Journal of Central European Agriculture, 2013, 14(2), p.1-10 DOI: 10.5513/JCEA01/14.2.1224

INFLUENCE OF THE PROLACTIN GENE POLYMORPHISM ON SELECTED REPRODUCTION TRAITS IN SOWS OF POLISH LARGE WHITE BREED*

WPŁYW POLIMORFIZMU GENU PROLAKTYNY NA WYBRANE CECHY REPRODUKCYJNE LOCH RASY WBP*

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ABSTRACT

The objective of our analyses was to identify prolactin gene polymorphism and determine its effect on reproduction traits in sows. The research included 138 Polish Large White sows from four nucleus herds supervised by the Polish Pig Breeders and Producers Association "POLSUS" based in the *kujawsko-pomorskie* province. Molecular analyses were conducted using the PCR-RFLP method. Three *PRL*/TaaI genotypes and two alleles were found in the examined sows. The allele frequency was as follows: PRL^{Ins} – 0.50; PRL^{Del} – 0.50, and the genotype frequency was at the following level: Del/Del – 0.27, Ins/Del – 0.45, Ins/Ins – 0.28. We determined the mean age at the first farrowing, and the mean number of piglets born alive and reared until 21 of their lives in two subsequent farrows. No statistically significant differences were found between the genotype variants within the analysed reproduction traits in Polish Large White pigs. The results we obtained prove that there is a need to continue research in this area using a larger group of animals.

KEYWORDS: domesticated swine, polymorphism, prolactin gene, reproductive traits

STRESZCZENIE

Celem przeprowadzonych analiz była identyfikacja polimorfizmu genu prolaktyny oraz określenie jego wpływu na cechy rozrodu loch. Badaniami objęto 138 loch rasy wielka biała polska, które pochodziły z czterech stad zarodowych objętych kontrolą Polskiego Związku Hodowców i Producentów Trzody Chlewnej "POLSUS„ z regionu kujawsko - pomorskiego. Analizy molekularne przeprowadzono za pomocą metody PCR – RFLP. W obrębie badanych loch zaobserwowano dwa allele oraz trzy genotypy *PRL/TaaI.* Frekwencja alleli wynosiła: PRL^{Ins} – 0,50; PRL^{Del} – 0,50 a frekwencja genotypów kształtowała się na poziomie: Del/Del – 0,27, Ins/Del – 0,45, Ins/Ins – 0,28. Określono średni wiek pierwszego oproszenia, średnią liczbę prosiąt żywo urodzonych i odchowanych do 21. dnia życia w kolejnych dwóch miotach. Nie stwierdzono statystycznie istotnych różnic między określonymi wariantami genotypów w obrębie analizowanych cech rozrodu świń rasy wielka biała polska. Uzyskane

wyniki wskazują na potrzebę prowadzenia dalszych badań w tym kierunku na większej liczbie zwierząt.

SŁOWA KLUCZOWE: świnia domowa, polimorfizm, gen prolaktyny, cechy reprodukcyjne

DETAILED ABSTRACT

The present work was conducted in order to identify prolactin gene polymorphic forms in domestic pigs and to determine the influence of prolactin gene variants on reproductive traits in domestic pigs. The research was conducted on Polish Large White sows, one of the maternal breeds, characterized by high fertility, fecundity and protectiveness over its offspring. The material for research was constituted by 138 sows from four herds supervised by the Polish Pig Breeders and Producers Association "POLSUS" based in the *kujawsko-pomorskie* province. Molecular tests were conducted on isolated genomic DNA, the prolactin gene genotypes were determined using the PCR – RFLP method. The frequency of alleles and genotypes was assessed and the genetic balance of the examined population was verified using the chi-square test. The influence of the genetic factor on the examined reproductive traits was examined performing the analysis of variance and the Student's t-test. Two alleles of prolactin gene were identified in the examined group: PRL^{Del} and PRL^{Ins}. The frequency of allele occurrence remained at the same level of 0.50. Three genotypes were identified, occurrence frequency of which was: 0.27 for Del/Del, 0.28 for Ins/Del and 0.45 for Ins/Ins. In respect of the mean age of first farrowing of all examined animals, the sows to give birth and rear piglets at the youngest age were the Ins/Ins genotype sows (360 days) and the oldest (370 days) were the Del/Del genotype sows (Table 1). In respect of the influence of the genetic factor on the examined reproductive traits in sows, no statistically relevant differences were observed.

