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Association of the polymorphism at defensin gene *loci* with dairy production traits and milk somatic cell count in Black-and-White cows*

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Because of the antimicrobial role that defensins play in humans and animals, genes encoding these peptides may be considered as molecular markers of a genetically determined susceptibility (or resistance) of the mammary gland to *mastitis*. Records were gathered of daily milk yield, fat, protein, and lactose content of milk, and milk somatic cell count (SCC) of 217 lactating Black-and-White cows. To determine the defensin gene polymorphism, DNA was isolated from blood and the RFLP method with enzyme *Taq*I was used. Twenty different polymorphic systems were revealed, possibly representing variants of genes encoding different defensins. Statistical evaluation included cows with more than seven records, and showing the 2.5% frequency of combined defensin genotypes (CDGs). In this way 13 different CDGs of 204 cows appeared available for statistical evaluation.
CDGs significantly affected all dairy performance traits studied, as well as SCC. The important message from these results is that the defensin(s) may probably be used as genetic marker(s) in the breeding programmes aiming at selecting highly productive dairy cattle with increased resistance to udder infections.

KEY WORDS: cows / defensin / milk traits / somatic cell count

Results from investigations conducted over the recent years point to the important role of peptides existing in various human and animal organs including the mammary gland [Exner *et al*. 2000]. Defensins is a group of antimicrobial peptides with antibiotic

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and cytotoxic activity against bacteria, viruses, and fungi [Tunzi *et al.* 2000]. Their antibacterial properties refer both to the Gram-positive, and Gram-negative bacteria [Ganz *et al.* 1985, Tunzi *et al*. 2000]. Two types of β-defensin are known [Harder *et al.*1997]. In humans, β-defensins type 1 are produced in the kidney epithelium, pancreas, salivary glands, respiratory tract [Ryan *et al*. 1998], urogenital tract and placenta [Zhao *et al*. 1999], while type 2 were found in the skin and tongue tissues and in female urogenital tract [Harder *et al.* 1997]. The expression of β-defensin gene was also shown in the cow mammary gland [Exner *et al.* 2000]. In the human genome the β-defensins are encoded by a group of genes localized to one *locus* on chromosome 8 [Bevins and Diamond 1997], while in cattle they have been mapped to chromosome 27 [Gallagher *et al*. 1995]. Moreover, the amino acid and genomic sequences are known of several cattle defensins [Zhang *et al*. 2000].

Due to the role played by defensins in defending humans and animals from bacterial, viral, or fungal infections, the genes encoding them seem to be potential markers of the genetically determined susceptibility (or resistance) of the mammary gland. It was shown that tissues of an infected human mammary gland contain β-defensin gene transcripts [Exner *et al*. 2000], and that mammary epithelial cells secrete β-defensins. Defensins are present not only in the mammary gland, but also in milk [Jia *et al*. 2001], as well as in leukocyte granules [Diamond *et al*. 2000; Frye *et al*. 2000] and in macrophages [Zhang *et al*. 1998], which constitute a part of milk cell population. Both cell types are responsible for phagocytosis of microbes [Fehlbaum *et al*. 2000]. Moreover, defensins are produced on all epithelial surfaces of the mammary gland [Kaiser and Diamond 2000]. In the light of these findings we suppose that defensins secreted into milk during lactation may protect the udder tissue from bacterial colonization and, consequently, affect both yield and quality of milk.

Mammary gland of a cow is highly susceptible to inflammation. During the gland tissue infection with bacteria the number of polymorphonuclear leucocytes in milk increases dramatically [Emanuelson *et al*.1988, Hogan *et al*. 1992, Lund *et al*. 1999]. The effect of bacterial infection on the yield, composition and quality of cow milk is well documented [Kehrli and Schuster 1994]. Defensins that are recognized as one of the innate defensive response systems against the pathogenic microorganisms can affect the quality of cow milk. This prompted us to investigate whether the defensin genotype affects the milk yield and quality in Black-and-White (BW) dairy cows. This hypothesis is even more attractive, as our earlier investigation showed a significant relation between defensin genotypes and SCC of bovine milk [Ryniewicz *et al*. 2002].

Material and methods

Animals

The studies were conducted in the years 1999-2002 on 217 lactating Black-and-White cows with a share of more than 80% of Holstein-Friesian blood. The cows were maintained on Polish Academy of Sciences Experimental Farm, Kosów. The animals

were randomly chosen from the herd, of which the annual mean milk yield amounted to 8300 kg milk, containing 4.01% fat and 3.45% total protein. As only 4-5 cows were daughters of one sire, the sire effect was not included in the statistical model. The cows were kept in loose barn with outside run, and fed complete total mixed ration (TMR) diet of corn silage, wilted grass silage, and concentrates, supplemented with minerals and vitamins, according to INRA feeding standards. Water was available *ad libitum*. The cows were milked twice a day. Milk samples were taken from each cow once a month during the whole lactation.

DNA isolation

Blood for DNA genotyping was collected from jugular vein by an authorized veterinarian to the tubes containing K_3EDTA and stored at -25°C for 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].

