

IMPACT OF PRL AND FST LOCI POLYMORPHISM **ON SEXUAL ACTIVITY OF PUŁAWSKA GILTS***

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Abstract

PRL (prolactin) and FST (follistatin) genes were investigated and analysed with regard to values of selected indices determining the reproductive activity of gilts in heat. The performed analyses comprised gilts of Puławska breed kept within the framework of the Genetic Resources Protection Programme of Farm Animals. Ninety-six gilts were phenotyped (one set for FST and one set for PRL). Each genotype (PRL, FST) group was represented by an equinumerous population of 32 gilts. The analysis took into consideration the second and third spontaneous oestrus during which the following parameters were assessed: age, body weight, duration of the tolerance reflex in the phase of true oestrus and intensity of reproductive responses during the oestrus period. The sexual behaviour was estimated with the assistance of: the manual method, mounting with a simultaneous pressure with knees of the lumbosacral area, and using a teaser boar in accordance with a 3-point score. The performed experiments revealed a significant impact of polymorphism in the FST locus on oestrous activity. On the other hand, the PRL gene was found to affect feeding behaviour, motor activity (including oestrous) and contributed to lack of motor activity treated as rest or sleep.

Key words: polymorphism, PRL, FST, sexual behaviour, Puławska breed

Novel research techniques made it possible to show potential genetic markers for swine reproductive traits, including such ones as: age at first oestrus, weight of reproductive organs, number of eggs ovulated, and number of piglets born (live and dead)

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(Bidanel and Rothschild, 2002; De Koning et al., 2001; Drögemüller et al., 2001; Hirooka et al., 2001; Rathje et al., 1997; Rohrer et al., 1999). The above-mentioned traits are very important with respect to swine production economy. Sexual activity of gilts also deserves mention since it is a factor indicating potential and true fertility (Babicz et al., 2011). Walkiewicz et al. (1990), when investigating fertility of Polish Landrace gilts in relation to the intensity of the tolerance reflex during the third oestrus, demonstrated higher fertility of gilts of equal temperament, clearly manifesting heat and tolerating the boar.

Therefore, identification of genetic preconditioning of gilt sexual behaviour can be considered as an important economic factor utilised in swine selection. The selection of the genetic marker should depend on the function a given gene plays in reproduction processes as well as on the product preconditioned by it. In this regard, prolactin and follistatin deserve attention. Prolactin belongs to a group of peptide hormones and is made up of 198 amino acids of 22.4 kDa mass. It is synthesised primarily by the frontal lobe of the pituitary gland but also in uterus, placenta and mammary gland. Prolactin is also believed to play an important role with respect to animal behaviour. It was demonstrated that this hormone, as one of the factors affecting neurohormonal management, affects female sexual behaviour during the oestrous cycle (Goncikowska, 2001; Fremann, 2000). Follistatin inhibiting FSH secretion (Esch et al., 1987; Li et al., 1997) is also an important constituent part of the oestrous cycle. It belongs to cytokinins secreted from the follicular fluid of swine ovaries and is characterised by multidirectional action. Follistatin functions in reproduction are associated with the regulation of processes taking place inside ovaries, development of ovarian cells, steroidogenesis, maturation of oocytes as well as the function of the yellow body (Knight and Glister, 2001). Blowe et al. (2006), who underlined the physiological role of follistatin, proposed the FST gene as a marker of reproductive traits of sows.

The aim of the performed investigations and analyses was to determine the effect of polymorphism in the *PRL* and *FST* loci on selected indices affecting the sexual activity of gilts during the oestrous cycle.

Material and methods

The analyses comprised Puławska gilts maintained within the framework of the Genetic Resources Protection Programme of Farm Animals. The housing and feeding conditions of experimental gilts were in keeping with animal welfare standards. Gilts were kept in groups of minimum 4 and maximum 5 animals in pens of 1.65 m²/animal in a system of shallow litter. One kg of the diet contained: 15.8% crude protein, 12.72 MJ/kg metabolisable energy and 4.08% crude fibre.

