Effect of estrogen receptor, follicle stimulating hormone and myogenin genes on the performance of Large White sows

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ABSTRACT: The study was designed to determine the associations between the Estrogen Receptor Gene (*ESR - Pvu*II), Follicle Stimulating Hormone Gene (*FSHB - Hae*III) and Myogenin Gene (*MYOG - Msp*I) with traits characterizing the sow efficiency comprehensively. Concretely, the age of the first conception (AFC), service period (SP), insemination interval (InI), total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets weaned (NW), lean meat content (LMC), backfat thickness (BFT) and average daily gain (ADG) were included in the study. Significant effects of *ESR* and *FSHB* genes on litter size (particularly in the second parity) and on reproduction traits were found in the investigated population. The influence of *MYOG* gene on reproduction traits was observed but there was no effect of this gene on litter size. None of the included genes had an effect on growth traits. The results outline the possible application of the above-mentioned genes in selection of Large White sows.

Keywords: litter size; growth; reproduction; *ESR*; *FSHB*; *MYOG*

We decided to study the effect of Estrogen Receptor Gene (*ESR*) *Pvu*II polymorphism, Follicle Stimulating Hormone Gene (*FSHB*) *Hae*III polymorphism and Myogenin Gene (*MYOG*) *Msp*I polymorphism because the effects of these polymorphisms on reproduction and growth traits were previously described. The *ESR* gene is considered as a candidate gene for reproduction. The relationship between the *Pvu*II polymorphism of *ESR* gene (Rothschild et al., 1991) and reproduction traits was studied by many authors, e.g. by Depuydt et al. (1999), Linville et al. (2001), Isler et al. (2002) and Van Rens et al. (2002). Similarly, the *FSHB* gene is considered to be a candidate gene because of its function in the maturation of small and medium follicles into ovulating large follicles (Wang and Greenwald, 1993). Korwin-Kossakowska et al.

(2003) studied the association of *FSHB* gene with reproduction traits. Li et al. (1998) found significant effects of *FSHB* gene on pig litter size in the population of Landrace, Yorkshire and Yorkshire hybrids crossed with the Erhualian breed. The myogenin gene (*MYOG*) plays a role during the terminal transformation of myoblasts into myofibres (Te Pas and Visscher, 1994). The effect of *MYOG* gene on reproductive traits was reported only by Horák et al. (2004). Te Pas et al. (1999) concluded that the myogenin gene influenced the growth of pigs and muscle mass. There are some divergences in results of previous studies which were focused on the genes of our interest. The divergences or rather antagonistic results may be caused by the disparity of studied populations. Hybrids or very divergent breeds such as Meishan and Large White are often

studied (Rothschild et al., 1991; Li et al., 1998). Our study tried to evaluate the effect of three candidate genes in one herd of Large White breed. All sows were bred under the same living and selection conditions, nutrition and a closed system of the herd was used in this breed.

MATERIAL AND METHODS

Animals

The information on sows was acquired during the last 6 years. All sows originated from one elite herd of Large White breed. In total, information on 915 litters and reproduction traits of 246 sows was collected. The levels of growth rate were known for all of them. A total number of 246 sows (in the case of *ESR* gene), 186 sows (in the case of *FSHB* gene) and 147 sows (in the case of *MYOG* gene) were genotyped by PCR-RFLP method.

Trait definition

In our set of animals, the following traits were analysed: backfat thickness (BFT, cm), average daily gain (ADG, g), lean meat content (LMC, %), age of the first conception (AFC, day), service period in the 1st to 6th and in the 2nd parity (SP, day), insemination interval in the 1st to 6th and in the 2nd parity (InI, day), total number of piglets born (TNB), number of piglets born alive (NBA) and number of piglets weaned at 21 days (NW). The traits of litter size (TNB, NBA, NW) were analyzed in the 1st, 2nd and 1st–6th parities. An independent analysis was carried out for service period and insemination interval in the second parities because the effects of genes on litter size in the second parities were found. Backfat thickness was determined as a mean of three measurements: BFT 1 – thickness to the middle of the second thoracic vertebra, BFT 2 – thickness to the middle of the last thoracic vertebra and BFT 3 – thickness to the middle of the first sacral vertebra. ADG was calculated from birth to 100 kg body weight.

