

# ANALYSIS OF MATERNAL TRAITS IN NATIVE PUŁAWSKA SOWS OF KNOWN GENOTYPE (INS/DEL) AT THE PRL LOCUS\*

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#### Abstract

The objective of the study was to analyse periparturient behaviour and the reproductive parameters of native Puławska sows with regard to polymorphism (insertion/deletion) at the PRL locus as components in the assessment of maternal value. The analysis included 45 sows of known genotype at the PRL locus, which were managed under a conservation breeding programme in individual farms. Behaviour was assessed by using an ethogram of sow responses to the stockpersons and piglets during the rearing period. The production parameters included litter size between days 1 and 21, mortality, body weight and daily weight gains of the piglets during that period. The performed analyses and observations show an association between the polymorphism of PRL gene and the maternal parameters of Pulawska sows. Del/Del homozygous sows exhibited significantly higher realised fertility, but the maternal characteristics were generally more favourable in sows of the PRL Ins/Ins genotype. These animals were more docile towards the stockpersons and piglets, and were characterised by lower crushing rates, generally lower litter mortality, and higher parameters of piglet growth and development.

Key words: pigs, native breed, PRL, maternal traits, behaviour

The native Puławska pig breed has been managed under conservation breeding since 1996. It has lower carcass muscle scores, but its reproductive merit is comparable to other breeds used as a maternal component of commercial crossbreeding schemes and it is worth noting the complex of traits referred to as maternal care. In this regard, it must be stressed that considering the currently high welfare standards,

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the determinants of these characteristics should be sought in the effects of sow genotype. The possibility of utilising genetic markers in pig breeding and production has captured the attention of researchers for many years (Ellegren, 1993; Rohrer et al., 1999; Buske et al., 2006), and it is believed that the results of these studies could be used in marker-assisted selection (MAS) (Søller, 1994). Prolactin (PRL) is one of the genes with a potential effect on maternal traits. The product of this gene is the hormone prolactin, one of the peptide hormones. Composed of 198 amino acids of 22.4 kDa mass, it is synthesised mainly by the anterior pituitary gland, but also in the uterus, placenta and mammary gland. By acting as a neurohormone, prolactin has been shown to influence female behaviour during the reproductive cycle, including maternal care (Freeman et al., 2000; Goncikowska and Sotowska-Brochocka, 2001). Maternal care is essential to the normal rearing of suckling piglets (Grandinson et al., 2003), because piglets lack immune and thermal resistance, which is negatively compounded by large litter size. Sows with a low level of maternal care neglect their offspring and even show aggression towards them, which leads to increased litter mortality. It is difficult to reduce piglet mortality during the rearing period due to the low heritability and considerable effect of various environmental factors (Grandinson et al., 2002). Current research suggests that the mortality of piglets per litter is influenced by the sows' ability to rear their offspring (Špinka et al., 2000; Andersen et al., 2005). Opinions are divided concerning the best method for assessing maternal characteristics in sows, but most often this type of analysis is based on the rearing performance of the piglets and behavioural tests of the dams (Grandinson et al., 2002; Grandinson et al., 2003; Løvendahl et al., 2005; Vangen et al., 2005).

DNA sequence analysis studies of the native Puławska breed identified an Insertion/Deletion (*Ins/Del*) mutation of the *PRL* gene on chromosome 7, in exon 5 coding for 3'UTR fragment (Babicz et al., 2008). This was followed by research aimed to determine the association between the polymorphism identified at the *PRL* locus and the productive traits of the Puławska sows, which provided a maternal component. The results of this research are shown in the present study.

The aim of the study was to analyse periparturient behaviour and selected reproductive traits of Puławska sows (number of piglets born alive, piglet mortality, piglet body weight, daily weight gains of piglets in the second parity) with regard to polymorphism (*Ins/Del*) at the *PRL* locus (rs789846153).

