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EFFECT OF POLYMORPHISM IN GENE *BTN1A1* ON SOMATIC CELL COUNT IN MILK OF JERSEY COWS

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The aim of the study was to analyse the dependence between P35Q and K468R polymorphism in the butyrophilin gene (*BTN1A1*) and somatic cell count in milk in cattle. The experiment was conducted on a population of 214 Jersey cows. Genotypes of the animals were determined using PCR-RFLP. The following allele sequences were found: P35Q – 0.85 (A) and 0.15 (C); K468R – 0.66 (A) and 0.34 (G). The association analysis was performed for the whole investigated population and with the division into age groups (primiparous and multiparous cows), and taking into consideration the stage of lactation (the first, the second and the third 100 days). In case of mutation K468R, milk of homozygous AA animals was characterised by the higher somatic cell count. Differences with the highest significance ($p \leq 0.01$) were found between genotypes AA and AG in the group of multiparous cows, especially in the initial period of lactation. In the population of primiparous cows the effect of polymorphism K468R was much lower. A significant difference ($p \leq 0.05$) was observed only between homozygotes AA and GG in the first stage of lactation. An analysis performed for polymorphism P35Q did not show significant associations with somatic cell counts in milk of cows.

INTRODUCTION

Considerable competition on the market forces producers to constantly lower the costs of milk production and to sell the raw material with high hygienic and microbial quality. The Jersey breed is the second dairy breed in the world in terms of the population size and it also exhibits advantageous functional traits, such as fertility, easy calving, health state of the udder and longevity [Larson, 2001].

The number of somatic cells in milk is an index of the health state of the udder and the hygienic value of the raw material for dairy industry. Diseases of the mammary gland cause a lowering of milk yields and disadvantageous changes in the chemical composition of milk, which in consequence leads to a deterioration of its nutritive and technological value. According to Rosochowicz *et al.* [2002], the introduction of somatic cell count to the selection index as a trait may restrict selection response in production traits, but it would improve the health state of the mammary gland.

Apart from pathological states, somatic cell count in milk is affected by several factors, such as the season of the year, breed, successive lactation, the stage of lactation, stress, feeding, *etc.* [Harmon, 1994; Ng-Kwai-Hang *et al.*, 1984; Sender *et al.*, 1987]. At present different methods are being sought to limit the incidence of mastitis, as manifested *e.g.* in the studies aiming at the utilisation in breeding practice of traits of hereditary immunity of cows to mastitis.

Somatic cell count in milk belongs to quantitative traits with low heritability, amounting most frequently to 0.07–0.11 [Schutz *et al.*, 1995]. Selection using genetic markers may serve a key function in the improvement of such traits. One of the methods to search for loci controlling quantitative traits is to determine candidate genes, *e.g.* on the basis of the known biological functions of the polypeptide they code for.

Butyrophilin (*BTN1A1*) is the primary protein component of the membrane surrounding fat molecules in milk, and its expression is limited to epithelial cells in the mammary gland at the final stage of pregnancy and during lactation [Ogg *et al.*, 1996]. Studies conducted recently have shown that functional protein *BTN1A1* is necessary for the proper secretion of milk components, especially fat [Ogg *et al.*, 2004]. Butyrophilin belongs to integral glycoproteins anchored in the cell membrane [Banghart *et al.*, 1998]. On the cytoplasmic side there is a C-end domain with a highly conservative region B30.2, probably involved in interactions with other proteins. In turn, within the N-end exoplasmic part of the protein the presence of two domains was found, contained in numerous proteins from the family of immunoglobulins (IgI, IgC1), suggesting a possible role of butyrophilin in immunity mechanisms.

Gene *BTN1A1* was located in cattle in chromosome 23 [Taylor *et al.*, 1996]. It is composed of 8 exons divided by 7 introns [Seyfert & Lüthen, 1998]. The sequence analysis showed the occurrence of numerous mutations, some of which leading to changes in coded proteins and potentially having an effect on its function [Husaini *et al.*, 1999; Seyfert

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& Lüthen, 1998; Taylor *et al.*, 1996]. The P35Q polymorphism consists in substitution C→A in exon 3 and leads to the substitution of prolin by glutamine within domain IgI [Seyfert & Lüthen, 1998], whereas K468R is a A→G mutation within exon 8 causing a substitution of lysine by arginine in region B30.2 [Taylor *et al.*, 1996].