PCR-RFLP

The PCR-RFLP methods described by Short et al. (1997) (restriction enzyme *Pvu*II), Rohrer et al. (1994) (restriction enzyme *Hae*III) and Te Pas et al. (1996) (restriction enzyme *Msp*I) were applied for detection of genotypes in the *ESR*, *FSHB* and *MYOG* genes, respectively. Two alleles: *B* (55; 65 bp) and *A* (120 bp); *A* (332 bp) and *B* (173; 159 bp), *A* (219; 134 bp) and *B* (353 bp) were detected in *ESR1*, *FSHB* and *MYOG* genes, respectively.

Statistical analysis

To evaluate the effect of the studied genes on selected traits a mixed linear model (procedure REML) in SAS for Windows 9.1.3 was used. Specifications of the used factors are shown in Table 1. Because of a low number of sows with *BB*

Table 1. Factors used in mixed linear models to evaluate polymorphism associations

Factor		Genotype	Ind.	Boar	Sire	Dam	YMB	YML	OL
BFT		F	–	–	R	R	F	–	–
ADG		F	–	–	R	R	F	–	–
LMC		F	–	–	R	R	F	–	–
AFC		F	–	–	R	R	F	–	–
SP		F	–	–	R	R	F	–	–
InI		F	–	–	R	R	F	–	–
TNB, NBA and NW	1st parity	F	–	R	–	–	–	F	–
	2nd parity	F	–	R	–	–	–	F	–
	1st–6th parity	F	R	R	–	–	–	F	F

F = fixed effect; R = random effect; BFT = backfat thickness; ADG = average daily gain; LMC = lean meat content; AFC = age of the first conception; SP = service period; InI = insemination interval; TNB = total number of piglets born; NBA = number of piglets born alive; NW = number of piglets weaned; Genotype = genotypes in the relevant gene; Ind. = the effect of an individual; Boar = sire of litter; Sire = effect of the sire of sows; Dam = effect of the dam of sows; YMB = year × months of sow birth; YML = year × months of litter

Table 2. Relative frequencies of genotypes and alleles of the estrogen receptor gene (*ESR*), follicle stimulating hormone gene (*FSHB*) and myogenin gene (*MYOG*)

Locus	Genotype	Frequency of genotype	χ^2-test	Allele	Frequency of alleles
	AA	0.27	0.06[NS]	*A*	0.51 ± 0.02
ESR (*n* = 246)	*AB*	0.49		*B*	0.49 ± 0.02
	BB	0.24			
	AA	0.09	7.80**	*A*	0.23 ± 0.02
FSHB (*n* = 186)	*AB*	0.28		*B*	0.77 ± 0.02
	BB	0.62			
	AA	0.52	0.97[NS]	*A*	0.73 ± 0.03
MYOG (*n* = 147)	*AB*	0.42		*B*	0.27 ± 0.03
	BB	0.05			

**Significant difference (the population is not in H-W equilibrium) $P \le 0.01$; [NS]non significant

genotype in *MYOG* gene this genotype was excluded from the association analysis.

RESULTS

The observed frequencies of genotypes are shown in Table 2. The number of sows with *AA* genotypes in the *FSHB* gene and particularly *BB* genotypes in the *MYOG* gene is remarkably low. The χ^2 tests show that the population was not in Hardy-Weinberg equilibrium in the *FSHB* gene. No significant influences of any genes on the growth traits (BFT, ADG, LMC) were found in our data set (Table 3). Results of the association of genes with AFC, SP and InI shown in Table 4 are more interesting. The genotypes of *FSHB* gene had no effect on AFC, SP and InI. On the contrary, the *ESR* and *MYOG*

genes have significant effects on SP ($P \le 0.05$) and InI ($P \le 0.01$ and $P \le 0.001$) in the 1st–6th parities. Sows with *AB* genotypes of *ESR* gene and *AB* genotypes of *MYOG* gene have demonstrably longer SP and InI than sows with *AA* genotypes. Similar results were obtained in the 2nd parity. The effect of the genes on SP and InI can correspond with the results of associations of genes with litter size (Tables 5–7). No significant effect of *ESR* gene on litter size in the 1st and 1st–6th parities was found. Reversely, in the 2nd parities, the *ESR* gene had an impact on the total number of piglets born, number of piglets born alive as well as on the number of piglets weaned. The effect of *FSHB* gene on the number of piglets weaned in the 1st–6th parities was not so convincing. The influence of *FSHB* gene on the number of piglets born alive in the 2nd parity was almost significant. Contrary to the

