POLYMORPHISM OF PROLACTIN RECEPTOR GENE (PRLR) IN THE POLISH LANDRACE AND POLISH LARGE WHITE SWINE POPULATION AND REPRODUCTIVE TRAITS

POLIMORFIZM GENU RECEPTORA PROLAKTYNY (PRLR) W POPULACJI ŚWIŃ RASY POLSKA BIAŁA ZWISŁOUCHA I WIELKA BIAŁA POLSKA A CECHY REPRODUKCYJNE

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ABSTRACT

Prolactin receptor gene was found in pig chromosome 16, and it is one of the genes with a significant effect on reproduction traits in sows. The objective of the research was to determine polymorphism of the prolactin receptor gene in pigs of two maternal breeds: Polish Landrace and Polish Large White, as well as analyse relations between particular allelomorphic variants, and reproduction traits of examined sows. Two PRLR gene alleles, A and B, were isolated, they were obtained after AluI restriction gene digestion of the PCR product with the length of 163 bp; furthermore, three genotypes were identified: $PRLR^{AA}$ – 85, 59, 19 bp; $PRLR^{AB}$ – 104, 85, 59, 19 bp; $PRLR^{BB}$ – 104, 59 bp. We assessed 122 sows, in terms of their age at the first farrowing, as well as the sizes of the two subsequent litters. No statistically significant differences were found in the examined reproduction traits in sows with different allelomorphic relations, both within each breed and between breeds. Obtained results indicate that it is necessary to conduct further research on a larger animal group.

KEY WORDS: polymorphism, PRLR gene, reproductive traits, swine

STRESZCZENIE

Gen receptora prolaktyny został zlokalizowany w 16 chromosomie świń i jest jednym z genów o dużym efekcie wpływającym na cechy rozrodu loch.

Celem badań było określenie polimorfizmu genu receptora prolaktyny w grupie świń dwóch ras matecznych (wbp i pbz), a także analiza związków między poszczególnymi wariantami allelomorficznymi i cechami rozrodu badanych loch.

Wyodrębniono dwa allele genu PRLR - A i B uzyskane po trawieniu enzymem restrykcyjnym AluI produktu PCR o długości 163 pz, oraz zidentyfikowano trzy genotypy $PRLR^{AA}$ – 85, 59, 19 pz, $PRLR^{AB}$ – 104, 85, 59, 19 pz oraz $PRLR^{BB}$ – 104 i 59 pz.

Oceniano 122 lochy pod względem wieku pierwszego oproszenia a także liczebności dwóch kolejnych miotów. Nie stwierdzono statystycznie istotnych różnic w ocenianych cechach rozrodu świń o różnych układach allelomorficznych zarówno w obrębie ras jak i między rasami. Otrzymane wyniki wskazują na konieczność przeprowadzenia dalszych badań na większej grupie zwierząt.

SŁOWA KLUCZOWE: polimorfizm, gen PRLR, cechy reprodukcyjne, trzoda chlewna

DETAILED ABSTRACT

Materiał badawczy stanowiło 87 loch rasy wielka biała polska (wbp) i 35 loch rasy polska biała zwisłoucha (pbz) pochodzących z czterech stad regionu kujawsko-pomorskiego.

Genomowe DNA wyizolowano z pełnej krwi, genotypy receptora prolaktyny (PRLR) określono metodą PCR-RFLP zgodnie z metodyką [3,16]. Produkt reakcji PCR poddano działaniu enzymu restrykcyjnego AluI, a fragmenty restrykcyjne rozdzielono elektroforetycznie w żelu agarozowym wobec markera molekularnego pUC19/MspI.

Określono częstości występowania genów i genotypów, równowagę genetyczą, współczynnik heterozygotyczności uwzględniając rasę świń [2]. Zgodność rozkładu frekwencji genotypów z regułą Hardy'ego-Weinberga określono wykorzystując test chi^2 [12]. Wpływ rasy i genotypu na badane cechy rozrodu loch oraz analizę statystyczną wykonano przy użyciu programu Statistica 8.0. ANOVA.

W badanej grupie świń zidentyfikowano dwa allele PRLR^A i PRLR^B oraz trzy genotypy – PRLR^{AA}, PRLR^{AB}, PRLR^{BB}. W badanych grupach rasowych loch obserwowano zróżnicowane częstości genotypów. W grupie loch rasy pbz obserwowano wyższy współczynnik heterozygotyczności w porównaniu z grupą loch rasy wbp. Najmłodsze podczas pierwszego oproszenia były lochy o genotypie PRLR^{AB} (336 dni) rasy wbp oraz o genotypie PRLR^{BB} (318 dni) rasy pbz. W pierwszym miocie obserwowano jednakową średnią liczbę urodzonych prosiąt we wszystkich badanych grupach genotypowych loch ras wbp i pbz. Lochy rasy pbz o genotypach PRLR^{AB}, PRLR^{BB} rodziły średnio o jedno prosię więcej w drugim miocie w porównaniu z pozostałymi grupami genotypowymi.