The aim of this study was to determine the dependence between the and K468R polymorphism of gene *BTN1A1*, and somatic cell counts in milk of Jersey cows.

MATERIALS AND METHODS

Experimental material consisted of blood and milk of 214 Jersey cows kept at the Iwno Stud Farm in the years 1997-2005. From the records of production value testing of dairy cattle information was collected on somatic cell counts in 5209 milk samples.

DNA for molecular analyses was isolated from peripheral blood using the standard phenol method. Genotypes were determined using PCR-RFLP. Starter sequences for PCR were established on the basis of the gene sequence available in the GenBank data base (accession no. Z93323), using PRIMER3 software [http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi]:

P35Q-F: 5' – TGGTAGGTCAGGAAGCCATC – 3'
P35Q-R: 5' – GTATTCAGCCATCTCCTCGC – 3'
K468R-F: 5' – TGGAGCTCTATGGAAATGGG – 3'
K468R-R: 5' – ACCCTTTGGGTTTTCTGCTT – 3'

PCR amplification was performed in a TGradient thermocycler (Biometra). The reaction mixture contained in 10 μL a total of 20–50 ng genomic DNA, 0.5 units of Taq polymerase (Fermentas), 1 μmol/L each starter (IBB PAN), 75 mmol/L Tris-HCl (pH 8.8), 20 mmol/L $(NH_4)2SO_4$, 0.01% Tween 20, 2 mmol/L $MgCl_2$, 5% DMSO and 200 mmol/L each dNTP (Fermentas). Reaction conditions included initial denaturation at 94°C (5 min), followed by 30 cycles consisting of denaturation (94°C, 30 s), annealing (58°C for P35Q or 56°C for K468R, 30 s) and synthesis (72°C, 40 s), followed by final synthesis at 72°C (5 min).

Amplified fragments of P35Q and K468R were digested at 37°C for at least 5 h using 5 units of a respective restriction enzyme – *Bcn*I or *Bsu*RI (*Hae*III) (Fermentas). Digestion products were subjected to electrophoresis in 2–3% agarose gel (*BASICA LE GQT*, Prona) stained with ethidium bromide. Separation products were observed under UV light. In the study dependencies were analyzed between P35Q and K468R polymorphisms of the *BTN1A1* gene and somatic cell count in the whole investigated population and presented in terms of the division into primiparous and multiparous cows including the stages of lactation (the first, second and third 100 days). Since somatic cell count does not have the normal distribution required in statistical methods, the trait was transformed according to the formula by Da *et al.* [1992]:

$$SCS = \log_2 (SCC/100) + 3$$

where: SCS – somatic cell count after transformation, SCC – somatic cell count from direct measurement (in thousands).

The effect of genotypes on somatic cell count (SCC) in milk was tested using the GLM procedure from the SAS statistical package [SAS, 2005]. The statistical model included the effects of *BTN1A1* genotypes, the sire effect, the effect of lactation number, as well as the effect of the year and season of calving.

RESULTS AND DISCUSSION

As a result of PCR products were obtained of 560 bp (P35Q) and 780 bp (K468R). Digestion of the K468R fragment gave bands with the lengths of 371, 162, 141, 83, 13 and 10 bp (allele A coding for lysine) or 338, 162, 141, 83, 33, 13 and 10 bp (allele G coding for arginine). In case of the P35Q mutation, digestion products were visible in the gel in the form of two (74 and 500 bp) or three (74, 96 and 404 bp) bands, respectively, for variant A coding for glutamine and C coding for prolin.

In the investigated group of 214 Jersey cows three possible genotypes were identified both for mutation P35Q and K468R. The P35Q polymorphism was characterised by a much higher frequency of incidence of variant A (0.85) in comparison to variant C (0.15). Homozygotes AA were found in the biggest number (153 animals), followed by heterozygotes AC (56 animals) and homozygotes CC (only 5 animals). In the other analysed *locus* (K468R) the numbers of animals with specific genotypes were as follows: AA –

TABLE 1. The effect of polymorphism P35Q of the *BTN1A1* gene on somatic cell counts in terms of the age of cows and the stage of lactation.

