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DEPENDENCIES BETWEEN *PRL* GENE POLYMORPHISM AND PRODUCTION PROGRESS IN POLISH HOLSTEIN-FRIESIAN BLACK-AND-WHITE COWS

ZALEŻNOŚCI MIĘDZY POLIMORFIZMEM GENU *PRL* A POSTĘPEM PRODUKCYJNYM U KRÓW RASY POLSKIEJ HOLSZTYŃSKO-FRYZYJSKIEJ ODMIANY CZARNO-BIAŁEJ

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Streszczenie. Celem badań była analiza zależności pomiędzy polimorfizmem genu *PRL* w obrębie 4 eksonu 23 bydlęcego chromosomu (*locus g.8398G>A*) a wielkością postępu produkcyjnego w zakresie wydajności mleka, tłuszczu i białka w mleku u krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej. Wyniki przeprowadzonych badań świadczą o występujących zależnościach między genotypem w *locus g.8398G>A* genu *PRL* a postępem produkcyjnym w zakresie wydajności mleka oraz tłuszczu i białka w mleku. Wykazano, że największym postępem produkcyjnym oraz postępem kumulowanym w wydajności mleka oraz wydajności tłuszczu i białka w mleku charakteryzowały się heterozygoty AG w *locus g.8398G>A*, natomiast najmniejsze wartości analizowanych parametrów uzyskały homozygoty *AA.*

Key words: cattle, polymorphism, *PRL*, production progress.
Słowa kluczowe: bydło, polimorfizm, *PRL*, postęp produkcyjny.

INTRODUCTION

The bovine prolactin gene (*PRL*) is located within chromosome 23, it is approx. 10 kb in length and comprises five exons and four introns (Camper et al. 1984). Prolactin is expressed mainly in the pituitary gland by a process regulated by many transcription factors (Toda et al. 2008). PRL serves numerous versatile functions, e.g. it stimulates mammogenesis, colostrogenesis, lactogenesis, galactopoesis and galactokinase activity. Li et al. (2006) presented an opinion that prolactin serves an important role in the development of the mammary gland and milk production. For this reason, this gene has been selected as a marker of milk production traits in cattle. Accorsi et al. (2002) stated that this hormone also plays a primary role in the controlled involution of epithelial cells of the udder in cattle. QTLs found at chromosome 23 in cattle are responsible for variation in the yield of milk protein (Georges et al. 1995) as well as yields and contents of milk fat and conformation traits (Ashwell and Van Tassell 1999).

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In cattle more than a dozen polymorphisms have been found within the *PRL* gene. Results reported to date concerning the dependence between prolactin genetic variants and production traits have been inconclusive. For this reason analyses proposed within this study may provide new insights into the dependence between genotypes at *locus g.8398G>A* of the *PRL* gene and the level of obtained production progress in dairy cows. From the point of view of milk production economics the most desirable solution is to obtain maximum production progress within a relatively short time, while at the same time ensuring no deterioration of performance traits of cows and no increase in financial outlays on milk production. In the opinion of Atashi et al. (2006), it is possible to simultaneously improve lactation persistency and milk yield. This objective may be realised on condition we find a respective genetic marker, which would be of great value both for dairy cattle breeders and milk producers. Literature sources lack reports concerning a dependence between genotype at *locus g.8398G>A* of the *PRL* gene and production progress in terms of milking performance traits. The authors decided to investigate this problem in view of its practical importance and a lack of literature sources on the subject.

The aim of the study was to analyse the dependence between the *PRL* gene polymorphism at *locus g.8398G>A* and the level of production progress in terms of milk yield, as well as the yield of fat and milk protein in Polish Holstein-Friesian Black-and-White cows.

MATERIAL AND METHODS

Analyses included 1099 Polish Holstein-Friesian Black-and-White cows. Cows were kept at six farms from the Wielkopolska region (Poland).

The study comprised analyses of the polymorphic site (*locus g.8398G>A*) of the *PRL* gene using PCR-RFLP.

