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VARIABILITY IN THE LEPTIN, LEPTIN RECEPTOR AND HEART FATTY ACID BINDING PROTEIN GENES IN RELATIONSHIP WITH MEAT QUALITY TRAITS IN PIGS

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Abstract

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The leptin (*LEP-Hinf*I), leptin receptor (*LEPR-Hpa*II) and heart fatty acid binding protein (*H-FABP-Hinf*I) genes and their genotypes combination (*LEP-Hinf*I **LEPR-Hpa*II) were tested for associations with the pH_1, pH_{24}, myoglobin content (mg/100 g), intramuscular fat content (%) and remission (%). The genotypes were determined in Large White, Landrace and Duroc breeds (n = 106, 56 and 4, respectively). The allele frequencies were: *LEP-Hinf*I: *C* = 0.133 T = 0.867; *LEPR-Hpa*II: *A* = 0.331 *B* = 0.669; *H-FABP-Hinf*I: *H* = 0.745 *h* = 0.255. The populations of breeds were in the genetic equilibrium according to the χ^2 test in the tested loci. The combinations of *LEP-Hinf*I and *LEPR-Hpa*II were significantly associated with the pH_{24} and remission. The *H-FABP-Hinf*I locus was significantly associated with intramuscular fat content.

genetic marker, *LEP*, *LEPR*, *H-FABP*, pig, associations, meat quality

The breeding programs in the pig production brought a real improvement in measurable characteristics in the last decade. It came to fruition mainly in the percentage of lean meat weight and back fat thickness. However, average daily gain and feed conversion were enhanced too. In the relative short time, there were cumulated desirable genes and their forms (alleles) in the contemporary breeds of pigs. On the other hand, several new problems had occurred there, for example the robustness reduction, leg weakness (tePAS and Visscher, 1994) and a porcine stress syndrome, which is considered as a causative factor for the PSE (pale, soft, exudative) meat quality. The causal mutation in the *RYR1 = CRC* (calcium release channel) gene, a key aspect for the stress susceptibility, was revealed by Fujii et al. (1991). The *CRC* genotyping is performed using the molecular genetics methods (PCR-RFLP) for selected animals, and this initiated the implementation of marker assisted selection (MAS) in pig breeding schemes. There are efforts to find the polymorphism of loci with impact on the cell and biochemical composition of tissues and on the variability of DNA candidate genes. The particular loci can be used in MAS after their statistical confirmation for associations with the production traits. Lately, the importance of meat quality in livestock is increased. Heritability coefficients (h^2) for meat quality characters are low with the exception of intramuscular fat content (IMF), for which the middle and high levels in the various populations were found (average $h^2 = 0.49$) (Kuciel and Lahučký, 1996). Later there were described several meat performance characteristics (characteristic for meat taste and technological and kitchen treatment important), their correlations and coefficient of heritability values (Steinhauser et al., 2000). The objective of this study was to determine the associations between genetic variability of *LEP*, *LEPR* and *H-FABP* genes and varia-

tion in meat quality traits (pH_1, pH_{24}, remission, myoglobin, IMF).

MATERIAL AND METHODS

Animals and data

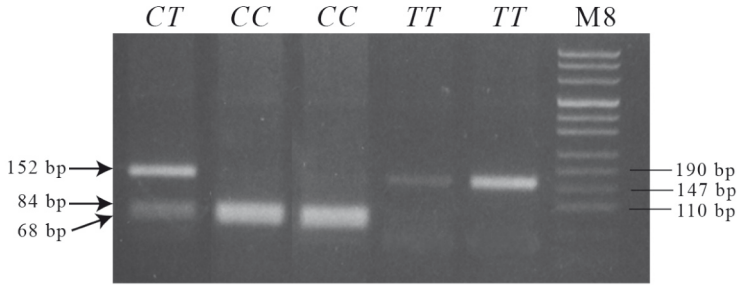
At the station for the meat performance testing control in Grygov were tested the pigs of Large White (LW), Landrace (LA) and Duroc (D) breeds, n = 106, 56 and 4, respectively. Experimental animals were housed in couples, boar and gilt. Sex ratio was 80 boars : 86 gilts. All animals were in intra-purebred breeding test evaluated, according to the CSN 466164, called Control for fattening ability and carcass value (Central herd book of pigs, 1994). Feed consumption data and the live weight of animals in 30–100 kg were recorded. The nutritious values of the mixture GENT were following: nitrogenous components (N): 18%, all digestible components: 74%, the metabolized energy (ME): 12 MJ / 1 kg, fiber: 3.3%, lysine: 1.1–1.3%, methionin + cystein: 0.6%, Ca: 0.85%, P: 0.65%.

