

## Effects of polymorphism at 5'-noncoding regions (promoters) of $\alpha$ S1- and $\alpha$ S2-casein genes on selected milk production traits in Polish Black-and-White cows\*

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The effects of cow's genotype at  $\alpha$ S1- and  $\alpha$ S2-casein gene 5'-noncoding regions (promoters) were determined on selected milk production traits of the 135 Polish Black-and-White (Polish Friesian) cows as related to the animal's age, lactation parity and stage, and somatic cell count. Cows of the *AA* genotype of  $\alpha$ S1-casein gene yielded more milk daily than *AG* heterozygotes. Also, the daily yield of solids-non-fat, protein and lactose was higher in *AA* genotype cows. Milk of the cows with genotype *CC* of  $\alpha$ S2-casein contained more lactose but less protein than that of the *CT* heterozygotes. The daily protein yield was slightly (but significantly) higher in the cows of the *CT*  $\alpha$ S2-casein genotype. In summary, the results showed that genetic variants of  $\alpha$ S1- and  $\alpha$ S2-casein 5'-noncoding regions had only a slight effect on milk production traits of the Polish Black-and-White cows. Nevertheless, the *AA* genotype of  $\alpha$ S1-casein seemed favourable for higher milk yield, as well as for lactose and protein content.

**KEY WORDS:** casein genes / cows / milk / polymorphism / promoter

In bovine milk six major protein fractions – caseins  $\alpha$ S1,  $\alpha$ S2,  $\beta$ ,  $\kappa$ ,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin – are coded by autosomal single-copy genes. Four genes coding for caseins are clustered within less than 300 Kb on bovine chromosome 6 in the following order:  $\alpha$ S1,  $\beta$ ,  $\alpha$ S2, and  $\kappa$  [Feretti *et al.* 1990, Rijnkels *et al.* 1997]. It was assumed

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that the three genes coding for the calcium-sensitive  $\alpha$ S1-,  $\alpha$ S2- and  $\beta$ -caseins evolved from one ancestral gene by exon shuffling and intra- and intergenic duplications [Jones *et al.* 1985, Yu-Lee *et al.* 1986, Bonsing and Mackinlay 1987]. In contrast, the  $\kappa$ -casein encoding gene evolved differently [Alexander *et al.* 1993]. Lien *et al.* [1993] supported a strong genetic linkage of the casein genes by using the “single sperm typing” method. So, it may be supposed that close localization and linkage of the casein genes might influence a common inheritance of these genes with only a little recombination. Moreover, the close proximity of the casein genes supported the hypothesis of common hormonal regulation of the entire casein gene complex [Rosen 1987].

Genetic polymorphism of milk proteins in cattle is well documented [Grosclaude 1988, Martin *et al.* 2002]. The proteins differ in amino acid substitutions or deletions resulting from the differences in the coding sequences of corresponding genes. Associations have been described between polymorphism in the coding regions of the milk protein genes and the level of expression of milk proteins in cattle [Van Eenennaam and Medrano 1991]. Variable sites were also identified in 5'-noncoding sequences of bovine milk protein genes [Schild and Geldermann 1996, Kamiński 1996, Bleck *et al.* 1996, Geldermann *et al.* 1996, Van Ehrmann *et al.* 1997]. At least some genetically variable sites are located in putative regulatory sequences, e.g. in transcription factor-binding sites [Schild and Geldermann 1996, Szymanowska *et al.* 2003], and thus they may affect the gene expression. It has been suggested that differential expression of various alleles of milk proteins possibly resulted from linkage between variants of coding and regulatory regions of their genes [Van Eenennaam and Medrano 1991].

The objective of this study was to examine the effect of polymorphism in the 5'-noncoding region of the bovine  $\alpha$ S1- and  $\alpha$ S2-casein genes on daily milk yield and composition in Black-and-White dairy cows during the whole lactation period. Moreover, the contribution of casein genotype and animal's age, lactation parity and stage, and somatic cell count were assessed, and their effects on the pleiotropic variation in milk production traits were estimated.

## Material and methods

### Animals

The Black-and-White (BW) cows were maintained at the Polish Academy of Sciences Experimental Farm, Kosów in a herd yielding on average 8,300 kg of milk containing 4.01% fat and 3.45% total protein. For this study 135 cows, with more than 80% of Holstein-Friesian (HF) blood, were randomly chosen. Only 4-5 cows were daughters of one sire and for this reason the sire effect was not included in the statistical model. During the whole period the cows were kept in loose barn with outside run. During the test period animals were fed complete TMR diet (total mixed ratio) of corn silage, wilted grass silage and concentrates, supplemented with mineral and vitamin mixture, according to the INRA system. Water was available *ad libitum*.

The cows were milked twice a day. Milk samples were taken from each cow once a month during three consecutive lactations, starting from lactation I.