The scheme of the experiment comprised several stages:

a) isolation of genetic material – DNA (from peripheral blood, taken from the jugular vein) was performed applying phenol extraction;
b) amplification of a DNA fragment (156 bp) – was conducted with a TGradient thermocycler (Biometra) using primer sequences provided by Mitra et al. (1995); PRLF (5'-CGAG TCCTTATGAGCTTGATTCTT-3') and PRLR (5-GCCTTCCAGAAGTCGTTTGTTTTC-3'). The reaction mixture of 15 μl consisted of 90 ng genomic DNA, 0.6 U *Taq* polymerase, 10 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM dNTP, 1.5 μl PCR buffer – $(NH_4)_2SO_4$ (10x) and 0.75 μl DMSO. Following initial denaturation (94°C/5 min) 30 cycles were run comprising denaturation (94°C/30 sec), primer annealing (53.7°C/30 sec) and synthesis (72°C/30 sec), followed by final synthesis (72°C/5 min);
c) digestion of PCR products using a restriction enzyme. The amplification product was digested for 3 h with a restriction enzyme (*Rsa*I) at 37°C with Buffer Tango™. The composition of the reaction mixture (11 μl) for one sample was as follows: 5 μl PCR product, 1 μl restriction enzyme at 10 U/μl (Fermentas), 1μl enzyme buffer (Fermentas) and 4 μl H_2O;
d) verification of digestion products using agarose gel electrophoresis. Following restriction enzyme digestion each sample was supplemented with 2 μl loading buffer (Gel Loading

Solution type I, 6x). Afterwards the digestion products were verified using electrophoresis in 3% agarose gel (BASICA GQT, Prona) in 1 x TBE buffer. The DNA Gene Ruler™ marker in the DNA Ladder Mix was applied in a mixture consisting of 2 μl loading buffer, 1.5 μl DNA marker and 10.5 μl H_2O. The settings for electrophoresis time and applied voltage were 30 minutes and 150 V, respectively. Digestion products were examined in UV light;

e) identification of genotypes. The amplified fragment of the *PRL* gene was 156 bp. The following genotypes were identified: GG – 156 bp (no site recognised by the *Rsa*I restriction enzyme), AG – fragments of 156, 82 and 74 bp, and AA – fragments of 82 and 74 bp;

f) statistical analysis. Frequency of alleles *A* and *G* was 0.2106 and 0.7894, respectively, while genotype frequencies were GG = 0.6087, AG = 0.3613 and AA = 0.0300.

Cows came from herds with an average milk yield in 305-day lactation ranging from 4500 to 8000 kg milk. Animals were in their 1st to 10th lactations. Milk recording data provided information on 3365 305-day lactations from the period of 2002–2009. Successive lactation ranks in the overall number of lactations accounted for the following shares: 1 – 28.4%, 2 – 25.5%, 3 – 20.1%, 4 – 13.4%, 5 – 7.0%, 6 – 3.3%, 7 – 1.5%, 8 – 0.5%, 9 – 0.2% and 10 – 0.1%. Milk yield as well as yields of milk fat and milk protein were recorded for each cow.

In this study annual production progress and cumulative progress in the period of 2002– 2009 were calculated for milk yield as well as yields of milk fat and milk protein for the investigated population of cows depending on genotypes at *locus g.8398G>A* of the *PRL* gene.

Statistical analyses were conducted using the following linear model:

$$Y_{ijklmnop} = \mu + H_i + R_j + S_k + L_l + G_m + \beta_1 hfn + \beta_2 wco + e_{ijklmnop}$$

where:

$Y_{ijklmnop}$ – phenotypic value of analysed trait,
μ - population mean,
H_i- fixed effect of herd (i = 1,…,6),
R_j- fixed effect of calving (j = 1,…,8),
S_k- fixed effect of calving season (k = 1,…,4),
L_l- fixed effect of lactation rank (l = 1,…,10),
G_m – fixed effect of genotype at *locus g.8398G>A* (l = 1,…,3),
β_1, β_2 – partial first-order linear regression coefficients:
hfn – share of HF genes in the genotype,
wco – age at first calving in days,
$e_{ijklmnop}$ – random residual effect.