Analyzed traits

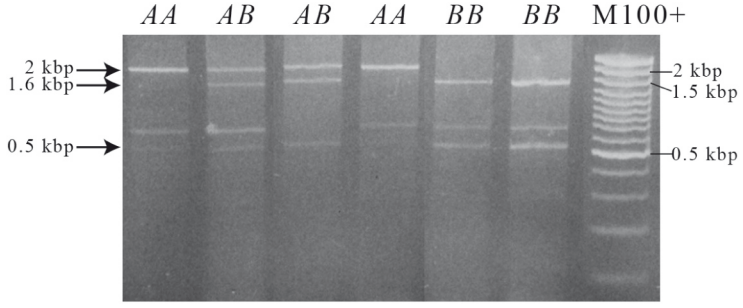
Meat quality characters were evaluated. The values of pH_1 were on the slaughterhouse determined into one hour *post mortem*. The value of the pH_{24}, remission (REM), myoglobin content (MYO) and intramuscular fat content (IMF) were measured in samples of *musculus longissimus lumborum at thoracis* at the Department of Food Technology MZLU in Brno. The percent remission was established on the fresh cut using adsorption spectroscopy. The myoglobin content in mg/100 g of meat was evaluated using Hornsey´s method and intramuscular fat content was established in %, using soxhleth-petrolether extraction.

Genotyping

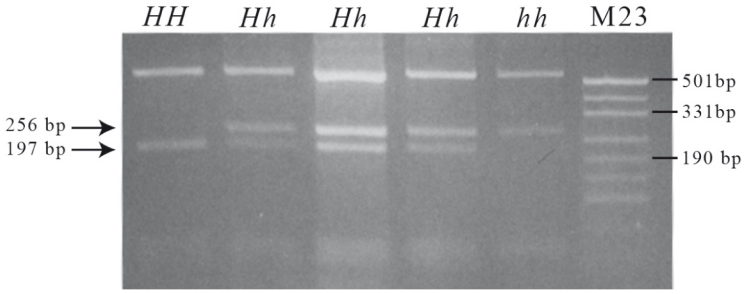
DNA testing was performed in the Molecular Genetics Laboratory at the Department of Genetics MZLU Brno using PCR–RFLP methods. The genotypes of the chosen polymorphic sites of candidate genes were determined according to the authors (Tab. I). DNA for analysis was isolated from the EDTA-treated blood, using the proteinase method according to Nebola et al. (1994) with modifications. The DNA quality was checked on the 1.2% agarose gel, stained by ethidium-bromide, before genotyping. The PCR was performed in the thermocycler with the heated lid (PTC-100 MJ Research). The RFLP products of *LEP* (N = 166), *LEPR* (N = 166) and *H-FABP* (N = 96) polymorphisms were established on the agarose gel using the UV lamp, Fig. 1, 2 and 3, respectively.



1: *Agarose gel electrophoresis (2.5%) showing genotypes at HinfI loci of LEP gene (see the Table Ib).*



2: *Agarose gel electrophoresis (2%) showing genotypes at HpaII loci of LEPR gene (see the Table Ib).*



3: *Agarose gel electrophoresis (3%) showing genotypes at HinfI loci of H-FABP gene (see the Table Ib).*

I: *DNA test conditions*
Ia: *PCR conditions*
Final components' concentrations in reactive mixture („master mix")

