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Effect of Prolactin Receptor (PRLR) and Beta-Casein (CSN2) Gene Polymorphism on the Chemical Composition of Milk Sows

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The objective of the studies was to evaluate the impact of the prolactin receptor and β-casein genes on the basic chemical composition and pH of the colostrum and milk of sows. Experiments were carried out on 103 Złotnicka White breed sows. These animals are under the Domestic Program of Protection of Genetic Resources. Analysis of the influence of polymorphism in the PRLR and CSN2 loci revealed that sows of the TT homozygote were characterised by the highest dry matter content. Analysis of polymorphism in the PRLR locus for protein showed that the highest values were in milk of sows of the TT genotype, and GG homozygotes in the case of the CSN2 locus. Inference of the impact of polymorphism in the PRLR and CSN2 loci on the fat and lactose content of sow milk demonstrated considerable variability. These differences were statistically significant at the level of $\alpha = 0.01$ and $\alpha = 0.05$. Periodical changes in individual pH values were apparent for particular genotypes in both loci (PRLR and CSN2). The perceptible changes that occurred between individual genotypes were statistically significant at the levels of $\alpha = 0.01$ and $\alpha = 0.05$. The investigations confirmed that the nutritive values of sow colostrum and milk were determined by genetic factors. This issue warrants comprehensive analysis, especially in terms of evaluation of the breeding value of maternal breeds.

Key words: Prolactin receptor, beta-casein, milk protein, Złotnicka White, sow.

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Prolactin receptor (PRLR) is a specific prolactin receptor of the peptide hormone of the anterior pituitary gland lobe which has a number of significant functions associated with animal reproduction (VINCENT *et al*. 1997; KMIEĆ & TERMAN 2006), including the regulation of gonad functions (KELLY *et al*. 1991), influence of sow behaviour during the perinatal period and maternity (HENNIGHAUSEN *et al*. 1997; MANN & BRIDGES 2001; BABICZ *et al*. 2014) and impact on the immunological system (BESEDOVSKY *et al*. 2012). Prolactin (PRL) participates in the development of the mammary gland and stimulates milk secretion directly after parturition which has a key role for the rearing of offspring (BOLE-FEYSOT *et al*. 1998; CAPUCO & AKERS 2009). Acting through its receptor (PRLR), prolactin regulates the expression of major proteins of mammalian milk, i.e. α and β casein, stimulating an increase in its production in the mammary gland of females (ONO & OKA 1980; NEVILLE & DANIEL 1987; LEE *et al*. 2008). The activity of the PRL hormone also increases lactose synthesis (OPPAT & RILLEMA 1988) as well as lipid metabolism (VEERNON & FLINT 1983; WATERS & RILLEMA 1988). The binding of lactation hormones to the promoter part of the β casein gene (CNS2) leads to its activation. Beta-casein can be augmented by signal transducers and transcription activators (STATs). Investigations conducted by LEE *et al*. (2008) demonstrated that STAT5s positively regulate the transcription of porcine β casein. Moreover, it is known that STAT transcription activators (Stat1, Stat3 and, primarily, Stat5) bind to the intracellular domain of the long isoform of the prolactin receptor (LPRLR) causing its activation (BOLE-FEYSOT *et al*. 1998).

The aim of this investigation was to evaluate the effect of the non-synonymous substitution in the c.1528A>T position of the PRL receptor gene as

well as the silent mutation in the c.294T>G position of the β casein gene on the basic chemical composition and pH of colostrum and milk of Złotnicka White breed sows in two consecutive lactations.

Material and Methods

Animal material

Experiments were carried out on 103 sows of White Złotnicka breed. The animals were kept on a farm situated in north-western Poland rearing native swine breeds within the framework of the Domestic Program of Protection of Genetic Resources. All sows were mated naturally and their progeny was derived from one father – a boar of the Złotnicka White breed. Throughout the duration of the trial, mothers' milk was the only food of piglets, and they were not given additional concentrate.

Authorisation was given by the Local Ethics Committee (number 109/2008) to perform experiments on animals.

Feeding

Investigations comprised two consecutive lactations of the experimental sows. The sows were kept in identical conditions in compliance with the appropriate welfare requirements. The animals were kept in individual parturition pens beginning with the 10th day before farrowing until the 4th week of lactation,. The sows were fed individually twice a day with standard mixtures according to NUTRIENT REQUIREMENTS FOR PIGS (1993). Water was available *ad libitum*. The composition of the experimental diet is presented in Table 1.

Table 1

Chemical composition and energetic value of experimental mixture for sows

Nutrients	Value
Metabolisable energy	12.94 MJ min.
Crude protein	≤ 16% min.
Crude fibre	≥ 7.5% max
Crude ash	4.8 -6.8% min. - max
Crude fat	4.2-6.2% min. - max
Ca	≤ 1.0% min.
P-digestible	≤ 0.32% min.
Na	≤ 0.20% min.
Lysine	≤ 0.93% min.
Methionine	≤ 0.26% min.
Met.+Cyst.	≤ 0.54% min.
Tryptophan	≤ 0.16% min.
Threonine	≤ 0.64% min.