## Material and methods

# Animals

The analysis included 45 Puławska sows, kept in five family farms as part of the Conservation Programme for Puławska Pig Genetic Resources. The feeding (Table 1) was adjusted to the age and physiological status of the sows. Housing conditions complied with welfare requirements. The sows were kept on litter in individual

pens, with the free movement of sows and with a separate lying area for the piglets. Sows were mated naturally to a Puławska boar according to the mating scheme outlined in the breeding programme for Puławska pigs.

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Components	Nutritive value of the diets	
Metabolizable energy	12.65 MJ/kg	
Crude protein	17.10%	
Lysine	0.81%	
Methionine	0.29%	
Met. + Cyst.	0.60%	
Calcium	0.85%	
Phosphorus	0.60%	
Sodium	0.22%	

Table 1. Nutrient content of the diet for suckling sows

# Analysis of prolactin gene (PRL) polymorphism

Prolactin gene polymorphism (rs789846153) was analysed. The biological material was represented by hair roots from the sows. DNA isolation was performed using a Sherlock AX kit (A&A Biotechnology) following the manufacturer's procedure. The genotype was verified by PCR RFLP analysis. PCR was carried out with a thermal cycler (PTC-200 Peltier DNA Engine, MJ Research). The primers and PCR conditions were designed based on literature data (Babicz et al., 2008). The primer sequences were synthesised at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. PCR was performed with a thermal cycler (PTC-200 Peltier DNA Engine, MJ Research). Each sample of 20 µl, prepared for PCR, contained: 5 µl of RedTaq<sup>TM</sup>ReadyMix<sup>TM</sup>PCR (SIGMA), 0.2 µl of each primer  $(0.2 \ \mu\text{M})$ , 3.6  $\mu\text{l}$  of water (SIGMA) and 2  $\mu\text{l}$  of DNA (20 ng/ $\mu\text{l}$ ). The PCR products were digested with HpyCH4III restriction enzyme. Electrophoretic separation of the PCR product for different genes, following digestion with restriction enzymes, was performed in agarose gel (Sigma). Three genotypes were obtained (PRL Ins/Ins, PRL Ins/Del, PRL Del/Del), each of which was represented by 15 sows. Sows with specific PRL genotypes were chosen based on genotyping of the Puławska population managed under a conservation breeding programme (unpublished data).

## Maternal behaviour

Observations were made by stockpersons in particular herds and recorded using an ethogram for 21 days after farrowing. Maternal behaviour was determined based on 6 traits measured with different scales, using a modified version of the diagram reported in Gäde et al. (2008):

# Attitude to the stockpersons present in the pen during farrowing and handling of piglets (AtS) (scale 1–3):

1	restless, fearful,
2	normal, tolerant,
3	aggressive, savaging attempts, biting.
Maternal care during	g farrowing (MC) (scale 1–5):
1 (best)	very calm behaviour during farrowing, careful observation of piglets, maternal reaction to piglet vocalisations, exposing all teats for suckling without crushing the lying piglets, strong defensive reaction (standing up) towards intense piglet vo- calisations, no crushed piglets,
• • •	
5 (poorest)	very restless during farrowing, abrupt postural changes from lying to standing, no reaction towards piglet vocalisations or aggressive reaction towards the litter, repeated crushed pig- lets (more than 1 animal).
<b>Crushing of suckling</b>	piglets (CSP) (scale 1–2):
1	no crushed piglets,
2	at least one crushed piglet.
Behaviour towards p	iglets during and after farrowing (BtP) (scale 1–2):
1	normal, caring,
2	aggressive, biting.
Attitude to piglets du	ring suckling (AtPS) (scale 1–2):
1	lying calmly in lateral position, exposing all teats for suck- ling, maternal vocalisations,
2	lying on the belly after piglets begin to suckle, aggressive, biting of piglets.
Reaction to piglets' v	ocalisations during rearing (RPVR) (scale 1–3):
1	contact with piglet, 'calming' vocalisations,
2	neutral reaction.
3	aggression, crushing of piglets during rapid movements.