Statistical calculations were performed using the SAS® statistical software package (2011) using the MEANS and GLM procedures.

Object means were compared using the Duncan multiple range test.

RESULTS

Table 1 presents the calculated annual production progress and cumulative progress for milk yield in the years of 2002–2009 for the investigated population of cows in terms of genotyping at *locus g.8398G>A* of the *PRL* gene.

Table 1. Production progress for milk yield [kg] in the years 2002–2009 for the studied population of Holstein-Friesian Black-and-White cows[a] with regard to genotyping at *locus g.8398G>A*

Tabela 1. Postęp produkcyjny dla wydajności mleka [kg] w latach 2002–2009 w badanej populacji krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej[a], z uwzględnieniem podziału na genotypy w *locus g.8398G>A*

Year Rok **	Genotype at *locus g.8398G>A* – Genotyp w *locus g.8398G>A*														
	GG *					AG *					AA *				
	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%
2002	5862.0			ABC DEF		5654.1	a		ABC DEFa		5846.2			ABC	
2003	6151.6 a	289.6 A	104.9	289.6	104.9	6064.9 b	410.8 Ab	107.3	410.8 a	107.3	5481.0 ab	–365.2	93.8	–365.2	93.8
2004	6595.4	443.8 AB	107.2	733.4 A	112.5	6436.6	371.7 Ab	106.1	782.5 A	113.8	6426.4	945.4	117.3	580.2	109.9
2005	7060.7 a	465.3 B	107.0	1198.7 B	120.4	7112.5 b	675.9 A	110.5	1458.4 B	125.8	6436.6 ab	10.2	100.2	590.4	110.1
2006	7104.1	43.4 C	100.6	1242.1 C	121.2	7240.3 a	127.8	101.8	1586.2 C	128.1	6649.8 a	213.2	103.3	803.6	113.7
2007	7635.4	531.3 CD	107.5	1773.4 D	130.3	7606.3	366.0 c	105.1	1952.2 D	134.5	7223.9	574.1	108.6	1377.7 A	123.6
2008	8263.0 a	627.6 D	108.2	2401.0 E	141.0	7994.7	388.4 c	105.1	2340.6 E	141.4	7694.9 a	471.1	106.5	1848.7 B	131.6
2009	8291.9 a	28.9	100.3	2429.9 F	141.5	8272.9 b	278.2	103.5	2618.8 F	146.3	7519.9 ab	–175.0	97.7	1673.7 C	128.6
$\bar{x}$		347.1					374.1					239.1			

$x_i–x_{i–1}$ – annual production progress – roczny postęp produkcyjny, $x_i–x_0$ – production progress in relation to the initial year (cumulative production progress) – postęp produkcyjny w stosunku do roku początkowego (kumulowany postęp produkcyjny); ** statistically significant effect at $P \leq 0.01$ – wpływ statystycznie istotny przy $P \leq 0,01$; * statistically significant effect at $P \leq 0.05$ – wpływ statystycznie istotny przy $P \leq 0,05$. Means marked with the same letters are statistically different – Średnie oznaczone tymi samymi literami różnią się statystycznie: A, B, C – at $P \leq 0.01$ – przy $P \leq 0,01$; a, b, c – at $P \leq 0.05$ – przy $P \leq 0,05$; with $x_i–x_{i–1}$ differences between years are marked – przy $x_i–x_{i–1}$ oznaczono różnice między latami; for $x_i–x_0$ differences in relation to the initial year within the group are indicated; mean differences between individual genotypes at *locus g.8398G>A* were determined – przy $x_i–x_0$ oznaczono różnice w stosunku do roku początkowego w obrębie grupy; przy średnich oznaczono różnice między poszczególnymi genotypami w *locus g.8398G>A*.

[a] until 2005, the Black-and-White name was used – do 2005 roku obowiązywała nazwa: czarno-biała.