All procedures carried out with the use of animals were approved by the Local Ethical Commission; permission No 67/2001).

**DNA isolation from whole blood**

Blood samples for DNA genotyping were collected from the jugular vein by an authorized veterinarian on K<sub>2</sub>EDTA and stored at -25°C for a few weeks, or at -75°C up to several months. The isolation of DNA from whole blood was done with a rapid method described by Kanai *et al.* [1994].

**Determination of αS1- and αS2-casein genotype with PCR-RFLP method**

Detection of restriction fragment length polymorphism (RFLP) based on the polymerase chain reaction (PCR) was carried out according to Koczan *et al.* [1993] and Schild and Geldermann [1996]. The sequence of primers and conditions of PCR are summarized in Table 1. The PCR was performed in a reaction volume of 25 µl containing approximately 100 ng of bovine genomic DNA, 0.25 µM of each primer, 160 µM dNTPs and 2 units of Taq polymerase (PolGen, Poland). All PCR reactions were performed in MJ Research PTC-225 Thermal Cycler. The PCR-amplified DNA fragment (from -1150 to -872 nt) of the αS2 casein gene was digested at 37°C for 3 hours with 5 units of the *MaeII* nuclease (FERMENTAS, Lithuania). For the αS1-casein, gene fragments: -1145/+101 or -372/+36, were amplified and digested with *SspI* or *MaeIII* restriction endonucleases, respectively. The digested products were

Table 1 PCR primers and conditions of DNA amplification used for RFLP genotyping in 5'-noncoding regions (promoters) of αS1- and αS2-casein genes in the Polish Black-and-white cows

Casein gene and mutation	Restriction enzyme	Sequence of primers	Location	Amplified fragment (bp)	Number of PCR cycles	Temperature time (min)
αS2-casein/-1150	<i>MaeII</i>	5'-TATGACATGTCGACGAAATGCGA-3' 5'-TATAGGAGATGAGTGGCTGGCAGGCA-3'	-1150/-872	278	7	94°C:10 54°C:10 72°C:10
αS1-casein/-175	<i>MaeIII</i>	5'-GAGGAGGCGGCGGAGCTGGCA-3' 5'-TGGCATGTTCTGATGATAGGCA-3'	-372/+36	110	7	94°C:10 60°C:10 72°C:10
αS1-casein/-101	<i>SspI</i>	5'-TATGACATGTCGACGAAATGCGA-3' 5'-TATGAGGAGATGAGTGGCTGGCAGGCA-3'	-1145/+101	1246	7	94°C:10 55°C:10 72°C:10

separated on 2% agarose (GIBCO-BRL, England) gels in  $1 \times$  TRIS-borate-EDTA (TBE) buffer. The gels were stained with ethidium bromide and visualised and scanned in FX Phosphoimager (Bio-Rad).

#### Milk composition

The fat, protein and lactose content was estimated in fresh milk samples using Milko Scan 104A/B, and somatic cell counts (SCC) were determined by means of Fossomatic apparatus. Per cent of total solids in each milk sample was expressed as a sum of per cent of fat, total protein, lactose and minerals. The concentration of minerals was calculated according to the Sherman equation:  $P = 0.1 \times$  per cent of total protein + 0.38.

The milk samples were divided according to the age of cows into three groups: cows in lactation I, II and III (or further). The whole lactation period was divided into three post-calving stages: 6-60 days, 61-210 days, and 211 days up to the end of lactation. According to the SCC the milk samples were divided into three classes: up to 400 thousand, 401-800 thousand and >801 thousand cells/ml. The SCC values were transformed to the natural logarithm scale.

#### Statistical

The data were evaluated by the analysis of variance according to Harvey [1990] using the following fixed model:

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

where:  $Y_{ijklm}$  – observed mean value of the trait;  
 $\mu$  – overall mean;  
 $a_i$  – effect of  $i$ -th age of cow ( $i = 1, 2, 3$ );  
 $b_j$  – effect of  $j$ -th stage of lactation ( $j = 1, 2, 3$ );  
 $c_k$  – effect of  $k$ -th  $\alpha$ S1- or  $\alpha$ S2-casein genotype ( $k = 1, 2, 3, 4$ );  
 $d_l$  – effect of  $l$ -th class of SCC ( $l = 1, 2, 3$ );  
 $e_{ijklm}$  – random error.

The reference genotypes were: *AA*, *AG* for  $\alpha$ S1-casein gene, and *CC*, *CT* for  $\alpha$ S2-casein gene.

