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Association of porcine growth hormone gene with growth performance in "Zovawk": A hill pig of Mizoram, India

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Abstract

In Mizoram, majority of local population are non-vegetarian and pork is the choice of meat. There is very high demand for the meat of Mizoram local pig "Zovawk" because of its deliciousness. The porcine growth hormone (GH) gene is considered to be one of the most important candidate genes that can influence porcine performance traits because of its crucial function in growth and metabolism. The study was undertaken to find association between growth hormone gene variants and growth rate in zovawk. The PCR-RFLP digested product revealed three genotypes in Mizo local pig population. The genotype AA was found predominantly (0.45) as compare to the other genotypes AB and BB in the population. The frequency of A and B alleles were 0.60 and 0.40 in the population. The different genotypes did not show any significant effect on the growth performance at different age groups in the population.

Keywords: Mizoram, Zovawk, growth hormone gene, performance traits, PCR-RFLP

Introduction

Pork is a favoured meat for people of Mizoram, a small hilly state in North eastern India. This part of the country is breeding tract to an indigenous semi-wild population of pig called "Zovawk". The meat of the animal is a delicacy to the local people. Swine production demands high growth rate together with efficient conversion of feed to meat. Important economic traits are usually controlled by genetic and non genetic factors like environmental temperature and nutrition. Exogeneous porcine somatotropin (GH) administration causes higher muscle mass and lower fatness in pigs. The effect of porcine somatotropin depends on the genetic potential for fat deposition of the breed, e.g. animals of German Landrace and the Schwerfurther Race showed a higher response to administrated somatotropin than Pietrain ^[1]. Furthermore, pigs selected for higher weights at slaughter have higher basal concentrations of GH than unselected animals ^[2, 3]. Growth hormone is a peptide hormone approx. 190 amino acids in length which is produced and released by the anterior pituitary in response to the hypothalamic peptide, GH-releasing factor ^[4]. Secretion of GH results in a range of metabolic actions, the end result of which is growth. Porcine growth hormone gene contains four introns and five exons ^[5], and the gene was assigned to porcine chromosome 12p1.2-p1.5 ^[6]. The effects of GH gene variations on performance traits have been described for cattle ^[7, 8] and mice ^[9]. The transcriptional activities between GH variants, which might eventually cause higher plasma GH concentrations and higher growth rates ^[10], however, their studies did not establish a direct cause and effect relationship. In the present study, the polymorphism of GH was investigated in Mizoram local pig and associations of GH genotypes with some performance traits.

Materials and Methods

Selection and maintenance of the animals: A total of 60 apparently healthy Zovawk pigs reared in the Livestock Farm, College of Veterinary Sciences and A.H., Selesih, Aizawl, Mizoram were selected. The animals were maintained along with other animals and fed as per the routine feeding schedule followed in the Livestock Farm of the college. Weaning of the pigs was done on the 56^{th} day of age.

Collection of blood sample: Blood samples were collected in 3 ml capacity K₃EDTA coated tubes by venipuncture of anterior venacava.

Adequate care and necessary steps were taken up to prevent any untoward stress to the animals. Cold chain was maintained for collected blood samples during the transit from the farm to the laboratory for further studies.

PCR-RFLP of GH gene

DNA was extracted using standard phenol/chloroform extraction method followed by ethanol precipitation ^[11]. Working dilutions of extracted DNA were prepared for each individual at a concentration of 20 ng/µl. The Primers sequences ^[12] consisting of forward 5' TTA TCC ATT AGC ACA TGC CTG CCA 3' and reverse 5' CTG GGG AGC TTA CAA CAT CCT T 3' were used for PCR (polymerase chain reaction) amplification between nucleotides -119 and +486 bp of the porcine growth hormone gene [5]. The PCR mixture contained 50 ng genomic DNA, 25 pmol of each primer, 25 µM of each dNTP, 1 unit of Taq DNA Polymerase and 10×reaction buffer in a 25 µl reaction volume. PCR was done using the Eppendorf thermocycler according to the following procedure: initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 59°C for 45 sec, 72°C for 30 sec and final synthesis at 72°C for 10 min

The PCR products were subsequently digested with ApaI restriction endonuclease enzymeby incubating at 37°C for 3 hours. The restriction digests were separated using 2.0% agarose gel in $1 \times TAE$ at a constant current of 80V. The gels were stained with ethidium bromide and the fragments were visualized using a UV transilluminator.

Statistical analysis

Data were analyzed using SPSS version 16. GLM test was applied to evaluate the effect of genotypes on growth performance trait.

