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Influence of MC4R mutations in traits of tested Danube white purebred pigs

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Abstract

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Restriction analysis (restriction fragment length polymorphism, RFLP) for establishing of MC4R polymorphisms with 81 Danube white purebred pigs was carried out. Genotypes and alleles frequencies of MC4R gene were classified as follows: the number of heterozygous animals $MC4R^{AB}$ -72.8% was the highest one and significantly lower for both homozygous genotypes 12.3-14.8%. The allele frequencies – $MC4R^{A}$ and $MC4R^{B}$ were close as the difference from 5.04% was in advantage for favorable $MC4R^{B}$ alleles.

It was established that as a result of the mutation of Asp298Asn pigs with genotype $MC4R^{BB}$ exceeded animals of the other genotypes as the differences for the following traits: back fat at point X_2, meat content and back fat MLD were significant ($P \leq 0.05$).

The present study conducted with pigs from the Danube white breed gives us reason to assert that the MC4R gene can be used as a reliable genetic marker for the selection of fattening and carcass traits measured during the testing period of 90 kg live weight.

Keywords: MC4R gene mutation; Danube white pigs

Introduction

Melacortin – 4 receptor genes (MC4R) regulates energy homeostasis (feed intake) and influences on the physical development in humans and animals. MC4R encodes a receptor involved in the neural circuits that regulate food intake (Krashes et al., 2016; Shen et al., 2017) in this respect, the role of MC4R gene was in regulating appetite and blocking feed intake (Barb et al., 2004). According to Zemel (1998) the interaction of MC4R gene with leptin as well as with other peptides from the hypothalamic regulation was significant for the relationship between live weight and feed intake.

A number of research studies were carried out assessing the MC4R polymorphisms with fattening ability traits and carcass qualities of pigs. Chen et al. (2004), Kim et al. (2006) and Meidtner et al. (2006) established that MC4R gene could be used as a genetic marker for the studied traits in the selection of purebred pigs. Van den Maagdenberg et al. (2007) and Magdalena (2010) demonstrated the effect of MC4R

gene mutations on the fattening and carcass qualities of pigs with different origin. On the other hand, Stachowiak et al. (2006) did not find any influence of these genetic polymorphisms on feed intake and back fat.

Iowa State University group, in collaboration with Pig improvement company (PIC) established that MC4R gene mutation increased the appetite (about 10%), the growth intensity (6-8%) and there was an exfoliation of more fat (6-10%). The use of this mutation was recommended for selection of feed intake in maternal lines where the back fat was at the desired level. Regarding the fathers' lines, the same mutation could be used for back fat reduction (Rothschild & Ruvinski, 2010).

The purpose of the present study was to investigate the influence of MC4R gene mutation of tested Danube white purebred pigs.

Material and Methods

Restriction analysis (RFLP) for establishing of MC4R polymorphisms with 81 Danube white purebred pigs was carried out in the Agricultural institute – Shumen. Polymorphisms of the same gene for traits of 90 kg live weight testing period were analyzed: back fat at points X_1 and X_2, growth intensity, back fat of *Musculus lonngissimus dorzi* (MLD) and lean meat percent. DNA isolation was performed according to the protocol of AccuPrep Genomic DNA Extraction Kit. Hair follicles of 90 kg tested purebred pigs were used. Primers were worked out by Kim, 2000 and could be presented as follows:

Forward primer: 5'- TAC CCT GAC CAT CTT GAT TG -3'
Reverse primer: 5'- ATA GCA ACA GAT GAT CTC TTT G -3'

PCR reaction was carried out with 80-100 ng DNA. The reaction composition consists master mix ($MgCl_2$, ddH_2O, buffer, dNTPs), ddH_2O – 21 µl, FP – 1 µl RP – 1 µl DNA – 2 µl. PCR profile includes: 2 min. to 94°C; 35 cycles of: 30 sec. denaturation at 94°C; 1 min. annealing at 56°C, 1.30 min. elongation at 72°C and 15 min. additional elongation at 72ºC performed with Gene Amp PCR System. PCR product was fragmented with Taq 1 enzyme. The identification of the isolated fragments was carried out on 2% of agarose gel. The used DNA control (DNA Ladder) was 100 bp for determination of the fragments' length.

The PCR profile included 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C; and a final 7-min extension at 72°C in a Gene Amp PCR System 9700/9600/2400.

Statistical data analysis was carried out by the software LSMLMW&MIXMDL (Harvey, 1990). Significances of differences between the separate genotypes were evaluated by Student´s t-test.