# Production parameters of maternal merit

As envisaged in the conservation programme, Puławska sows in the second reproductive cycle were mated to purebred boars. The parameters of maternal merit included those measured during farrowing and until day 21 of lactation:

- number of piglets born alive per litter (NPBA) and at days 7 (NP7), 14 (NP14) and 21 (NP21) of age (head),

- piglet mortality (PM) during the period from day 1 to day 7, from day 8 to day 14, and from day 15 to day 21 (%),

- piglet body weight (PW) at days 1, 7, 14 and 21 (g),

- daily weight gains of piglets (DGP) during the period from day 1 to day 7 of lactation, from day 8 to day 14 of lactation, and from day 15 to day 21 of lactation. Body weight was measured in the morning, between 7:00 and 9:00.

### Statistical analysis

The effect of genotype on the level of the analysed traits was verified using the following mixed model:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklm}$$

where:

 $y_{iiklm}$  – value of the observed trait,

 $\mu$  – mean value of a trait,

 $a_i$  – fixed effect of genotype at the analysed locus; (i=1–3),

 $b_i$  – random effect of the herd (j=1–5),

 $c_k$  - random effect of the season (k=1-4, where 1- winter, 2- spring, 3- summer, 4- autumn),

 $d_i$  – random effect of the sire of the litter,

 $f_{\rm m}$  – random effect of the mother of the litter,

 $e_{iiklm}$  – random error.

The calculations were made with the SAS statistical package. The level of the analysed traits was presented as least square means (LSM) while giving the standard errors (SE). Differences between traits were analysed at P $\leq$ 0.01 and P $\leq$ 0.05 levels of significance.

# Results

The reactions of the farrowing sows to the stockperson's presence and handling (AtS) on the testing scale ranged from  $1.87\pm0.19$  (*PRL Del/Del*) to  $2.45\pm0.22$  (*PRL Ins/Ins*) (Table 2), which means that the sows of the *Del/Del* genotype were anxious but tolerant of the stockperson's presence, while *Ins/Ins* homozygous sows more often (P $\leq$ 0.05) showed signs of aggression. Also, the maternal care of the sows (MC) at farrowing was differentiated by prolactin genotype. On a 5-point scale, maternal care was highest ( $2.47\pm0.48$  pts.) for the *Ins/Ins* homozygous sows and poorest ( $3.54\pm0.61$  pts.) for the sows of the *Del/Del* genotype. The differences were statistically significant (P $\leq$ 0.05).

Piglet crushing (CSP) during the rearing period was relatively infrequent, and the values obtained for the different genotypes remained at similar levels.

The behaviour of the sows towards the piglets during suckling was divided into three weekly periods (AtPS). In the first week of life, the most favourable maternal traits according to the proposed evaluation scale were observed in the *PRL Ins/Ins* sows. A significantly higher value (P $\leq$ 0.05), associated with the sow lying frequently in sternal recumbency (which prevented suckling) and the incidence of aggression towards the piglets, was found in the *PRL Del/Del* animals.

Our own observations of the sow's response to piglet vocalisations (RPVR) were differentiated by both the mother sow genotype and the piglet rearing period. During the first week of the piglets' lives, the most favourable reactions were noted in sows of the *PRL Ins/Ins* genotype, and the least favourable in the group of the *PRL Del/Del* sows, and the differences proved statistically significant (P $\leq$ 0.05).

		0 0	0 10
	PRL Ins/Ins	PRL Del/Del	PRL Ins/Del
Behavioural indicators	n 15	15	15
	LSM±SE	LSM±SE	LSM±SE
AtS	2.45±0.22 a	1.87±0.19 b	2.08±0.20
MC	2.47±0.48 a	3.54±0.61 b	2.89±0.54
CSP	1.20±0.34	1.33±0.28	1.22±0.42
BtP	1.05±0.24	1.20±0.31	1.07±0.36
AtPS, d 1 to 7 of lactation	1.09±0.22 a	1.57±0.49 b	1.12±0.30
AtPS, d 8 to 14 of lactation	1.34±0.43	1.10±0.37	1.19±0.32
AtPS, d 15 to 21 of lactation	1.11±0.39	1.49±0.53	1.20±0.41
RPVR, d 1 to 7 of lactation	1.06±0.31 a	2.19±0.53 b	1.41±0.40
RPVR, d 8 to 14 of lactation	1.34±0.28	1.28±0.32	1.22±0.31
RPVR, d 15 to 21 of lactation	1.50±0.42	1.47±0.52	1.53±0.48

