

EFFECTS OF GENOTYPES *LEPR* AND *MC4R* ON PIGS PRODUCTION

EFFECTUL GENOTIPURILOR *LEPR* ȘI *MC4R* ASUPRA PRODUCȚIEI DE SUINE

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*The aim of this study was to identify polymorphism in *LEPR* and *MC4R* genes and evaluate some production traits in 102 pigs. Genotyping of pigs was done by PCR – RFLP methods. Into the set of 102 pigs were found three genotypes AA(14), AB(32) and BB(56) for *LEPR* gene and AA(46), AG(37) and GG(19) for *MC4R*. Differences between genotypes were detected. The high determination coefficient was confirmed (0,68 – 0,79). Choises model was statistically highly significant and highly significant *LEPR* and *MC4R* genes effect was confirmed for backfat thickness (BFT). Also this study published polymorphism of these genes and their effect on production traits of pigs (mainly lean meat - LM and average daily gain - ADG). The results were obtained on small number of animals and the results have to be confirmed on larger number of animals.*

Key words: pig production, *LEPR*, *MC4R*

Introduction

In process of pig interbreeding, as well as of other farming animals, the aim of breeders is to achieve the best possible productive results and traits derived from several molecular-genetic methods. Demand for lean pork meat results in breeding programmes aimed at fat reduction. Therefore we have decided to focus on candidate genes effective in pork-meat fat content, among which we include: *LEPR* and *MC4R*. Leptin, product of *LEP* gene, is secreted in adipose tissue and signals to hypothalamus that the body had been saturated. Expression and secretion of leptin is obviously related to food intake regulation, energy metabolism and body weight (Campfield et al., 1995, Remesar et al., 1997). Its receptor (leptin receptor, *LEPR*) has been localized to several hypothalamic nuclei, including the ventromedial

hypothalamus, the medial preoptic area, the paraventricular nucleus, and the arcuate nucleus (Keen-Rhinehart et al. 2004). Vincent (1997) identified a *HinfI* polymorphism in *LEPR* gene and mapped its location to pig chromosome 6. Emmett et al. (2001), Mindeková and Trakovická (2006) confirmed *LEPR* (Mbol) gene effect on average daily gain. Melanocortin-4 receptor (*MC4R*) is famous for their roles in feeding behavior, energy homeostasis and HPA axis regulation (Salajpal, 2007). Kim et al. (2000) studied *MC4R* as a candidate gene for the control of economically important growth and performance traits in the pig. Melanocortin-4 receptor (*MC4R*) has been found to play a significant role in regulating leptin's effects on food intake and body weight (Fan et al., 1997). Kim et al. (2000) demonstrated that a missense mutation in *MC4R* was associated with backfat thickness, growth, and feed intake in different genetic lines of pigs. Two alleles were detected by PCR-RFLP using restriction enzyme *TaqI*(allele 1-Asp298, allele 2-Asn298) by Kim et al. (2000). The aim of this contribution was to analyse productive traits following polymorphism of genes *LEPR* and *MC4R*.

Materials and Methods

We used 102 young boars and sows of hybrid combination Large White and Landrass for our experiment. DNA was isolate using by commercial kit nucleospin blood from Macherey-Nagel. For amplification of specific fragments for *LEPR* gene detection the following oligonucleotide primers FOR and REV are used taken from Stratil et al., (1998):

FOR 24 mer: 5' - GGA AGG CAT TTG TTT CAG CAG TAA - 3'

REV 24 mer: 5' - CAA GTC CTC TTT CAT CCA GCA CTG - 3'

Pig specific primers for amplification of specific fragments for *MC4R* gene (Kim, 2000):

FOR: 5'-TTA AGT GGA GGA AGA AGG-3'

REV: 5'-CAT TAT GAC AGT TAA GCG G-3'

Genotyping of pigs was done by PCR – RFLP methods. After amplification, the PCR product was digested with *HpaII* (*LEPR*) and *TaqI*(*MC4R*). Statisticall analyse individual genes to production traits were done by linear model GLM with fixed and variable effects using SAS (2000) programme.

$$Y_{ijkl} = \mu + LEPR_i + MC4R_j + LEPR^*MC4R + Sex_k + e_{ijkl}$$

Y_{ijkl}	- boduated trait (average daily gain, back fatt thickness, lean meat, MLD)
μ	- average of population
$LEPR_i$	- effect at genotype <i>LEPR</i> gene (i = AA, AB, BB)
$MC4R_j$	- effect of genotype <i>MC4R</i> gene (j = AA, AG, GG)
$LEPR^*MC4R$	- effect of genotype interaction <i>LEPR</i> and <i>MC4R</i>
Sex_k	- effect of sex
e_{ijkl}	- random mistake

Results and Discussion

Into the set of 102 pigs were found three genotypes AA(14), AB(32) and BB(56) for *LEPR* gene. Frequencies of genotypes and genes are presented in Table 1. Genotype frequencies at all polymorphic loci nonfollowed Hardy-Weinberg equilibrium ($p \leq 0,001$). There were founded differences in frequencies of alleles both of locies. High frequency of *LEPR* gene was confirmed for allele A ($0,7058 \pm 0,0535$) against allele B ($0,2942 \pm 0,0535$). There were founded lower differences in frequency of *MC4R* (A = $0,9324 \pm 0,0512$, B = $0,3676 \pm 0,0512$).

