GENETIC MARKERS *MYF4* AND *FSHB* IN RELATION TO PERFORMANCE OF BOARS

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ABSTRACT

The aim of this research was to study of variability of two genes *MYF4* and *FSHB* in the population of Czech Large White boars and analyze theirs associations with production traits (backfat thickness, average daily gain, average daily gain test and lean meat). We studied 170 boars of Czech Large White boars from one herd. The genomic DNA was isolated from blood samples. The genotypes were determined by PCR-RFLP. Genotype deviation from the Hardy–Weinberg equilibrium was evaluated by chi-square test. Association analysis of tested genotype was performed by general linear model with fixed effects of genes. In this studied the significant associations of *MYF4* gene with lean meat and *FSHB* gene with all traits were observed. The associations of interaction of genes with all studied traits were significant.

Key words: pig, *MYF4*, *FSHB*, production traits, Czech Large White

INTRODUCTION

Pig production profitability is strongly influenced by the achieved level of reproduction and production traits. The objective of this research was to determine the influence of myogenin gene *MYF4* and follicle-stimulating hormone gene *FSHB* gene on the productive trait in Czech Large White – CLW herd. In this study we evaluated the significant effects of two candidate genes on production traits with great economic impact on the pig industry.

Te Pas and Visscher (1994) concluded that the myogenin gene plays an important role during the terminal transformation of myoblasts into myofibres. The study of Te Pass et al. (1999) suggested that the myogenin gene has an impact on birth weight, growth rate and lean yield but no impact on backfat thickness. The follicle-stimulating hormone gene are considered to be candidate genes for reproduction. *FSHB* genes code the β subunit that is specific for all animals (Humpoliček et al., 2009).

In this study, the following traits were analysed: backfat thickness BFT (cm), average daily gain ADG (g), average daily gain test (g) and lean meat LM (%). The results of statistical evaluation are shown in Table 6 and represent the analysis of possible relations between the genotypes of *MYF4* and *FSHB* locus and studied traits in Czech Large White.

MATERIAL AND METHODS

We studied 170 boars of Czech Large White (CLW) boars from one herd. The genomic DNA was isolated from blood samples by using QIAamp®DNA Blood Mini Kit (QIAGEN GMBH).

Gen myogenin (PCR/RFLP *Msp*I)

Genotype of the myogenin gene was established by Te Pas et al. (1996). It is a polymorphism at the 3' side. Allele *A* is cleaved with restriction endonuclease *Msp*I, PCR-RFLP for the product is made up of fragments of alleles of a length of 219-bp and 134-bp. Allele *B* is not cleaved with the length fragment of 353 bp. The mixture of 50 µl containing 150 ng genomic DNA, 1x PCR buffer, 200 µM of each dNTP, 4 pmol of each primer, 1.5 mM Mg 2 +, 0.3 U Taq polymerase. PCR reaction carried out under conditions: an initial denaturation of 95°C / 4 min, 30 cycles at temperatures of 95°C/60 s, 60 °C/60 s, 72°C/60 s, final elongation of 72°C / 5 min.

Follicle-stimulating hormone gene (PCR / RFLP *Hae*III)

Genotype in the gene follicle-stimulating hormone (*FSHB*) was determined by Rohrer et al. (1994). Using the restriction endonucleases *Hae*III two alleles were detected. Allele fragment comprising a length of 332 bp and allele B split into two fragments with a length of 173 and 159 bp. The reaction mixture contained 150 mg of genomic DNA, 1x PCR buffer, 200 µM of each dNTP, 0.5 µM of each primer, 1.5 mM Mg 2 +, 1.3 U Taq polymerase. PCR reaction carried out in next

the conditions: initial denaturation of 95°C / 3 min; 30 cycles at temperatures of 95 °C/40 s, 58 °C/60 s, 72° C/90 s, final elongation of 72°C / 5 min.

Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (Hartl and Clark, 1997). Tested genotypes were used for association analysis performed by general linear model (PROC GLM) with fixed effects in SAS for Windows 9.1.4 (SAS Institute, Cary, NC, USA) using the equation:

$$y_{ijkl} = \mu + MYF4_i + FSHB_j + MYF4 \times FSHB_k + e_{ijkl}$$

Where: y_{ijkl} = the phenotypic value of the analysed trait, μ = the population mean, $MYF4_i$ = the fixed effect of the i^{th} genotype of *MYF4* gene (i = *AA*, *AB* and *BB*), $FSHB_j$ = the fixed effect of the j^{th} genotype of *FSHB* gene (j = *AA*, *AB* and *BB*), $MYF4 \times FSHB_k$ = effect of k^{th} interaction of *MYF4* and *FSHB* genes, e_{ijkl} = the random error effect of each observation.

RESULTS AND DISCUSSION

In the studied populations of pigs the frequencies of genotypes and alleles *MYF4* in chosen loci were evaluated. In the group of CLW pigs the frequency of $MYF4^A$ allele was 0.59 and of $MYF4^B$ allele 0.43. Čechová and Mikule (2004) reported similar frequencies of $MYF4^A$ and $MYF4^B$ allele in the Czech Large White (0.66 and 0.34, respectively). Three genotypes $MYF4^{AA}$ 0.4059, $MYF4^{AB}$ 0.3824 and MYF4^{BB} 0.2118 with frequencies were detected (Table 1).

Table 1. Relative frequencies (R) of genotypes and alleles at the myogenin gene MYF4 and follicle stimulating hormone gene FSHB loci. χ^2 – test of Hardy-Weinberg genetics equilibrium.

n	Genotype	R	Chi-square test	Allele	R
69	$MYF4^{AA}$	0.4059	7.17 *	$MYF4^A$	0.59
65	$MYF4^{AB}$	0.3824		$MYF4^B$	0.43
36	$MYF4^{BB}$	0.2118			
24	$FSHB^{AA}$	0.1412	20.15**	$FSHB^A$	0.27
44	$FSHB^{AB}$	0.2588		$FSHB^B$	0.72
102	$FSHB^{BB}$	0.6000			

Significant differences: * P < 0.05; ** P < 0.01

By evaluation of *FSHB* gene allele frequency we found out the frequency of allele $FSHB^A$ 0.27 and allele $FSHB^B$ 0.72. It corresponds to a very high frequency of $FSHB^B$ allele compared to $FSHB^A$ allele. The genotypes frequencies were $FSHB^{AA}$ 0.1412, $FSHB^{AB}$ 0.2588 and $FSHB^{BB}$ 0.6000.

The frequency of the allele $FSHB^B$ gene was found to be higher than the frequency of allele $FSHB^A$. There were more boars with $FSHB^{BB}$ genotypes than $FSHB^{AB}$ heterozygotes in the population of CLW. The frequency of genotypes $FSHB^{AA}$ was clearly the lowest.

The chi-square χ^2 tests show that the population was not in the Hardy-Weinberg Equilibrium for both studied loci.

The observed frequencies of genotypes of both studied genes are shown in Table 2. In the group of CLW pigs with higher frequencies of combinations of genotypes $MYF4^{AA}/FSHB^{BB}$ and $MYF4^{AB}/FSHB^{BB}$ and lower frequencies of combinations of genotypes $MYF4^{AA}/FSHB^{AA}$ and $MYF4^{AB}/FSHB^{AA}$ were observed.

Table 2. Frequency, row percent and column percent of combinations of genotypes of MYF4 by FSHB loci

	$FSHB^{AA}$	$FSHB^{AB}$	$FSHB^{BB}$	
$MYF4^{AA}$	7 10.14 29.17	23 33.33 52.57	39 56.52 38.24	69
$MYF4^{AB}$	4 6.15 16.67	12 18.46 27.27	49 75.38 48.4	65
$MYF4^{BB}$	13 36.11 54.17	19 25.00 20.45	14 38.89 13.73	46
	24	44	102	170 100.00