Zależność między genotypami PRLR a wiekiem pierwszego oproszenia oraz liczbą prosiąt żywo urodzonych w dwóch kolejnych miotach badanej grupy loch ras wbp i pbz okazały się statystycznie nieistotne.

Ze względu na brak stwierdzenia jednoznacznego wpływu alleli PRLR^A i PRLR^B na cechy rozrodcze loch wskazane jest kontynuowanie tego typu badań.

INTRODUCTION

Improvement of fleshiness and meat quality, development of animal growth rate, and better utilization of feedstuff by pigs had until recently been the major objectives of breeding efforts based on breeding programmes. Currently, pig development programmes also pay attention to improvement of reproduction performance traits.

Reproduction traits are characterized by low heritability indices, and their improvement based solely on performance assessment results is not very effective. Improvement of reproduction traits through animal selection, assisted by genetic markers, creates new opportunities in this area [8, 10].

The prolactin receptor gene was mapped in pig chromosome 16, it is directly associated with reproduction. Despite promising results in numerous researches, we have been unable to confirm significant relationships between particular variants of this gene and the level of examined reproduction traits in several pig breeds, including Polish maternal breeds [3, 14, 18].

The product of the prolactin receptor gene was found in the majority of tissues and organs, including in the ovary, uterus, additional sex glands, and mammary gland cells. In pigs, protein being the PRLR gene product also occurs in granular cells, corpora lutea, and theca externa folliculi cells, regulating a number of biological processes taking place in the ovaries and uterus. These proteins may stimulate various signal transduction pathways inside cells, which may in turn lead to activation of the set of genes specific for a given tissue, species, or reproduction period [1, 4]. Moreover, the research proved that protein being the product of the prolactin receptor gene is transmembrane protein from type I cytokine receptor family, and is characterized by strong similarity to protein produced in matrices of the growth hormone receptor (GHR) gene [13, 15].

The objective of the research was to analyse polymorphism of the prolactin receptor gene, and its association with reproduction traits of the examined Polish Large White and Polish Landrace swine.

MATERIAL AND METHODS

The research material was constituted by 87 Polish Large White (wbp) and 35 Polish Landrace (pbz) sows from four herds maintained by the Polish Pig Breeders and Producers Association POLSUS in the Kujawy and Pomorze region.

The genomic DNA was isolated from whole blood in accordance with relevant methods, using Epicentre MasterPure™ DNA Purification Kit.

The prolactin receptor (PRLR) genotypes were determined by means of the PCR-RFLP method, using specific oligonucleotide sequences. PCR amplification was performed using 100 ng of genomic DNA, 200 μM each dNTP, 1 UI Tag polymerase, 10 pM each primer, 2 mM $MgCl_2$ and PCR buffer (MBI Fermentas). Thermal cycling began with an initial cycle of 95°C for 4 min followed by 35 cycles of 94°C for 30 s, 58°C for 45 s,

and 72°C for 1 min, and concluded with a final extension at 72°C for 5 min, and hold at 15°C [3, 16]. The PCR reaction product was exposed to 5 units of the AluI restriction enzyme (MBI Fermentas) for 12 hours at the temperature of 37°C. The DNA restriction fragments were separated electrophoretically in 3% agarose gels containing ethidium bromide against the pUC19/MspI molecular marker (MBI Frementas), and the results were visualized in UV light.

Based on the identified polymorphism of the PRLR gene, genotype and allele frequencies of occurrence were determined, and genetic equilibrium was established in accordance with the Hardy-Weinberg principle for the examined swine group, taking their breeds into account [2]. The chi-square test was used to verify compliance of the genotype frequency distribution in accordance with the Hardy-Weinberg principle [12]. Utilising formulas provided by Charon and Świtoński [2], we calculated the heterozygosity index, taking the breeds into account.

The statistical analysis of the results was performed with the use of the Statistica 8.0 software (ANOVA). In order to establish the effect of the breed and genotype on the examined sow reproduction traits, the analysis of variance was applied.

RESULTS AND DISCUSSION

In the examined swine population two alleles of the PRLR gene were identified: $PRLR^{A}$ and $PRLR^{B}$, as well as three genotypes – $PRLR^{AA}$, $PRLR^{AB}$ and $PRLR^{BB}$. The genotype identification is shown in Fig. 1.