Statistical analysis showed that year (at $P \leq 0.01$) and *PRL* genotype (at $P \leq 0.05$) had a significant effect on milk yields of cows. In the analysed period the greatest mean annual production progress for milk yield (374.1 kg) was found for the AG heterozygotes, while it was lowest (239.1 kg) for the AA homozygotes. For the GG homozygotes this value was 347.1 kg. In terms of cumulative progress obtained at the last observation it was found to be greatest (2618.8 kg) in the group of the AG heterozygotes, with a lower value recorded for the GG homozygotes (2429.9 kg) and the lowest (1673.7 kg) recorded for the cows with the AA genotype.

When comparing milk yields of cows with specific genotypes at *locus g.8398G>A* in individual years of observations it was shown that the lowest value of this trait was recorded most frequently for cows with the AA genotype. Statistically in 2003, 2005 and 2009 the AA homozygotes differed in milk yield at $P \leq 0.05$ from the two other genotypes of cows. In turn, in 2006 and 2008 they differed (at $P \leq 0.05$) in terms of the same traits from the AG heterozygotes and the GG homozygotes, respectively. No statistically significant differences were recorded in milk yields between the GG homozygotes and the AG heterozygotes.

Table 2 presents the calculated annual production progress and cumulative progress for the yield of milk fat for cows differing in their genetic variants at *locus g.8398G>A*. It was shown that year and genetic variant at the specific polymorphic site of the *PRL* gene influenced ($P \leq 0.01$) the analysed milking performance trait. The greatest average progress in the production of milk fat (15.3 kg) during the analysed period was observed in the AG heterozygotes, while the level by 3 kg lower was recorded in the GG homozygotes, while it was lowest (8.5 kg) in cows with the AA genotype. At the last observation time identical ordering of genotypes was observed for the value of cumulative production progress calculated for the yield of milk fat. The values of this parameter for the AG, GG and AA genetic variants amounted to 107.3 kg, 86.2 kg and 59.8 kg, respectively. When analyzing the yield of milk fat in individual years of observations depending on the genotype at *locus g.8398G>A* it was shown that except for 2002 the greatest value of this milking performance parameter was obtained for the AG heterozygotes, while it was lowest for the AA homozygotes. Statistical analysis showed that in 2005 cows with the AA genotype differed at the significance level $P \leq 0.05$ in their yields of milk fat from the other genetic variants of cows. In 2007 and 2009 for the discussed milking performance trait statistically significant differences were recorded between the AG heterozygotes and the AA homozygotes at $P \leq 0.01$ and $P \leq 0.05$, respectively.

Table 3 gives values of calculated production progress and cumulative progress obtained in the period of 2002–2009 for the yield of milk protein for cows differing in their *PRL* genotype. The year of observation and genotype at *locus g.8398G>A* had a significant effect (at $P \leq 0.01$) on the analysed milking performance parameter. The average production progress during the period of observations for the yield of protein was highest (12.6 kg) in cows with the AG genotype, followed by the GG genotype (with 10.7 kg), whereas it was lowest (7.5 kg) in the AA homozygotes. At the last observation time the value of cumulative production progress for the yield of milk protein in cows with the GG, AG and AA genetic variants increased in relation to the initial level by 138.3%, 146.9% and 126.3%, respectively, amounting to 10.7 kg, 12.6 kg and 7.5 kg. In terms of the yield of milk protein recorded in individual years of observations it may be stated that cows with the AA genotype produced the lowest amounts of this milk component (except for 2002).

Table 2. Production progress for fat yield [kg] in the years 2002–2009 for the studied population of Holstein-Friesian Black-and-White cows[a] with regard to genotyping at *locus g.8398G>A*

Tabela 2. Postęp produkcyjny dla wydajności tłuszczu [kg] w latach 2002–2009 w badanej populacji krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej[a], z uwzględnieniem podziału na genotypy w *locus g.8398G>A*