Identification of defensin genotypes

Basing on the sequence of the β1-defensin (enteric; GenBank, accession no. AF016539) and using the Primer 3 software (www.genome.wi.mit.edu) the following primers were designed for the amplification of 1638-bp β-defensin genes:

BBD1S – 5'-GCCAGCATGAGGCTCCAT-3'and

BBD2A – 5'-AACAGGTGCCAATCTGT-3'.

The PCR reactions were performed in a MJ RESEARCH PTC-225 thermal cycler (DNA denaturation – 94°C; annealing – 63.5°C, elongation – 72°C; 34 cycles). The quality of the polymerase chain reaction (PCR) product was tested electrophoretically in 2% agarose gel (GibcoBRL) with ethidium bromide. To determine the polymorphism of defensin genes the restriction fragment length polymorphism (RFLP) method was used. The PCR products were digested with *Taq*I restrictase (FERMENTAS, Lithuania) for 3 h, with 10 units/20 μl, at 37°C) and restriction fragments were analysed electrophoretically in 2% agarose gel in TBE buffer.

In order to confirm the identity of the PCR products the "nested" PCR was carried out. Both the major PCR-amplified fragments of the defensin genes – approx. 1650 and 1300 bp – were eluted from the agarose gel. "Nested" PCR products were re-amplified using the following primers:

DEF1A – 5'-TGCTCCCATTGCTCCATAGAT-3' and

DEF1B – 5'-CCCCCTCCTCCTCCCTTTGT-3',

designed to amplify a 429 bp fragment of the β-defensin (enteric) gene, from nt 1441 to 1869 (GenBank AF016539). The resulting PCR products were sequenced. Then, a sequence alignment was made with that of the β-defensin genes available in GenBank database using BLAST programme (http://www.ncbi.nlm.nih.gov/blast).

The DNA fragments were eluted from agarose gels using NucleoSpin (MACHERY-NAGEL) and then purified with Gene Elute PCR DNA Purification Kit (SIGMA) according to manufacturer's instruction manual.

Restriction maps of the amplified defensin gene fragments were done using HIBIO DNASIS, HITACHI programme, version 2.6. In the PCR-amplified 1638 bp fragment of the bovine β1-defensin (enteric) gene one *Taq*I restriction site was found at position 2363, while two sites at positions 1438/2366 and 1674/2596 in β4- and β5-defensin genes, respectively (positions of nucleotides are given according to sequences obtained from the GenBank).

Dairy performance traits

Cows were in lactations from I to VII. The daily milk yield was recorded monthly and fat, protein, and lactose content, and somatic cell count (SCC) were determined every month during the whole lactation. Fat, protein and lactose content was determined in fresh milk samples using Milko Scan 104A/B, while SCC with Fossomatic apparatus. Both SCC and lactose levels were considered as indicators of the udder health.

Statistical

A total of 4632 records of daily milk yield and milk fat content, 4583 and 3907 of protein and lactose content, respectively, and 3787 of SCC (Tab. 1) were included in the statistical evaluation of results. The SCC values (expressed in thousands) were transformed to the natural logarithm scale (log SCC).

In order to evaluate the significance of relationship between the polymorphism of defensin genes and investigated traits an analysis of variance was performed according to the GLM method [SAS, 1999-2001]. The following model was used:

$$Y_{ijklm} = \mu + c(CDG)_i + CDG_j + P_k + YS_l + \beta_1(x_1 - \bar{x})_{ijklm} + \beta_2(x_2 - \bar{x})_{ijklm} + e_{ijklm}$$

where:
Y_{ijklm} – observed mean value of the trait;
μ – overall mean;
$c(CDG)_i$ – random effect of cow nested in combined defensin genotype – CDG (i = 1...204);
CDG_j – fixed effect of CDG (j = 1...13);
P_k – fixed effect of parity (k = I...≥V);
YS_l – fixed effect of year-season of calving (l = 1....48);
$\beta_1(x_1 - \bar{x})_{ijklm}$ – regression of number of days passed from calving to sampling on all traits investigated;
$\beta_2(x_2 - \bar{x})_{ijklm}$ – regression of milk yield on fat, protein and lactose content, and SCC;
e_{ijklm} – random error.

The interaction year-season of calving (each month considered as a separate class)

was evaluated by dividing the animals into 48 interaction classes. For subsequent lactations five classes were established, with the fifth class including lactation V, VI and VII. The number of records classified by month and year of calving, and parity are given in Table 1.