The experiment was conducted in two stages. The first stage encompassed investigations of *PRL* and *FST* gene polymorphism. The biological material comprised hair bulbs of gilts. DNA isolation was carried out employing the Sherlock AX (A&A Biotechnology) kit according to the procedure provided by the manufacturer.

Polymerase chain reaction (PCR) was performed with the assistance of a thermocycler Engine MJ Research (PTC–200 Peltier thermal cycler). Starters and PC reaction conditions were designed on the basis of data from relevant literature and GeneBank base: FST gene – Shimasaki et al. (1988) and Blowe et al. (2006); *PRL* gene – Babicz et al. (2008). Ninety-six gilts were phenotyped (one set for *FST* and one set for *PRL*). The gilts were genotyped early and then only 32 gilts of each genotype were selected to be phenotyped. The analyses took into consideration the second and third spontaneous oestrus during which the following parameters were assessed: age, body weight, duration of the tolerance reflex during the phase of true oestrus, intensity of sexual reactions during the oestrus period.

The evaluation of behaviour was assessed by: manual method (an attempt of pressure in the lumbosacral area), mounting with a simultaneous pressure with the knees of the lumbosacral area, using a teaser boar. A 3-point scale was applied in each of the above-mentioned methods: 1 - gilts characterised by very distinct heat symptoms, responding to an attempt of pressure, mounting and teaser boar for the period of at least 60 seconds – score of 3 points; 2 - gilts characterised by distinct heat symptoms, responding to an attempt of pressure, mounting and teaser boar for the period of 30 seconds – score of 2 points; 3 - gilts characterised by poor heat symptoms, responding to an attempt of pressure, mounting and teaser boar for the period of 5 seconds – score of 1 point. Occurrence of oestrus was monitored using a teaser boar twice a day after morning and afternoon feeding.

During the period when the tolerance reflex occurred, direct observations were conducted using an ethogram regarding: feeding behaviour (feed and/or water consumption), excretory behaviour (excretion of faeces and/or urine), and activities: moving in the pen, mounting other animals or being mounted by other animals, reactions showing readiness to accept the boar. The time of observation was referred to as the duration of the tolerance reflex for a given animal. The results were expressed in percentages.

The statistical analysis (SAS) was performed for genotypes of individual genes with reference to the behavioural responses of gilts in the second and third oestrus. The influence of the genotype on the level of analysed traits was verified using mixed models in which the following factors were taken into account:

$$y_{iikl} = \mu + G_i + R_k + i(hys) + a + e_{iikl}$$

where:

 y_{ijkl} – value of the observed trait; μ – mean value of the trait; G_i – constant effect of the genotype in the examined locus; R_k – constant effect of the genotype in locus RYR1; i(hys) – herd – year – season interaction; a – regression coefficient on gilt's age; e_{iikl} – random error.

Results

Tables 1 and 2 present numerical values referring to age and body weight parameters which were recorded for individual genotypes of gilts. These values remained at a similar level and the occurring differences turned out to be statistically non-significant. With respect to the *FST* and *PRL* gene polymorphism, a trend was recorded showing that in the case of gilts with the A/A genotype, the second and third oestrus occurred earliest. Body weights that were recorded during those periods fell within the range of breeding standards.

	Genotype			
Trait	A/A	A/B	B/B	
ITal	LSM	LSM	LSM	
	SE	SE	SE	
Age of gilt at second oestrus (days)	188.79	191.66	190.08	
	5.24	<i>6.41</i>	<i>4.97</i>	
Age of gilt at third oestrus (days)	209.72	212.68	211.80	
	6.47	5.49	<i>8.23</i>	
Weight of gilt at second oestrus (kg)	109.49	107.56	110.04	
	<i>7.02</i>	5.47	<i>4.58</i>	
Weight of gilt at third oestrus (kg)	121.63	120.01	123.97	
	<i>8.47</i>	5.34	6.57	

Table 1. Age and body weights of gilts at second and third oestrus with regard to the genotype in the FST locus

Table 2. Age and body weights of gilts at second and third oestrus with regard to the genotype in the PRL locus