Table 2. Behavioural indicators of the sows during farrowing and rearing of piglets

a, b – means in rows with different letters are significantly different:  $P \le 0.05$ , AtS – attitude to stockpersons present in the pen during farrowing and handling of piglets, MC – maternal care during farrowing, CSP – crushing of suckling piglets, BtP – behaviour towards piglets during and after farrowing, AtPS – attitude to piglets during suckling, RPVR – reaction to piglets' vocalisations during rearing.

	PRL Ins/Ins	PRL Del/Del	PRL Ins/Del
	n 15	15	15
	LSM±SE	LSM±SE	LSM±SE
NPBA	10.94±1.01 a	11.86±1.04 b	11.42±1.16
NP7	10.62±1.09	11.09±1.17	10.86±1.03
NP14	10.22±1.16	10.71±1.05	10.47±0.97
NP21	10.11±0.98	10.59±1.04	10.35±1.12

## Table 3. Litter size at birth and on successive days of rearing

a, b – means in rows with different letters are significantly different:  $P \le 0.05$ , NPBA – number of piglets born alive per litter, NP7, NP14, NP21 – number of piglets at d 7, 14, 21 of age, respectively.

Table 4. Eight mortanty in successive periods of rearing			
	PRL Ins/Ins	PRL Del/Del	PRL Ins/Del
	n 15	15	15
	LSM±SE	LSM±SE	LSM±SE
PM, d 1 to 7 of age (%)	3.09±0.29 a	6.51±0.71 b	4.88±0.51
PM, d 8 to 14 of age (%)	3.74±0.20	3.41±0.42	3.56±0.32
PM, d 15 to 21 of age (%)	1.12±0.11	1.09±0.09	$1.00 \pm 0.08$

Table 4. Piglet mortality in successive periods of rearing

a, b - means in rows with different letters are significantly different: P≤0.05, PM - piglet mortality.

A comparison of realised fertility with regard to different sets of alleles (Table 3) revealed significant differences (P $\leq$ 0.05). Sows of the *Del/Del* genotype gave birth to more live piglets per litter compared to those with the *Ins/Ins* genotype. Analysis of the mortality in the Puławska piglets during the three successive weeks after birth showed that polymorphism at the *PRL* locus is associated with piglet mortality (Table 4). During the first week of life, the highest piglet mortality (PM) was noted in litters from the sows of the *PRL Del/Del* genotype. This value (6.51%±0.71) was significantly higher (P $\leq$ 0.05) than in sows with *Ins/Ins* alleles. In the other weeks, the differences between the sow genotypes were not significant.

Our study (Table 5) demonstrated that the sow genotype had a significant effect ( $P \le 0.05$ ) in three successive weeks of rearing. As regards the *PRL Ins/Ins* homozygous sows, higher DGP was observed in the first and third weeks of the piglets' lives, whereas the *PRL Del/Del* genotype turned out to be more beneficial for achieving high DGP values in the second week of rearing.

Tuble 5. Durly gains of sucking piglets in successive periods of rearing			
PRL Ins/Ins	PRL Del/Del	PRL Ins/Del	
n 15	15	15	
LSM±SE	LSM±SE	LSM±SE	
305±30 a	162±21 b	230±26 b	
209±25 a	428±51 b	323±37 b	
346±44 a	216±20 b	277±27	
	Of sacking pigets in 3   PRL Ins/Ins   n 15   LSM±SE   305±30 a   209±25 a   346±44 a	PRL Ins/Ins PRL Del/Del   n 15   LSM±SE LSM±SE   305±30 a 162±21 b   209±25 a 428±51 b   346±44 a 216±20 b	

Table 5. Daily gains of suckling piglets in successive periods of rearing

a, b – means in rows with different letters are significantly different: P $\leq$ 0.05, DGP – daily gains of the piglets.