#### Results and discussion

Frequencies of genotypes and alleles at 5'-noncoding regions (promoters) of the  $\alpha$ S1- and  $\alpha$ S2-casein genes in the studied group of cows are shown in Table 2. RFLP at the bovine  $\alpha$ S2 casein gene promoter was investigated at the site previously reported as polymorphic by Schild and Geldermann [1996]. The polymorphisms occurred at posi-

**Table 2.** Frequency of genotypes and alleles in 5'-noncoding regions (promoters) of  $\alpha$ S1- and  $\alpha$ S2-casein genes in the Polish Black-and-White cows

Locus/position of polymorphic site	Restriction nuclease	Genotype	Number of animals	Allele frequency
$\alpha$ S2-casein <sup>-1084</sup>	MaeII	CC	100	
		CT	42	C: 0.85
		TT	1	T: 0.15
			total: 143	
$\alpha$ S1-casein <sup>-175</sup>	MaeIII	AA	126	A: 0.97
		AG	9	G: 0.03
			total: 135	
$\alpha$ S1-casein <sup>-728</sup>	SspI	(-/-)	126	(-): 0.97
		(T/-)	9	(T): 0.03
			total: 135	

tion -1084 relative to the transcription start point. It was a single nucleotide substitution (SNP) – C/T (transition) – recognizable by PCR-RFLP with the nuclease MaeII. All three genotypes were found, although the TT genotype was rare and appeared only in one animal. The CC genotype and allele C were the most frequent – 70 and 85%, respectively. The results showed that the C→T transition in the  $\alpha$ S2-casein gene promoter at position -1084 was rare, similarly as in other cattle breeds. Previously, Schild and Geldermann [1996] among seven cattle breeds showed C→T mutation in one Zebu (*Bos indicus*) and in one German Simmental individual only. In our earlier study on Polish native cattle breeds [Klauzińska *et al.* 2003] among 195 Polish Red cows no TT genotype was identified.

In this study the RFLP of the bovine  $\alpha$ S1 casein gene promoter was investigated at two polymorphic sites reported by Koczan *et al.* [1993] and Schild and Geldermann [1996]. The mutation in  $\alpha$ S1-casein gene found at position -728 was a deletion/insertion of T nucleotide (Tab. 2). An amplified DNA fragment was digested with restriction endonuclease SspI, and two genotypes: (-/-) and (T/-) were observed. The frequency of allele (-) was 97% (Tab. 2). No TT animals were found in the studied group of cows. The high frequency of the allele (-) was also reported by Schild and Geldermann [1996] who found only two TT animals out of 13 belonging to seven cattle breeds. We also found no TT genotype in Polish Red cattle [Klauzińska *et al.* 2003], but the allele T appeared slightly more frequent in Polish Red than in BW cattle (0.12 and 0.03, respectively). Another polymorphism studied in the  $\alpha$ S1-casein gene 5'-noncoding region was A/G transition at position -175 [Koczan *et al.* 1993]. Since A→G substitution creates new restriction site for MaeIII nuclease it can be analysed by the PCR-RFLP method. While studying polymorphism of casein genes in individual animals we noticed the complete associations between specific variants of  $\alpha$ S1-casein at positions -175 and -728. All animals of genotype (-/-) at position -728 appeared AA for nucleotide at position -175,

Table 3. Least squares means (LSM) and their standard errors (SE) for daily milk yield, milk energy and per cent of major milk constituents as affected by OS1 and OS2 groups in the Polish Black-and-white cows