	Genotype			
Trait	A/A	A/B	B/B	
	LSM	LSM	LSM	
	SE	SE	SE	
Age of gilt at second oestrus (days)	189.91	190.67	191.71	
	<i>4.18</i>	5.47	<i>7.12</i>	
Age of gilt at third oestrus (days)	210.87	211.68	212.61	
	<i>4.87</i>	5.14	5.23	
Weight of gilt at second oestrus (kg)	110.07	109.12	107.56	
	<i>6.47</i>	4.85	<i>3.25</i>	
Weight of gilt at third oestrus (kg)	122.38	121.34	121.39	
	<i>5.13</i>	5.91	6.56	

The performed statistical analysis revealed a statistically significant impact of the *FST* gene polymorphism on the duration of the tolerance reflex. Gilts of the *FST* B/B genotype exhibited the lordosis reflex of 49.78 h, whereas *FST* A/A gilts showed the duration of the tolerance reflex to be shorter by 9.52 h (P \leq 0.05). Identical trends were

registered when analysing the *PRL* gene polymorphism. The recorded differences between the longest (*PRL*, B/B) and the shortest (*PRL*, A/A) duration of the tolerance reflex amounted to 9.42 h and were statistically significant ($P \le 0.05$). The duration of the tolerance reflex in the third oestrus for all genotypes of *FST* and *PRL* loci was longer in comparison with the second oestrus (Tables 3 and 4).

		Genotype	
Trait	A/A	A/B	B/B
nut	LSM	LSM	LSM
	SE	SE	SE
Duration of tolerance reflex at second oestrus (h)	40.26 a	44.58 ab	49.78 b
	3.14	5.13	<i>4.87</i>
Duration of tolerance reflex at third oestrus (h)	51.24	52.47	51.01
	2.23	2.14	<i>3.26</i>
Symptom intensity at second oestrus – "pressure" (pts.)	1.78	1.91	2.15
	0.25	0.19	0.21
Symptom intensity at second oestrus – "mounting" (pts.)	1.85 a	1.98 ab	2.61 b
	0.21	0.18	0.24
Symptom intensity at second oestrus - "boar" (pts.)	2.18	2.01	2.59
	0.24	0.28	0.14
Symptom intensity at second oestrus - mean (pts.)	1.95	1.98	2.46
	0.23	0.22	0.20
Symptom intensity at third oestrus – "pressure" (pts.)	1.88	1.74	2.01
	0.18	0.21	0.26
Symptom intensity at third oestrus – "mounting" (pts.)	2.11	2.05	2.13
	0.11	0.29	0.20
Symptom intensity at third oestrus – "boar" (pts.)	2.20	2.31	2.48
	0.20	0.27	0.19
Symptom intensity at third oestrus – mean (pts.)	2.07	2.04	2.21
	0.17	0.25	0.22

Table 3. Results of sexual activity of gilts at second and third oestrus with regard to the genotype in the FST locus

a, b – values in rows with different letters differ significantly (P≤0.05).

From among the applied monitoring methods of heat symptoms, the highest values were shown for gilts which were in direct contact with the boar. The performed analysis of the results obtained for polymorphism of the *FST* gene revealed significant differences in the intensity of heat symptoms checked with the assistance of "mounting" in homozygotic A/A and B/B gilts. Gilts of *FST* B/B genotype were found to manifest heat symptoms more clearly. This value amounted to 2.62 pts and was higher in comparison with the *FST* A/A genotype by 0.76 pts (P \leq 0.05).

<i>I AL</i> IOCUS		Genotype	
Trait	A/A	A/B	B/B
Han	LSM	LSM	LSM
	SE	SE	SE
Duration of tolerance reflex at second oestrus (h)	42.03 a	50.21 b	51.45 b
	3.64	4.14	3.58
Duration of tolerance reflex at third oestrus (h)	52.14	57.87	56.28
	<i>3.97</i>	<i>4.24</i>	<i>4.01</i>
Symptom intensity at second oestrus – "pressure" (pts.)	1.64 a	2.01	2.20 b
	0.20	0.24	<i>0.19</i>
Symptom intensity at second oestrus – "mounting" (pts.)	1.72	1.97	2.01
	0.18	0.21	0.24
Symptom intensity at second oestrus - "boar" (pts.)	2.04	2.12	2.34
	0.26	0.18	0.23
Symptom intensity at second oestrus – mean (pts.)	1.81	2.04	2.18
	0.22	<i>0.20</i>	0.21
Symptom intensity at third oestrus – "pressure" (pts.)	1.84	1.75	2.01
	0.21	0.18	<i>0.22</i>
Symptom intensity at third oestrus – "mounting" (pts.)	2.08	1.87	2.07
	0.18	0.19	0.24
Symptom intensity at third oestrus – "boar" (pts.)	2.15	2.67	2.74
	0.27	0.23	0.26
Symptom intensity at third oestrus – mean (pts.)	2.02	2.11	2.28
	0.20	0.19	0.23