The analysis of particular herds allows to observe slightly different relationships between the genotype and the examined reproductive trait (Table 2). The Ins/Ins homozygotes in herd I first gave birth at the youngest age, but the second litter was the smallest in number. The Ins/Del sows in the herd from Błędowo give birth and reared the biggest number of piglets in the first litter, while the case was the opposite in the second litter (Table 2). The Ins/Ins homozygotes form the second herd furrowed at the oldest age, which influenced the increase in the number of piglets born and reared in the first litter. The Ins/Del genotype sows form Gościeradz gave birth to the highest number of piglets, but reared the fewest (Table 2). The Ins/Ins genotype sows from the herd number three furrowed at the earliest age, while the Ins/Del heterozygotes furrowed at the oldest age. However, the latter give birth to and reared the highest number of piglets in the first litter (Table 2). The Ins/Del heterozygotes from the fourth herd reared in the first litter the lowest number of piglets up to 21 days from birth (Table 2). The differences between the examined herds proved to be statistically irrelevant.

INTRODUCTION

Prolactin gene in the domestic pig was mapped on chromosome seven, and it constitutes one of many genes connected with reproduction traits in sows

(Colenbrander B. et. al. 1998, Van Rens B. et. al. 2002). The gene encoding prolactin (protein hormone) is of high importance in the breeding of farming animals, and significantly influences proper reproduction, i.e. it affects the farrow size, lactation, and maternal instinct (Farmer C. et. al. 1999). Prolactin is synthesised by lactotropic cells of the preceding lobe of the pituitary. It was also located in the uterus, thymus, and immune system tissues. We also found it in bodily fluids, e.g. in milk, sweat, and tears (Freeman M. E. et. al. 2000, Kelly P. A. et. al. 1991). The hormone produced by the *PRL* gene reaches, by endocrine route, numerous receptors located in the central membrane of various organs, and when it merges with them, it creates a characteristic effect (Terman A et. al. 2007). Prolactin ejections, which run in cycles, have a positive effect on the development of yellow bodies, and their transformation into pregnancy bodies. Moreover, *PRL* stimulates progesterone production, which is a necessary hormone for proper pregnancy (Freeman M. E. et. al. 2000).

Apart from playing key part in reproduction, *PRL* was also observed to influence immunity in animals, participate in water-electrolyte regulation, as well as to have favourable influence on proper growth and development of cells (Bole – Feysot C. et. al. 1998). Prolactin has a positive effect on the growth and development of the mammary gland, as well as lactogenesis and lactopoesis (Dusza L. et. al. 2007). Prolactin strengthens synthesis of major proteins in milk, coordinates activity of enzymes which synthesize lipids, and participates in stimulating the synthesis of lactose (Freeman M. E. et. al. 2000).

The objective of the research was to analyze prolactin gene polymorphism in a group of maternal breed sows (Polish Large White), as well as to determine the influence of particular *PRL* genotypes on reproduction traits of the farming animals group under analysis.

MATERIAL AND METHODS

The material for research was constituted by 138 unrelated sows from the Polish Large White breed. The animals were taken from four nucleus herds (I – from Błędowo, II – from Gościeradz, III – from Borzymie, IV – from Dobrzejewice) supervised by the Polish Pig Breeders and Producers Association “POLSUS” based in the *kujawsko-pomorskie* province. The examined sows were kept in equal environmental conditions (group housing, grating system), and their physiological condition was the same (multiparous).

Genetic tests were conducted on genomic DNA isolated from peripheral blood. Isolation was based on the Master Pure DNA Purification Kit procedure prepared by Epicentre Technologies. Prolactin genotypes were determined using the PCR-RFLP method according to M Babicz et al. (2008).

The product of *PCR* gene prolactin, containing 298 base pairs, was exposed to *TaaI* restrictase for 3 hours at 65°C. DNA restriction fragments were split electrophoretically in 3.5% agarose gel with the presence of ethidium bromide. Then the gel was analysed in a transilluminator and UV light, and enotypes were identified against molecular pBR322 DNA/BsuRI.