	PRL Ins/Ins	PRL Del/Del	PRL Ins/Del
	n 15	15	15
	LSM±SE	LSM±SE	LSM±SE
PW, d 1 of age (g)	1366±173	1259±183	1310±198
PW, d 7 of age (g)	3195±208 a	2231±244 b	2690±241
PW, d 14 of age (g)	4450±502	4799±502	4633±519
PW, d 21 of age (g)	6523±597	6093±471	6298±567

Table 6. Body weight of suckling piglets in successive days of rearing

a, b - means in rows with different letters are significantly different: P≤0.05, PW - piglet body weight.

Statistically significant differences were also found for piglet weight (Table 6). At 7 days of lactation, sows of the *PRL Ins/Ins* genotype reared significantly heavier piglets than the *Del/Del* homozygous and *Ins/Del* heterozygous gilts.

## Discussion

The domestic pig has a mothering instinct that is as strong as in carnivorous animals (Empel, 2005). In sows kept outdoors or in free movement pens it is especially visible before farrowing, because in this environment animals are able to express their natural behaviour (Nowicki et al., 2012). Breed is one of the genetic factors differentiating the level of maternal characteristics in sows (Knap and Merks, 1987; Vangen et al., 2005). In this respect, individual genotypes should also be counted as a genetic determinant of the complex of maternal traits. Genes coding for products involved directly in the manifestation of maternal behaviour in sows could have a particularly significant impact. These include the hormone prolactin (encoded by the PRL gene), which is a determinant of behaviour and reproductive behaviour in females, and its increased concentration in the blood is essential to the complex of maternal traits being manifested prior to parturition (Widowski et al., 1990; Castrén et al., 1993). As is evident from our study (Table 2), the attitude of farrowing sows to the presence of, and handling by, stockpersons (AtS) as well as their maternal care (MC) during farrowing varied according to the analysed PRL locus polymorphism. The Ins/Ins sows showed aggressive behaviour towards the handlers while demonstrating maternal responsiveness towards their piglets. Based on these two traits it is concluded that sows from the PRL Ins/Ins group are more similar to primitive pigs, in which maternal characteristics are associated with greater maternal care for the young and fear of, or aggression towards, humans (Knap and Merks, 1987; Vangen et al., 2005).

Lactation is the period during which sow behaviour has a significant impact on litter size (Beattie et al., 1996). Piglet mortality may reach 80% on the farrowing day and within 3–4 days of birth, and most life-threatening situations occur within the first 24–36 hours of birth as a result of the mother sow's reactions (Marchant et al., 2001). Wechsler and Hegglin (1997) demonstrated that the sow's response to the screams of crushed piglets is an individual characteristic. In farrowing pens, piglets are most often crushed when the sow rolls from side to side, and in traditional pens additionally by different body movements of the sow (Weary et al., 1996). In our study, the crushing of piglets by sows with a specific genotype were not different and assumed minimal values (Table 2, CSP). Such a low level of crushing, with relatively large litters and management in conventional pens with free movement of the sows, reflects the high level of maternal characteristics in the Puławska sows under analysis.

An important aspect of rearing the piglets is that they can suckle their mother without the risk of being crushed or savaged by the sow. Based on our own practical observations, sows may behave abnormally towards their piglets within 1–2 days of farrowing, after which their maternal behaviour returns to the optimal level. There are also cases when a sow shows normal maternal characteristics after farrowing but her behaviour becomes abnormal over subsequent days. Therefore, in our study we divided this period into three seven-day intervals (Table 2, AtPS). The observations of sow behaviour revealed that it was more favourable in the *Ins/Ins* homozygotes only during the first week of lactation (Table 2). Environmental conditions are criti-

cal to the normal behaviour of suckling sows. Low standards often lead to abnormal behaviour, which is manifested in stereotyped or aggressive behaviour. Meunier-Salaün and Dantzer (1990) report that the level of stereotypies in sows is much higher during the prepartum period, after which this phenomenon is positively reduced by the piglets. While stereotypies are not a significant threat to the litter, behavioural abnormalities characterised by aggression are a significant factor in the mortality of suckling piglets. As reported by Chen et al. (2008), savaging in suckling sows is a more generalised behavioural pathology and is not specifically piglet directed.