Table 3. The associations of the estrogen receptor gene (*ESR*), follicle stimulating hormone gene (*FSHB*) and myogenin gene (*MYOG*) with growth traits of sows (Least-Squares Means and Standard Errors, LSM ± SE)

Gene	Genotype	BFT	ADG	LMC
	AA	0.82 ± 0.02	650.13 ± 7.18	61.63 ± 0.25
ESR	*AB*	0.81 ± 0.02	653.45 ± 6.20	61.66 ± 0.21
	BB	0.80 ± 0.02	654.55 ± 7.58	61.53 ± 0.27
	AA	0.80 ± 0.04	646.31 ± 11.90	62.12 ± 0.50
FSHB	*AB*	0.76 ± 0.03	665.40 ± 7.95	62.12 ± 0.30
	BB	0.79 ± 0.02	655.95 ± 6.50	62.12 ± 0.30
	AA	0.81 ± 0.02	664.46 ± 6.79	61.78 ± 0.28
MYOG	*AB*	0.78 ± 0.03	656.86 ± 7.56	62.16 ± 0.33
	BB	–	–	–

BFT = backfat thickness; ADG = average daily gain; LMC = lean meat content. Values with different superscripts show a significance level within the rows: $P \le 0.05$ (a,b); $P \le 0.01$ (a,b); $P \le 0.001$ (c,d); $P \le 0.1$ (*,**)

Table 4. The associations of the estrogen receptor gene (*ESR*), follicle stimulating hormone gene (*FSHB*) and myogenin gene (*MYOG*) with reproduction traits (Least-Squares Means and Standard Errors, LSM ± SE)

Gene	Genotype	SP 1st–6th parities	SP 2nd parity	InI 1st–6th parities	InI 2nd parity	AFC
ESR	*AA*	39.51 ± 3.11a	41.26 ± 4.51	32.03 ± 1.61a	31.19 ± 1.84a*	248.81 ± 7.24
	AB	47.70 ± 2.76b	49.55 ± 4.13	37.27 ± 1.42b	37.96 ± 1.69b	252.76 ± 6.09
	BB	42.57 ± 3.46	42.70 ± 5.02	34.70 ± 1.78	36.55 ± 2.05**	243.27 ± 7.82
FSHB	*AA*	38.47 ± 5.84	43.38 ± 7.97	33.70 ± 2.80	36.27 ± 3.49	253.42 ± 14.07
	AB	43.41 ± 4.32	39.62 ± 5.15	32.69 ± 2.06	34.76 ± 2.25	254.22 ± 8.71
	BB	47.10 ± 3.39	50.25 ± 3.88	34.06 ± 1.73	35.45 ± 1.70	246.20 ± 7.11
MYOG	*AA*	40.00 ± 3.37a	42.19 ± 4.18a	30.14 ± 1.83c	31.73 ± 1.98a	250.39 ± 7.86
	AB	53.97 ± 3.93b	55.21 ± 5.17b	37.64 ± 2.06d	39.26 ± 2.44b	241.79 ± 8.91
	BB	–	–	–	–	–

AFC = age of the first conception; SP = service period; InI = insemination interval. Values with different superscripts show a significance level within the columns: $P \leq 0.05$ (a,b); $P \leq 0.01$ (a,b); $P \leq 0.001$ (c,d); $P \leq 0.1$ (*,**).

ESR and *FSHB* gene, the *MYOG* gene has no impact on litter size.

