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Prolactin Gene Polymorphism and Somatic Cell Count in Dairy Cattle

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Abstract: Mammary infections are one of the most serious problems in dairy cow farming. The factors that reduce the incidence of mastitis may include prolactin-one of the most multifunctional hormones in the body. Prolactin's biological activity consists of various roles in reproduction, lactation and a number of homeostatic biological functions including immune functions. In view of this fact, it is reasonable to investigate in pursuit of any associations between PRL polymorphism and somatic cell count (susceptibility/resistance to mastitis). The study included a herd of 720 Holstein-Friesian cows, Red-and-White variety. The frequencies of prolactin alleles and genotypes (recognized by endonuclease *Rsa*I) were determined. The statistical analysis also included a search for associations between prolactin polymorphism and SCC in milk. Parity, barn, date of study, stage of lactation, Holstein-Friesian gene share and cow were also included as sources of variability. Two prolactin alleles: A and B were found in the studied population of dairy cows, Statistically significant association was found between SCC and PRL genotype. It was also confirmed that statistically significant associations existed between SCC and the barns, date of study, parity, lactation stage and cow. The highest SCC (transformed to a logarithmic scale) was recorded in the milk of BB cows while the lowest one-in AA cows. The differences were confirmed statistically.

Key words: Prolactin, polymorphism, somatic cell count, milk, holstein-friesian cow

INTRODUCTION

Mammary infections are one of the most serious problems in dairy cow farming. Every year, farmers suffer financial losses caused by medical expenses and culling due to mastitis. Milk from infected cows does not qualify for consumption, which leads to decreased profits of dairy cow farms.

The factors that reduce the incidence of mastitis may include prolactin-one of the most multifunctional hormones in the body. Prolactin's biological activity consists of various roles in reproduction, lactation and a number of homeostatic biological functions including immune functions (Brand *et al*., 2004).

The prolactin molecule is arranged in a single chain of amino acids with three intramolecular disulfide bonds between 6 cysteine residues (Cys^{4}-Cys^{11}, Cys^{58}-Cys^{174} and Cys^{191}-Cys^{199} in humans) (Cooke *et al*., 1981). In cattle, the prolactin chain consists of 199 amino acids with a molecular mass of ~23 kDa (Wallis, 1974). Based on its genetic, structural, binding and functional properties, prolactin belongs to the prolactin/growth hormone/placental lactogen family (group I of the helix bundle protein hormones) (Boulay and Paul, 1992; Horseman and Yu-Lee, 1994). The Prolactin gene (PRL) is 10 kb long and is composed of 5 exons and 4 introns (BTA23) (Cooke *et al*., 1981). The gene was found to contain several polymorphic sites. One of the mutations is a point mutation A↓G in exon III detected by Mitra *et al*. (1995). The mutation is recognized by endonuclease *Rsa*I and it does not affect the protein structure.

Prolactin is synthesized and secreted in the anterior pituitary gland, brain, placenta, amnion, decidua and uterus. Moreover, prolactin is synthesized in the epithelial cells of the lactating mammary gland and can be detected in the milk itself (Grosvenor and Keenan, 1992; Lkhider *et al*., 1997). Extensive evidence suggests that lymphocytes can be a source of prolactin as well (Gala and Shevach, 1994; Jurcovicová *et al*., 1993; Montgomery *et al*., 1990; Russell, 1989). Although the control of pituitary prolactin secretion differs from that of lymphocytic origin, there is abundant evidence that lymphocytes contain dopamine receptors that may be involved in the regulation of lymphocytic prolactin production/release (Devins *et al*., 1992).

Prolactin is best known for the multiple effects it exerts on the mammary gland. The varied effects of prolactin on the mammary gland include growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis) and maintenance of milk

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secretion (galactopoiesis). Prolactin is also a common mediator of the immunoneuroendocrine network, where nervous, endocrine and immune systems communicate with each other (Gofin *et al*., 1998). Prolactin plays a significant role in regulating the humoral and cellular immune responses in physiological as well as pathological states, such as autoimmune diseases (Buskila and Shoenfeld, 1996; Neidhart, 1998; Walker *et al*., 1993).

Somatic Cell Count (SCC) in milk represents a good diagnostic tool that allows early detection of either subclinical or acute form of mastitis (Green *et al*., 2004; Haas *et al*., 2004). SCC is a valuable component of monitoring programs (Schukken *et al*., 2003). SCC is genetically associated with clinical mastitis (r_g = 0.3-0.7) and its heritability is higher (h^2 = 0.10-0.14) than that of clinical cases of mastitis (Mrode *et al*., 1998).

Besides the udder infection, a number of other factors influence SCC, namely lactation number, lactation stage, season, or genotype of the cows (Harmon, 1994). Changes in SCC are also associated with molecular genetic markers (Ashwell *et al*., 1997; Zhang *et al*., 1998; Klungland *et al*., 2001).