Table 1. Number of observations within classes of effects

Class effect	Trait				
	milk (kg)	fat (%)	protein (%)	lactose (%)	SCC
Month of calving					
I	330	330	325	260	270
II	298	298	296	229	237
III	323	323	320	283	291
IV	351	351	330	285	297
V	387	387	381	298	308
VI	469	469	465	388	393
VII	543	543	444	333	344
VIII	364	364	363	318	323
IX	399	399	392	328	340
X	419	419	417	343	353
XI	405	405	404	345	361
XII	434	434	426	377	390
Year of calving					
1999	1199	1199	1191	515	516
2000	1249	1249	1240	1240	1240
2001	1630	1630	1611	1535	1611
2002	554	554	541	497	540
Parity					
I	1802	1802	1786	1449	1407
II	1320	1320	1306	1097	1060
III	803	803	793	716	702
IV	437	437	431	391	371
≥V	270	270	267	254	247
Total	4632	4632	4583	3907	3787

Results and discussion

The primers BBD-1S and BBD-2A were used for amplification of β-defensin gene fragment. Theoretically, with these primers the 1638 bp β1-defensin (enteric) gene fragment, encompassing parts of exons 1 and 2 and the interweaving intron, was to be amplified. However, in addition to the bovine β1-defensin, these primers match by 100% the sequence of β4-defensin gene (AF008307; 1651 bp PCR product) and β12-defensin cDNA (AH007938), and the forward primer differs by only one nucle-

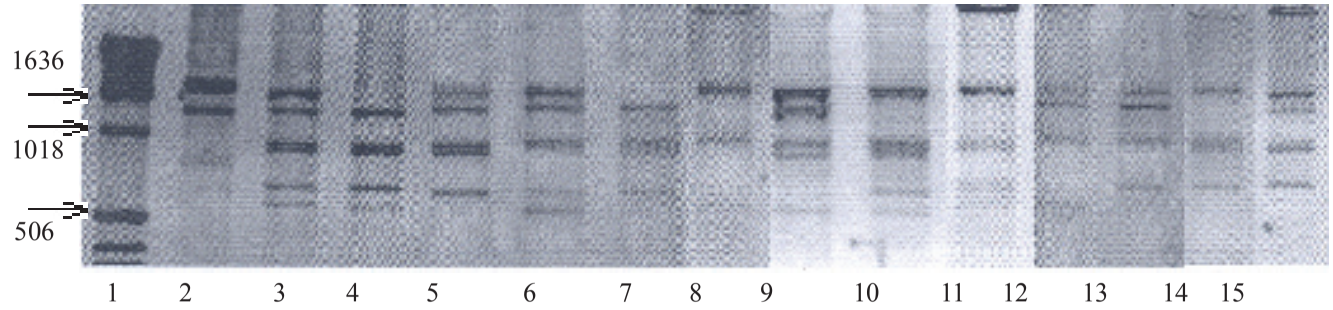

Fig. 1. Restriction fragment length polymorphism (RFLP) of the bovine defensin genes obtained after digesting of the amplification products with *Taq*I restrictase; 2% agarose gel electrophoresis. Different combined defensin genotypes (CDGs) are shown.

1 – 1Kb DNA marker;
2 – non-digested PCR product;
3 – CDG No. 13;
4 – CDG No. 2;
5 – CDG No. 12;
6 – CDG No. 10;
7 – CDG No. 1;
8 – CDG No. 3;
9 – CDG No. 11;
10 – CDG No. 6;
11 – CDG No. 4;
12 – CDG No. 8;
13 – CDG No. 9;
14 – CDG No. 5;
15 – CDG No. 7.

Individual CDGs are specified in Table 2.

otide from the sequence of β5-defensin (AJ278799; 1646 bp PCR product). Actually two major – approx. 1650 and 1300 bp – and several less abundant PCR amplification products appeared (Fig. 1). This indicates that in addition to β1-defensin (enteric) the genes coding for other defensins were amplified.

The identity of the PCR-amplified β-defensin gene fragments was confirmed using re-amplification and sequencing of a shorter fragment located within the 1650 bp and 1300 bp PCR products. The "nested" PCR of the 1650 bp-band DNA with primers DEF1A and DEF1B resulted in a 429 bp PCR product that was identical with the fragment of the β1-defensin (enteric) gene (position 1441-1869; GenBank AF016539) except for a G/A transition at position 1615, the nucleotide substitution indicating the new defensin gene polymorphism. The sequence similarity of the "nested" PCR product to β4-defensin (AF008307) was 93.4% with 19 single nucleotide substitutions or deletions, and to β5-defensin (AJ278799) – 90.8% – with 23 single nucleotide substitutions or deletions. The sequence similarity of the "nested" 429 bp PCR DNA product of the 1300 bp band to the β1-defensin (enteric), β4-defensin, and β5-defensin was 100, 92, and 90%, respectively.