Table1
Genotype and allele frequencies of *LEPR* and *MC4R* genes in the LWxLa population (N= 102)

Locus	Genotype			Allele		χ^2 - test	H _e	PIC
	AA	AB	BB	A	B			
<i>LEPR</i>	14	32	56	0,2942	0,7058	6,0948 ⁺⁺	0,4153	0,3249
<i>MC4R</i>	46	37	19	0,6324	0,3676	4,9298 ⁺	0,4649	0,3569

P>0,05⁺, P>0,01⁺⁺, P>0,001⁺⁺⁺,

The results of GLM analyses are presented in Tables 2, 3, 4. The high determination coefficient was confirmed (0,68 – 0,79). The references evaluation of individual genotypes of marker gene with phenotypic parameters of meat are presented in Tables 2, 3. Average daily gain according to genotype *LEPR* was found in heterozygote genotype AB. Difference of trait was significant against genotype AA. Significant differences were found between genotypes for BFT and LM. Least significance was found in BB as in AB and AA genotype, for BFT(AA > AB > BB). Highest significance was found in AA as in AB and BB genotype, for LM. Non statistically significant differences were found between genotypes for MLD. On the base of genotype *MC4R* differences followed similar tendency for BFT and LM. Highly significant was least backfat thickness, while tendency of that trait was following: AA > AG > GG. Statistically significant differences were found between genotypes for LM, highly significance was found in AA genotype. Average daily gain was found in important lower value at heterozygote composition of young pigs. Choices model was statistically highly significant and highly significant *LEPR* and *MC4R* genes effect was confirmed for BFT. Significant interaction of this genes was confirmed too. The results of GLM analyses are presented in Table 4. Our results corespond with results of Salajpal *et al.* (2007), Kim *et al.* (2000), that *MC4R* control economic important traits and Mindeková, Trakovická (2006), that *LEPR* is candidate genes with effect to fatt content. Emmett *et al.* (2001) found influence of *LEPR* (Mbol) gene to daily gain.

Table2

LSM analysis for carcass quality traits of pigs by the *LEPR* genotype

Meat parameters*	AA (14)	AB (32)	BB (56)
	LSM \pm s _e	LSM \pm s _e	LSM \pm s _e
ADG (g)	596,30^a \pm 28,16	604,29^a \pm 24,23	600,09 \pm 30,97
BFT (mm)	10,77^a \pm 0,43	10,02^{ab} \pm 0,53	8,98^b \pm 0,53
LM (%)	57,64^{ab} \pm 1,18	58,91^a \pm 1,75	59,98^b \pm 2,25
MLD(cm ²)	45,07 \pm 3,90	45,85 \pm 2,42	46,64 \pm 1,19

*ADG = average daily gain (g), BFT = average backfat thickness (mm), LM = lean meat (%), MLD = area of *musculus longissimus lumborum et thoracis* (cm²)

**Significance of the differences: ^a = P \leq 0,05, ^A P = \leq 0,01, ^A = P \leq 0,001

Table3

LSM analysis for carcass quality traits of pigs by the *MC4R* genotype

Meat parameters*	AA (46)	AG (37)	GG (19)
	LSM \pm s _e	LSM \pm s _e	LSM \pm s _e
ADG (g)	601,32 \pm 23,26	595,46^a \pm 28,76	607,36^a \pm 24,23
BFT (mm)	10,74^a \pm 0,94	10,01^{ab} \pm 0,56	8,08^B \pm 0,85
LM (%)	57,90^a \pm 1,41	58,11 \pm 1,37	60,04^a \pm 2,25
MLD(cm ²)	45,38 \pm 2,31	46,46 \pm 2,51	46,21 \pm 3,39

*ADG = average daily gain (g), BFT = average backfat thickness (mm), LM = lean meat (%), MLD = area of *musculus longissimus lumborum et thoracis* (cm²)

**Significance of the differences: ^a = P \leq 0,05, ^A P = \leq 0,01, ^A = P \leq 0,001

Table4

Effects of *LEPR*, *MC4R* and thiers interaktions on carcass meat quality traits

Meat parameters*	R ²	Model GLM	<i>LEPR</i>	<i>MC4R</i>	Interaction <i>LEPR-MC4R</i>
ADG (g)	0,68	0,001 ⁺⁺	0,087	0,051	0,0701
BFT (mm)	0,79		0,0142⁺	0,002⁺⁺	0,008 ⁺⁺
LM (%)	0,71		0,0527	0,045	0,0945
MLD(cm ²)	0,73		0,712	0,594	0,0924

*ADG = average daily gain (g), BFT = average backfat thickness (mm), LM = lean meat (%), MLD = area of *musculus longissimus lumborum et thoracis* (cm²)

Significance of the effect: ⁻ P \geq 0,05, ⁺ P \leq 0,05, ⁺⁺ P \leq 0,01, ⁺⁺⁺ P \leq 0,001

R²-determination coefficient

Conclusion

The results presented here confirming the existence associations of *LEPR* and *MC4R* genes to backfat thickness (BFT) index. Also this study published polymorphism of these genes and their effect on production traits of pigs (mainly

lean meat - LM and average daily gain - ADG). The results were obtained on small number of animals. Their verification needs further study.

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