Table 1 presents results concerning the genetic structure of the examined group of sows. The observed genotype frequencies differed between breed groups of the examined sows. Among the Polish Landrace sows the highest frequency was noted for the $PRLR^{AB}$ heterozygotes (0.5002), and the least numerous were the $PRLR^{AA}$ homozygotes (0.2401). In the Polish Large White group of sows the frequencies of occurrence of particular genotypes were more similar, and they equalled 0.3032 for sows with the $PRLR^{AA}$ genotype (the lowest frequency), and 0.3927 for sows with the $PRLR^{BB}$ genotype (the highest frequency) (Table 1). We established that there was no genetic equilibrium in the examined group of Polish Large White sows. Among the Polish Landrace sows the genotype distribution met the Hardy-Weinberg principle. In the examined group of the Polish Landrace sows we observed higher heterozygosity index as compared to the group of the Polish Large White sows (Table 1).

The Polish Large White and Polish Landrace sows were nearly the same age at the time of the first littering, 359 and 358 days respectively. However, among the Polish Landrace sows higher variability was noted (Table 2). Within the group of Polish Large White sows, the youngest at the first farrowing were sows with the $PRLR^{AB}$ genotype (336 days), and in the case of the Polish Landrace those with the $PRLR^{BB}$ genotype (318 days) (Table 2). Both these groups were at the same time

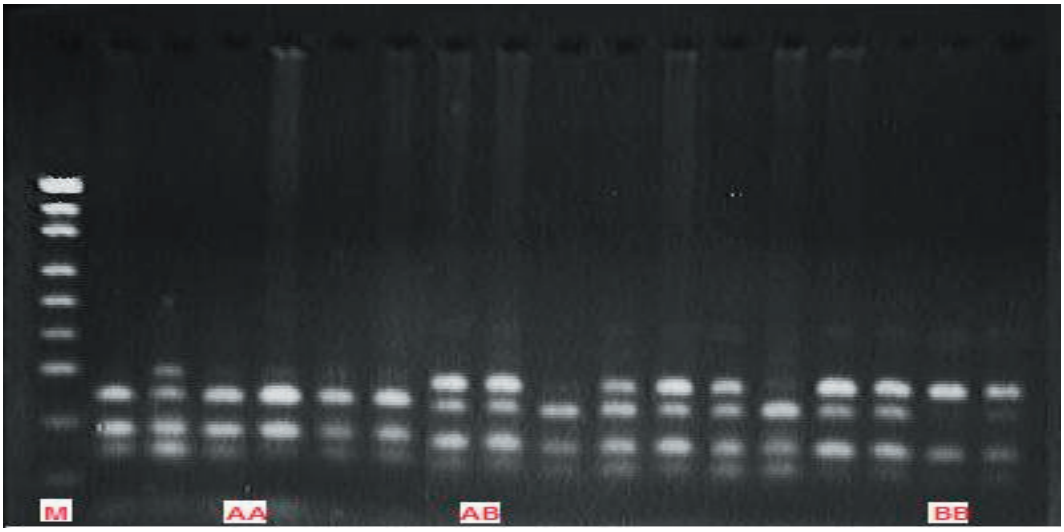

Figure 1. PRLR gene genotype identification (molecular M marker pUC19/MspI; AA, AB, BB – PRLR gene genotypes).

Rysunek 1. Identyfikacja genotypów genu PRLR (M-marker molekularny pUC19/MspI; AA, AB, BB – genotypy genu PRLR).

Table 1. Genetic structure of examined group of sows
Tabela 1. Struktura genetyczna badanej grupy loch

Structure of genetic population Struktura genetyczna populacji		Polish Large White wielka biała polska n=87	Polish Landrace polska biała zwisłoucha n=35	Total n=122
Allele frequency	*A*	0.4553	0.4897	0.4684
Frekwencja alleli	*B*	0.5448	0.5103	0.5316
Observed genotype	*AA*	0.3032	0.2401	0.2907
Frequency	*AB*	0.3041	0.5002	0.3611
	BB	0.3927	0.2597	0.3482
Obserwowana frekwencja genotypów				
Expected genotype	*AA*	0.2073	0.2398	0.2194
Frequency	*AB*	0.4961	0.4998	0.4980
	BB	0.2968	0.2604	0.2826
Oczekiwana frekwencja genotypów				
Computational chi-square Test χ^2		14.97	0.00	7.60
Heterozygosity Index Współczynnik hererozygotyczności		0.6109	0.7510	0.6545

Chi-square tab. p≤0.05 value of 5.99; p≤0.01 value of 9.21

characterized by the lowest variability.