Mammary glands

Colostrum and milk were obtained from mammary glands of sows following earlier intramuscular injection of oxytocin in the amount of 2 to 4 ml (Oxytocinum 10 IU/ml). The quantity of the administered oxytocin depended on the day of lactation a given sow was in, and not on the weight of the animal. The later the period of lactation, the higher the dose of oxytocin; i.e. on days 1, 2 and 3 of lactation sows were given 2 ml of the hormone, on days 7 and 14-3 ml and on days 21 and 28-4 ml. Colostrum and milk from each sow were milked manually in the amount of 7 ml into specially labelled test tubes containing a special preservative (Milkostat) from 6 randomly selected and active teats. All samples were cooled down to the temperature of -20^{o}C. Samples were collected on days 1, 2, 3, 7, 14, 21 and 28 of lactation (6 teats x 7 days in each lactation x 2 lactations x 103 sows). Colostrum was determined on days 1, 2 and 3 of lactation, while the remaining days, i.e. 7, 14, 21 and 28 refer to milk. Designations L_I and L_{II} were used to refer to lactation one and lactation two.

Determination of the chemical composition and active acidity of colostrum and milk

Chemical composition and active acidity of colostrum and milk were determined in all samples collected from experimental sows in the course of the entire trial. Basic chemical composition of colostrum and milk (fat, protein, lactose, dry matter) was determined with the assistance of a Milkoscan FT 120 (Foss Electric) apparatus. The examined colostrum and milk samples were diluted using zero S-6060 Liquid (Floss Electric) at 1:3 ratio. Active acidity (pH) of colostrum and milk was determined by the electrometric method by measuring the activity of hydrogen ions using, for this purpose, a pH meter (Mettler Toledo) equipped in an electrode specific for milk measurements after calibration of the apparatus against three buffers of 4.01, 7.01 and 10.01 pH.

DNA sampling

The material for DNA isolation comprised hair bulbs collected from experimental sows. The isolation was carried out with the assistance of a Sherlock AX (A&A Biotechnology) commercial kit commonly used for DNA isolation with the addition of 20 μl DTT (dithiothreitol) in accordance with the procedure recommended by the manufacturer. After the isolation, DNA was suspended in a TE buffer and stored at a temperature of -20^{o}C. The quality

and purity of the isolated DNA samples was checked spectrophotometrically (A_{260}/A_{280}) using a SmartSpec^{TM} 3000 (BIO-RAD) spectrophotometer.

Genotyping

Polymorphism examination in the prolactin receptor (PRLR) and β casein (CSN2) genes was conducted with the assistance of the PCR-RFLP method using a PTC-200 Peltier (MJ Research) thermocycler. Primers for the prolactin receptor (PRLR) gene F: 5'-TCCCATGAACCAAGCTCTCACTGAA-3' and R:5'-CGGGGAAGGAAGGGCAACCG-3' were designed for the NC_010458.3 (GenBank) sequence using the PrimerBLAST program, whereas primers for analyses of polymorphism in the CSN2 gene comprised F: 5'-GGGTAGAACCCTTGGAGAGG-3' and R: 5'-CGCCAGAATAAAATCCACCA-3' proposed by CIEŚLAK *et al*. (2012). In both cases, primers were designed for the largest exons, i.e.: exon 8 (PRLR) and exon 7 (CSN2).

The entire volume of the PCR mixture amounted to 15 μl and contained: 7.5 μl of the reagent RED-Taq® ReadyMix™ PCR Reaction Mix (Sigma-Aldrich), 0.2 μl (5 μM) of each primer (Sigma-Aldrich), 5.9 μl water free of nucleases (Sigma-Aldrich) and 1.2 μl (20 mg/μl) DNA. The thermal conditions of the PCR were as follows: 95°C, 5 min, 36 cycles (95°C, 45 s, 62°C, 45 s, 72°C, 45 min), 72°C, 5 min, 4°C (PRLR) and 95°C, 5 min, 36 cycles (95°C, 40 s, 61°C, 40 s, 72°C, 40 s.), 72°C, 5 min, 4°C (CSN2). Upon the termination of the amplification reaction, 10 μL of the PCR mixture was used for digestion with restriction enzymes. NebCutter V2.0 (New England BioLabs® Inc.) was applied in order to adjust restriction enzymes to polymorphic sites. The following enzymes were employed to digest PCR products: N*co*I (EURx Ltd.) – PRLR; M*sp*I (Fermentas International Inc.) – CSN2. Following enzymatic digestion, the obtained fragments were separated on a 2% agarose gel containing ethidine bromide as a fluorescent marker.