Results

The Danube white breed is a meat selection with a limited area and one of the tasks for her breeding is to maintain genetic diversity in population threatened by extinction. The information characterizing the values of the traits of the self-estimation productivity is presented in Table 1. The analysis of the results shows that purebred pigs from the Danube white breed are of satisfactory fattening ability and carcass qualities, which was in conformity with traditional feeding and breeding conditions. Danube white pig breeds compared to high-yielding breeds are characterized by thicker back fat and lower meat percent. Traits: back fat in two points X_1 and X_2 ranged from 12 to 16 mm, lean meat percent – 56%. Variation is in normal rates 5-13%. There are high values of coefficients of determination $R^2 = 0.797 – 0.942$ except for the trait back fat of *Musculus longissimus dorsi* (MLD). High values of determination indicate that studied factors (genotypes) have a precise effect on the variation of the examined traits in the model.

Table 1. Average values, variation and coefficient of determination for traits of the self-estimation productivity

Traits	Total average, LSM	Variation coefficient	Coefficient of determination
	n=81	C%	R^2
Back fat:			
point X_1, mm.	16.32	10.39	0.896
point X_2, mm.	12.16	13.94	0.797
Muscle depth of MLD, mm.	44.06	10.47	0.163
Lean meat %	56.04	1.21	0.972
Age, days	211.35	5.25	0.821

Genotyping of MC4R gene, situated in the first chromosome, has been carried out. The fragmentation of PCR product with Taq I enzyme causes the following allocation: PCR fragments of 226 bp correspond to $MC4R^B$ allele, of 70 bp and 156 bp – to $MC4R^A$ allele. There are fragments from both of the alleles with allocation of 226bp+156bp+70bp (Figure 1) in animals with heterozygous genotype $MC4R^{AB}$.

Genotype and allele frequencies of the MC4R gene are presented in Table 2. The number of heterozygous animals is the highest one $MC4R^{AB}$ -72.8% and for both homozygous genotypes is significantly lower 12.3-14.8%. Allele frequencies – $MC4R^A$ and $MC4R^B$ are close as the difference from 5.04% is in favor of the $MC4R^B$ allele.

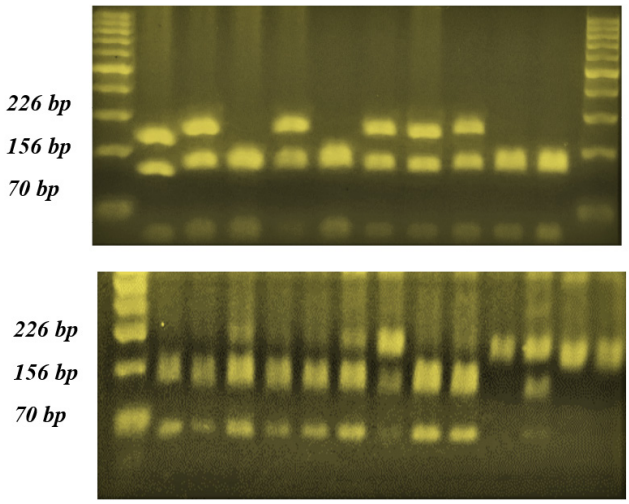

Fig. 1. PCR fragments

Table 2. Frequency of genotypes (%) and alleles (%) for melanocortin-4 receptor gene in a group of Danube white pigs (n=81)

		Frequency of genotypes, n	
	AA	10	12.35
	BB	12	14.81
	AB	59	72.84
MC4R locus			
		Frequency of allels	
	A		48.77
	B		51.23

The characteristic of the studied genotypes of the MC4R gene for the measured traits of the tested animals is presented in Figures 2 and 3. Data from the analysis of the back fat at points X_1 and X_2 indicates that the back fat of pigs with genotype $MC4R^{BB}$ is significantly thinner as the difference between the homozygous animals from the two genotypes measured at point X_2 is 3.8 mm ($P \leq 0.05$). Significant differences are also established between homozygous animals for the trait back fat of *MLD*. Pigs with genotype $MC4R^{BB}$ differ from genotypes of $MC4R^{AA}$ ($P \leq 0.05$) with higher percent of muscle thickness – 4.6 mm. Regarding the lean meat percent measured for live pigs, a significant superiority of the animals with genotype $MC4R^{BB}$ is established as differences with genotype $MC4R^{AA}$ (4.02%) and genotype $MC4R^{AB}$ (1.8%) are significant ($P \leq 0.05$). The fattening ability of pigs from the three genotypes expressed by the trait age at reaching of 90 kg live weight is within close range (199-205 days). There is a tendency for extensive growth for heterozygous animals – $MC4R^{AB}$.

