VARIABILITY OF LEPR GENE AND HIS ASSOCIATION WITH INDICATORS OF PRODUCTION PORK

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ABSTRACT

The aim of this thesis, which is focused on *Variability of LEPR gene and his association with indicators of production pork* is confirm or confute hypothesis about relation between polymorphisms in *LEPR* gene and indicators of production pork. The *LEPR* gene mediate effects of leptin in organism. Is supposed, that *LEPR* influences traits related to growth and body composition, especially backfat thickness and rate of muscle.

In this thesis polymorfisms in exon 6 and exon 18 on *LEPR* gene were studied in view of quality of pork, in exon 6 by using restriction endonuclease *HpaII*, in exon 18 by application restriction endonuclease *AvaII*. In population of 82 animals which were tested, only genotypes *bb* and *Bb* for exon 6, *DD* and *Dd* for exon 18 were found. Sows are crossbreeds of Large White and Landrace, boars are crossbreeds of Duroc, Pietraine and White paternal pig.

In molecular-genetic laboratory samples were analysed, genotypes of animals were estimated and results were evaluated by association analysis.

It was setting: weight in 28 days (kg), weight of slaughter in warm state (kg), weight of slaughter in cold state (kg), rate of muscle (%), backfat thickness (mm), muscle thickness (mm). These indicators were compared with genotypes. Association analysis didn't prove conclusive influence of *LEPR* gene exon 6 and 18 on indicators of production pork. These results can be cause by insufficiently large population. It would be suitable to do other analysis with bigger population of tested animals.

Key words: pork, genetic polymorphism, *LEPR*, leptin, exon 6, exon 18

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INTRODUCTION

Several factors influence quality of pork. As main indicator of quality of pork can be use marbling score. Fat influences sensorial traits, especially taste, juiciness and edibleness after heat treatment. In animal breeding programs these traits get attention, because of DNA technologies can improve marbling score of pork. By combination of traditional selection method and marker assisted selection we can increase amount of intramuscular fat (IMF) without of his storage on the other place in body (Óvilo C. *et al.* 2002).

Methods of molecular genetics showed, that genes which are related with quality of meat and influence IMF occur on *Sus scrofa* chromosome 6 (Xiaoping L. *et al.* 2010). Testing of DNA make possible calculate estimate of genomic breeding value. Is supposed, that genomic selection will improve genetic progress for selected traits (Ježková A. 2012).

Polymorfismus of *LEPR* gene assigne association with phenotyp diversity in growth, thickness and quality of carcass. *LEPR* gene can be accept as candidate gene and can be potentially use in process of genomic selection (Chen C. *et al.* 2004). Analysis of candidate genes suppose, that polymorphisms in *LEPR* gene could be responsible to effects on quantitative trait loci on chromosome 6. Alelles *B* and *b* are in exon 6, alelles *D* and *d* are in exon 18. (Muñoz G. *et al.* 2009).

The aim of this thesis was confirm or confute hypothesis about relation between polymorphisms in *LEPR* gene and indicators of production pork. In molecular-genetic laboratory analyse samples, estimate genotypes of animals in exon 6 and in exon 18 of *LEPR* gene. Get together information about production traits of tested population of animals. Evaluate results by association analysis.

MATERIAL AND METHODS

Animals

Samples of blood were taken from 82 animals from two farms. Conditions of farming were different. Animals were final hybrids which originate from three or four breeds. 7 boars were crossbreeds of Pietrain × Duroc, Pietrain × White paternal pig or pure-bred Pietrain. And 37 sows were crossbreeds of Large White × Landrace.

Molecular-genetic methods

Isolation of genomic DNA from blood was made by QIAamp DNA Blood Mini Kit from company Qiagen. PCR-RFLP issue from study CHEN C. C., CHANG T., SU H. Y., 2004: *Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits*. Animal biotechnology, (15) 1: 89–102.

Classification of carcass

Classification of carcass was made by method Neddle IS-D-15.

Statistical assessment

For statistical assessment was used mixed linear model MLM, mixed procedure, method of estimate was REML (Restricted Maximum Likelihood), programme SAS. Equation for determination of association among genotypes of *LEPR* gene and indicators of production pork:

$y_{ijklmn} = \mu + gen1_i + gen2_j + sex_k + porvrh_l + odchov_m + denpor_n + e_{ijklmn}$

y_{ijklmn}	= useful trait
μ	= average value of observe trait
$gen1_i$	= influence of genotypes of exon 6, fixed efect, i = Bb, bb
$gen2_j$	= influence of genotypes of exon 18, fixed efect, j = DD, Dd
sex_k	= sex, fixed efect, k = 1, 2
$porvrh_l$	= order of litter, fixed efect, l = 1, 2, 3, 4, 5, 6, 7
$odchov_m$	= location of pigs during feed period, fixed efect, m = 1, 5
$denpor_n$	= day of birth after insemination, fixed efect, n = 1, 2, 3, 4, 5
e_{ijklmn}	= rezidue

RESULTS AND DISCUSSION

Frequency of genotypes and alleles

Tab. 1 presents absolute and relative frequency of genotypes in exon 6. In population of 82 animals, which were tested, only genotypes *bb* and *Bb* for exon 6 were found. Dominant homozygous *BB* were absent absolutly.