In the first litter we observed identical mean number of piglets born in all examined genotypic groups of the Polish Large White and Polish Landrace sows. In spite of the same mean size of the 1st litter, differences in variability were found. The highest variability was observed in sows with the $PRLR^{BB}$ genotype, both in the Polish Large White as well as the Polish Landrace (Table 2). In the 2nd litter, the highest variability was observed among sows with the $PRLR^{AB}$ genotype in both examined breeds (Table 2). The Polish Landrace sows with the $PRLR^{AB}$ and $PRLR^{BB}$ genotypes gave birth on average to one more piglet in the second litter as compared to the other genotypic groups (Table 2).

The relationship between the PRLR genotypes and the age at the first farrowing, as well as the number of piglets born alive in 2 subsequent litters in the examined group of Polish Large White and Polish Landrace sows proved statistically insignificant.

Research based on analysing DNA confirmed existence of the polymorphic spot, identified by AluI restriction enzyme, in the prolactin receptor gene being candidate as reproduction traits marker. Research projects carried out all over the world concerns polymorphism analysis in the PRLR gene in relation to the reproduction traits of sows of various breeds. Examinations focus on the effect of the A allele presence on the increase in the number of piglets in a litter [9, 11, 19].

Research has been, for instance, conducted on the PIC line swine population, where the results showed significant effect (p≤0.05) of the said polymorphism on the number of offspring in a litter, and on the number of piglets born alive. The AA homozygotes were characterized by more offspring in the first (by 0.25 piglets) and subsequent litters in comparison with animals with the BB genotype [15].

Van Rens and Van der Lende [17] also established in the research they conducted that there was a positive effect of the PRLR gene polymorphism. Animals with the $PRLR^{AA}$ genotype had higher litter sizes, more implantation spots, increased number of embryos and embryos alive. They proved that reproduction traits of sows with the $PRLR^{AA}$ genotypes increase, which confirms the effect of the PRLR polymorphism on the physiological grounds of reproduction processes taking place in ovaries, uterus, and placenta.

Kmieć et al. [6], examining white swine breeds, found differences between animals with the $PRLR^{AA}$ and $PRLR^{BB}$ genotypes. Sows with the $PRLR^{AA}$ genotypes were characterized by greater sizes of the first litter, and their results were at the level of 10.51, whereas the $PRLR^{AB}$ heterozygotes gave birth to 10.44 piglets in the first litter, and the $PRLR^{BB}$ homozygotes 10.16. The observed differences proved statistically significant at

Table 2. Characteristics for reproduction traits of examined group of sows
Tabela 2. Charakterystyka statystyczna cech rozrodu badanej grupy loch

Examined traits Analizowana cecha	Statistical measures Miara statystyczna	Polish Large White wielka biała polska				Polish Landrace polska biała zwisłoucha			
		Genotype Genotyp			Total Ogólnie	Genotype Genotyp			Total Ogólnie
		AA	*AB*	*BB*		*AA*	*AB*	*BB*	
	n	26	27	34	87	8	18	9	35
Age at first ferrowing (days) Wiek pierwszego oproszenia (dni)	X	377	336	364	359	326	385	318	358
	Sx	46.89	15.75	45.64	42.30	32.62	64.90	28.70	5 8.50
Number of piglets born alive in 1st litter Liczba prosiąt żywo urodzonych w pierwszym miocie	X	11	11	11	11	11	11	11	11
	Range Rozstęp	9-13	9-12	8-13	8-13	10-12	8-14	8-14	8-14
	Sx	1.04	0.89	1.20	1.13	0.74	1.77	1.94	1.63
Number of piglets born alive in 2nd litter Liczba prosiąt żywo urodzonych w drugim miocie	X	11	11	11	11	11	12	12	11
	Range Rozstęp	10-14	10-14	10-14	10-14	11-13	9-16	7-12	7-16
	Sx	1.14	1.21	1.06	1.13	0.74	1.73	1.50	1.51

$p \leq 0.01$.

Dissimilar results, indicating positive influence of the B allele, were obtained while analysing the *PRLR* gene polymorphism in the 990 line and duroc swine bred in Poland [7].

Drogemuller et al. [3] also established positive influence of the B allele on litter sizes in the duroc breed. Isler et al. [5] claim, on the other hand, that the B allele may have a favourable effect on the increase of the number of foetuses and their weight in the Yorkshire pigs crossed with the Large White.