Based on the results we obtained, we determined the genetic structure of the examined population for the analysed restriction sites, in accordance with the Hardy-Weinberg principle. We verified the genetic balance of the examined group of sows using the chi-square test (Charon K. et. al. 2009, Ruszczyc Z.1981). Taking genetic sow groups into consideration, we determined the mean age at the first farrowing, mean number of piglets born alive and reared until day 21 of their lives in two

subsequent farrows. We examined the effect of the genetic factor on selected reproduction traits, performing the analysis of variance and the Student`s t-test. The statistical analysis of obtained results was conducted with the use of Statistica 9.0 software.

RESULTS AND DISCUSSION

In the examined swine population two alleles of the PRL gene were identified: PRL^{Ins} and PRL^{Del}, as well as three genotypes – *PRL*: Del/Del (287bp, 11bp), Ins/Del (287bp, 212bp, 86bp, 11pz), Ins/Ins (212bp, 86bp).

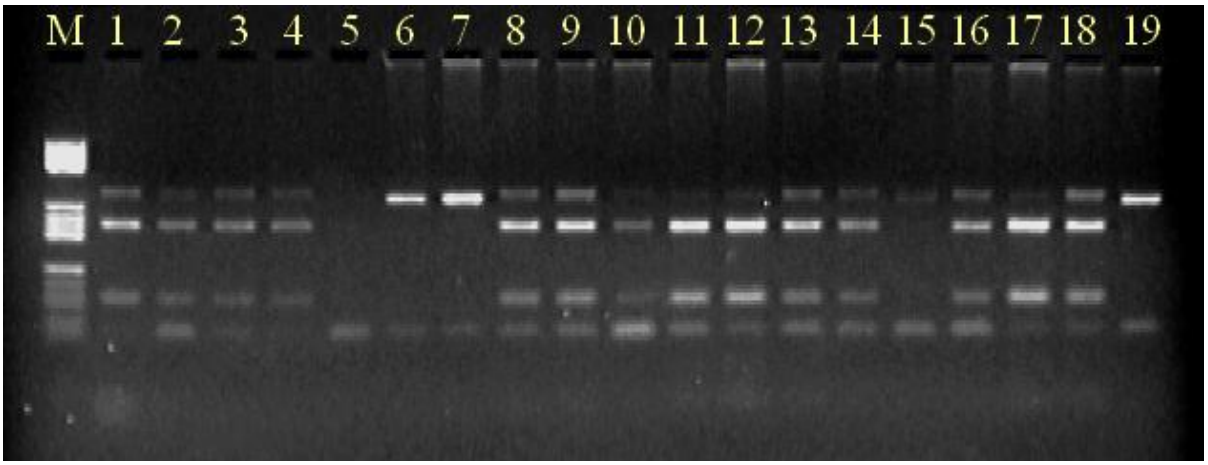

Figure 1. *PRL* gene genotype identification (molecular M marker pBR322 DNA/BsuRI;
genotypes: Del/Del – 6,7,15,19; Ins/Del – 1,2,3,4,8,9,13,14,16,18; Ins/Ins – 10,11,12,17).

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Fot. 1 Identyfikacja genotypów genu *PRL* (M – marker molekularny pBR322 DNA/BsuRI: genotyp Del/Del – 6,7,15,19; genotyp Ins/Del – 1,2,3,4,8,9,13,14,16,18; genotyp Ins/Ins – 10,11,12,17).

The frequency of *PRL* gene alleles occurrence was at the same level for Ins – 0.50, and Del – 0.50. The Ins/Del genotype frequency was the highest, equalling 0.27 for Del/Del, and 0.28 for Ins/Ins. Babicz et al. (2008), examining *puławska* sows, observed different allele frequency (Ins 0.34; Del 0.66). Among genotypes, they found the highest percentage of Del/Del (frequency 0.54). Ziółkowska et al. (2010), examining Polish Landrace and Polish Large white breeds, also obtained different results. In Polish Large white group the allele distribution was Del – 0.42, and Ins – 0.58. Whereas as regards Polish Landrace, the frequency for the Del allele was 0.63, and 0.37 for the Ins allele. The examined Polish Large White sow group in *PRL* locus had a distribution in accordance with the Hardy-Weinberg genetic balance principle (chi-square tab.: $p \leq 0.55\text{-}5.99$; $p \leq 0.01\text{-}9.21$).

Sows with the Del/Del genotype gave birth to their first piglets as the latest, i.e. on average at 370 days; they also had the highest diversity of this trait (320-550) (Table 1). Animals with the Ins/Del combination were the youngest at the time of the first farrowing, as well as had the lowest variability of this trait (Table 1). In herd II, we

observed that sows with the Ins/Ins genotype gave birth no later than at the age of $\bar{x}$ 386 days, and they had the highest variability of this reproduction trait (Table 2).