Milk component	n	Milk yield (kg)		Milk energy (MJ/kg)		Total solids (%)		Solid non-fat (%)		Protein (%)		Lactose (%)	
		OS1	OS2	OS1	OS2	OS1	OS2	OS1	OS2	OS1	OS2	OS1	OS2
OS1 groups													
A1	1026	17.3 <sup>d</sup>	17.1 <sup>d</sup>	1.71 <sup>d</sup>	1.71 <sup>d</sup>	11.5	11.5	9.12	9.02	1.9 <sup>d</sup>	1.9 <sup>d</sup>	1.60	1.61
A2	166	16.0 <sup>b</sup>	17.1 <sup>d</sup>	1.40 <sup>b</sup>	1.40 <sup>b</sup>	11.6	11.6	9.05	9.05	1.5 <sup>b</sup>	1.5 <sup>b</sup>	1.55	1.55
OS2 groups													
C1	1585	16 <sup>a</sup>	17.1 <sup>d</sup>	1.7 <sup>a</sup>	1.7 <sup>a</sup>	11.6	11.6	9.10	9.01	1.50	1.50	1.55 <sup>b</sup>	1.55 <sup>b</sup>
C2	607	16.8	17.1 <sup>d</sup>	1.7 <sup>a</sup>	1.7 <sup>a</sup>	11.5	11.5	9.07	9.01	1.51	1.51	1.60 <sup>a</sup>	1.60 <sup>a</sup>
Lactation days													
6-60 days	196	17.9 <sup>d</sup>	17.9 <sup>d</sup>	1.70 <sup>d</sup>	1.70 <sup>d</sup>	11.6 <sup>d</sup>	11.6 <sup>d</sup>	9.13 <sup>d</sup>	9.01	1.7 <sup>d</sup>	1.7 <sup>d</sup>	1.61 <sup>d</sup>	1.61 <sup>d</sup>
61-120 days	105	16.8 <sup>b</sup>	17.1 <sup>d</sup>	1.70 <sup>d</sup>	1.70 <sup>d</sup>	11.5 <sup>b</sup>	11.5 <sup>b</sup>	9.10 <sup>b</sup>	9.01	1.6 <sup>b</sup>	1.6 <sup>b</sup>	1.57 <sup>b</sup>	1.57 <sup>b</sup>
> 120 days	7=1	17.0 <sup>c</sup>	17.1 <sup>d</sup>	1.7 <sup>c</sup>	1.7 <sup>c</sup>	11.7 <sup>c</sup>	11.7 <sup>c</sup>	9.17 <sup>c</sup>	9.01	1.7 <sup>c</sup>	1.7 <sup>c</sup>	1.62 <sup>c</sup>	1.62 <sup>c</sup>
Lactation													
I	1=1	15.5 <sup>a</sup>	16.4 <sup>d</sup>	1.41 <sup>a</sup>	1.41 <sup>a</sup>	11.7 <sup>a</sup>	11.7 <sup>a</sup>	9.13 <sup>a</sup>	9.05	1.57	1.57	1.54 <sup>a</sup>	1.54 <sup>a</sup>
II	601	16.0 <sup>b</sup>	16.4 <sup>d</sup>	1.4 <sup>b</sup>	1.4 <sup>b</sup>	11.6 <sup>b</sup>	11.6 <sup>b</sup>	9.14 <sup>b</sup>	9.01	1.5 <sup>b</sup>	1.5 <sup>b</sup>	1.61 <sup>b</sup>	1.61 <sup>b</sup>
III and further	11=6	18.1 <sup>b</sup>	17.1 <sup>d</sup>	1.71 <sup>b</sup>	1.71 <sup>b</sup>	11.7 <sup>b</sup>	11.7 <sup>b</sup>	9.19 <sup>b</sup>	9.01	1.6 <sup>b</sup>	1.6 <sup>b</sup>	1.57 <sup>b</sup>	1.57 <sup>b</sup>
SE (SEM)													
1=100 thousand	1=1	17.1 <sup>d</sup>	17.1 <sup>d</sup>	1.7 <sup>d</sup>	1.7 <sup>d</sup>	11.5	11.5	9.14 <sup>d</sup>	9.01	1.7 <sup>d</sup>	1.7 <sup>d</sup>	1.57 <sup>d</sup>	1.57 <sup>d</sup>
1=1-100 thousand	601	16.7 <sup>b</sup>	17.1 <sup>d</sup>	1.7 <sup>d</sup>	1.7 <sup>d</sup>	11.6	11.6	9.14 <sup>d</sup>	9.01	1.7 <sup>d</sup>	1.7 <sup>d</sup>	1.60 <sup>d</sup>	1.60 <sup>d</sup>
1=101 thousand	11=6	16.1 <sup>b</sup>	17.1 <sup>d</sup>	1.7 <sup>d</sup>	1.7 <sup>d</sup>	11.5	11.5	9.14 <sup>d</sup>	9.01	1.5 <sup>b</sup>	1.5 <sup>b</sup>	1.60 <sup>d</sup>	1.60 <sup>d</sup>

<sup>a, b, c, d</sup> Number of milk samples analyzed  
<sup>1, 2, 3</sup> Milk in the columns and lactation stages bearing different letters are significantly different (small letters – P < 0.05; capital letters – P < 0.01)

Table 4. Least square means (LSM) and their standard errors (SE) for daily yield of major milk components as referred to Q51 or Q52 genotype in the Polish Black-and-white cows