DISCUSSSION

We found the *BB* genotype of *ESR* gene as the least widespread in our population. It corresponds with the results of Omelka et al. (2005) and Matoušek et al. (2005), who also studied the same polymorphism in the Large White population. The majority of the preliminary studies with respect to the *ESR* locus focused primarily on reproductive traits. Not so many works evaluated the effect on growth traits. A significant effect of *ESR* on backfat thickness was determined by Leeds et al. (2001). Favourable pleiotropic effects for BFT and no difference between genotypes for ADG were detected by Short et al. (1997). This result was not repeated in our study where the *ESR* gene had no effect on the growth traits (ADG, BF, LM). Drogemuller et al. (2001) did not find any association of the *ESR* gene with backfat thickness or with average daily gain. Rothschild et al. (1996) suggested the effect of the favourable *B* allele of *ESR* might be antagonistic relative to BFT. It indicates that the effect of *ESR* gene on growth traits is miscellaneous, which can be affected by different studied populations.

Table 5. The associations of the estrogen receptor gene (*ESR*) with litter size (Least-Squares Means and Standard Errors, LSM ± SE)

Genotypes of *ESR*	TNB	NBA	NW
1st parity			
AA	12.24 ± 0.36	12.04 ± 0.36	10.36 ± 0.29
AB	12.35 ± 0.28	12.13 ± 0.28	10.62 ± 0.26
BB	11.81 ± 0.37	11.56 ± 0.37	10.58 ± 0.30
2nd parity			
AA	12.48 ± 0.47a	12.49 ± 0.44*	12.04 ± 0.36a
AB	13.07 ± 0.37*	12.78 ± 0.34	11.75 ± 0.28a
BB	14.15 ± 0.50b**	13.65 ± 0.47**	10.95 ± 0.37b
1st–6th parities			
AA	13.33 ± 0.30	13.12 ± 0.28	10.18 ± 0.22
AB	13.28 ± 0.27	13.07 ± 0.25	10.16 ± 0.21
BB	13.45 ± 0.31	13.09 ± 0.29	10.11 ± 0.23

TNB = total number of piglets born; NBA = number of piglets born alive; NW = number of piglets weaned. Values with different superscripts show a significance level within the columns: $P \leq 0.05$ (a,b); $P \leq 0.01$ (a,b); $P \leq 0.001$ (c,d); $P \leq 0.1$ (*,**)

Table 6. The associations of the follicle stimulating hormone gene (*FSHB)* with litter size (Least-Squares Means and Standard Errors, LSM ± SE)

Genotypes of *FSHB*	TNB	NBA	NW
1st parity			
AA	11.68 ± 0.58	11.38 ± 0.59	10.93 ± 0.43
AB	11.68 ± 0.37	11.49 ± 0.38	10.81 ± 0.34
BB	12.36 ± 0.29	12.16 ± 0.29	10.33 ± 0.29
2nd parity			
AA	12.68 ± 0.90	12.14 ± 0.84	11.94 ± 0.61
AB	12.40 ± 0.61	12.09 ± 0.57*	11.51 ± 0.43
BB	13.71 ± 0.46	13.47 ± 0.43**	11.67 ± 0.36
1st–6th parities			
AA	12.91 ± 0.45	12.42 ± 0.43	10.67 ± 0.27a
AB	12.88 ± 0.35	12.71 ± 0.33	10.32 ± 0.24
BB	13.28 ± 0.30	13.02 ± 0.28	10.05 ± 0.21b

TNB = total number of piglets born; NBA = number of piglets born alive; NW = number of piglets weaned. Values with different superscripts show a significance level within the columns: $P \leq 0.05$ (a,b); $P \leq 0.01$ (a,b); $P \leq 0.001$ (c,d); $P \leq 0.1$ (*,**)