Year Rok **	Genotype at *locus g.8398G>A* – Genotyp w *locus g.8398G>A*														
	GG **					AG **					AA **				
	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%
2002	243.5	A		ABC DEFG		234.6 a	A		ABC DEFG		255.8 a			ABC	
2003	254.0	10.5 AB	104.3	10.5 A	104.3	250.3	15.7 AB	106.7	15.7 A	106.7	246.2	–9.6	96.2	–9.6	96.2
2004	274.5	20.5 B	108.1	31.0 B	112.7	268.6	18.3 BC	107.3	34.0 B	114.5	271.1	24.9	110.1	15.3	106.0
2005	280.2 a	5.7	102.1	36.7 C	115.1	282.4 b	13.8 C	105.1	47.8 C	120.4	267.2 ab	–3.9	98.6	11.4	104.5
2006	279.0	–1.2 C	99.6	35.5 D	114.6	289.7	7.3 D	102.6	55.1 D	123.5	275.5	8.3	103.1	19.7	107.7
2007	307.8	28.8 CD	110.3	64.3 E	126.4	318.9 A	29.2 Da	110.1	84.3 E	135.9	294.7 A	19.2	107.0	38.9 A	115.2
2008	331.0	23.2 D	107.5	87.5 F	135.9	328.9	9.9 Ea	103.1	94.3 F	140.2	312.3	17.6	106.0	56.5 B	122.1
2009	329.7	–1.3	99.6	86.2 G	135.4	341.9 a	13.0 E	104.0	107.3 G	145.7	315.6 a	3.3	101.1	59.8 C	123.4
$\bar{x}$		12.3					15.3					8.5			

Explanations see Table 1 – Objaśnienia zob. tab. 1.

Table 3. Production progress for protein yield [kg] in the years 2002–2009 for the studied population of Holstein-Friesian Black-and-White cows[a] with regard to genotyping at *locus g.8398G>A*

Tabela 3. Postęp produkcyjny dla wydajności białka [kg] w latach 2002–2009 w badanej populacji krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej[a], z uwzględnieniem podziału na genotypy w *locus g.8398G>A*

Year Rok **	Genotype at *locus g.8398G>A* – Genotyp w *locus g.8398G>A*														
	GG **					AG **					AA **				
	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%	$\bar{x}$	$x_i–x_{i–1}$	%	$x_i–x_0$	%
2002	195.6	A		ABC DEFG		187.3 a	A		ABC DEFG		198.8 A	a		ABCDE Fa	
2003	204.5 B	8.9 AB	104.6	8.9 A	104.6	203.0 A	15.7 AB	108.4	15.7 A	108.4	186.4 AB	–12.4 Aa	93.8	–12.4 a	93.8
2004	225.7	21.2 BC	110.4	30.1 B	115.4	220.1	17.1 BC	108.4	32.8 B	117.5	221.5	35.1 A	118.8	22.7 A	111.4
2005	237.7 B	12.0 C	105.3	42.1 C	121.5	238.1 A	18.0 C	108.2	50.8 C	127.1	213.3 AB	-8.2	96.3	14.5 B	107.3
2006	237.6 B	–0.1 D	99.9	42.0 D	121.5	242.0 A	3.9 D	101.6	54.7 D	129.2	220.3 AB	7.0 B	103.3	21.5 C	110.8
2007	254.6 B	17.0 DE	107.1	59.0 E	130.2	253.1 A	11.1 DE	104.6	65.8 E	135.1	237.5 AB	17.2 BC	107.8	38.7 D	119.5
2008	272.2 B	17.6 E	106.9	76.6 F	139.2	265.3 A	9.9 EF	104.8	78.0 F	141.6	253.9 AB	16.4 C	106.9	55.1 E	127.7
2009	270.5 B	–1.7	99.4	74.9 G	138.3	275.2 A	9.9 F	103.7	87.9 G	146.9	251.0 AB	–2.9	98.6	52.2 F	126.3
$\bar{x}$		10.7					12.6					7.5			

Explanations see Table 1 – Objaśnienia zob. tab. 1.

Cows with that genetic variant, except for 2002 and 2004, differed in terms of this milking performance trait from the other genotypes at the significance level $P \leq 0.01$. Statistically the AG heterozygotes and the GG homozygotes did not differ in terms of their yields of milk protein.