Variation source	n	Q51 (kg <sup>2</sup> )		Total solids (kg)		Substance (kg)		Fat (g)		Protein (g)		Lactose (g)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
<b>Q51 genotype</b>													
A-A	1016	1.10 <sup>a</sup>	0.1	11.56	0.1	1.01 <sup>a</sup>	0.05	7.11	0.1	6.05 <sup>a</sup>	0.1	8.17 <sup>a</sup>	0.1
A-C	166	1.9 <sup>b</sup>	0.6	3.1 <sup>a</sup>	0.1	1.39 <sup>b</sup>	0.1	7.15	0.6	5.98 <sup>b</sup>	0.6	7.95 <sup>b</sup>	0.6
<b>Q52 genotype</b>													
C-C	1585	1.01	0.1	11.90	0.1	1.01	0.05	7.18	0.1	5.71 <sup>a</sup>	0.1	7.80	0.1
C-T	607	1.07	0.1	11.10	0.1	1.01	0.05	7.10	0.1	5.91 <sup>b</sup>	0.1	7.87	0.1
<b>Lactation stage</b>													
6-60 days	196	1.5 <sup>a</sup>	0.1	11.17 <sup>a</sup>	0.1	1.901 <sup>a</sup>	0.1	9.15 <sup>a</sup>	0.1	7.18 <sup>a</sup>	0.1	10.11 <sup>a</sup>	0.1
61-90 days	105	1.01 <sup>b</sup>	0.1	11.71 <sup>b</sup>	0.1	1.531 <sup>b</sup>	0.1	7.10 <sup>b</sup>	0.1	5.98 <sup>b</sup>	0.1	8.01 <sup>b</sup>	0.1
91-0 days	791	1.5 <sup>c</sup>	0.1	11.81 <sup>c</sup>	0.1	1.011 <sup>c</sup>	0.1	5.18 <sup>c</sup>	0.1	5.39 <sup>c</sup>	0.1	5.05 <sup>c</sup>	0.1
<b>Lactation</b>													
I	101	1.91 <sup>d</sup>	0.1	10.75 <sup>d</sup>	0.1	1.79 <sup>d</sup>	0.1	6.81 <sup>d</sup>	0.1	5.11 <sup>d</sup>	0.1	7.79 <sup>d</sup>	0.1
II	601	1.99 <sup>d</sup>	0.1	11.73 <sup>d</sup>	0.1	1.01 <sup>d</sup>	0.1	6.9 <sup>d</sup>	0.1	5.65 <sup>d</sup>	0.1	7.99 <sup>d</sup>	0.1
III and further	1166	1.11 <sup>a</sup>	0.1	11.91 <sup>a</sup>	0.1	1.63 <sup>a</sup>	0.1	7.65 <sup>a</sup>	0.1	6.19 <sup>a</sup>	0.1	8.51 <sup>a</sup>	0.1
<b>SS CVel</b>													
100 thousand	1501	1.10	0.1	11.86 <sup>a</sup>	0.1	1.601 <sup>a</sup>	0.1	7.11	0.1	6.01 <sup>a</sup>	0.1	8.17 <sup>a</sup>	0.1
101-100 thousand	150	1.01	0.1	11.67 <sup>a</sup>	0.1	1.71 <sup>a</sup>	0.1	6.96	0.1	5.75 <sup>a</sup>	0.1	7.95 <sup>a</sup>	0.1
101 thousand	101	1.01	0.1	11.07 <sup>a</sup>	0.1	1.39 <sup>a</sup>	0.1	7.11	0.1	5.67 <sup>a</sup>	0.1	7.00 <sup>a</sup>	0.1

<sup>a</sup> Number of milk samples analyzed  
<sup>b</sup> Q51 > 0.05; daily yield (kg) = 1.66 x fat (kg) + 15.74 x protein (kg), actual area of 1000 l  
<sup>c</sup> Means within column and variation source bearing different letters are significantly different: small letters - P < 0.01, capital - P < 0.05

and all (*T/-*) animals were *AG* heterozygotes (Tab. 2). These results suggest a tight linkage between both polymorphic sites located within a distance of 553 bp and the existence of intragenic haplotypes within the  $\alpha$ S2-casein gene. Therefore, only one polymorphism of the  $\alpha$ S1-casein gene – the *A/G* transition at position -175 – was included in our further calculations.

The individual effects of  $\alpha$ S1- and  $\alpha$ S2-casein genotypes on the milk yield and composition are shown in Tables 3 and 4. Cows of the *AA* genotype of  $\alpha$ S1-casein yielded more milk daily than *AG* heterozygotes (Tab. 3), the difference being 1.2 kg milk/day in favour of the *AA* genotype. However, the milks of *AA* cows contained significantly less gross energy (by 0.07 MJ/l) and fat (by 0.22 per cent units). This resulted in a not significant difference in FCM yield between cows of genotypes *AA* and *AG*. The difference in dairy performance between *AA* and *AG* cows appeared evident when the milk corrected for both fat and protein (VCM) was considered (Tab. 4). The difference at VCM value was significant and amounted to 1.1 kg in favour of the  $\alpha$ S1 *AA* genotype. This was due to the higher (though statistically not significant) concentration of protein in the milk of the *AA* genotype cows. In addition, the *AA* homozygotes yielded significantly more milk protein and lactose daily than those of *AG* genotype; the daily yield of fat was nearly the same in both groups (Tab. 4).

The  $\alpha$ S2-casein genotype influenced only the protein and lactose per cent of milk (Tab. 3). Milk of the cows with genotype *CC* of  $\alpha$ S2-casein contained more lactose but less protein than that of the *CT* heterozygotes. The *TT* genotype was not considered in the calculations since only one animal of this genotype was identified. The genotype of  $\alpha$ S2-casein affected only the daily protein yield, which was slightly, but significantly, higher in the *CT* cows (Tab. 4).