	LEP	***LEPR***	***H-FABP***
Taq pol. buffer (10x)	1.05 x	1.04 x	1 x
Mg^{2+} (25mM)	1.26 mM	1.33 mM	1.28 mM
dNTP (10 mM)	210 µM	208µM	200 µM
Primer 1 (5pmol/µl)	0.37 µM	0.5 µM	0.5µM
	TGCAGTCTGTCTCCTCCAAA	GGAAGGCATTTGTTTCAGCAGTTA	GGACCCAAGATGCCTACGCCG
Primer 2 (5pmol/µl)	0.37 µM	0.5 µM	0.5µM
	CGATAATTGGATCACATTTCTG	CAAGTCCTCTTTCATCCAGCACTG	CTGCATCTTTGACCAAGAGG
Taq pol. (5U/µl)	3.07U / 100µl	3.07U / 100µl	4.52U / 100µl

Temperature conditions:

Loci	**Denaturation**	**Annealing**	**Extension**	**PCR fragment length**	**Authors**
LEP	95 °C / 40 sec	55 °C / 60 sec	72 °C / 60 sec	152 bp	Stratil et al. (1997)
LEPR	94 °C / 60 sec	55 °C / 60 sec	72 °C / 120 sec	2000 bp	Stratil et al. (1998)
H-FABP	95 °C / 60 sec	57 °C / 60 sec	72 °C / 120 sec	700 bp	Gerbens et al. (1997)

Ib: *RFLP conditions*

Loci	**Restriction endonuclease**	**RFLP products in bp = final genotypes**		
LEP	*Hinf*I	152 = *TT*	152 + 84 + 68 = *CT*	84 + 68 = *CC*
LEPR	*Hpa*II	2000 = *AA*	2000 + 1450 + 550 = *AB*	1450 + 550 = *BB*
H-FABP	*Hinf*I	197 + 59 = *HH*	256 + 197 + 59 = *Hh*	256 = *hh*

Statistical analyses

Least square means (LSM) were evaluated for all animals with the four models of GLM procedures using program SAS 8.2. The meat quality traits were defined as dependent variables. The genotypes of evaluation candidate genes (*LEP*, *LEPR*, *H-FABP*) and their interaction (*LEP* x *LEPR*), sex, breed and period of time (months of slaughter), were defined as fixed factors. The three models (I, II and III) contained only one locus as a fixed factor. The model IV included the *H-FABP* genotype and the combination of *LEP* x *LEPR* genotypes, because we supposed that both loci influence fatness and are in the functionally dependence.

Model I, II and III: $y_{ijklm} = \mu + L_i + S_j + B_k + M_l + e_{ijklm}$

Model IV: $y_{ijklmn} = \mu + H\text{-}FABP_i + LEP*LEPR_m + S_j + B_k + M_l + e_{ijklmn}$

where:

$y_{ijklm(n)}$ - observed values;
μ - general mean;
L_i - effect of the *LEP* (i = 1, 2, 3), *LEPR* (i = 1, 2, 3), or *H-FABP* (i = 1, 2, 3) genotypes, in model I, II or III, respectively;
S_j - effect of the sex (j = 1, 2);
B_k - effect of the breed (k = 1, 2, 3);
M_l - months of slaughter (l = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12);
$LEP*LEPR_m$ - interaction between *LEP* and *LEPR* genotypes and
$e_{ijklm(n)}$ - residual error.

RESULTS AND DISCUSSION

The genotypes and allele frequencies in the analyzed population of chosen polymorphisms and the value of χ^2 test are in the Tab. II. All tested genes and genotypes were in the Hardy-Weinberg equilibrium.

The *LEP-Hinf*I polymorphic site showed high frequency of *T* allele (0.86) and the *CC* genotypes were at 2.41% animals of all. The *T* allele frequency was 0.835, 0.946 and 0.625 at Large White, Landrace and Duroc breeds, respectively. Stratil et al. (1997) described analogous allelic frequencies in Large White, Landrace and Hampshire breeds. In this study the animals of Meishan breed were monoallelic in the *LEP-Hinf*I polymorphic site, on the other hand. Křenková et al. (1999) established even the higher *T* allele frequencies, 0.91 and no *CC* genotype, in the population of hybrid pigs (Large White, Landrace, Pietrain) in the Czech Republic.