As a result of PCR-RFLP analysis with *Taq*I restrictase a total of 20 polymorphic patterns (genotypes) were obtained of which 13 (Fig. 1) were used for statistical evaluations. Considered were only the CDGs with frequency of ≥0.025, as well as cows with more than seven records (Tab. 2). Each pattern was characterized by bands arranged specifically in pairs, arbitrarily marked A_1A_2, B_1B_2, and C_1C_2. Basing on these results we cannot specify whether we were dealing with polymorphism at one or several gene *loci* of various defensins, or which particular combined defensin genotypes (CDGs)

Table 2. Frequency of RFLP patterns of combined defensin genotypes (CDGs), number of cows and observations gathered for each CDG

No.	CDG			No. of cows	CDG frequency	No. of data sets
1.	__A_1	B_1B_1	C_1__	6	0.030	113
2.	__A_1	B_1B_1	C_1C_1	12	0.059	221
3.	A_1__	B_1__	__C_1	7	0.034	145
4.	A_1__	B_1__	C_1C_1	7	0.034	168
5.	A_1__	B_1B_1	C_1__	6	0.030	149
6.	A_1A_1	B_1B_1	C_1C_1	12	0.059	301
7.	A_1A_1	__B_1	C_1__	5	0.025	135
8.	A_1A_1	B_1__	__C_1	16	0.078	386
9.	A_1A_1	B_1__	C_1__	8	0.039	115
10.	A_1A_1	B_1__	C_1C_1	17	0.083	336
11.	A_1A_1	B_1B_1	__C_1	7	0.034	185
12.	A_1A_1	B_1B_1	C_1__	28	0.137	702
13.	A_1A_1	B_1B_1	C_1C_1	73	0.358	1676
	Total			204	1.00	4632

represented homozygotes or heterozygotes. Nevertheless, the results show clear differences in the frequency of individual band patterns (genotypes). Frequency of RFLP patterns of CDG and number of cows and performance data sets collected for each CDG are shown in Table 2. The most frequent were CDGs No. 13 ($A_1A_2B_1B_2C_1C_2$) and No. 12 ($A_1A_2B_1B_2C_1$–) with the frequency of 0.358 and 0.137, respectively.

Means and standard deviations for all traits investigated are shown in Table 3. Standard deviations except for protein and lactose contents, appeared rather high, and that for SCC even very high. The mean daily milk yield and fat, protein and lactose content of milk in investigated group of cows amounted to 25.4 kg, 4,13%, 3,54% and 4,83%, respectively. Relatively high was mean SCC, exceeding 713 thousand. However, very high lactose content indicated that the cows suffered neither from sub-, nor clinical *mastitis*.

Table 4 summarizes the results of variance analysis of effects on traits investigated.

Table 3. Frequency, means and their standard deviations (SD) for traits investigated

Trait	No. of data sets	Mean	SD
Milk (kg)	4632	25.4	8.40
Fat (%)	4632	4.13	0.91
Protein (%)	4583	3.54	0.40
Lactose (%)	3907	4.83	0.31
SCC (thousands)	3787	713	135
SCC (log)	3787	5.45	1.49

Table 4. Effect of factors analysed on milk yield, composition, and somatic cell count

Effect	Trait				
	milk (kg)	fat (%)	protein (%)	lactose (%)	SCC (log)
Covariated in CDG[1]	***	***	***	***	***
CDG[1]	***	***	***	***	***
Parity	***	ns	***	**	ns
Year-season of calving	***	***	***	***	***
Interval from calving to sampling (days)	***	***	***	ns	***
Milk yield (kg)	–	ns	**	***	***
R^2	0.64	0.42	0.64	0.55	0.58

[1] Combined defensin genotype.
*P≤0.05; ** P≤0.01; *** P≤0.001.

Almost all effects were found significant except for that of parity on milk fat content and SCC, the day after calving on lactose content, and milk yield on fat content.

The CDGs most favourable for daily milk yield were No. 4 (A_1– B_1– C_1C_2) and No. 9 (A_1A_2 B_1– C_1–); both yields differed significantly from almost all others (Tab. 5). However, in the investigated group of cattle their frequency was low – 0.034 and 0.039, respectively (Tab. 3). The most frequent CDGs – No. 12 (A_1A_2 B_1B_2 C_1–) and No. 13 (A_1A_2 B_1B_2 C_1C_2) – were associated with mean daily milk yield. On the other hand, CDGs No. 12 and 13 contributed to the quite high fat, protein, and lactose content of

Table 5. Effects of combined defensin genotypes (CDGs) on milk yield (kg)

No.	CDG	LSM	SE	Different from CDG No.	
				P≤0.05	P≤0.001
1.	__A_1 B_1B_1 C_1__	24.78	0.64	8, 10	3, 4, 6, 9, 12
2.	__A_2 B_1B_1 C_1C_2	25.49	0.71	3, 12	4, 6, 9
3.	A_1__ B_1__ __C_2	27.11	0.71	2, 9, 13	1, 4, 11
4.	A_1__ B_1__ C_1C_2	29.67	0.79		1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 13
5.	A_1__ B_2B_2 C_1__	26.19	0.52	6, 9	4
6.	A_1A_2 B_1B_1 C_1C_2	27.79	0.43	5, 8, 10, 12	1, 2, 4, 7, 11, 13
7.	A_1A_2 __B_2 C_1__	25.72	0.61		4, 6, 9
8.	A_1A_2 B_1__ __C_2	26.38	0.61	1,6, 11	4, 9
9.	A_1A_2 B_1__ C_1__	29.38	1.18	3, 5	1, 2, 7, 8, 10, 11, 12, 3
10.	A_1A_2 B_1__ C_1C_2	26.27	0.69	1, 6, 11	4, 9
11.	A_1A_2 B_1B_1 __C_2	24.96	0.62	8, 10	3, 4, 6, 9, 12
12.	A_1A_2 B_1B_2 C_1__	26.61	0.48	2, 6	1, 4, 9, 11, 13
13.	A_1A_2 B_1B_2 C_1C_2	25.73	0.44	3	4, 6, 9, 12