Age group of cows	Genotype	Total		Stage of lactation (days)					
				≤100		101–200		>200	
		$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Primiparous	AA	3.54	1.35	3.64	2.35	3.36	2.16	3.58	1.86
cows	AC	3.40	1.01	3.55	1.83	3.10	2.22	3.53	1.36
	CC	2.71	0.97	3.18	0.62	2.41	2.28	2.42	0.58
Multiparous	AA	3.59	1.49	3.49	2.24	3.53	1.74	3.76[a]	1.60
cows	AC	3.62	1.46	3.65	2.09	3.57	1.83	3.80	2.19
	CC	4.30	2.84	4.13	2.79	4.13	3.98	5.13[a]	1.39
Total	AA	3.58	1.44	3.56	2.28	3.47	1.89	3.82	1.71
	AC	3.57	1.31	3.61	1.99	3.40	2.01	3.69	1.87
	CC	3.81	2.72	3.81	2.17	3.66	3.86	4.02	3.02

Means denoted with identical letters differ statistically within the age group of cows: A, B, C – highly significantly ($p \leq 0.01$); a, b, c – significantly ($p \leq 0.05$).

TABLE 2. The effect of polymorphism K468R of the *BTN1A1* gene on somatic cell count in terms of the age of cows and the stage of lactation.

Age group of cows	Genotype	Total		Stage of lactation (days)					
				≤100		101–200		>200	
		$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Primiparous	AA	3.52	1.06	3.72^{a}	1.97	3.31	2.08	3.50	1.59
cows	AG	3.43	1.38	3.64	2.33	3.27	2.30	3.58	1.76
	GG	3.19	1.55	3.07^{a}	2.21	3.14	2.28	3.54	2.20
Multiparous	AA	3.95^{A}	1.78	3.96^{Aa}	2.21	3.75^{ab}	2.18	4.05^{a}	1.91
cows	AG	3.41^{A}	1.24	3.40^{A}	2.10	3.34^{a}	1.59	3.61^{a}	1.58
	GG	3.58	1.52	3.44^{a}	2.49	3.21^{b}	1.18	4.02	1.95
Total	AA	3.74^{a}	1.54	3.75^{ab}	2.12	3.59	2.18	3.85	1.85
	AG	3.42^{a}	1.28	3.48^{a}	2.18	3.38	1.82	3.63	1.65
	GG	3.43	1.54	3.35^{b}	2.38	3.25	1.56	3.74	2.07

Means denoted with identical letters differ statistically within the age group of cows: A, B, C – highly significantly ($p \leq 0.01$); a, b, c – significantly ($p \leq 0.05$).

93 cows, AG – 95 cows and GG – 26 cows, respectively. Frequencies for alleles A i G were 0.66 and 0.34, respectively.

Mean somatic cell counts found in the populations of primiparous and multiparous cows were 3.46 and 3.63, respectively. The lowest SCC in the first lactation and its increasing values in successive lactations were also reported by *e.g.* Kiiman & Saveli [1998], Borkowska & Januś [2001] and Pytlewski & Dorynek [2000]. An increased risk of thw development of new infections in older cows is probably a consequence of high milk yields and gradual wearing out of the teat sphincter muscle. Irrespective of the division of animals into age groups the highest number of somatic cells was shown for the first and third stage of lactation. Similar results were also reported by Sender *et al.* [1987]. In contrast, studies by other authors indicate an upward trend for SCC along with the course of lactation [Sawa *et al.*, 2000] or a lack of the effect of lactation stage on this trait [Antkowiak *et al.*, 2003].

Results concerning the effect of polymorphism in gene *BTN1A1* on somatic cell count in milk in terms of age and stage of lactation are presented in Tables 1 and 2. The conducted analysis did not show the effect of the P35Q mutation of the investigated trait. Only one statistically significant difference was observed between genotypes AA and CC in the third stage of lactation in the group of primiparous cows (Table 1). However, due to the very low number of homozygotes CC (5 cows) the obtained result could have been incidental. While analysing the dependence between polymorphism K468R and somatic cell count, the highest number of somatic elements was found in milk of cows with genotype AA (Table 2). In the group of primiparous cows a statistically significant difference was observed only between homozygotes AA and GG in the initial stage of lactation. In the population of multiparous cows the effect of mutation K468R was much more tangible. Genotype AA was characterised, especially in comparison with heterozygotes AG, by a higher SCC in milk at all stages of lactation, although the biggest differences were found in its initial stage.