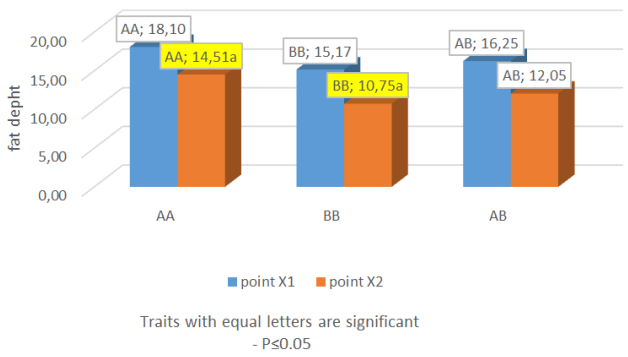

Fig. 2. Fat depth at 90kg live weight

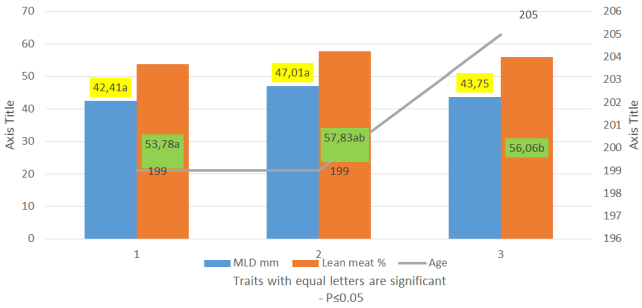

Fig. 3. Depth of MLD, lean meat and age at 90 kg live weight

Discussion

Selection is a major factor determining genetic diversity in populations and the long-lasting team increases their homozygosity i.e. reduces the variation of selection traits and leads to a selection plateau. Houston et al. (2004) noted that the application of a selection press for the traits meat content and feed intake over several generations for two groups of animals selected from the same population indicated to some diversity between them (the two groups) with respect to the frequency of the alleles of MC4R gene.

In the present study, single nucleotide polymorphism (SNP) was indicated where some substitution of nucleotides (G → A) for the 298th amino acid (Asp298Asn – substitution of Aspartic amino acid to Asparagine) was available from the MC4R gene protein. The allele frequencies we have found were in close proximity with little superiority to the more favorable $MC4R^{B}$ allele. Magdalena et al. (2010) established higher differences between the frequencies of the two alleles (15.2%). In similar studies with Large white

pig breed, Kim et al. (2000) and Hernandez-Sanchez et al. (2003) have found almost identical frequencies between the MC4R gene alleles. The same authors noted that the investigated Landrace breed population achieved higher frequencies of the $MC4R^B$ allele. This tendency for superiority of the $MC4R^B$ allele also appeared in Stachowiak et al. (2006) surveys with the Polish Large White and Polish Landrace breeds.

Our research has shown that homozygous $MC4R^{BB}$ pigs are characterized by better fattening and slaughtering qualities than other genotypes. It was established that the $MC4R^B$ allele presented from a fragment of 226 bp, significantly influenced on the back fat at point X_2, of MLD and on the percentage of meat measured in born alive ($P < 0.05$). Our results were similar to Chen et al. (2004) with pigs of local breeds and F_2 crosses where the genotype $MC4R^{BB}$ pigs significantly grew up with a higher intensity of up to 100 kg live weight, the back fat is thinner and the meat content is higher ($P \leq 0.05$). Magdalena et al. (2010) in a study on the carcass characteristic of the Pula breed pig (which is included in a canning breed program) established identical results. Animals with $MC4R^{AA}$ genotype have got a proven higher fat content in the carcass (mainly for the thickness of back fat, weight and fat in the fillet and neck) and lower meat content in the carcass. Davoli et al. (2012) established similar results in genotyping of the MC4R gene for two Italian breeds where G → A polymorphism has a favorable effect on average daily gain, feed conversion and ham weight. Concerning the meat content in the carcass, it was established a favorable influence of $MC4R^{BB}$ genotype on the Italian Duroc and on the thickness of back fat of Italian Large white. Van den Broeke et al. (2015) in a boar study, as far as the residual scent of the meat was concerned, animals with the genotype $MC4R^{AA}$ showed a significantly higher androstenone content ($P = 0.044$), skatol ($P = 0.049$) and indole ($P = 0.006$). Concerning the carcass traits: lower yield ($P = 0.005$), shorter ham width ($P = 0.024$), smaller thickness of MLD ($P = 0.011$), higher back fat thickness ($P < 0.001$) and lower meat percentage in the carcass ($P < 0.001$).

Conclusion

The allele frequencies – $MC4R^A$ and $MC4R^B$ were close as the difference from 5.04% was in favor of the desired $MC4R^B$ allele. It was established that as a result of the mutation of Asp298Asn, pigs with genotype $MC4R^{BB}$ exceeded animals of the other genotypes as the differences for the following traits: back fat at point X_2, muscle depth (MLD) were significant ($P \leq 0.05$).