Table 3. Basic statistical characteristics of particular traits in population

Trait	n	Mean	Vx	Sx	Xmin	Xmax
BF (mm)	170	0.87	0.02	0.15	0.50	1.35
ADG (%)	170	633.52	2776.67	52.69	457.00	752.00
ADGT (%)	39	1019.69	18843.96	137.27	585.00	1206.00
LM (%)	170	61.01	2.55	1.59	57.10	65.50

BF – backfat thickness (cm), ADG – average daily gain (g), ADGT (g) – average daily gain on test, LM – lean meat content (%). V_x – Variance, S_x – Standard deviation, X_{min} – minimum, X_{max} – maximum

Table 4 shows the description statistics for genotypes of *MYF4* gene with the following observations: the mean of backfat thickness was calculated at 0.88 cm in $MYF4^{AA}$, that trait is lower in genotype $MYF4^{BB}$. The ADG was higher in homozygotes individuals. The mean lean meat is 60.90 % of $MYF4^{AA}$, and is approximately similar to the other genotypes.

Table 4. Basic statistical characteristics of particular traits according to determined genotypes of MYF4 in CLW pigs

Trait	Genotype	n	Mean	V_x	S_x	X_{min}	X_{max}
BF (mm)	AA	69	0.88	0.02	0.14	0.57	1.16
	AB	65	0.88	0.02	0.16	0.50	1.35
	BB	36	0.83	0.01	0.14	0.50	1.08
ADG (g)	AA	69	633.62	3656.47	60.46	457.00	752.00
	AB	65	630.76	2471.43	49.71	532.00	726.00
	BB	36	638.33	1746.69	41.79	569.00	747.00
ADGT(g)	AA	10	928.10	29130.99	170.67	585.00	1190.00
	AB	5	1052.20	31485.20	177.44	842.00	1196.00
	BB	24	1051.08	9353.30	96.71	814.00	1206.00
LM (%)	AA	69	60.90	2.35	1.53	58.30	64.20
	AB	65	60.87	2.74	1.65	57.10	65.10
	BB	36	61.46	2.45	1.56	58.70	65.50

BF – backfat thickness (cm), ADG – average daily gain (g), ADGT (g) – average daily gain on test, LM – lean meat content (%). V_x – Variance, S_x – Standard deviation, X_{min} – minimum, X_{max} – maximum

The evaluation of the studied carcass value traits in detected genotypes of *FSHB* gene in Czech Large White pigs indicated small differences in favour of homozygotes $FSHB^{AA}$ and $FSHB^{BB}$ in comparison with heterozygotes $FSHB^{AB}$.

Table 5. Basic statistical characteristics of particular traits according to determined genotypes of FSHB in CLW pigs

Trait	Genotype	n	Mean	V_x	S_x	X_{min}	X_{max}
BF (mm)	AA	24	0.81	0.02	0.14	0.57	1.14
	AB	44	0.85	0.02	0.16	0.50	1.27
	BB	102	0.89	0.02	0.14	0.50	1.35
ADG (g)	AA	24	652.62	1576.77	39.70	578.00	726.00
	AB	44	642.77	3805.53	61.68	457.00	752.00
	BB	102	625.04	2470.52	49.70	486.00	747.00
ADGT(g)	AA	15	1089.93	7341.78	85.68	956.00	1206.00
	AB	15	999.73	22391.35	149.63	585.00	1196.00
	BB	9	935.88	19577.11	139.91	714.00	1196.00
LM (%)	AA	24	61.60	2.40	1.55	58.80	64.50
	AB	44	61.21	2.65	1.62	57.50	65.50
	BB	102	60.79	2.44	1.56	57.10	65.10

BF – backfat thickness (cm), ADG – average daily gain (g), ADGT (g) – average daily gain on test, LM – lean meat content (%).

V_x – Variance, S_x – Standard deviation, X_{min} – minimum, X_{max} – maximum

R-square value of linear models, used for association analysis, ranged from 0.11 (lean meat), 0.07 (average daily gain), and 0.09 (backfat thickness) to 0.48 (average daily gain in test).

In table 6 are described the results of association analysis (least square means, standard errors and statistical significance among genotypes of studied loci).