Gene *BTN1A1* in cattle is located in the region of chromosome 23, in which the presence was detected of hypothetical QTLs (*quantitative trait loci*) controlling milk yield and composition [Ashwell *et al.*, 1997; Zhang *et al.*, 1998] and somatic cell count [Ashwell *et al.*, 1996; Heyen *et al.*, 1999]. Butyrophilin is one of candidate genes with a potential effect on the variation in mapped QTLs. Its polymorphism has not been the subject of intensive studies aiming at the detection of an association with quantitative traits. Studies including the effect of *BTN1A1* on somatic cell count in milk were only conducted by Zegeye *et al.* [1999]. They analysed the effect of five mutations, including four intron ones and K468R, on milk producing characters and health state in Holstein – Friesian cattle. For K468R no significant dependence was identified.

The results of this study indicate that genotype AA in *locus* K468R may be related to an increased content of somatic cells in milk. Mutation K468R is found within region *BTN1A1* coding for the cytoplasmic domain B30.2, present also in numerous other proteins [Henry *et al.*, 1998]. The function of B30.2 remains to a large degree unknown. In case of the butyrophilin gene, it probably participates in the secretion of milk lipid compounds by binding, through such proteins as xanthinoxidase, intercellular fat of the udder glandular tissue [Mather & Keenan, 1998]. The K468R polymorphism may thus be connected with the variation in milk composition. Its effect on the health state of animals seems rather less probable. The effect of the other investigated mutation (P35Q) would be easier to justify, due to its location within the IgI domain, showing homology with certain proteins contained in the immune system [Vernet *et al.*, 1993; Linsey *et al.*, 1994]. However, the conducted analysis did not show any dependence between somatic cell count and the P35Q polymorphism.

The effect detected for K468R might not be a direct consequence of this mutation, but it might rather result from a linkage with another locus controlling immunity in animals. In this aspect attention needs to be paid to genes of the major histocompatibility complex (MHC), located in cattle in chromosome 23 in the vicinity of *BTN1A1* [Fries *et al.*, 1986], whose relationship with the level of somatic cells in milk has been described in several studies [Rupp & Boichard, 2003].

CONCLUSIONS

1. The conducted analysis did not show any dependence between somatic cell count and the P35Q polymorphism.

2. The results of this study indicate that genotype AA in

locus K468R may be related to an increased content of somatic cells in milk. In the population of multiparous cows the effect of mutation K468R was much more marked. Genotype AA was characterised, especially in comparison with heterozygotes AG, by a higher SCC in milk at all stages of lactation, although the biggest differences were found in its initial stage.

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WPŁYW POLIMORFIZMU GENU *BTN1A1* NA ZAWARTOŚĆ KOMÓREK SOMATYCZNYCH W MLEKU KRÓW RASY JERSEY

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Butyrofilina (*BTN1A1*), ze względu na funkcje jakie pełni podczas laktacji oraz podobieństwo do genów z rodziny immunoglobulin, może być uważana za jeden z genów kandydujących o potencjalnym wpływie na cechy mleczności oraz odporność zwierząt. Celem obecnej pracy była analiza zależności między dwoma mutacjami *BTN1A1* prowadzącymi do zmian w kodowanym białku (P35Q i K468R) a zawartością komórek somatycznych (SCS) w mleku, będących wskaźnikiem zdrowotności wymienia u bydła. Doświadczenie przeprowadzono w populacji 214 krów rasy jersey. Genotypy zwierząt określono metodą PCR-RFLP. Uzyskane frekwencje alleli były następujące: P35Q – 0.85 (A) i 0.15 (C); K468R – 0.66 (A) i 0.34 (G). Analizę asocjacji przeprowadzono dla całej badanej populacji oraz z podziałem na grupy wiekowe (pierwiastki i wieloródki), z uwzględnieniem faz laktacji (pierwsze 100 dni, drugie i trzecie). Dla polimorfizmu P35Q nie wykazano istotnych asocjacji z liczbą komórek somatycznych w mleku krów (tab. 1). W przypadku mutacji K468R, mleko osobników homozygotycznych AA charakteryzowało się najwyższą zawartością SCS (tab. 2). Różnice o największej istotności ($P \leq 0.01$) uzyskano między genotypami AA i AG w grupie wieloródek, szczególnie w początkowym okresie laktacji. W populacji pierwiastek efekt polimorfizmu K468R był znacznie mniejszy. Istotną różnicę ($P \leq 0.05$) zaobserwowano między homozygotami AA i GG jedynie w pierwszej fazie laktacji. Istnieje możliwość, że wykryty efekt nie jest bezpośrednim skutkiem mutacji K468R, ale wynika ze sprzężenia z innymi loci kontrolującymi odporność zwierząt, takimi jak np. geny głównego kompleksu zgodności tkankowej (MHC).