DISCUSSION

Literature on the subject lacks conclusive results indicating a relationship of a specific genotype at *locus g.8398G>A* of the *PRL* gene with milking performance traits in cows. Mehmannavaz et al. (2009) in their studies on Holstein bulls showed a significant effect of the *G* gene on the yields of milk and milk protein. A similarly significant effect of the GG genotype on the above-mentioned milking performance traits in Red-and-White cows was reported by Oprządek (2007). An advantageous dependence between the GG genetic variant and milk production was also shown by Ghasemi et al. (2009). In turn, Brym et al. (2005) stated the highest milk yields for the AG heterozygotes. Neja et al. (2013) for the period from 2001 to 2009 in Poland for milk yields of the population of Black-and-White cows and Polish Holstein-Friesian Black-and-White cows covered by the milk recording programme recorded an increase by 1423 kg, while the mean annual production progress was 158 kg. In comparison to the level of annual production progress and cumulative progress for milk yields in the active population of Black-and-White cows and Polish Holstein-Friesian Black--and-White cows, published by KCHZ (2003, 2004, 2005) and PFHBiPM (2006, 2007, 2008, 2009, 2010) with the results recorded in this study, for most years the annual increment in milk production and cumulative production progress irrespective of the *PRL* genotype was greater in the group of cows in this study. Production progress in the investigated period in relation to the initial year of the study (2002) in cows with the AG and GG variants of the *PRL* gene was by 1128.9 kg and 1317.8 kg greater, while in the AA genotype it was by 372.7 kg greater in comparison to cumulative progress recorded in 2009 (1301 kg) for cows from the entire active Polish population.

When analysing results given by KCHZ (2003, 2004, 2005) and PFHBiPM (2006, 2007, 2008, 2009, 2010) it may be stated that Polish Holstein-Friesian Black-and-White cows with the AG genotype in the investigated period (except for 2002) had a greater annual production progress in terms of the yield of milk fat in comparison to the results obtained by the active population of that cattle breed in an analogous period. Cumulative progress for the yield of milk fat in cows with the AG, GG and AA genetic variants was higher when compared to that in the active population by 55.3 kg, 34.2 kg and 7 kg, respectively.

The most advantageous values of annual production progress for the yield of milk protein, exceeding means calculated by KCHZ (2003, 2004, 2005) and PFHBiPM (2006, 2007, 2008, 2009, 2010) for the entire active population of the Black-and-White and Polish Holstein-Friesian Black-and-White breeds, were recorded in the investigated period for cows with the AG genotype, followed by AA and GG. In turn, in the case of cumulative progress in the analogous period it was similarly greatest for the AG heterozygotes, followed by the GG and AA homozygotes. At present in view of consumer preferences, breeding work on dairy cattle is focused on increasing the production of milk protein. In many countries it is assumed in the

implemented breeding programmes to reduce the discrepancy between contents of milk fat and milk, while the ratio of these milk parameters varies, e.g. from a relatively small difference of 0.3% for fat, to an absolute balance of 1 : 1 (Neja et. al. 2013).

CONCLUSIONS

Results of studies on the population of Polish Holstein-Friesian Black-and-White cows indicate dependencies between genotype at *locus g.8398G>A* of the *PRL* gene and production progress in milk yield as well as the yield of milk fat and milk protein.

It was shown that the greatest production progress and cumulative progress for milk yield as well as yields of milk fat and milk protein were found for the AG heterozygotes, whereas the lowest values of these parameters were recorded for the AA homozygotes.

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Abstract. The aim of this study was to analyse dependencies between *PRL* gene polymorphism within exon 4 of bovine chromosome 23 (*locus g.8398G>A*), and the level of production progress in milk yield, yields of milk fat and milk protein in Polish Holstein-Friesian Black-and-White cows. Results of this study indicate dependencies between genotype at *locus g.8398G>A* of the *PRL* gene and production progress in terms of milk yield as well as the yields of milk fat and milk protein. The greatest production progress and cumulative progress for milk yield as well as yields of milk fat and milk protein were shown for the AG heterozygotes at *locus g.8398G>A*, while the lowest values of analysed parameters were recorded for the AA homozygotes.