Te Pas et al. (1999) noted that in the population of Large White pigs the $MYF4^{BB}$ genotype was associated with increased birth weight, higher growth rate and lean meat elevated content. According to Horák et al. (2004) the polymorphism at the ´3 side of the *MYF4* gene impacts on carcass traits and lean meat content in pigs. In our study the significant associations ($P \leq 0.05$) between $MYF4^{AB}$ and $MYF4^{BB}$ in lean meat were observed. The backfat thickness of animals with genotype $MYF4^{AB}$ was greater than that of the animals with genotype $MYF4^{BB}$ and $MYF4^{AA}$.

The significant effect of *FSHB*^{AB} and *FSHB* ^{BB} genotypes in backfat thickness and lean meat considered. The values of ADG were significantly different between genotypes *FSHB*^{AA} and *FSHB*^{BB}, wherein the homozygous genotype *FSHB*^{AA} showed highly significant greater value of ADG. Interesting is differences in values of ADG between the homozygous and heterozygous genotype of *MYF4* gene because even with the results of the large standard errors can not be defined as statistically significant. The homozygous and heterozygous genotypes of *FSHB* gene were shown high significant differences ($P \leq 0.01$) for average daily gain test. The ADGT of animals with genotype *FSHB*^{AA} was greater than that of animals with genotype *FSHB*^{AB} and *FSHB*^{BB}.

The high significant effect ($P \leq 0.01$) on backfat thickness, average daily gain test and lean meat between following genotypes *MYF4*^{AA}/*FSHB*^{AB}, *MYF4*^{AA}/*FSHB*^{BB} and *MYF4*^{BB}/*FSHB*^{AB} were observed. In our results high significant differences were identified in genotype *MYF4*^{BB}/*FSHB*^{AA} in ADGT and LM. The series of the significant differences ($P \leq 0.05$) in backfat thickness, average daily gain, average daily gain test and lean meat are identified in this research.

The interaction genotype *MYF4*^{AB}/*FSHB*^{AB}, not shown statistically significant differences on BF and ADG. The genotype combination *MYF4*^{AB}/*FSHB*^{BB} has no significant effect on ADGT. We identified the significant effects of genotypes *MYF4*^{AA}/*FSHB*^{AA}, *MYF4*^{AA}/*FSHB*^{BB}, *MYF4*^{AB}/*FSHB*^{AA} and *MYF4*^{AB}/FSHB^{BB} on ADG. The genotypes combination *MYF4*^{BB}/*FSHB*^{BB} affected only the LM ($P \leq 0.01$).

Table 6. Interaction of genes MYF4 and FSHB (Least-Squares Means and Standard Errors, LSM±SE)

Genotype	BF	ADG	ADGT	LM
$MYF4^{AA}$	0.85±0.02	643.48±7.98	983.85± 47.46	61.25±0.23
$MYF4^{AB}$	0.89±0.02	651.12±10.31	1022.66±52.36	60.70±0.30^{a}
$MYF4^{BB}$	0.81±0.02	638.67±8.83	1036.36±24.17	61.59±0.26^{a}
$FSHB^{AA}$	0.83±0.03	662.83±11.8^{A}	1143.86±46.59^{A,B}	61.36±0.35
$FSHB^{AB}$	0.83±0.02^{a}	642.28±8.45	964.09±41.98^{A}	61.44±0.25^{a}
$FSHB^{BB}$	0.89±0.01^{a}	628.17±5.94^{A}	934.93±40.56^{B}	60.74±0.17^{a}
$MYF4^{AA}/FSHB^{AA}$	0.76±0.05^{a,b,c,d}	664.42±19.66^{a}	1190.00±111.09^{a,b,c}	62.24±0.58^{a,b,c,d,e}
$MYF4^{AA}/FSHB^{AB}$	0.89±0.03^{a,A}	644.13± 10.84	913.57±41.98^{a,A,B,C}	60.78±0.32^{a,A}
$MYF4^{AA}/FSHB^{BB}$	0.90±0.02^{b,e,B}	621.89±8.33^{a,b}	848.00±78.55^{b,d,e,D}	60.73±0.24^{b,f,B}
$MYF4^{AB}/FSHB^{AA}$	0.92±0.07^{f}	689.00±26.01^{b,c}	1174.00±78.55^{A,d,f,g}	60.15±0.77^{c,g}
$MYF4^{AB}/FSHB^{AB}$	0.86±0.04	640.83±15.01	875.00±111.09^{f}	61.08±0.44^{h}
$MYF4^{AB}/FSHB^{BB}$	0.88±0.02^{c,g}	623.55±7.43^{c}	1019.00±78.55	60.88±0.22^{d,i}
$MYF4^{BB}/FSHB^{AA}$	0.81± 0.04^{e,f}	635.07±14.42	1067.58±32.06^{B,e,h}	61.70±0.42^{f,C}
$MYF4^{BB}/FSHB^{AB}$	0.74±0.04^{A,B,g}	641.88±17.34	1103.71±41.98^{b,C,D,i}	62.46±0.51^{A,B,g,h,i}
$MYF4^{BB}/FSHB^{BB}$	0.90±0.03^{d}	639.07±13.90	937.80±49.68^{c,g,h,i}	60.61±0.41^{e,C}