According to Nowicki and Klocek (2004) and Grandinson et al. (2003), the sensitivity of sows to cues from piglets decreases litter mortality, and a sow's response to piglet vocalisations may be an important test for assessing the sow's maternal characteristics. In this regard analysis has been made of the behaviour of sows after their temporary separation from the piglets, or of their reactions to piglet vocalisation (Grandinson et al., 2003; Løvendahl et al., 2005). The latter may reflect the sow's behaviour towards the screams of a crushed piglet. In our study, the reaction of the sows to the piglets' vocalisations during rearing (RPVR) were more favourable, especially in the first period of rearing, in the *Ins/Ins* homozygous sows (Table 2). This distribution of values suggests that the *Del/Del* homozygous sows more often showed a negative response to vocalisations of the litter, which is a stress factor for the piglets, leading as a consequence to increased litter mortality. During successive rearing periods, the reactions of sows of different genotypes remained at a similar level.

Another important aspect of the sow's maternal characteristics is the level of litter size and its biological value expressed as weight gains and body weight. Analysis of sow reproductive results showed higher realised fertility in sows of the *Del/Del* genotype (Table 3).

The findings of other authors yield inconsistent results concerning the effect of polymorphism at the *PRL* locus on litter size. Mazurowski et al. (2013) found no significant effect of the *PRL* gene polymorphism (indel) on the number of piglets born and reared from Polish Large White sows. Also, for the polymorphism of the *PRL*/BstU1 gene, localised in intron 2, Korwin-Kossakowska et al. (2003) failed to show its significant effect on litter size in line 990 sows.

Reducing piglet mortality plays a significant role when shaping production efficiency in the farrowing section, expressed by the number of piglets weaned per sow (Röhe and Kalm, 2000). It should be stressed that current breeding work with modern pig breeds has failed to account for traits such as maternal care and periparturient behaviour of the sow. Other authors (Grandinson et al., 2002, 2003; Løvendahl et al., 2005) point out that the application of well-designed behavioural tests for the mother sow could be an effective breeding tool for improving the rearing performance of piglets. Analysis of the mortality in Puławska piglets showed significant associations with the PRL locus polymorphism in the first week of life. The highest mortality (PM) was noted in litters obtained from sows of the *Del/Del* genotype (Table 4).

An important element determining the survival of piglets and their suitability for further fattening is the body weight and growth rate during the initial period of their lives (Johansen et al., 2003). Daily gains of the piglets (DGP) are a direct indicator

of the sow's milk yield while indirectly showing the maternal care of the females, the normal behaviour of which is necessary for the piglets to suckle. The distribution of the DGP values (Table 5) observed in our study in successive weeks of lactation may suggest that, in general, sows with the *PRL Ins/Ins* alleles exhibit a higher level of maternal traits (Table 5).

The overall tendency for increasing piglet body weight in successive rearing periods was also more advantageous for sows of the *PRL Ins/Ins* genotype, as confirmed by their higher maternal care (Table 6).

# Conclusions

The performed observations and analyses suggest that the complex of traits referred to as 'maternal care' is genetically determined in the studied group of Puławska sows. In general, sows of the *PRL Ins/Ins* genotype exhibited a more favourable set of maternal traits in terms of both behaviour and production parameters. On the other hand, the *Del/Del* homozygous sows showed a significantly higher realised fertility. However, before these components are introduced into breeding and production practice and put to appropriate use, the conclusions need to be validated on a larger number of animals.

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