Statistical analysis

The results of the above calculations were presented as least squares mean (LSM) and standard errors (SE). Frequency of occurrence of genotypes and alleles for PRLR and CSN2 was calculated in accordance with Hardy-Weindberg's law of trait distribution. The chi – square test (χ^2) for independence was applied to verify statistical hypotheses. Apart from the examined major effects, also other effects such as: season (autumn, winter, spring, summer) as well as milking of colostrum and milk (hourly determination) were also taken into consideration.

The obtained data were processed statistically using SAS ver. 8.11 (2007) and employing a test for standard normal distribution (UNIVARIATE) as well as multifactorial analysis of variance (PROC GLM LSM).

Results and Discussion

Prolactin receptor (PRLR) and beta casein (CSN2) gene genotyping

Following the digestion of the PRLR gene fragment with the N*co*I restriction enzyme (*PRLR* gene), two alleles: A (369 bp and 114 bp) and T (483 bp – absence of digestion) and three genotypes: AA, AT and TT were obtained. CSN2 gene fragment digestion using the E*sp*I restriction enzyme made it possible to identify two alleles: G (367 bp and 112 bp) as well as T (479 bp – absence of digestion) and three genotypes: TT, TG and GG (Table 2).

Figures 1 and 2 present the results of the electrophoretic separation of PCR products after enzymatic digestion.

The non-synonymous substitution c.1528A>T in the PRLR receptor gene identified with the N*co*I restriction enzyme led to a change in the amino acid sequence of the p.Met510Leu protein. On the other hand, the synonymous substitution c.294T>G in gene CSN2, identified with the M*sp*I enzyme, failed to cause a change in the amino acid sequence of the beta-casein protein in position 98. Proline

Table 2

Products of PCR and digestion with restriction enzymes NcoI and MspI

Gen	PCR	RE*	Allele	Length of fragments after digestion	SNP (dbSNP)
PRLR	483 bp	N*co*I	A T	369 bp, 114 bp, 483 bp (lack of digestion)	rs45435438
CSN2	479 bp	M*sp*I	G T	367 bp, 112 bp, 479 bp (lack of digestion)	rs55619222

*RE – restriction enzyme, bp – base pair.

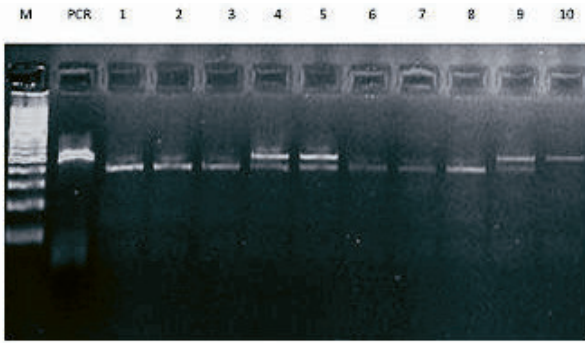

Fig. 1. PCR-RFLP products of porcine PRLR gene digested by N*co*I restriction enzyme . PCR product of PRLR gene: M – 100 bp DNA marker, PCR product (483 bp), PCR-PRLR polymorphism (N*co*I): 1, 2, 3, 6, 7, 8 – AA genotype (369/114 bp); 4, 5, 9 – AT genotype (483/369/114 bp) and 10 – TT genotype (483 bp; no cut).

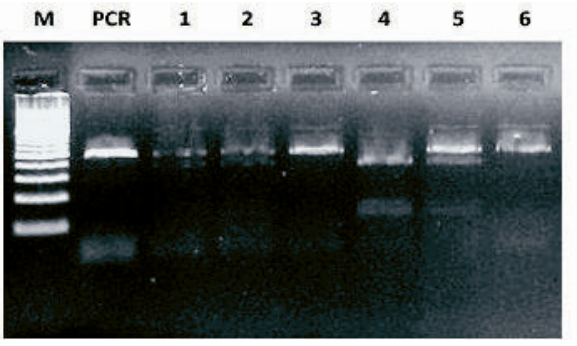

Fig. 2. PCR-RFLP products of porcine CSN2 gene digested by M*sp*I restriction enzyme. PCR product of CSN2 gene: M – 100 bp DNA marker, PCR product (479 bp), PCR-PRLR polymorphism (MspI): 3, 6 – TT genotype (479 bp; no cut), 1, 2, 5 – TG genotype (479/367/112 bp); and 4 – GG genotype (367/112 bp).

coded by both CCT and CCG codons occurred in this position. Table 3 presents the location of polymorphism and type of changes in the DNA sequence.