BF – backfat thickness (cm), ADG – average daily gain (g), ADGT – average daily gain on test, LM – lean meat (%).

Values with the same superscripts in columns show significant differences: $^{A, B, C, D}$ ($P \leq 0.01$), and $^{a, b, c, d, e, f, g, h, i}$ ($P \leq 0.05$)

An important indicator of the pig's body condition is measuring backfat thickness and has become since this has a direct relationship with its body fat content. A decrease in the backfat thickness is directly related to the inter and intramuscular fat content. It corresponds to the lowest backfat thickness in pigs with *MYF4*^{BB} genotype. Kahánková and Dvořák (1998) analysed backfat thickness and lean meat percentage in sows of Czech Large White and Landrace breeds according to genotypes of *MYF4* gene and she noted that pigs with *MYF4*^{AA} genotype had higher lean meat percentage and lower backfat thickness in comparison with animals with *MYF4*^{AB} genotype

In pig selection and breeding, the application of *FSHB* gene may be recommended only if significant effect is confirmed in a given specific population (Humpoličeк et al., 2009). The significant effect of *FSHB*^{AB} and *FSHB*^{BB} genotypes in backfat thickness and lean meat considered.

In their study Wyszyńska - Koko et al. (2006) presented serious statistical differences ($P \leq 0.01$) in the average daily weight gain among the genotypes *MYF4*^{AA} and *MYF4*^{BB}, and *MYF4*^{AB}. In this work the individuals with genotype *MYF4*^{AA} showed a higher average daily weight gain than those with genotype *MYF4*^{BB}. At the same time, the individuals with genotype *MYF4*^{AB} showed higher average daily weight gain than those with genotype *MYF4*^{BB}.

The lean meat content of the carcass, significantly affects the economic efficiency of pig production (Vališ et al. 2009). The animals with the highest lean meat deposition rates that accomplish their potential growth rates will produce much better food conversion efficiencies throughout their life. The lean meat growth is very efficient and requires much less energy than fat deposition.

CONCLUSION

The most important breeding goals in pigs production is genetic improvement of productive performance and discovering individual genes. Molecular biology methods provide excellent opportunities the identification of genes or genetic markers associated with production traits which influenced quantity of meat.

Therefore, many significant associations with productive traits were detected. We evaluated the significant associations of *MYF4* gene only with lean meat. The significant associations *FSHB* gene with backfat thickness and lean meat were observed. Also for the same gene we identified highly significant effects ($P \leq 0.01$) on average daily gain and average daily gain test. Only the interaction of genotype *MYF4*^{AB}/*FSHB*^{AB} has not shown statistically significant differences on backfat thickness and average daily gain. The genotype combination *MYF4*^{AB}/*FSHB*^{BB} has not shown effect on average daily gain in test. This research has shown high significant differences between analyzed genotypes for all traits.

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