Table 6. Effects of combined defensin genotypes (CDGs) on fat content

No.	CDG	LSM	SE	Different from CDG No.	
				P≤0.05	P≤0.001
1.	__A_1 B_1B_1 C_1__	4.20	0.09	5	3, 4, 8, 9, 10
2.	__A_2 B_1B_1 C_1C_2	4.00	0.10	8, 10, 13	3, 5, 9
3.	A_1__ B_1__ __C_2	3.70	0.10		1, 2, 5, 6, 7, 11, 12, 13
4.	A_1__ B_1__ C_1C_2	3.88	0.11	9, 12	1, 5, 13
5.	A_1__ B_2B_2 C_1__	4.43	0.07	1	2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13
6.	A_1A_2 B_1B_1 C_1C_2	4.05	0.06	8, 10	3, 5, 9
7.	A_1A_2 __B_2 C_1__	4.09	0.08	10	3, 5, 8, 9
8.	A_1A_2 B_1__ __C_2	3.85	0.08	2, 6, 11	1, 5, 7, 12, 13
9.	A_1A_2 B_1__ C_1__	3.63	0.16	4, 10	1, 2, 5, 6, 7, 11, 12, 13
10.	A_1A_2 B_1__ C_1C_2	3.85	0.09	2, 6, 7, 9, 11	1, 5, 12, 13
11.	A_1A_2 B_1B_1 __C_2	4.05	0.09	8, 10	3, 5, 9
12.	A_1A_2 B_1B_2 C_1__	4.05	0.07	4, 13	3, 5, 8, 9, 10
13.	A_1A_2 B_1B_2 C_1C_2	4.14	0.06	2, 12	3, 4, 5, 8, 9, 10

Table 7. Effects of combined defensin genotypes (CDGs) on protein content

No.	CDG	LSM	SE	Different from CDG No.	
				P≤0.05	P≤0.001
1.	__A_1 B_1B_1 C_1__	3.52	0.03	2, 8	5, 7
2.	__A_1 B_1B_1 C_1C_1	3.60	0.03	1	3, 6, 8, 10, 11, 12
3.	A_1__ B_1__ __C_1	3.47	0.03		2, 4, 5, 7, 13
4.	A_1__ B_1__ C_1C_1	3.59	0.04	6, 10, 12	3, 8, 11
5.	A_1__ B_1B_1 C_1__	3.64	0.02		1, 3, 6, 8, 10, 11, 12
6.	A_1A_1 B_1B_1 C_1C_1	3.51	0.02	4, 8	2, 5, 7, 13
7.	A_1A_2 __B_1 C_1__	3.61	0.03		1, 3, 6, 8, 10, 11, 12
8.	A_1A_2 B_1__ __C_1	3.45	0.03	1, 6, 9, 10	2, 4, 5, 7, 12, 13
9.	A_1A_1 B_1__ C_1__	3.54	0.06	8	
10.	A_1A_2 B_1__ C_1C_1	3.51	0.03	4, 8	2, 5, 7, 13
11.	A_1A_1 B_1B_1 __C_1	3.46	0.03	12	2, 4, 5, 7, 13
12.	A_1A_2 B_1B_1 C_1__	3.52	0.02	4, 11	2, 5, 7, 8, 13
13.	A_1A_1 B_1B_1 C_1C_1	3.57	0.02		3, 6, 8, 10, 11, 12

Table 8. Effects of combined defensin genotypes (CDGs) on lactose content

No.	CDG	LSM	SE	Different from CDG No.	
				P≤0.05	P≤0.0001
1.	__A_1 B_1B_1 C_1__	4.88	0.04	12, 13	4, 10
2.	__A_2 B_1B_1 C_1C_1	4.84	0.05	11	4, 10
3.	A_1__ B_1__ __C_1	4.83	0.05	10, 11	4
4.	A_1__ B_1__ C_1C_1	4.67	0.05		1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13
5.	A_1__ B_1B_1 C_1__	4.79	0.03	11	
6.	A_1A_1 B_1B_1 C_1C_1	4.82	0.03		4, 11
7.	A_1A_2 __B_1 C_1__	4.83	0.04	10, 11	4
8.	A_1A_2 B_1__ __C_1	4.82	0.04	10	4, 11
9.	A_1A_1 B_1__ C_1__	4.85	0.09		4
10.	A_1A_2 B_1__ C_1C_1	4.76	0.05	3, 7, 8, 13	1, 2, 4, 11
11.	A_1A_1 B_1B_1 __C_1	4.91	0.04	2, 3, 5, 7	4, 6, 8, 10, 12, 13
12.	A_1A_2 B_1B_1 C_1__	4.81	0.04	1	4, 11
13.	A_1A_1 B_1B_1 C_1C_1	4.82	0.03	1, 10	4, 11