The present study conducted with pigs from the Danube white breed gives us reason to assert that the MC4R gene can be used as a reliable genetic marker for the selection of fattening and carcass traits measured during the testing period of 90 kg live weight.

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References

Barb, C. R., Robertson, A. S., Barrett, J. B., Kraeling, R. R. & Houseknecht, K. L. (2004). The role of melanocortin-3 and -4 receptor in regulating appetite, energy homeostasis and neuroendocrine function in the pig. *Journal of Endocrinology, 181*, 39-52.

Chen, M., Wang, A., Fu, J. & Li, N. (2004). Different allele frequencies of MC4R gene variants in Chinese pig breeds. *Archiv fuer Tierzucht Dummerstorf, 47(5),* 463-468.

Davoli, R., Braglia, S., Valastro, V., Annaratone, C., Comella, M., Zambonelli, P., Nisi, I., Gallo, M., Buttazzoni, L. & Russo, V. (2012). Analysis of MC4R polymorphism in Italian Large White and Italian Duroc pigs: association with carcass traits. *Meat Sci., 90(4),* 887-92.

Hernande, J., Visscher, P., Plastow, G. & Haley, C. (2003). Candidate gene analysis for quantitative traits using the transmission disequilibrium test: the example of the melanocortin-4 receptor in pigs. *Genetics, 164*, 637-644.

Houston, R. D., Cameron, N. D. & Rance, K. A. (2004). A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected Large White pig populations. *Animal Genetics, 35(5),* 386-90.

Kim, K. S., Larsen, N., Short, T., Plastow, G. & Rothschild, M. F. (2000). A missense variant of the melanocortin 4 receptor (MC4R) gene is associated with fatness, growth and feed intake traits. *Mammal. Genom, 11,* 131-135.

Kim, K. S., Lee, J. J., Shin, H. Y., Choi, B. H., Lee, C. K., Kim, J. J., Cho, B. W. & Kim, T. H. (2006). Association of melanocortin 4 receptor (MC4R) and high mobility group AT -hook 1 (HMGA1) polymorphisms with pig growth and fat deposition traits. *Animal Genetics, 37(4),* 419-21.

Krashes, M. J., Lowell, B. B. & Garfield, A. S. (2016). Melanocortin-4 receptor-regulated energy homeostasis. *Nat. Neurosci., 19(2),* 206-219. https://doi. org/10.1038/nn.4202.

Meidther, K., Wermter, A. K., Hinne,y A., Remschmidt, H., Hebebrand, J. & Fries, R. (2006). Association of the melanocortin 4 receptor with feed intake and daily gain in F2 Mangalitsa x Pietrain pigs. *Animal Genetics 37(3),* 245-247.

Rothschild, M. F. & Ruvinski, A. (2010). The genetics of the pig. 2nd edition, 342-348.

Shen, W. J., Yao, T., Kong, X., Williams, K. W. & Liu, T. (2017). Melanocortin neurons: Multiple routes to regulation of metabolism. *Biochim. Biophys. Acta, 1863(10 Pt A),*

2477-2485.
https://doi. org/10.1016/j.bbadis.2017.05.007

Stachowiak, M., Szydłowski, M., Obarzanek-Fojt, M. & Świtoński, M. (2006). An effect of a missense mutation in the porcine melanocortin-4 receptor (MC4R) gene on production traits in Polish pig breeds is doubtful. *Animal Genetics, 37(1),* 55-57.

Szyndler-Nędza, M., Tyra, M., Blicharski, T. & Piórkowska, K. (2010). Effect of mutation in MC4R gene on carcass quality in Pulawska pig included in conservation breeding programme. *Animal Science Papers and Reports, 28 (1),* 37-47.

Van den Broeke, A., Aluwé, M., Tuyttens, F. A., Ampe, B., Vanhaecke, L., Wauters, J., Janssens, S., Coussé, A., Buys, N. & Millet, S. (2015). An intervention study demonstrates effects of MC4R genotype on boar taint and performances of growing-finishing pigs. *J. Anim. Sci., 93(3),* 934-43.

Van den Maagdenberg, K., Stinckens, A., Claeys, E., Seynaeve, M., Clinquart, A., Georges, M., Buys, N. & De Smet, S. (2007). The Asp298Asn missense mutation in the porcine melanocortin-4 receptor (MC4R) gene can be used to affect growth and carcass traits without and effect on meat quality. *Animal, 1(8)*, 1089-1098.

Zemel, M. B. (1998). Agouti/melanocortin interaction with leptin pathways in obesity. *Nutrition Reviews, 56,* 271-281.

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