Tab. 1: Absolute and relative frequency of genotypes in exon 6.

	bb	*Bb*	*BB*	total
Absolute frequency (pcs)	64	18	0	82
Relative frequency	0.78	0.22	0.00	1.00

Tab. 2 presents absolute and relative frequency of alelles in exon 6. It is evident, that alelle *b* is prevalent in our population.

Tab. 2: Absolute and relative frequency of alelles in exon 6.

	b	*B*	total
Absolute frequency (pcs)	146	18	164
Relative frequency	0.89	0.11	1.00

Tab. 3 presents absolute and relative frequency of genotypes in exon 18, Tab. 4 shows frequency of alelles in exon 18.

Tab.3: Absolute and relative frequency of genotypes in exon 18.

	dd	*Dd*	*DD*	total
Absolute frequency (pcs)	0	17	65	82
Realtive frequency	0.00	0.21	0.79	1.00

Tab.4: Absolute and relative frequency of alelles in exon 18.

	d	*D*	total
Absolute frequency (pcs)	17	147	164
Relative frequency	0.89	0.11	1.00

These results corresponded with results Chen C. *et al.* 2004, when they tested breeds Yorkshire, Landrace, Duroc. All of these breeds showed very similar frequency alelles as well as genotypes in common with population of pigs, which were tested in this thesis. In spite of fact, that Chen C. *et al.* 2004 genotyped population from 97 to 482 individuals, frequency stayed same. Is it possible to suppose, that fact appeearance of *Dd*, *DD* and *bb*, *Bb* genotypes was not caused by small tested population.

Influence of genotype on classification to the class by rate of muscle

Tab. 5 presents absolute and relative distribution of carcass in classification class in relation to genotypes. Because of *LEPR* gene mediates effect of leptin in organism, is suppose his influence on rate of muscle. Rate of muscle is one of criterions for classification of carcass and coin. Rate of muscle is important indicator in economy of farming. Carcass with genotypes *Bb*, *bb* and *DD* were included to class E and U, whereas in class U was included cca half of carcass. While in genotype *Dd* was number in class E and U almost balanced. From these results followed, that animals with genotype *Dd* showed less rate of muscle. But it is complex trait, which is influenced by several factors.

Tab.5: Influence of genotype on classification to the class by rate of muscle.

	S	E	U	R	O	P	total
Bb	0	11	5	1	0	0	17
	0.00%	15.49	7.04%	1.41%	0.00%	0.00%	23.94%
bb	4	32	17	0	1	0	54
	5.63%	45.07%	23.04%	0.00%	1.41%	0.00%	76.06%
DD	2	34	15	1	1	0	53
	2.82%	47.89%	21.13%	1.41%	1.41%	0.00%	74.65%
Dd	2	9	7	0	0	0	18
	2.82%	12.68%	9.86%	0.00%	0.00%	0.00%	23.35%

Influence of genotype on production traits

Results of statistical analysis did not prove significant influence of *LEPR* gene exon 6 and exon 18 on production traits. It is possible observe certain tendency in influence on production traits. In economically important traits, for examle rate of muscle and backfat thickness, exon 6 showed influence.

Tab. 6 presents association polymorphism of *LEPR* gene exon 6 and 18 with production pork. Is it possible to suppose, that not significant results were caused by small tested population. Significant differences were observed in bigger tested population by other authors. Chen C. *et al.* 2004 detected significant difference in polymorphism in exon 18 at Duroc and Yorkshire. In polymorphisms in exon 6 they found significant difference among genotypes *bb* and *Bb* at Landrace. Difference among breeds and among genotypes too were only by tenths of millimetres. Group of tested pigs was choosen randomly, however sample was relatively small. Normal distribution of date could be break.

Tab.6: Influence of genotype on production traits.

	Rate of muscle (%)	Backfat thickness (mm)	Muscle thickness (mm)
	LSM ± SE	LSM ± SE	LSM ± SE
Bb	54.53 ± 0.98	17.89 ± 1.23	62.07 ± 2.16
bb	53.99 ± 0.87	18.54 ± 1.09	60.93 ± 1.87
DD	54.16 ± 0.87	18.14 ± 1.09	60.22 ± 1.82
Dd	54.36 ± 0.98	18.29 ± 1.24	62.79 ± 2.21

For all traits $p > 0.05$ – not significant.

CONCLUSION

Variability of *LEPR* gene and his association with indicators of production pork was not proved. Production traits as rate of muscle and backfat thickness were probably influenced by exon 6. It confer about traits of carcass, which play important role in coin pork. They are basic for economy of farming. Place of farming considerable influenced muscle thickness.

Association analysis did not prove conclusive influence of *LEPR* gene in exon 6 and 18 on indicators of production pork. These results can be cause by insufficiently large population or by irregular distribution of genotypes. It would be suitable to do other analysis of polymorphisms in LEPR gene with bigger population of tested animals.

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