milk, well above the mean values for all the cows considered (Tab. 6, 7, 8). The animals carrying the A_1_ B_1B_2 C_1_ combination (No. 5, frequency of 0.030) had highest milk fat and protein content (Tab. 6 and 7). The CDG best for lactose content (No. 11) appeared at low frequency of 0.034 (Tab. 8). As expected, the CDG No. 4 – A_1_ B_1B_2 C_1C_2 – appearing most favourable for milk yield (Tab. 5) was related to low or very low fat (Tab. 6) and lactose (Tab. 8) content of milk due to the well known negative genetic correlation between milk yield and milk components content. Interestingly, however, CDG No. 4 was associated both with the highest milk yield (Tab. 5) and very high protein content (Tab. 7). The two CDGs associated with high milk yield (Nos. 4

Table 9. Effects of combined defensin genotypes (CDGs) on log SCC

No.	CDG			LSM	SE	Different from CDG No. P≤0.05	Different from CDG No. P≤0.001
1.	__A_4	B_1B_1	C_1__	5.01	0.17	11	3, 6, 8, 10, 12, 13
2.	__A_4	B_1B_1	C_1C_1	5.06	0.23	6, 11	3, 8, 10, 12, 13
3.	A_4__	B_1__	__C_1	5.47	0.21	8, 9, 12	1, 2, 4
4.	A_4__	B_1__	C_1C_1	4.82	0.24	7	3, 6, 8, 10, 11, 12, 13
5.	A_4__	B_1B_1	C_1__	5.18	0.14	8, 12	
6.	A_4A_4	B_1B_1	C_1C_1	4.42	0.12	2, 8	1, 4, 12
7.	A_4A_4	__B_1	C_1__	5.17	0.16	4, 13	8, 10, 12
8.	A_4A_4	B_1__	__C_1	5.77	0.20	3, 5, 6	1, 2, 4, 7, 9, 11, 13
9.	A_4A_4	B_1__	C_1__	4.87	0.40	3, 13	8, 10, 12
10.	A_4A_4	B_1__	C_1C_1	5.74	0.24	11, 13	1, 2, 4, 7, 9
11.	A_4A_4	B_1B_1	__C_1	5.37	0.17	1, 2, 10	4, 8, 12
12.	A_4A_4	B_1B_1	C_1__	5.79	0.17	3, 5	1, 2, 4, 6, 7, 9, 11, 13
13.	A_4A_4	B_1B_1	C_1C_1	5.46	0.16	7, 9, 10	1, 2, 4, 8, 12

and 9), were simultaneously related to quite low SCC (Tab. 9). The cows carrying the two most frequent CDGs, *i.e*. Nos. 12 and 13 showed rather high SCC (Tab. 9), while those with CDG No. 6, having the lowest SCC, appeared moderately high in milk yield, milk fat, lactose and protein content. Unfortunately, the frequency of favourable CDG No. 6 was quite low (0.059).

The SCC of milk seems to have an important impact on cattle breeding and production traits, as it reflects the health status of the udder [Philipson *at al*. 1995]. In the present report traits reflecting the dairy performance, such as daily milk yield, were found highly correlated with the health status of the mammary gland (Tab. 4). In this context, defensin genotypes associated with low SCC of milk, may be considered as indicators of a healthy udder.

With the global overproduction of milk and dairy products the consumer's interest is shifting now to healthy and good quality foods. Investigations are carried out aiming at identifying genetic markers related to the health status of the udder, and particularly to the resistance to *mastitis* [Ashwell *et al.* 1996, Kelm *et al.* 1997, Ashwell and Van Tassell 1999, Tunzi *et al*. 2000, Zhang *et al*. 2000]. Klungland *et al*. [2001] using veterinary records and SCC of Norwegian dairy cattle localized putative quantitative trait *loci* (QTLs) for clinical *mastitis* to chromosomes 3, 4, 14, and 27. They concluded that comparison of SCC with QTLs affecting clinical *mastitis* should be of great interest since SCC is often used in selection against the udder inflammation when direct information on clinical mastitis is not available. QTLs affecting milk production and health of dairy cattle were mapped in a very large Holstein granddaughter design [Zhang *et al.* 1998]. It was shown that chromosome 17 likely harbours two QTLs affecting milk yield; other chromosomes showed some evidence for two linked QTLs affecting the same trait. Ashwell *et al*. [1996] searched for a QTL for somatic cell score in a Holstein population using the granddaughter design and 20 autosomal microsatellites from chromosomes

4, 8, 13, 17 and 23. Based on these results the most likely position of a somatic cell score QTL was shown to be near marker 513, on chromosome 23. Ashwell *et al*. [1998, 1999] have identified several QTLs affecting body conformation and milk yield traits of US Holsteins using the granddaughter design and 16 microsatellite markers. The most significant effect had marker BM203 (chromosome 27) for dairy form. A multivariate analysis for dairy form and milk yield was also conducted, indicating that segregating QTL (or QTLs) affecting dairy form and milk yield could exist near BM203 on chromosome 27. Thus, several QTLs have been found, some of them located to the bovine chromosome 27, the same on which the group o genes coding for defensins has been localized. This makes β-defensin genes attractive candidates for genetic markers of the udder health traits and perhaps its suscectibility to inflammations. Nevertheless, despite many investigations, such markers had not been found yet.