Frequency of alleles and genotypes of PRLR and CSN2 genes

Table 4 shows the frequency of occurrence of alleles and genotypes of prolactin receptor (PRLR) and beta-casein (CSN2) in the examined swine population of White Złotnicka breed calculated in accordance with the Hardy-Weinberg law. The frequency of A and T alleles in the PRLR locus (rs45435438) amounted to 0.694 and 0.306, respectively, while that of the identified genotypes – 0.495 (AA), 0.398 (AT) and 0.107 (TT). The frequency of T and G alleles in the CSN2 locus

Table 3

Site of polymorphism, type of changes in DNA sequence

Gene	Exon	Type of variation	SNP	Position in CDS	Position in AA sequence	Amino acid	Codon
PRLR	8	non-synonymous substitution	A>T	1528	510	M/L	ATG/TTG
CSN2	7	synonymous substitution	T>G	294	98	P	CCT/CCG

Table 4

Frequency of genotypes and alleles controlling PRLR and CSN2 polymorphism in accordance with Hardy-Weinberg's law in the examined population of Złotnicka White breed swine

Genotype	Number of genotypes	Frequency of genotypes	Frequency of alleles	χ^2	Critical value for significance levels	
					0.05	0.01
PRLR						
AA	51	0.495	A=0.694	0.0395		
AT	41	0.398		0.1716		
TT	11	0.107	T=0.306	0.1885		
Σ	103	1	1	0.3996	5.991	9.210
CSN2						
TT	72	0.699	T= 0.821	0.096		
TG	25	0.243		0.921		
GG	6	0.058	G= 0.179	2.209		
Σ	103	1	1	3.226	5.991	9.210

χ^2 – tabl. n-1= 2; α= 0.01; χ^2 – tabl. n-1= 2; α= 0.05.

(rs55619222) amounted to: 0.821 and 0.179, respectively, whereas that of genotypes: 0.699 (TT), 0.243 (TG) and 0.058 (GG).

Dry matter and protein in colostrum and milk of examined sows

The impact of the genotypes identified in PRLR and CSN2 loci on the content of dry matter (Table 5) and protein (Table 6) in the colostrum and milk of Złotnicka White breed sows was analysed.

The percentage content of dry matter differed in both lactations (L_I and L_{II}).

The performed statistical analysis did not show a significant influence of the PRLR locus polymorphism (rs45435438) on dry matter content in the colostrum and milk of sows during the first lactation (L_I). An impact was observed only on the 21st and 28th days of the second lactation (L_{II}), when the highest dry matter content was recorded in the case of TT homozygote sows (20.60% and 20.22%). The observed significant variability in the remaining days failed to confirm any noticeable tendency in favour of any of the examined genotypes.

The identified polymorphism in CSN2 (rs55619222) locus significantly differentiated dry matter content on the 7th day of the first lactation (L_I) as well as on days 1 and 2 of the second lactation (L_{II}) at the level of $\alpha = 0.01$. The 21st and 28th days of lactation also significantly differentiated the content of dry matter in milk at the level of $\alpha = 0.05$. The highest values were observed in colostrum and milk of homozygote CSN2-TT sows.

The PRLR locus polymorphism (rs45435438) also turned out to be significant for protein content (Table 6). The highest significant level of protein was obtained on days 1 and 14 of the first lactation (L_I) as well as on days 1, 3 and 7 of the second lactation (L_{II}). Colostrum and milk characterised by the highest protein levels was derived from homozygote TT sows.

A significant impact of the CSN2 locus polymorphism on protein levels (Table 6) was recorded on days 1, 2 and 14 (L_I) as well as on days 1 and 14 (L_{II}) of lactation.

High protein content was determined in CSN2-GG homozygote sows on the 1st (11.36%) and 2nd (8.18%) days of the first lactation (L_I). Also in the second lactation (L_{II}), CSN2-GG homozygote sows yielded colostrum and milk of the highest percentage protein content. These differences were significant at the level of $\alpha = 0.01$ and $\alpha = 0.05$.

Earlier reports corroborate a significant role for the identified polymorphisms in the PRLR locus (BRYM *et al*. 2005; VIITALA *et al*. 2006; LU *et al*. 2011) as well as in the locus CSN2 locus (NG-KWAI-HANG *et al*. 1984; CERIOTTI *et al*. 2004; COMIN *et al*. 2008; CAROLI *et al*. 2009; BONFATTI *et al*. 2010; SKRZYPCZAK *et al*. 2012) in milk production and chemical composition.