Table 1. Influence of *PRL* genotype on reproduction traits in sows of polish large white breed.
Tabela 1. Wpływ genotypu *PRL* na cechy rozrodu loch rasy wbp.

Genotype Genotyp	Measures Miary	Feature Cecha				
			I litter I miot		II litter II miot	
		Age at I farrowing (days) Wiek I oproszenia	Number of piglets born alive Liczba prosiąt żywo urodzonych	Number of piglets brought up to 21. of day of live Liczba prosiąt odchowanych do 21. dnia	Number of piglets born alive Liczba prosiąt żywo urodzonych	Number of piglets brought up to 21. of day of live Liczba prosiąt odchowanych do 21. dnia
Del/Del (n=37)	$\bar{x}$	370,27	10,92	10,51	10,89	10,70
	SD	50,25	1,28	1,30	1,15	1,08
	R	302-550	8-14	8-14	7-14	7-12
Ins/Del (n=63)	$\bar{x}$	360,35	11,10	10,89	11,35	10,94
	SD	41,30	1,33	1,32	1,23	1,05
	R	302-512	8-14	8-13	9-16	9-13
Ins/Ins (n=38)	$\bar{x}$	365,32	11,24	10,92	11,61	11,24
	SD	48,32	1,05	1,15	1,15	1,05
	R	304-548	8-13	8-13	10-14	10-13

SD- standard deviation
R- spread

The number of piglets born alive in the first farrow was the lowest among sows with the Del/Del genotype. It was the highest ($\bar{x}$ 11.24), meaning that also the variability was the lowest, in sows with the Ins/Ins genotype. Variability among animals having the Ins/Del genotype was the highest (close to variability in sows with the Del/Del genotype), and the differentiation for these animals was identical as in Del/Del animals (Table 1). Among animals from Błędowo, the least number of piglets were born from heterozygous pigs, they were also characterised by the highest variability (Table 2). In the herd from the village of Borzymie, the highest number of piglets were born from sows with the Ins/Del genotype. This was also the herd in which we noted the highest variability. In the case of homozygous pigs the value of the trait and variability were similar. In herd number III, higher variability positively translated into the value of the trait (Table 2). In the subsequent farrow, the highest number of piglets were born from sows with the Ins/Ins genotype, also having the lowest variability and differentiation of the trait. The value of the trait for animals with the Ins/Del genotype was only slightly different to that measured for Ins/Ins animals.

However, both the variability and differentiation of the trait were the highest (Table 1). In herd number I, we observed that Ins/Ins sows had the lowest value of the trait, and the lowest differentiation (Table 2). In herd number II, the highest number of piglets were born from Ins/Del sows, whereas the value of the trait in homozygous pigs was similar (Table 2). Babicz et al. (2011) report that primaparous sows give birth to and rear one piglet more as compared to multiparous sows (Babicz M. et. al. 2011). In our research, we observed a rising trend, but the difference was only 0.20 of a piglet (Table 1).

Another trait we analysed was the number of piglets reared until day 21 of their lives. As regards the first farrow, the highest value was obtained by the sows with the Ins/Ins genotype. They also had the lowest variability. The value in the sows with the Ins/Del genotype was only slightly lower than in the Ins/Ins animals. The difference was merely 0.03 of a piglet (Table 1). Within the herd from Błędowo, sows with the Ins/Del genotype reared the lowest number of piglets until day 21. The sows with the Ins/Ins genotype had the highest value for this trait. In herd number III, the highest value of the trait was found among the heterozygous pigs, whereas the trait value in homozygous pigs was similar (Table 2). In the subsequent farrow, the sows with the Ins/Ins genotype also reared the highest number of piglets until day 21. The lowest number of piglets were reared by the sows with the Del/Del genotype. This group had the highest variability and the highest differentiation of the trait (Table 1). Among animals from Błędowo, the lowest number of piglets were reared by the sows with the Ins/Ins genotype, and also the lowest variability of the trait was present there. In herd number II, the highest number of piglets reared until day 21 were those born from the sows with the Del/Del genotype. They also had the highest variability, and the value of the trait (Table 2). Among the animals from Dobrzejowice, the sows with the Ins/Ins genotype had the highest variability and the value of the trait. The sows with the Ins/Del genotype from this herd had the lowest value of the trait and the lowest variability (Table 2).