Table 4. Results of sexual activity of gilts at second and third oestrus with regard to the genotype in the *PRL* locus

a, b – values in rows with different letters differ significantly (P≤0.05).

Observations of the intensity of heat symptoms during the second and third oestrous cycle demonstrated higher mean values for the *FST* B/B genotype. Also in the case of the *PRL* locus, more favourable values were characteristic of B/B homozygotes. Differences registered between the examined genotypes turned out to be statistically significant (Table 4).

Tables 5 and 6 present results of behavioural observations of gilts during the period of lordosis reflex. Characteristic sexual behaviours of gilts during the true oestrus period allow their effective fertilisation. Therefore, the kind of behaviour described in this experiment as oestrous motor activity was most interesting. The performed investigations of *FST* and *PRL* gene polymorphism showed that this activity influenced significantly the proportion of heat behaviours in general gilt behaviour in the course of showing the lordosis reflex. With respect to *FST* and *PRL* genes, the highest proportion of oestrous motor activity was observed for the B/B genotypes. The value was 12.73% for the *FST* locus (higher by 8.41% in comparison with the *FST* genotype A/A) and 14.47% for the *PRL* locus (8.35% higher in comparison with the *PRL* genotype A/B) (P \leq 0.05). In comparison with homozygotes, heterozygous

gilts exhibited in the *PRL* locus a significantly lower motor activity in the course of lordosis reflex during true oestrus.

	• •			
		FST genotype		
Trait	A/A	A/B	B/B	
mun	LSM	LSM	LSM	
	SE	SE	SE	
Feeding behaviour	5.26	4.13	4.97	
	0.48	0.32	0.51	
Excreting behaviour	2.14	2.84	1.12	
	0.24	0.20	0.15	
Motor activity	70.51	68.29	73.15	
	4.15	5.24	6.14	
a) maintenance	66.32	60.72	61.47	
	3.58	2.56	2.41	
b) oestrous	4.32 a	8.12 ab	12.73 b	
,	0.31	0.94	0.84	
Absence of motor activity	23.42	26.54	22.13	
-	1.48	1.94	1.14	

Table 5. Results of observations of gilts showing the lordosis reflex (% of lordosis reflex duration)

a, b – values in rows with different letters differ significantly (P \leq 0.05).

		PRL genotype			
Trait	A/A	A/B	B/B		
mut	LSM SE	A/B LSM SE 3.54 b 0.15 1.47 0.11 54.41 a 3.87 48.51 2.14 6.12 a 0.24	LSM SE		
Feeding behaviour	6.14 a 0.38		3.97 b 0.16		
Excreting behaviour	2.34 0.25		1.83 0.11		
Motor activity	69.57 ab 3.14		75.12 b 3.89		
a) maintenance	58.48 2.87		61.34 3.01		
b) oestrous	11.12 ab 0.54		14.47 b 0.64		
Absence of motor activity	23.47 a 1.12	42.81 b 1.57	21.47 a 1.43		

Table 6. Results of observations	of gilts showing the lordosis reflex (% of lordosis reflex du	ration)

a, b – values in rows with different letters differ significantly ($P \le 0.05$).

Discussion

The intensity of perioestrous behaviour constitutes an important factor which may be decisive with regard to mating efficiency (Babicz et al., 2011). As a rule, gilts and sows with silent heats exhibit longer periods between litters which is attributed to low efficiency of fertilisation. Most researchers working in this field point to a significant influence of environmental factors (Canaday et al., 2013; Auvigne et al., 2010; Peltoniemi et al., 2000). However, it appears that the impact of genes whose products are associated with the oestrous cycle or/and sexual behaviour may also be an important element.