Table 5

Impact of PRLR and CSN2 genotypes on dry matter content in colostrum and milk of sows

Trait		DL	PRLR						CSN2					
			AA		AT		TT		TT		TG		GG	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Dry matter (%)	L_I	1	27.47	0.50	27.28	0.56	27.15	1.12	27.37	0.71	27.28	0.42	28.44	1.58
		2	25.67	0.43	25.47	0.48	26.72	0.95	26.07	0.60	25.54	0.36	26.02	1.34
		3	24.05	0.38	23.91	0.42	23.93	0.84	24.23	0.52	23.86	0.31	24.45	1.11
		7	23.28	0.31	23.41	0.35	23.63	0.70	24.33^{A}	0.43	23.00^{B}	0.26	23.50	0.97
		14	21.80	0.31	22.32	0.34	21.40	0.69	22.43	0.43	21.87	0.26	21.06	0.97
		21	21.40	0.29	21.88	0.22	21.33	0.55	22.32	0.33	21.17	0.21	20.88	1.09
		28	20.83	0.31	20.81	0.35	21.08	0.69	21.19	0.44	20.73	0.26	20.63	0.98
	L_{II}	1	25.90	0.52	25.82	0.58	25.65	1.17	27.40^{A}	0.72	25.29^{B}	0.43	25.78	1.61
		2	22.77	0.45	23.70	0.51	23.29	1.01	24.26^{A}	0.64	22.72^{B}	0.38	24.17	1.42
		3	21.83	0.47	22.18	0.12	21.99	1.04	22.59	0.65	21.85	0.39	20.82	1.46
		7	21.32	0.44	20.60	0.49	21.10	0.98	21.79	0.61	20.76	0.37	20.51	1.38
		14	19.89	0.38	20.37	0.42	19.84	0.84	20.77	0.53	19.83	0.31	20.17	1.19
		21	19.24^{a}	0.26	19.13^{a}	0.29	20.60^{b}	0.58	20.01^{a}	0.37	19.12	0.22	18.79^{b}	0.81
		28	19.11^{a}	0.21	19.09^{a}	0.30	20.22^{b}	0.42	19.77^{a}	0.43	19.01	0.12	18.30^{b}	0.87

DL – day of lactation; L_I, – first lactation; L_{II} – second lactation;
In rows, means designated with different capital letters (A, B) differ statistically at the level of $\alpha = 0.01$In rows, means designated with different small letters (a, b) differ statistically at the level of $\alpha = 0.05$.

Table 6

Impact of PRLR and CSN2 genotypes on protein content in colostrum and milk of sows

Trait		DL	PRLR						CSN2					
			AA		AT		TT		TT		TG		GG	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Protein (%)	L_I	1	8.89^{A}	0.43	9.22^{A}	0.48	12.52^{B}	0.95	7.74^{Ab}	0.60	9.83^{a}	0.36	11.36^{B}	1.35
		2	6.47	0.25	6.71	0.28	7.24	0.55	6.04^{A}	0.34	6.74	0.20	8.18^{B}	0.77
		3	5.06	0.16	5.40	0.17	5.20	0.35	5.14	0.22	5.24	0.13	5.11	0.49
		7	4.44	0.11	4.74	0.13	4.31	0.25	4.49	0.16	4.57	0.09	4.51	0.36
		14	4.16	0.07	4.04^{a}	0.08	4.46^{b}	0.17	3.89^{A}	0.10	4.23	0.06	4.24^{B}	0.23
		21	4.08	0.12	4.00	0.10	4.17	0.22	3.99	0.12	4.10	0.11	3.90	0.34
		28	3.99	0.08	4.01	0.09	4.23	0.18	3.90	0.11	4.07	0.07	3.88	0.26
	L_{II}	1	9.70	0.40	8.72^{a}	0.44	11.17^{b}	0.88	7.48^{Aa}	0.53	10.1^{b}	0.32	10.17^{B}	1.19
		2	6.24	0.22	6.33	0.25	7.15	0.50	6.20	0.32	6.37	0.19	7.08	0.71
		3	5.28^{a}	0.13	5.73	0.14	5.90^{b}	0.29	5.56	0.19	5.51	0.11	5.48	0.42
		7	4.70^{a}	0.08	5.00	0.09	5.28^{b}	0.19	4.89	0.12	4.86	0.07	5.09	0.28
		14	4.30	0.07	4.37	0.08	4.64	0.16	4.23^{a}	0.09	4.37^{a}	0.06	4.85^{b}	0.22
		21	4.32	0.06	4.37	0.05	4.53	0.13	4.30	0.08	4.38	0.05	4.31	0.18
		28	3.99	0.10	3.98	0.09	4.22	0.08	4.23	0.24	4.01	0.13	3.99	0.11

DL – day of lactation; L_I, - first lactation; L_{II} – second lactation;
In rows, means designated with different capital letters (A, B) differ statistically at the level of $\alpha = 0.01$;
In rows, means designated with different small letters (a, b) differ statistically at the level of $\alpha = 0.05$.

However, this role is analysed, primarily, with respect to cattle as the main farm animal species producing milk for consumption purposes. In the case of pigs, the role of milk is limited to the rearing of piglets for which it is the sole source of nutrients indispensible for life. Therefore, it can be said that its quantity and quality is decisive for the proper development of piglets during the first critical period of life which translates into achieved production results (DEVILLERS *et al*. 2004; SKRZYPCZAK *et al*. 2013).