Nevertheless, the effect of *ESR* gene on the reproduction traits and litter size is notable. Previous studies were focused on litter size in the 1st and later parities. Thus Rothschild et al. (1996) and Short et al. (1997) found the significantly lowest number of TNB and NBA in the 1st and later parities in sows with *AA* genotypes in *ESR* gene. Only Noguera et al. (2003) analyzed the effect in the 2nd parities. They did not find any effect of *ESR* gene on TNB and NBA. Our results indicate that the *ESR* gene is associated with service period and insemination interval. In our study the sows with *AA* genotype in the *ESR* gene had provably the shortest service period and insemination interval in the 1st–6th as well as in the 2nd parities. The litter sizes in the 1st and 1st–6th parities were similar in sows with different genotypes. However, the litter size in the 2nd parities was different according to genotypes in the *ESR* gene. It is to note that sows with *BB* genotype of *ESR* gene had the significantly shortest SP, lowest number of TNB and NBA in the 2nd parities but they had the highest NW. It can be caused by the exhaustion of sows after the first parity. A significant effect of *ESR* gene on the number of

Table 7. The associations of the myogenin gene (*MYOG*) with litter size (Least-Squares Means and Standard Errors, LSM ± SE)

Genotypes of *ESR*	TNB	NBA	NW
1st parity			
AA	11.96 ± 0.33	11.74 ± 0.33	10.45 ± 0.37
AB	12.46 ± 0.36	12.21 ± 0.37	10.62 ± 0.36
BB	–	–	–
2nd parity			
AA	13.56 ± 0.42	13.20 ± 0.42	11.64 ± 0.29
AB	12.96 ± 0.50	12.68 ± 0.50	11.68 ± 0.40
BB	–	–	–
1st–6th parities			
AA	13.03 ± 0.27	12.76 ± 0.26	10.28 ± 0.23
AB	13.38 ± 0.32	13.10 ± 0.31	10.32 ± 0.24
BB	–	–	–

TNB = total number of piglets born; NBA = number of piglets born alive; NW = number of piglets weaned. Values with different superscripts show a significance level within the columns: $P \leq 0.05$ (a,b); $P \leq 0.01$ (a,b); $P \leq 0.001$ (c,d); $P \leq 0.1$ (*,**)

piglets weaned in the Large White population was observed by Omelka et al. (2005). The sows with *AA* genotype had the significantly lowest total number of piglets born and number of piglets born alive but they had a significantly higher number of piglets weaned. These results can be determined by the length of service period.

We observed a low number of sows with *AA* genotype in the *FSHB* gene or generally low presence of allele *A* in our population. Similar allele frequencies in the *FSHB* gene were reported by Linville et al. (2001). Li et al. (1998) found major effects on litter size associated with a marker within *FSHB*. Korwin-Kossakowska et al. (2003) did not find any significant association of the *FSHB* locus with litter size and piglet weights in the population of 444 sows of the Polish synthetic line. No association of the *FSHB* gene either with reproduction or with growth traits was demonstrable in our population. The highest number of piglets born alive in the 2nd parity was associated with *BB* genotype. On the contrary, the numbers of piglets weaned in the 1st to 6th parities were the biggest piglets weaned had sows with *AA* genotype. The results can be ruled by a low number of sows with *AA* genotype.

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. Our study did not confirm these effects. The influence of the myogenin gene on reproductive traits was studied only by Horák et al. (2004) in the population of Přeštice Black-Pied sows. They found significant differences between the numbers of piglets weaned in sows with *AA* and *AB*, *BB* genotype in the 1st–6th parities and in the 2nd to 6th parities. The *AA* genotype proved the largest litter size. Our study indicates that the myogenin gene influences the reproduction traits such as service period and insemination interval; nevertheless we did not confirm the results of Horák et al. (2004). Because of such a low number of sows with *BB* ge-notypes further study is necessary to say the last word about the usefulness of the *MYOG* gene in practical breeding.

CONCLUSIONS

In our study we evaluated the effect of three candidate genes on traits of great economic impact on the pig industry. Many previous studies were focused on some of these traits but only few of them were focused on all the traits together in order to evaluate the effect comprehensively. Contrary to expectations, the *MYOG* gene had no effect on growth traits, nevertheless the effect of this gene on reproduction traits was observed. Both the *ESR* and the *FSHB* gene had a significant effect on litter size and on the other reproduction traits. The results outlined that selected candidate genes may be useful in practical breeding. Nevertheless, the effect of genes must be evaluated in the concrete population and breeding conditions while the effect on the other important traits must be taken into consideration.

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