The results presented so far show that genetic variants at the 5'-noncoding region of  $\alpha$ S1-casein gene have a marked effect on daily milk yield and yield of milk main components, as well as their content of milk. The *AA* genotype of  $\alpha$ S1-casein seemed favourable for higher milk yield, and higher lactose and protein content. On the other hand, the  $\alpha$ S2-casein gene genotype affected only lactose and protein content of milk.

Effects of lactation stage, cow's age (lactation parity), and somatic cell count (SCC) on milk yield and composition are shown in Tables 3 and 4. Older cows (lactation III) produced more milk – both total and fat-corrected – than did the younger ones. The milk yield depended significantly on lactation stage, being highest in early lactation (6-60-days period). Milks from the late stage of lactation (>240 days) contained more gross energy, total solids, fat, and protein than those from earlier lactation stages. High SCC was adversely related to milk yield (but not to FCM yield). Milks of cows with high SCC (>801 thousand) contained less solids-non-fat and lactose but significantly more fat and protein. The yield of milk components – total solids, protein, and lactose – was much smaller ( $P<0.01$ ) in cows with high than in those with low SCC (Tab. 4).

The contribution of different fixed (genotype) and random (lactation parity, lactation stage, SCC) factors to the variation in daily milk yield and composition is shown



in Table 5. The calculated contribution of casein genotype to milk yield was low and for  $\alpha$ S1 amounted to 0.79%, while for  $\alpha$ S2-casein to 0.24% of total phenotypic variation. The  $\alpha$ S1-casein genotype contributed mostly to fat content (10.10%), while the  $\alpha$ S2 was shown to affect mostly the phenotypic variation of lactose (16.36%). The contribution of lactation parity was high and varied from 44% for fat per cent, to more than 60% for milk yield, and up to 93% for per cent of protein. SCC mostly affected the lactose content of milk (50% of contribution of all factors analysed).

Very few studies have been done so far of the nucleotide sequence polymorphism in the 5'-regulatory regions of the milk protein genes and its effects on milk yield and composition. Voelker *et al.* [1997] examined 5'-flanking region of the bovine  $\alpha$ -lactalbumin gene for potential sequence variations. They identified a single base pair difference, the A/G transition, located at position -1689 from the transcription start point. A higher expression of allele A has been described in heterozygous (AG) animals by Graml *et al.* [1989]. Lum *et al.* [1997] investigated the role of the G to C transversion within a consensus binding site for activator protein-2 (AP2) at position -430 in bovine  $\beta$ -lactoglobulin gene. A possible regulatory role of AP2 in the transcriptional regulation of the  $\beta$ -lactoglobulin gene has been proposed [Lum 1997]. Folch *et al.* [1999] showed differential expression of a reporter gene fused to bovine  $\beta$ -lactoglobulin A or B promoter variants in transiently transfected HC11 cells, the A variant driving more efficient expression of the reporter than the B

Table 5. Contribution of different traits and number effects to the phenotypic variation in daily milk yield and composition in red medona  $\alpha$ S1- and  $\alpha$ S2-casein in the Polish Black-and-white cows

Variation source	Milk (kg)	Fat content (ml/l)	% CM	Protein (%)	Lactose (%)	Total solids (kg)	Solids non-fat (kg)	Cream energy (MJ/kg)
$\alpha$ S1-Casein genotype	0.79	0.1*	0.71	0.60	0.36	1.76	1.0	1.17
$\alpha$ S2-Casein genotype	0.24	0.01	0.37	1.11	16.76	1.81	0.7	1.72
Lactation parity	61.06	6* 17	50.00	91.06	1.31	79.79	15.37	72.41
SCC (lactation parity)	11	1.76	0.36	1.17	22.76	6.36	0	6.1*
SCC (ml)	1.00	0.1	0.50	1.57	50.31	0.11	0.9	1.06
Remainder (e.g. year of calving)	12.75	11.56	1.31	39.66	6.36	10.76	1.0	1.90
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

\* Least squares mean for each trait; 00 = sum of least squares mean for all traits

variant. Polymorphism within 5'-flanking region of the bovine  $\beta$ -lactoglobulin gene was also studied by Kamiński and Zabolewicz [1998] in the dairy Polish BW cattle. Several point mutations were found by SSCP analysis. Statistical evaluation revealed significant associations between the  $\beta$ -lactoglobulin genotypes and protein content of milk during the first complete lactation [Kamiński and Zabolewicz 2000].

Kamiński [1996] identified a *DdeI* RFLP polymorphism within the 5'-upstream region of the bovine  $\kappa$ -casein gene, containing five potential consensus sequences for different transcription factors. Associations between individual  $\kappa$ -casein genotypes and milk production traits were investigated and significant differences were found for protein content of milk [Kamiński 2000].