Immunoglobulins found in colostrum ensure appropriate resistance to pathogenic factors and promote maturation of the immunological system, particularly during the first days of newly born animals. Easily digestible proteins provide a source of valuable amino acids which are essential for growth and development (ZIMECKI & ARYTM 2005; WHEELER *et al*. 2007; SANGILD 2003; STELWAGEN *et al*. 2009). In addition, together with calcium and vitamin D, milk proteins play an important role in maintaining proper bone structure (BONJOUR 2005).

Fat and lactose in colostrum and milk of sows

Analysis of the impact of polymorphism in the PRLR and CSN2 loci on the fat content of sow milk revealed considerable variability (Table 7).

The highest percentage fat content in the PRLR locus was reached by AA homozygotes on days 1 (L_I) and day 7 (L_{II}) of lactation and by TT homozygotes on the 3rd day of lactation (L_{II}). These differences were significant at the level of $\alpha = 0.01$ and $\alpha = 0.05$. On the other hand, in the case of the CSN2 locus, the highest significant percentage fat content was recorded in TG homozygotes on days 1, 2 and 3 (L_I) as well as on day 3 (L_{II}) of lactation.

An identical correlation was observed in the case of lactose (Table 8). In addition, it demonstrated considerable variability.

The influence of the PRLR locus polymorphism on lactose content, despite the observed significant differences in both analysed lactations (L_I and L_{II}), was not clear as indicated by variation in levels of this constituent without any discernible tendency in favour of any of the identified genotypes.

Sows with the GG genotype in locus CSN2 were characterised by the lowest lactose level during both examined lactations. The only exception was milk derived from the 7th day of both lactations. Then sows of CSN2-TT homozygotes produced milk characterised by the lowest lactose value. The recorded differences were highly significant.

It is worth stressing that GG homozygotes in locus CSN2, in which the lowest percentage content of fat and lactose were determined, were characterised by the highest protein content in colostrum and milk. This correlation was also reported in earlier investigations carried out by VELMALA *et al*. (1995), FREYER *et al*. (1999) and IKONEN *et al*. (2001).

Table 7

Impact of PRLR and CSN2 genotypes on fat content in colostrum and milk of sows

Trait		DL	PRLR						CSN2					
			AA		AT		TT		TT		TG		GG	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Fat (%)	L_I	1	5.52^{a}	0.19	4.93^{b}	0.22	5.08	0.43	4.71	0.26	5.49^{a}	0.16	4.39^{b}	0.60
		2	6.42	0.19	6.35	0.21	6.54	0.42	6.32	0.25	6.53^{a}	0.15	5.11^{b}	0.58
		3	6.50	0.20	6.07	0.22	6.56	0.44	5.92^{a}	0.26	6.61^{Ab}	0.16	4.47^{Bb}	0.57
		7	6.57	0.15	6.70	0.17	6.62	0.35	6.30	0.21	6.72	0.12	6.92	0.48
		14	7.30	0.17	7.06	0.19	7.20	0.38	7.26	0.24	7.15	0.14	7.45	0.54
		21	7.55	0.23	7.29	0.11	7.86	0.27	7.38	0.22	7.20	0.18	7.99	0.66
		28	8.07	0.17	7.94	0.19	8.01	0.38	7.96	0.23	8.00	0.14	8.37	0.53
	L_{II}	1	4.85	0.18	4.89	0.20	5.26	0.41	4.70	0.25	5.03	0.15	4.23	0.57
		2	6.30	0.18	6.64	0.21	6.27	0.52	6.60^{a}	0.24	6.48^{a}	0.11	5.05^{b}	0.44
		3	6.25^{a}	0.19	5.80^{A}	0.21	7.19^{Bb}	0.42	5.80^{Aa}	0.26	6.44^{Ab}	0.15	4.06^{B}	0.58
		7	6.64^{a}	0.15	6.16^{b}	0.17	6.63	0.34	6.30	0.21	6.46	0.13	7.04	0.48
		14	7.17	0.18	6.63	0.20	6.44	0.40	6.83	0.26	6.90	0.16	6.92	0.58
		21	7.40	0.14	7.31	0.16	7.70	0.33	7.52	0.21	7.36	0.12	7.28	0.46
		28	7.88	0.16	7.55	0.21	8.04	0.17	7.55	0.20	7.45	0.18	7.33	0.33

DL – day of lactation; L_I, – first lactation; L_{II} – second lactation;
In rows, means designated with different capital letters (A, B) differ statistically at the level of $\alpha = 0.01$;
In rows, means designated with different small letters (a, b) differ statistically at the level of $\alpha = 0.05$

Table 8

Impact of PRLR and CSN2 genotypes on lactose content in colostrum and milk of sows