In *Hpa*II polymorphic site of *LEPR* the *A* allele had frequency 0.331 and approximately the same part were heterozygous *AB* and homozygous *BB*. The allele *A* frequency was 0.340, 0.321 and 0.250 at Large White, Landrace and Duroc breeds, respectively. Estany et al. (2002) established the allele frequency *A* = 0.234 in the two stress negative lines of the Landrace breed. Other frequencies were determined by Hardge et al. (2000), the set of 383 animals F2 (Berlin Miniature Pig x Duroc) showed the frequency of allele *A* = 0.62.

The genotyping in the *H-FABP* locus was performed only at 96 animals, which had determined IMF content. The frequencies of allele *H* = 0.75 and *h* = 0.25 were determined. The allele *H* frequency was 0.677 at Large White and 0.868 at Landrace, no animal of Duroc were tested for the *H-FABP* locus. Huang et al. (2001) described the frequencies allele *Hinf*I at ten breeds of pigs. The animals of Duroc, Landrace and Great Yorkshire breeds were not far in these allele frequencies from animals in our study, whereas breeds with origin in Asia, Meishan and Erhulian, showed a higher frequencies of h allele. Gerbens et al. (1997) published the following allele frequencies *H* = 0.70; 0.70; 0.97; 0.33; 0.45; 0.70; 0.90 at the Dutch Landrace, Duroc, Great Yorkshire, Hampshire, Meishan, Pietrain and Wild Pig, respectively.

II: *The genotype and allele frequencies*
II a: *The genotype and allele frequencies in all animals*

Gene		Frequencies of genotypes			Frequencies of alleles		χ^2 value
LEP		*CC*	*CT*	*TT*	*C* = 0.133 ± 0.019	*T* = 0.867 ± 0.019	0.535^{NS}
	N	4	36	126			
	rel.	0.024	0.217	0.759			
LEPR		*AA*	*AB*	*BB*	*A* = 0.331 ± 0.026	*B* = 0.669 ± 0.026	0.388 ^{NS}
	N	20	70	76			
	rel.	0.120	0.422	0.458			
H-FABP		*HH*	*Hh*	*hh*	*H* = 0.745 ± 0.032	*h* = 0.255 ± 0.032	0.018 ^{NS}
	N	53	37	6			
	rel.	0.552	0.385	0.063			

Note: NS – non significant difference

II b: *The genotype and allele frequencies according to breeds*

	Gene	*LEP*			*LEPR*			*H-FABP*		
Breeds	**Genotypes**	***CC***	***CT***	***TT***	***AA***	***AB***	***BB***	***HH***	***Hh***	***hh***
LW	freq.	0.038	0.255	0.707	0.142	0.396	0.462	0.419	0.616	0.065
	allele freq.		$C = 0.165 \pm 0.025$			$A = 0.340 \pm 0.033$			$H = 0.677 \pm 0.042$	
			$T = 0.835 \pm 0.025$			$B = 0.660 \pm 0.033$			$h = 0.323 \pm 0.042$	
LA	freq.	0	0.107	0.823	0.071	0.500	0.429	0.794	0.147	0.059
	allele freq.		$C = 0.054 \pm 0.021$			$A = 0.321 \pm 0.044$			$H = 0.868 \pm 0.041$	
			$T = 0.946 \pm 0.021$			$B = 0.679 \pm 0.044$			$h = 0.132 \pm 0.041$	
D	freq.	0	0.75	0.25	0.25	0	0.75	no date		
	allele freq.		$C = 0.375 \pm 0.171$			$A = 0.250 \pm 0.153$				
			$T = 0.625 \pm 0.171$			$B = 0.750 \pm 0.153$				

Note: χ^2 test for all loci was in all breeds non-significant

The frequency of *LEP* x *LEPR* genotypes combination is described in the Tab. III. In relation to combination single genotype of the leptin and leptin receptor genes, *LEP* x *LEPR*, at the individual, in our set was no combination *CC*/*AA* and low occurrences (1.2%) were at the other combinations with the genotype *CC* in the *LEP* locus, *CC*/*AB* and *CC*/*BB*. The combinations *TT*/*AB* and *TT*/*BB* were in the highest frequencies, practically about 33% (Tab. III). These results could not be confronted with other authors.