This report describes the first ever polymorphism in the bovine defensin genes. The significant effect of the polymorphism of defensin genes on SCC and on dairy performance traits found in this study may lead to the use of defensins as genetic marker(s) in the breeding programmes aiming at selecting highly productive dairy cattle with increased resistance to udder infections. However, to prove this, needed are further investigations including bigger cattle populations of different breeds as well as the studies aiming at demonstrating a polymorphism at individual defensin *loci* and its association with production traits and health status of the mammary gland.

REFERENCES

1. ASHWELL M.S., DA Y,, VANRADEN P.M., REXROAD C.E., JR, MILLER R.H., 1998 – Detection of putative *loci* affecting conformational type traits in an elite population of United States Holsteins using microsatellite markers ***Journal of Dairy Science*** 81 (4), 1120-1125.
2. ASHWELL M.S., REXROAD C.D., MILLER R.H., VAN RADEN P.M., 1996 – Mapping economic trait *loci* for somatic cell score in Holstein cattle using microsatellite markers and selective genotyping. ***Animal Genetics*** 27, 235-242.
3. ASHWELL M.S., VAN TASSELL C.P., 1999 – Detection of putative *loci* affecting milk, health and type traits in a US Holstein population using 70 microsatellite markers in genome. ***Journal of Dairy Science*** 82, 2497-2502
4. BEVINS C.L., DIAMOND G., 1997 – Molecular biological strategies in the analysis of antibiotic peptide gene families: the use oligonucleotides as hybridization probes. ***Methods in Molecular Biology*** 78, 151-166.
5. DIAMOND G., LEGARDA D., RYAN LK., 2000 – The innate immune response of the respiratory epithelium. ***Immunological Reviews*** 173, 27-38.
6. EMANUELSON U., DANELL B., PHILIPSON J., 1988 – Genetic parameters for clinical mastitis somatic cell counts and milk production estimated by multiple-trait restricted maximum likelihood. ***Journal of Dairy Science*** 71, 467-476.
7. EXNER K., THOMSEN P.D., PAUL S., ROOSEN S., KALM E., LOOFT CHR., 2000 – Characterization of β-defensins – a family of peptide antibiotics also expressed in the epithelium of the bovine mammary gland. ISAG Conference, Minnesota, July 22-26, Book of Abstracts, Abstract C006, p. 60.
8. FEHLBAUM P., RAO M., ZASLOFF M., ANDERSON M., 2000 – An essential amino acid induces epithelial β-defensin expression. ***Proceedings of the National Academy of Sciences, USA*** 97 (23), 12723-12728.