Results and Discussion

The PCR-RFLP ApaI digested product revealed allele A (fragments of 449 bp, 101 bp and 55 bp) and allele B (fragments of 316 bp, 133 bp, 101 bp and 55 bp). The fragment patterns representing three genotypes viz. AA (449 bp, 101 bp and 55 bp), AB (449 bp, 316 bp, 133 bp, 101 bp and 55 bp) and BB (316 bp, 133 bp, 101 bp and 55 bp) were observed in Mizo local pig population (Figure 1). The genotypes and the allele frequencies of GH are listed in Table 1. The higher frequency (0.45) of AA genotype was found as compare to the other genotypes AB and BB in the population. Comparatively higher frequency (75.8%) of AA genotype was reported i in Chinese native pig breeds ^[13]. In earlier study, it was reported that western pigs carried AA genotype with a low frequency ^[14], while Chinese native pig breeds had higher frequencies ^[12]. The frequency of A and B alleles were 0.60 and 0.40, respectively in the population. Similar finding of allele frequency was reported in Chinese native pigs [13]. Meanwhile, the allele B frequency in European pig breeds was high, especially in Pietrain where its frequency was 79.1% ^[13]. The genotypic frequencies observed in the population were within the Hardy-Weinberg expectation for the growth hormone locus as revealed by the chi-square test (p < 0.01). In Landrace pigs from Russian farms, the frequency of the AA genotype was the highest, and the lowest was the GG genotype. In this case, the A (0.52) allele had the highest frequency ^[15]. Testing this Landrace population for the Hardy-Weinberg ratio revealed a significant deviation of the observed genotype frequencies from the expected ones ($\chi 2 =$ 9.08, *p*<0.05).

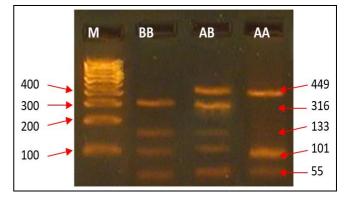


Fig 1: Genotypes of growth hormone gene in Mizo local pig revealed by PCR-RFLP in 3% agarose gel. (M: 100bp ladder; AA, AB & BB: Genotpes)

 Table 1: Gene and genotype frequencies of growth hormone gene in Mizo local pig

	Number	Frequency		
Allele				
А	96	0.60		
В	64	0.40		
Total	160	1.00		
Genotype				
AA	36	0.45		
AB	24	0.30		
BB	20	0.25		
Total	80	1.00		
Chi-Square	0.	.93 ^{NS}		
ISNT	•			

^{NS}Not significant

Weekly growth profile according to the genotypes is given in Table 2. The different genotypes did not show any significant effect on the growth performance at different age groups in the population. Similar to our finding, in Nanchang White pigs, no significant difference was observed between three genotypes and the trait. But the pigs with AA genotype had more birth weight, 2 months body weight, 4 months body weight and 6 months body weight. As far as corrected backfat thickness and average back-fat thickness were concerned, pigs with BB genotype were the thinnest, although no significant difference was observed ^[13]. In the offsprings of four purebred sires and two crossbred sires, no significant association was observed between growth hormone genotype and growth traits ^[16]. However, in Duroc, Landrace and Tao-Yun pig breeds the growth performance traits were highly correlated with their growth hormone genotype ^[17]. In a study on crossbred pigs in humid tropical environment, the genotype GA had higher growth trait values than genotype GG at birth, weaning and 16 weeks ^[18].

 Table 2: Effects of different genotypes on growth performance in

 Mizo local pig

Age	Genotype		
	AA	AB	BB
Birth weight ^{NS}	0.52 ± 0.03	0.47 ± 0.04	0.55 ± 0.04
1 st week ^{NS}	0.99 ± 0.08	0.81 ± 0.06	0.92 ± 0.05
2 nd week ^{NS}	1.55 ± 0.12	1.32 ± 0.13	1.51 ± 0.06
3 rd week ^{NS}	2.13 ± 0.14	2.01 ± 0.10	2.08 ± 0.07
4 th week ^{NS}	2.49 ± 0.20	2.27±0.17	2.39 ± 0.05
5 th week ^{NS}	2.83 ± 0.22	2.62 ± 0.13	2.93 ± 0.07
6 th week ^{NS}	3.39 ± 0.25	3.22 ± 0.18	3.44 ± 0.09
7 th week ^{NS}	3.88 ± 0.31	3.69 ± 0.38	3.91 ± 0.20
8 th week ^{NS}	4.30 ± 0.37	4.23 ± 0.34	4.34 ± 0.27

Note: Values are Mean±SE; ^{NS}Not significant

Conclusion

It is indicated the GH locus plays a major role in defining the genetic difference between breeds of pig and regards the GH gene as a candidate gene for fatness in pigs. So it is important to build several resource families and identify the single-nucleotide polymorphism (SNP), to find out which SNP has high relationship to production performance, and to provide a basis for developing successfully mark-assisted selection programmes. In our study the finding may further verified by including more samples and other regions of GH gene.

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