In 2007, among the Polish Large White sows, analysed as to their reproduction performance, the number of piglets from the same sow reared until day 21 was 10.58 on average in a single farrow (Blicharski T. et. al. 2008). The following year, a higher value was noted for a given trait: on average 10.80 piglets reared from a farrow (Blicharski T. et. al 2009). In the examined group of sows, animals with the Del/Del genotype had a lower value of the trait in two subsequent farrows as compared with the reproduction performance assessment conducted by “POLSUS” for 2008. Whereas the sows with the Ins/Del and Ins/Ins genotype had a higher value of the trait in two subsequent farrows as compared with statistics available in the literature on the subject. There were no significant differences between animals from various genotypic groups in terms of *PRL* locus considering the reproduction traits under examination (Table 1).

Table 2. Results breeding performance of race wbp considering individual genotype and herds.
Tabela 2. Wyniki użytkowości rozpłodowej loch rasy wbp z uwzględnieniem genotypu i stada.

Feature Cecha		Age at first farrowing (days) Wiek I oproszenia			I litter I miot						II litter II miot					
					Number of piglets born alive Liczba prosiąt żywo urodzonych			Number of piglets brought up to 21. of day of the life Liczba prosiąt odchowanych			Number of piglets born alive Liczba prosiąt żywo urodzonych			Number of piglets brought up to 21. Of day of the life Liczba prosiąt odchowanych		
Measures Miary		$\bar{x}$	SD	R	$\bar{x}$	SD	R	$\bar{x}$	SD	R	$\bar{x}$	SD	R	$\bar{x}$	SD	R
Genotype Genotyp	Herd Stado															
Del/Del	1 (n=15)	375,07	68,04	302-550	11,00	1,60	8-14	10,60	1,68	8-14	10,67	1,29	7-12	10,53	1,30	7-12
	2 (n=7)	380,29	43,77	316-439	10,43	1,40	8-12	10,14	1,21	8-12	11,14	0,90	10-12	11,00	1,15	9-12
	3 (n=8)	357,63	19,09	332-381	11,13	0,83	10-12	10,63	1,06	9-12	11,13	1,36	10-14	10,75	0,71	10-12
	4 (n=7)	364,43	39,75	308-412	11,00	0,82	10-12	10,57	0,79	10-12	10,86	0,90	10-12	10,71	0,95	10-12
Ins/Del	1 (n=17)	354,41	45,20	311-512	10,53	1,77	8-14	10,29	1,57	8-13	12,00	1,66	9-16	11,18	1,19	9-13
	2 (n=16)	372,25	54,98	313-484	10,75	1,06	9-13	10,56	1,15	9-13	11,44	1,03	10-13	10,81	0,98	10-13
	3 (n=15)	358,00	28,02	306-397	11,87	1,06	10-13	11,73	1,10	10-13	11,33	0,98	10-13	11,13	1,13	9-12
	4 (n=15)	356,73	31,15	302-402	11,33	0,82	10-13	11,07	0,96	9-13	11,07	1,16	10-14	10,60	0,83	10-12
Ins/Ins	1 (n=2)	344,50	2,12	343-346	12,00	1,41	11-13	11,00	0,00	11-11	10,50	0,71	10-11	10,50	0,71	10-11
	2	386,33	65,13	321-	11,08	1,38	8-13	10,83	1,47	8-13	11,17	1,11	10-	10,92	1,00	10-

	(n=12)			548									14			13
	3 (n=13)	351,46	37,90	304-415	11,15	0,90	10-13	10,69	1,11	9-13	11,77	1,09	10-13	11,46	1,05	10-13
	4 (n=11)	362,55	37,10	308-415	11,36	0,81	10-12	11,27	0,90	10-12	12,09	1,14	10-14	11,45	1,13	10-13

*ACKNOWLEDGMENTS

Paper in part financed by the European Social Fund, the Budget, and the budget of the Kujawsko – Pomorskie province within the Human Capital Operational Programme – priority 8, Activity 8.2, Sub – activity 8.2.2 "Regional Innovation Strategies", of the systematic project of the Kujawsko – Pomorskie province´s Local Government – "Step into the Future – PhD student grants, 3nd edition".

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