III: *The frequencies of LEP x LEPR genotypes combination*

Loci	**Combination of genotypes**								
LEP	*CC*	*CC*	*CC*	*CT*	*CT*	*CT*	*TT*	*TT*	*TT*
LEPR	*AA*	*AB*	*BB*	*AA*	*AB*	*BB*	*AA*	*AB*	*BB*
%	0	1.2	1.2	3.01	7.23	12.65	8.43	33.73	32.53

No effect of the tested loci *LEP* and *LEPR* was confirmed with models I-II. The significant difference was determined only for IMF in pigs between *H-FABP* homozygous genotypes (model III). The *HH* animals had significantly higher ($P < 0.001$) content of IMF (1.518 ± 0.204) than homozygous pigs *hh* (1.013 ± 0.294), however the *hh* genotype was only in six animals.

The GLM results for model IV, with the *H-FABP* genotypes and *LEP*LEPR* genotypes combinations as fixed factors, are present in the Tab. IV and V. Determinant coefficients for the traits variability explanation were at level from 0.226 (IMF) to 0.666 (pH_{24}). The *H-FABP* association with IMF and significant differences in this trait between homozygote *HH* and *hh* were not confirmed in the model IV. However, the results of the model III are in accordance with the results from Gerbens et al. (1998), who established the associations for three polymorphic loci in *H-FABP* gene at Duroc breed and the highest IMF content in animals with *HH* genotypes.

IV: *The association of H-FABP genotypes with production traits (LSM ± SE) from model IV*

Traits	Genotypes		
	HH	***Hh***	***hh***
pH_1	6.01 ± 0.12	5.97 ± 0.12	5.87 ± 0.16
pH_{24}	5.33 ± 0.04	5.31 ± 0.06	5.24 ± 0.05
MYO	75.69 ± 6.89	81.43 ± 6.84	74.69 ± 8.88
REM	22.78 ± 1.74	21.453 ± 1.73	21.27 ± 2.24
IMF	1.50 ± 0.24	1.38 ± 0.24	1.08 ± 0.32

Note: pH_1= pH value measured one hour *post mortem*, pH_{24} = pH value measured 24 hours *post mortem*, MYO = myoglobin content (mg/100 g), IMF = intramuscular fat content (%), REM = remission (%).

The associations of our study did not approve the results that were described by Emnett et al. (2001) for set of stress negative pigs of Berkshire, Duroc, Hampshire and Landrace breeds. Nevertheless, their study was performed for the assessment *Hae*III locus of *H-FABP* on meat quality traits, e.g. pH_{24}, quality index, percentage of IMF and color of meat. *Hae*III locus was associated with pH_{24} and IMF, and near of significance (0.09) was color of meat. Gerbens et al. (2000) realized study to confirm the influence *H-FABP* polymorphisms on IMF and back fat thickness, the F2 generation from Meishan and Western Pigs were used. The *H-FABP* localization in the QTL for IMF was confirmed; therefore the genetic variability in that region may be the causation for phenotypic variability in IMF. In any case, Gerbens et al. (2000) established large but not statistically significant influence of the microsatellite sequences variability within the *H-FABP* loci on IMF.

The significant influence of *LEP* x *LEPR* genotypes combination on pH_{24} variability was confirmed with the model IV. The *CT*/*AA* x *TT*/*AA* and *TT*/*BB* x *TT*/*AB* combinations were significantly different and the *CT*/*BB* x *TT*/*AA* and *TT*/*AA* x *TT*/*AB* were highly significantly different (Tab V). However, the model IV did not confirm the influences of chosen candidate genes on IMF. Hardge et al. (2000) established also no *LEP-Hinf*I and *LEPR-Msp*I polymorphic sites associations on marbling in the loin.