According to Rothschild et al. [11], the effect of particular polymorphic variants of the PRLR gene on the increase in the number of piglets in subsequent litters was estimated as being 0.25 piglets. It failed to result in any statistically significant differences between the examined animal groups.

CONCLUSION

The results obtained in the authors' own research on the Polish Large White and Polish Landrace breeds, combined with an analysis of the literature, indicate that there is a connection between the PRLR gene polymorphism with sows' reproduction traits. In the light of this research, it seems that the effect of the $PRLR^B$ allele on these traits is more favourable, which is corroborated by what other authors say. Due to the fact that no effect of the $PRLR^A$ and $PRLR^B$ alleles of the PRLR gene on reproduction traits (Age at first ferrowing (days), Number of piglets born alive in 1st litter, Number of piglets born alive in 2nd litter) has been confirmed definitively, it is advisable that such research should be continued.

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REFERENCE

[1] Ben-Jonathan N., Mershon J. L., Allen D. L., Steinmetz R. W. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocr. Rev., (1996) 17, 639-669.

[2] Charon K.M., Świtoński M. Genetics of animals, PWN Warszawa (2005).

[3] Drögemüller C., Hamann H., Distl O. Candidate gene markers for litter size in different German pig lines. J. Anim. Sci., (2001) 79: 2565-2570.

[4] Freeman M. E., Kanyicska B., Lerant A., Nagy G. Prolactin: structure, function, and regulation of secretion. Mol. Cell. Endocrinol., (2000) 80, 1523-1631.

[5] Isler, B. J., K. M. Irvin, M. F. Rothschild, and G. J. Evans. Association between the prolactin receptor gene and reproductive components in swine. In: Proc. 27th Int. Conf. Anim. Genet., Minneapolis, MN (2000) p 67.

[6] Kmieć M., Dybus A., Terman A. Prolactin receptor gene polymorphism and its association with litter size In Polish Landrace. Arch. Tierz., Dummerstorf (2001) 44: 5, 547-551.

[7] Korwin-Kossakowska A., Kamyczek M., Cieślak D., Pierzchała M., Kurył J. Candidate gene markers for reproductive traits in polish 990 pig line. J. Anim. Breed. Genet., (2003) 120, 181-191.

[8] Korwin-Kossakowska A., Sender G., Kamyczek M., Kurył J. Examining the polymorphism by beloveds of genes and seeking their connections with reproduction features of sows. Medycyna Wet., (2006) 62 (4) 468-470.

[9] Linville R. C., Pomp D., Johnson R. K., Rotshschild M. F. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. J. Anim. Sci., (2001) 79: 60-67.

[10] Rempel L. A., Nonneman D. J., Wise T. H., Erkens T. , Peelman L. J., Rohrer G. A. Association analyses of candidate single nucleotide polymorphisms on reproductive traits in swine. J. Anim. Sci. (2010) 88: 1-15.

[11] Rothschild M. F., Vincent A.L, Tuggle C. K., Evans G., Short T.H, Southwood O. I., Wales R., Plastow, G. S. A mutation in the prolactin reeeptor gene in associated with increasing litter size in pigs. Anim. Genet., (1998) 29 (suppl. 1), 60 – 74.

[12] Ruszczyc Z. Methodology of zootechnics experience. PWRiL Warszawa (1981).

[13] Sakai S., Katoh M., Berthon P., Kelly P., A. Characterization of prolactin receptors in pig mammary gland. Biochem. Journal, (1985) 224, 911-922.

[14] Terman A. Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. J. Anim. Breed. Genet., (2005) 122, 400-404.

[15] Terman A., Kmieć M., Kowalewska-Łuczak I. PRLR gene – marker of features reproductive traits dungeon? Medycyna Wet., (2007) 63 (2) 145-146.

[16] Thuy N. T. D., Thuy N. T., Cuong N. V. Genetic polymorphism of prolactin receptor gene in Mong Cai pig. Proceedings of International Workshop on Biotechnology in Agriculture (2006) 40-42.

[17] Van Rens, B., Van der Lende T. Litter size and piglet traits of gilts with different prolactin receptor genotypes. Theriogenology, (2002) 57: 883-893.

[18] Vincent A. L., Wang L., Tuggle C. K., Robic A., Rothschild M. F. Prolactin receptor maps to pig Chromosome 16. Mamm. Gen., (1997) 8, 793-794.

[19] Vincent, A. L. G., Evans T. H., Short O. I., Southwood, G. S., Plastow C. K., Tuggle, and Rothschild M. F., The prolactin receptor gene is associated with increased litter size in pigs. In: Proc.6th World Congr. Genet. Appl. Livest. Prod., Armidale, NSW, Australia (1998) 27:15–18.