, accounting for 62.79 %. The most common genotype in the WPxS group of pigs is C1/C2 (58.82 %).

Table 4: Genotypes of GH gene in five groups

Genotypes	WP % (n)	WPxS % (n)	WPxMC % (n)	MC % (n)	S % (n)
C1/C1	2.33 (1)			5.26(2)	6.67(2)
C1/C2	4.65 (2)	58.82 (20)	4.88(2)	13.16(5)	20.00(6)
C1/C3					
C1/C4	62.79 (27)	29.41 (10)	29.27(12)	18.42(7)	23.33(7)
C2/C2					
C2/C3			2.44 (1)		
C2/C4					
C3/C3					
C3/C4					
C4/C4	30.23 (13)	11.76 (4)	63.41 (26)	63.16 (24)	50.00 (15)
Total	100.00 (43)	100.00 (34)	100.00 (41)	100.00 (38)	100.00 (30)

In a previous study, Thuy et al. (2004) showed that the genotype GH gene in Mong Cai pigs, the highest frequency was genotype C2C4 with 51 %, and the lowest rate was C2C2 with 11 %. In the groups of Wild Boar x Piétrain pigs, the prevalence of the C2C4, C3C4 and C4C4 genotypes was 0.34 %; 28.42 % and 68.84 %; in Meishan x Piétrain crossbred pigs, genotypes are C1C4, C2C4, C3C4, and C4C4: 15.49%; 29.36%; 5.48% and 15.8 % (Knorr et al., 1997).

4. Conclusions

In this study, the crossbreeding with native wild pigs with the indigenous breeding pig was not effective in infertility and growth performance. The genotypes (AA, AG, and GG) of the FUT1 gene and (C1/C1. C1/C2, C1/C4, and C4/C4) of the GH gene were recorded in all groups. The favourable genotype of the FUT1 gene and GH gene appeared in low frequency. This result suggests that a marker-assisted selection program using FUT1 and GH alleles should be questioned in future studies.

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