In our earlier study [Martin *et al.* 2002] based on SDS-PAGE and HPLC techniques, differences were shown in casein content between milks obtained from cows carrying specific mutations at position -1084 and -728 of the  $\alpha$ S2- and  $\alpha$ S1-casein genes, respectively. Milk of BW cows with (*T/-*) genotype of  $\alpha$ S1-casein gene promoter was found to contain on average 11% more  $\alpha$ S1-casein than that of cows with (*-/-*) gene. Also the polymorphism within  $\alpha$ S2-casein gene promoter was shown to affect the content of the relevant protein of milk; milk from BW cows with *CT* genotype of  $\alpha$ S2-casein gene at position -1084 contained twice as much  $\alpha$ S2-casein than that from *CC* animals. Moreover, differences were found in the expression rates of the different  $\alpha$ S2-casein alleles using semi-quantitative RT-PCR analysis performed on RNAs isolated from biopsies of mammary glands or from somatic cells derived from cow's milk [Szymanowska *et al.* 2003]. These results show that nucleotide sequence variations in the 5'-noncoding regulatory regions of milk protein genes may influence expression of individual proteins in the cow's milk.

The results presented here show that such genetic variants in the bovine casein genes may also be related to the milk yield, as well as to milk components yield and content.

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#### REFERENCES

1. ALEXANDER L.G., HAYES G., BAWDEN W., STEWART A.F., MACKINLAY A.G., 1993 – Complete nucleotide sequence of the bovine beta-lactoglobulin gene. *Animal Biotechnology* 4, 1-10.
2. ARBEL R., BIGUN Y., EZRA E., STURMAN H., HOJMAN D., 2001 – The effect of extended calving intervals in high lactating cows on milk production and profitability. *Journal of Dairy Science* 84, 600-608.
3. BLECK G.T., CONROY J.C., WHEELER M. B., 1996 – Polymorphisms in the bovine  $\beta$ -casein 5' flanking region. *Journal of Dairy Science* 79, 347-349.
4. BONSING J., MACKINLAY A.G., 1987 – Recent studies on nucleotide sequences encoding the caseins. *Journal of Dairy Research* 54, 447-461.

5. EHRMANN S., BARTENSCHLAGER H., GELDERMANN H., 1997 – Polymorphism in the 5' flanking region of the bovine-lactoglobulin-encoding gene and its association with  $\beta$ -lactoglobulin in the milk. *Journal of Animal Breeding and Genetics* 114, 49-53.
6. FERRETTI L., LEONE P., SGARAMELLA V., 1990 – Long range restriction analysis of the bovine casein genes. *Nucleic Acids Research* 18, 6829-6833.
7. FOLCH J.M., DOVC P., MEDRANO J.F., 1999 – Differential expression of bovine  $\beta$ -lactoglobulin A and B promoter variants in transiently transfected HC11 cells. *Journal of Dairy Research* 66, 537-544.
8. GELDERMANN H., GOGOL J., KOCK M., TACEA G., 1996 – DNA variants within the 5' flanking region of bovine milk protein encoding genes. *Journal of Animal Breeding and Genetics* 113, 261-267.
9. GRAML R., WEISS G., BUCHBERGER J., PIRCHNER F., 1989 – Different rates of synthesis of whey protein and casein by alleles of the  $\beta$ -lactoglobulin and  $\alpha_{s1}$ -casein locus in cattle. *Genetics, Selection, Evolution* 21, 547-554.
10. GROSCLAUDE F., 1988 – Le polymorphisme genetique des principales lactoproteines bovines. *INRA Production Animale* 1, 5-17.
11. HARVEY W.R., 1990 – User's guide for LSMLMW. Ohio State University.
12. JONES W.K., YU-LEE L.Y., CLIFT S.M., BROWN T.L., ROSEN J.M., 1985 – The rat casein multigene family. II. Fine structure and evolution of the beta-casein gene. *Journal of Biological Chemistry* 260, 7042-7050.
13. KAMIŃSKI S., 1996 – Dde I RFLP at the 5' region of bovine kappa-casein gene. *Journal of Applied Genetics* 37, 173-178.
14. KAMIŃSKI S., 2000 – Association between polymorphism within regulatory and coding fragments of bovine kappa-casein gene and milk performance traits. *Journal of Animal and Feed Sciences* 9, 435-446.
15. KAMIŃSKI S., ZABOLEWICZ T., 1998 – SSCP polymorphism within 5' region of bovine lactoglobulin (LGB) gene. *Journal of Applied Genetics* 39 (1), 97-102.
16. KAMIŃSKI S., ZABOLEWICZ T., 2000 – Associations between bovine  $\beta$ -lactoglobulin polymorphism within coding and regulatory sequences and milk performance traits. *Journal of Applied Genetics* 41, 91-99.
17. KANAI N., FUJII T., SAITO K., TOKOYAMA T., 1994 – Rapid and simple method for preparation of genomic DNA from easily obtainable clotted blood. *Journal of Clinical Pathology* 47, 1043-1044.
18. KLAUZIŃSKA M., ŻURKOWSKI M., SIADKOWSKA E., SZYMANOWSKA M., GROCHOWSKA R., ZWIERZCHOWSKI L., 2003 – Comparison of polymorphism in selected gene loci between polish red cattle and Polish Black-and-White cattle. *Journal of Animal Breeding and Genetics* (in press).
19. KOCZAN D., HOBORN G., SEYFERT H.-M., 1993 – Characterization of the bovine  $\alpha_{s1}$ -casein gene C-allele, based on a MaeIII polymorphism. *Animal Genetics* 24, 74.
20. LIEN S., KAMIŃSKI S., ALESTROEM P., ROGNE S., 1993 – A simple and powerful method for linkage analysis by amplification of DNA from single sperm cells. *Genomics* 16, 41-44.
21. LUM L.S., DOVC P., MEDRANO J.F., 1997 – Polymorphisms of bovine  $\beta$ -lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. *Journal of Dairy Science* 80, 1389-1397.
22. MARTIN P., SZYMANOWSKA M., ZWIERZCHOWSKI L., LEROUX C., 2002 – The impact of genetic polymorphisms on the protein composition of ruminant milk. *Reproduction, Nutrition, Development* 42, 433-459.