Trait		DL	PRLR						CSN2					
			AA		AT		TT		TT		TG		GG	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Lactose (%)	L_I	1	3.95	0.14	4.04	0.16	3.52	0.31	4.17^{a}	0.20	3.91	0.12	3.12^{b}	0.44
		2	4.05^{a}	0.16	4.57^{b}	0.17	4.23	0.35	4.96^{A}	0.21	4.12^{Ba}	0.11	2.99^{Bb}	0.46
		3	4.42	0.12	4.15	0.13	4.49	0.26	4.02^{Aa}	0.15	4.50^{B}	0.09	3.24^{Ab}	0.35
		7	5.73^{A}	0.12	4.92^{Bb}	0.14	5.59^{a}	0.27	4.93^{a}	0.18	5.56^{b}	0.11	5.38	0.40
		14	5.55^{a}	0.12	5.97^{b}	0.13	5.29^{a}	0.28	6.03^{a}	0.18	5.61^{b}	0.10	5.04^{b}	0.39
		21	5.32	0.17	5.66	0.09	5.40	0.13	5.58	0.12	5.59	0.09	5.44	0.22
		28	5.54	0.10	5.73	0.11	5.69	0.23	5.63	0.14	5.60	0.09	6.04	0.32
	L_{II}	1	3.73	0.14	3.71	0.15	3.44	0.31	4.03^{A}	0.19	3.63	0.11	2.81^{B}	0.42
		2	3.83	0.15	4.27^{a}	0.17	3.43^{b}	0.33	4.62^{A}	0.20	3.82^{Ba}	0.12	2.65^{Bb}	0.44
		3	4.16	0.12	4.06	0.13	4.57	0.26	4.13^{A}	0.16	4.27^{A}	0.09	2.83^{B}	0.35
		7	5.53^{A}	0.01	4.78^{Ba}	0.11	5.37^{b}	0.22	4.83^{A}	0.15	5.36^{B}	0.08	5.02	0.33
		14	5.41	0.08	5.39	0.09	5.38	0.19	5.29	0.12	5.44	0.07	5.32	0.27
		21	5.46	0.06	5.41	0.07	5.59	0.15	5.40	0.09	5.44	0.06	5.85	0.21
		28	5.51	0.10	5.55	0.11	5.62	0.16	5.55	0.18	5.67	0.17	5.92	0.33

DL – day of lactation; L_I, – first lactation; L_{II} – second lactation;
In rows, means designated with different capital letters (A, B) differ statistically at the level of $\alpha = 0.01$;
In rows, means designated with different small letters (a, b) differ statistically at the level of $\alpha = 0.05$.

In the case of both examined lactations, an increase in the percentage content of fat and lactose with each consecutive day of lactation was apparent. The highest values were recorded during the last days of lactation irrespective of the genotype of the examined sows.

This situation can be explained by growing requirements of piglets for energy whose main source is fat and lactose. Moreover, lactose is important in the restoration of glycogen reserves utilised for thermoregulation which is particularly important during the early stage of piglet development (NENAUDEAU *et al*. 1997; RENAUDEAU *et al*. 2001; LABUSSIERE *et al*. 2011).

pH in colostrum and milk of sows

In both analysed loci (PRLR and CSN2), periodical changes in pH values for individual genotypes were visible (Figs 3 and 4). The impact of PRLR and CSN2 polymorphism was not unequivocal and exhibited massive variability in relation to genotypes. None of the examined