9. FRYE M., BARGON J., LEMBCKE B., WAGNER TOF., GROPP R., 2000 – Differential expression of human α and β-defensins mRNA in gastrointestinal epithelia. ***European Journal of Clinical Investigations*** 30, 695-701.
10. GALLAGHER D.S., RYAN J.A. M., DIAMOND G., BEVINS C.L., WOMACK J.E., 1995 – Somatic cell mapping of beta-defensin genes to cattle synthetic group U25 and fluorescence in situ localization to chromosome 27. ***Mammalian Genome*** 6, 554-556.
11. GANZ T., SELSTED M.E., SZKLAREK D., HARTWIG S.S., DAHER K., BAINTON D.F., LEHRER R.I., 1985 – Defensins. Natural peptide antibiotics of human neutrophils. ***Journal of Clinical Investigation*** 76, 1427-1435.
12. HARDER J., BARTELS J., CHRISTOPHERS E., SCHRODER J.M., 1997 – A peptide antibiotic from human skin. ***Nature*** 387, 861-862.
13. HOGAN J.S., WEISS W.P., TODHUNTER D.A., SMITH K.L., SCHOENBERGER P.S., 1992 – Efficacy of an Escherichia coli 15 mastitis vaccine in an experimental challenge trial. ***Journal of Dairy Science*** 75, 415-422.
14. JIA H.P., STARNER T., ACKERMANN M., KIRBY P., TACK B., MC CRAY B., 2001 – Abundant human β-defensin-1 expression in milk and mammary gland epithelium. ***Journal of Pediatrics*** 138, 109-112.
15. KAISER V., DIAMOND G., 2000 – Expression of mammalian defensin genes. ***Journal of Leukocyte Biology*** 68 (6) 779-784.
16. KANAI N., FUJII T., YOKOYAMA T., 1994 – Rapid and simple method for preparation of genomic DNA from easily obtained clotted blood. ***Journal of Clinical Pathology*** 47, 1043-1044.
17. KEHRLI M.E., SHUSTER D.E., 1994 – Factors affecting milk somatic cells and their role in health of the bovine mammary gland. ***Journal of Dairy Science*** 77 (2), 619-627.
18. KELM S.C., DETILLEUX J.C., FREEMAN A.E., KEHRLI M.E., DIETZ A.B., FOX L.K., BUTLER J.E., KASCKOVICS D.H., KELLEY., 1997 – Genetic association between parameters in innate immunity and measures of mastitis in periparturient Holstein cattle. ***Journal of Dairy Science*** 80 (8), 1767-1775.
19. KLUNGLAND H, SABRY A., HERINGSTAD B., OLSEN H.G, GOMEZ-RAYA L, VAGE D.I., OLSAKER I., ODEGARD J., KLEMETSDAL G., SCHULMAN N., VILKKI J., RUANE J., AASLAND M., RONNINGEN K., LIEN S., 2001 – Quantitative trait *loci* affecting clinical mastitis and somatic cell count in dairy cattle. ***Mammalian Genome*** 12 (11), 837-842.
20. LUND M.S., JENSEN J., PETERSEN P.H., 1999 – Estimation of genetic and phenotypic parameters for clinical mastitis, somatic cell production deviance, and protein yield in dairy cattle using Gibbs sampling. ***Journal of Dairy Science*** 82 (5), 1045-1051.
21. PHILIPSON J., RAL G., BERGLUND B., 1995 – Somatic cell counts as a selection criterion for mastitis resistance in dairy cattle. ***Livestock Production Science*** 41, 195-200.
22. RYAN L.K., RHODES J., BHAT M., DIAMOND G., 1998 – Expression of beta-defensin genes in bovine alveolar macrophages. ***Infection and Immunity*** 66, 878-881.
23. RYNIEWICZ Z., ZWIERZCHOWSKI L., BAGNICKA E., KRZYŻEWSKI J., STRZAŁKOWSKA N., 2002 – Preliminary investigations on the polymorphism of defensin genes in cattle – relation with milk somatic cell count. ***Animal Science Papers and Reports*** 20 (2), 125-131.
24. SAS, SAS/STAT 1999-2001 – User's Guide Release 8.E. SAS Institute Inc., Cary, NC, USA.
25. TUNZI Ch.R., HARPER P.A., BAR-OZ B., VALORE E.V., SEMPLE J.L., WATSON-MACDONELL J.L., GANZ T., ITO S., 2000 – β-defensin expression in human mammary gland epithelia. ***Pediatric Research*** 48, 30-35.
26. ZHANG G.L., ROSS C.R., BLECHA F., 2000 – Porcine antimicrobial peptides: New prospects for ancient molecules of host defense. ***Veterinary Research*** 31 (3), 277-296.

27. ZHANG Q., BOICHARD D., HOESCHELE I., ERNST C., EGGEN A., MURKVE B., PFISTER-GENSKOW M., WITTE L.A., GRIGNOLA F.E., UIMARI P., THALLER G., BISHOP M.D., 1998 – Mapping quantitative trait *loci* for milk production and health of dairy cattle in a large outbred pedigree. ***Genetics*** 149 (4), 1959-1973.
28. ZHAO CH., NGUYEN T., LIU L., SHAMOVA O., BROGDEN K., LEHRER R.I., 1999 – Differential expression of caprine β-defensins in digestive and respiratory tissues. ***Infection and Immunity*** 67 (11), 6221-6224.

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Związek polimorfizmu w *loci* genów defenzyn z cechami produkcji mlecznej i liczbą komórek somatycznych w mleku krów rasy cb

S t r e s z c z e n i e

Ze względu na fakt, że defensyny pełnią istotną rolę w obronie organizmów przed zakażeniami bakteryjnymi, geny kodujące te peptydy mogłyby stać się dobrymi markerami genetycznej wrażliwości lub odporności gruczołu mlekowego na stany zapalne wywoływane przez drobnoustroje (*mastitis*). Wykorzystano dane o dziennej wydajności, procencie tłuszczu, białka i laktozy w mleku oraz o liczbie komórek somatycznych mleka 217 krów dojnych. Z krwi krów izolowano DNA, a następnie metodą RFLP, używając enzymu *Taq*I, określono polimorfizm genów defensyn. Uzyskano 20 polimorficznych układów (*combined defensin genotypes* – CDG), reprezentujących warianty genów różnych defenzyn. Do analizy statystycznej użyto danych, pochodzących od krów, których mleko badano co najmniej osiem razy i o układach genotypów o częstości większej niż 2,5%. W rezultacie analizę przeprowadzono dla 13 różnych układów genotypów, stwierdzonych u 204 krów. Wykazano wysoce istotny wpływ układów genów defensyn (CDG) na wszystkie badane cechy produkcyjne i liczbę komórek somatycznych (SCC) w mleku. Wnioskuje się, że uzyskane wyniki dają nadzieję na wykorzystanie w selekcji bydła mlecznego polimorfizmu genów defensyn jako markera wysokiej produkcyjności i zwiększonej odporności krów na bakteryjne zakażenia wymienia. Konieczne są jednak dalsze badania.