23. RIJNKELS M., KOOIMAN P.M., DE BOER H.A., PIEPER F.R., 1997 – Organization of the bovine casein gene locus. *Mammalian Genome* 8, 148-152.
24. ROSEN J.M., 1987 – Milk protein gene structure and expression. In: The Mammary Gland. (M.C. Neville, C.W. Daniel, Eds.), Plenum Publ. Corp., New York, p. 301.
25. SCHILD T.A., GELDERMANN H., 1996 – Variants within the 5'-flanking regions of bovine milk-protein-encoding genes. III. Genes encoding the Ca-sensitive casein  $\alpha_{s1}$ ,  $\alpha_{s2}$  and  $\beta$ . *Theoretical and Applied Genetics* 93, 887-893.
26. SZYMANOWSKA M., MALEWSKI T., ZWIERZCHOWSKI L., 2003 – Transcription factor binding to variable nucleotide sequences in 5' flanking regions of bovine casein genes. *International Dairy Journal* (in press).
27. VAN EENENNAAM A.L., MEDRANO J.F., 1991 – Differences in allelic protein expression in the milk of heterozygous  $\kappa$ -casein cows. *Journal of Dairy Science* 74, 1491-1496.
28. VOELKER G.R., BLECK G.T., WHEELER M.B., 1997 – Single-base polymorphisms within the 5' flanking region of the bovine  $\alpha$ -lactalbumin gene. *Journal of Dairy Science* 80, 194-197.
29. YU-LEE L.Y., RICHTER-MANN L., COUCH C.H., STEWART A.F., MACKINLAY A.G., ROSEN J.M., 1986 – Evolution of the casein multigene family: conserved sequences in the 5' flanking and exon regions. *Nucleic Acids Research* 14, 1883-1902.

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## Wpływ polimorfizmu w rejonie niekodującym 5' (promotorze) genów kazein $\alpha$ S1 i $\alpha$ S2 na wybrane cechy mleczności polskich krów rasy czarno-białej

### Streszczenie

Zbadano wpływ polimorfizmu w rejonie niekodującym 5' genów kazein  $\alpha$ S1 i  $\alpha$ S2 na cechy charakteryzujące mleczność i skład mleka 135 polskich krów rasy cb (wydajność dzienna, zawartość suchej masy, suchej masy beztłuszczowej, tłuszczu, białka i laktozy) zależnie od wieku zwierząt, stadium laktacji i liczby komórek somatycznych (SCC) w mleku. Stwierdzono, że krowy o genotypie *AA* kazeiny  $\alpha$ S1 produkowały więcej mleka niż heterozygoty *AG*. Także dzienna produkcja niektórych składników, w tym białka i laktozy, była większa w mleku krów o genotypie *AA*. Mleko krów o genotypie *CC* kazeiny  $\alpha$ S2 zawierało więcej laktozy, lecz mniej białka niż mleko heterozygot *CT*. Dzienna produkcja białka była nieznacznie, ale istotnie większa w mleku krów o genotypie *CT* kazeiny  $\alpha$ S2. Uzyskane wyniki świadczą, że w porównaniu z wyraźnym wpływem okresu laktacji, wieku krów i SCC, warianty genetyczne niekodujących rejonów 5' genów kazein  $\alpha$ S1 i  $\alpha$ S2 wpływają w niewielkim stopniu na cechy produkcji mleka krów rasy cb. Tym niemniej, genotyp *AA* kazeiny  $\alpha$ S1 wyraźnie korzystnie wpływa na wydajność mleka oraz zawartość w nim laktozy i białka.