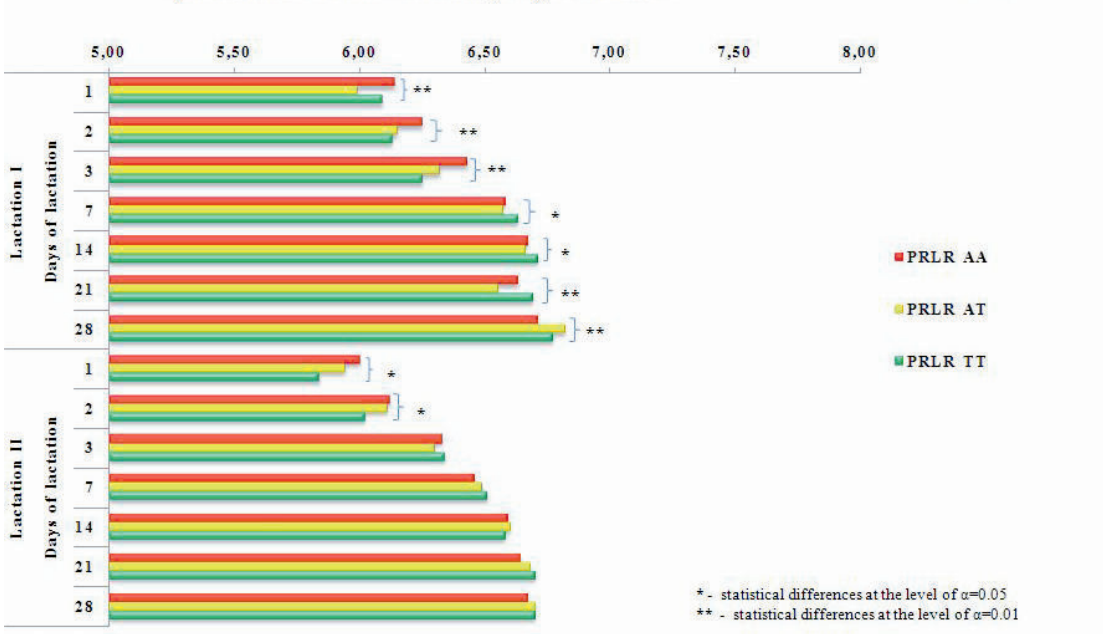

Fig. 3. pH of colostrum and milk in relation to genotypes in PRLR loci.

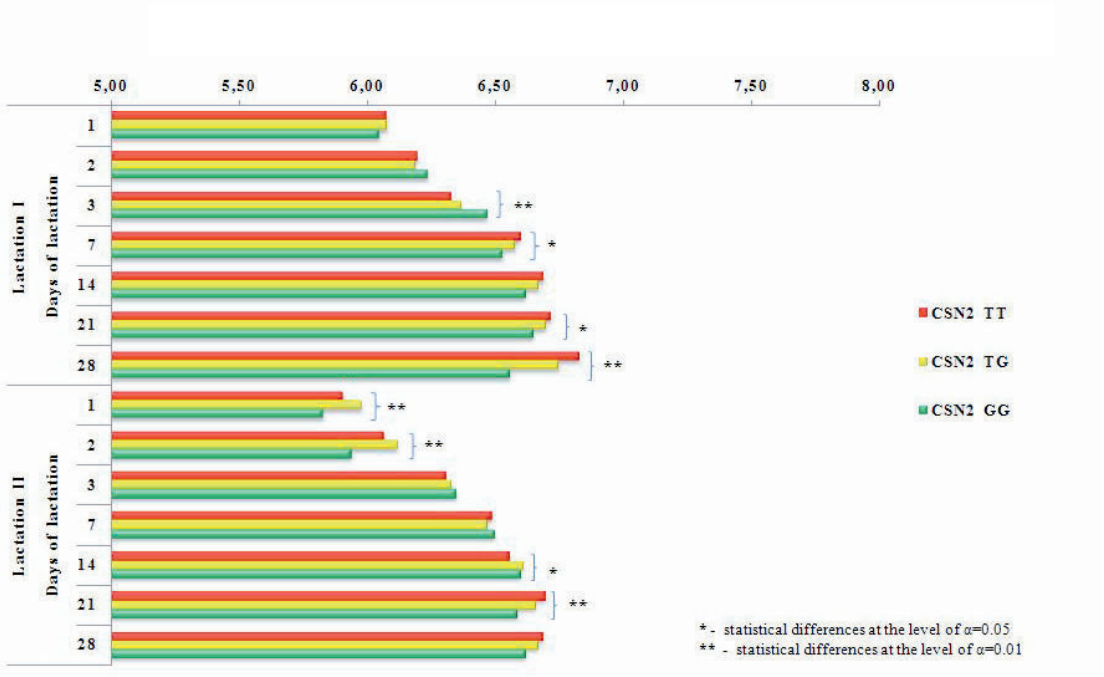

Fig. 4. pH of colostrum and milk in relation to genotypes in CSN2 loci.

genotypes showed a significant advantage. The apparent changes that occurred between individual genotypes were significant at the level of $\alpha = 0.01$ and $\alpha = 0.05$.

During the period of two consecutive lactations, pH of colostrum and milk exhibited a growing tendency with each successive day.

After birth, sucklings are incapable of manufacturing appropriate quantities of gastric juices and, therefore, it is recommended to avoid feeds which might increase pH in the gastrointestinal tract as this may contribute to a number of health problems. In addition, hydrochloric acid produced in the stomach not only acidifies the chyme protecting it against multiplication of undesirable microflora, but it also initiates digestive processes occurring in the stomach lumen (CANIBE *et al*. 2010; REZAEI *et al*. 2013).

Conclusion

The genetic potential of piglets inherited from parents is insufficient to reach high body weight gains without ensuring simultaneously optimal maintenance conditions and high feed quality. Environmental conditions are particularly important during the initial, critical period of life of the young when mother's colostrum and milk provide the sole nutrients responsible for maintaining proper growth and development of the organism and also immunizing it against ubiquitous pathogens. It is clear from these investigations that the nutritive value of colostrum and milk of sows is determined by genetic factors. Therefore this issue should be subject to a more comprehensive analysis, especially in terms of an assessment of the breeding value of maternal breeds in which lactation yield expressed by litter of piglet weight is one of the selection criteria of the breeding work.

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