With respect to the gilt sexual behaviour, the following three significant elements should be distinguished (Beach, 1976): attractiveness – described as the capability of the female to elicit sexual response on the part of the male; proceptivity – understood as sexual activity of the female in response to stimuli sent by the male; receptivity – considered as the female behaviour allowing the male to mate effectively.

Jointly, the three elements can be described as a complex of external symptoms or physiological changes occurring during the heat period. Walkiewicz et al. (2003) estimated the age of occurrence of the first spontaneous heat in the examined gilt population of domestic breeds to range between 150 and 160 days. However, this does not mean that at this age they achieve their reproductive maturity. Observations and experiments carried out in a group of Large White and Landrace gilts of domestic types revealed that the most favourable service age for the above breeds falls on day 211 at body weight exceeding 130 kg (Knauer, 2009). In our study (Tables 1 and 2), it was demonstrated that the second heat in the case of Puławska gilts occurred at the age interval of 188.79 – 191 days at body weight of 107.56–123.97 kg (depending on genotype), which falls within the range of breeding standards. This confirms that this breed is of the fat-meat type whose characteristic feature is earlier achievement of sexual and breeding maturity in comparison with the other domestic breeds (Stasiak et al., 2006). On the other hand, no impact of the *FST* and *PRL* loci on values of the above-mentioned traits was demonstrated.

One of the specific sexual reactions is the lordosis reflex, also known as the tolerance reflex. According to the definition, this reflex constitutes the response of the gilt or the sow to a sexual stimulus and manifests itself as readiness to accept the boar. The duration of the lordosis reflex increases the chance of gilt fertilisation thanks to the application of two or even three matings of insemination operations.

Puławska gilts subjected to observations exhibited significantly different durations of the tolerance reflex in the second oestrus depending on the polymorphism of their *FST* (Table 3) and *PRL* (Table 4) genes. From this point of view, the highest values were characteristic of B/B genotypes. Moreover, the intensity of the oestrous symptoms monitored using "mounting" in the second consecutive oestrus was significantly influenced by the polymorphism in the *PRL* locus. This can be attributed to the role of prolactin which does not only stimulate the development of lactic glands and milk production but also affects female sexual behaviour (Goncikowska, 2001).

Investigations conducted so far indicate that the polymorphism of the discussed genes can be linked primarily to the number of piglets in litter, which is justified by the economic value of this trait (Blowe et al., 2006). However, bearing in mind the fact that at the present time many gilts lack symptoms of heat or manifest them poorly, which makes fertilisation of gilts difficult or even impossible, the undertaken studies appear to be also economically justified.

Gilt behaviour during the oestrus period is affected by a number of environmental factors, e.g. housing conditions, presence of the boar, etc (Pedersen et al., 1997). From the point of view of mating effectiveness, it is important that gilts manifest clearly and sufficiently long their oestrous symptoms with respect to feeding, motor and excretory behaviours. Observations from our investigations demonstrated a significant influence of the polymorphism in the *FST* locus on oestrous activity (Table 5), whereas in the case of the *PRL* gene – on the feeding behaviour, motor behaviour (including oestrous behaviour) as well as on lack of motor activity treated as rest or sleep (Table 6). As in the case of the lordosis reflex, this effect may confirm basic influence of prolactin on the behaviour of gilts during the stage of true heat (Goncikowska, 2001; Fremann, 2000).

The performed investigations made it possible to show significant interrelationships with respect to FST and PRL gene polymorphism as well as selected traits associated with gilt sexual behaviour. It was demonstrated that gilts with FST B/B and PRL B/B genotypes were characterised by a significantly longer duration of the tolerance reflex in the second oestrus, i.e. during the period used most frequently by breeders and producers alike for female mating. In addition, the proportion of oestrous activity in these gilts also turned out highest. The research results obtained as well as the performed analyses point to FST and PRL genes as markers of sexual behaviour of Puławska gilts in conservation breeding.

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