V: *Association the combination genotypes LEP x LEPR with production traits (LSM ± SE) from model IV*

Combinations of genotypes	Traits				
	pH_1	**pH_{24}**	**MYO**	**REM**	**IMF**
CC/AB	6.10 ± 0.23	no date	no date	21.07 ± 3.49	1.22 ± 0.49
CC/BB	5.90 ± 0.22	5.29 ± 0.11	no date	22.53 ± 3.22	1.23 ± 0.45
CT/AA	no date	5.35^{a} ± 0.06	73.05± 11.71	18.0^{a} ± 2.96	1.52 ± 0.42
CT/AB	5.95 ± 0.16	5.26 ± 0.06	81.26 ± 9.54	22.97 ± 2.41	1.21 ± 0.32
CT/BB	5.93 ± 0.14	5.38^{A} ± 0.05	71.58 ± 7.94	22.31 ± 2.01	1.26 ± 0.28
TT/AA	5.91 ± 0.16	5.10^{ABa} ± 0.10	77.12 ± 9.26	22.18 ± 2.34	1.45 ± 0.33
TT/AB	5.96 ± 0.11	5.38^{Bb} ± 0.03	77.18 ± 6.22	22.14 ± 1.57	1.31 ± 0.22
TT/BB	5.96 ± 0.11	5.28^{b} ± 0.04	77.69 ± 6.50	23.44^{a} ± 1.64	1.36 ± 0.23

Note: Values with the same exponents show significance level within columns: $^{A, B}$ = P ≤ 0.01; $^{a, b}$ = P ≤ 0.05; pH_1= pH value measured one hour *post mortem*, pH_{24} = pH value measured 24 hours *post mortem*, MYO = myoglobin content (mg/100 g), IMF = intramuscular fat content (%), REM = remission (%).

SUMMARY

The estimation of the associations between three polymorphic candidate genes and meat quality traits was the aim of this study. The genotypes of leptin (*LEP-Hinf*I), leptin receptor (*LEPR – Hpa*II) and heart-fatty acid binding protein (*H-FABP-Hinf*I) genes were determined for the pigs of Large White, Landrace and Duroc breeds. The PCR- RFLP methods were used for genotyping. The animals in the testing station for fattening capacity and carcass value were raised. The meat quality was determined with the follows parameters: pH_1, pH_{24}, myoglobin content, intramuscular fat content and remission. Allele frequencies of the three polymorphic sites were determined for each breed and for whole set. The influence on the meat quality traits was evaluated in this study for combination of *LEP-Hinf*I and *LEPR-Hpa*II genotypes. This *LEP*LEPR* genotypes combination was associated with pH_{24} on the high significant level, and significant association was estimated for remission. The significant effect of the tested loci was not estimated for the chosen meat quality traits, with exception the *H-FABP-Hinf*I associations on intramuscular fat content.

SOUHRN

Variabilita v genech pro leptin, leptinový receptor a protein vázající mastné kyseliny ve vztahu ke kvalitě masa u prasat

Cílem práce bylo zjistit vztah tří kandidátních genů, respektive vybraných polymorfních míst genů leptinu (*LEP-Hinf*I), receptoru leptinu (*LEPR-Hpa*II), genu pro protein vázající mastné kyseliny (*H-FABP--Hinf*I) a charakteristik důležitých pro kvalitu masa. V analýze byl použit soubor prasat plemen bílé ušlechtilé, landrace a durok pocházející ze stanice pro kontrolu výkrmnosti a jatečné hodnoty. Parametry kvality svaloviny, zjištěné na jatkách a v laboratoři Ústavu technologie potravin MZLU v Brně, byly: pH_1, pH_{24}, obsah myoglobinu, procento remise a obsah vnitrosvalového tuku. Z krve testovaných prasat byly genotypy stanoveny metodou PCR-RFLP. Frekvence alel jednotlivých polymorfismů byly stanoveny u celého souboru a také podle jednotlivých plemen. Dle χ^2 testu byly genotypy ve sledovaných lokusech v genetické rovnováze. Vyhodnocována byla také kombinace genotypů *LEPR* x *LEP*, neboť se stále zkoumá vliv polymorfních míst těchto genů nejen na ukazatele tučnosti a je předpoklad také funkční závislosti u různých variant těchto genů. Výsledkem je potvrzení statisticky vysoce průkazného vlivu kombinace genů *LEPR* x *LEP* na hodnotu pH_{24} a statisticky průkazného vlivu na remisi. Průkazný vliv jednotlivých lokusů na vybrané ukazatele kvality svaloviny nebyl zjištěn, s výjimkou vlivu *H-FABP–Hinf*I na vnitrosvalový tuk.

genetický marker, *LEP*, *LEPR*, *H-FABP*, prase, asociace, kvalita masa

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