In view of the facts mentioned above, it is reasonable to investigate in pursuit of any associations between PRL polymorphism and SCC (susceptibility/resistance to mastitis).

MATERIALS AND METHODS

The study included a herd of 720 Holstein-Friesian cows, Red-and-White variety, kept in 7 barns belonging to one farm located in south-western Poland. The cows were fed standard feed rations and seasonally (in spring and summer) were put out to pasture. The cows were milked twice a day with the use of a pipeline milking machine. The herd's milk yield was evaluated with the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR). The data concerning SCC in milk was collected in the years 1998-2004 on the basis of monthly milking tests, representatively sampled from both of the two milkings (15,436 samples) performed at the same day time for each cow. SCC in the samples was determined with an instrumental method in compliance with the PN-EN ISO/IEC 17025 standard, using Combifoss equipment (Foss, Hillerod, Denmark).

Peripheral blood to be used for DNA isolation was collected from all the cows and placed in test tubes containing EDTA as anticoagulant. DNA was isolated from whole blood by a method described by Kanai. Afterwards, the PCR-RFLP method was used to analyze the polymorphism of a 156-bp gene fragment (exon III) recognized by restriction enzyme *Rsa*I, which cuts the sequence GT↓AC.

The isolated DNA was used for PCR-amplification of the 156-bp PRL gene fragment (III exon) using the following primers:

Forward: – 5'-CGA GTC CTT ATG AGC TTG ATT CTT -3'

Reverse: – 5'-GCC TTC CAG AAG TCG TTT GTT TTC-3'

The PCR was carried out according to Mitra *et al*. (1995). Restriction analysis of the amplified fragment was done with the RFLP method using *Rsa*I enzyme (for 3 h, with 5 units/20 mL, at 37°C) and the restriction fragments were analyzed electrophoretically on a 2% agarose gel in TBE buffer. The *Rsa*I digestion produced a mixture containing fragments of 156 bp (allele A, no sequence recognized by the restriction enzyme), 82 bp and 74 bp (allele B).

Next, the frequencies of prolactin alleles and genotypes were determined. The statistical analysis also included a search for associations between prolactin polymorphism and SCC in milk. Parity, barn, date of study, stage of lactation (number of days from calving to the day of the study-DIM the day in milking), Holstein-Friesian gene share and cow (random factor nested in the PRL genotype) were also included as sources of variability. Lactation number 5 and the subsequent ones were considered as one category. SCC was transformed to a logarithmic (ln) scale in order to balance the distribution. The following statistical model was applied:

$$(\ln SCC)\ y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m(a_i) + g(HF) + n(DIM) + e_{ijklm}$$

Where,

- y_{ijklm} = Somatic Cell Count (ln SCC).
- μ = Mean Somatic Cell Count for herd (ln SCC).
- a_i = Effect of PRL genotype.
- b_j = Effect of parity.
- c_k = Effect of barn.
- d_l = Effect of the date of study.
- $f_m(a_i)$ = Effect of cow, random nested factor in PRL genotype.
- g(HF) = Coefficient of regression of HF gene share on somatic cell count in milk.
- n(DIM) = Coefficient of regression of the day in milking on somatic cell count in milk.
- e_{ijklm} = Random error.

The results of the analyses were processed statistically using STATISTICA data analysis software, version 7.0 (StatSoft, Inc. 2001) with GLM multiple-factor mixed nested model. The influence of PRL genotype on

daily milk yield and fat, protein, lactose and dry matter content in milk was also analyzed. The model applied was the same as the one described for the analysis of associations between PRL genotype and SCC in milk.

RESULTS AND DISCUSSION

Two prolactin alleles: A and B were found in the studied population of dairy cows and their frequencies were 58.22 and 41.78%, respectively. The alleles controlled the occurrence of three genotypes of the following frequencies: AA-18.46%, BB-2.01% and AB-79.53%.

Allele A was found to be the most frequent allele in the studied herd. This result corresponds to the results reported by other researchers. A high frequency of the PRL allele A (0.95) was found in Holstein cows by several authors (Chrenek *et al*., 1998; Chung *et al*., 1996). A similar frequency of this allele was found in Russian Ayrshire (0.86), Gorbatov Red (0.91-0.92) (Udina *et al*., 2001; Chrenek *et al*., 1998), Argentinean Holstein (0.87), Polish Red and Polish Black-and-White cattle (Klauzinska *et al*. 2004) and in 4 Lithuanian cattle breeds (0.77-0.97).

Statistically significant association was found between SCC and PRL genotype ($p = 0.01$). It was also confirmed that statistically significant associations existed between SCC and the barns ($p = 0.001$), date of study ($p = 0.001$), parity ($p = 0.001$), DIM ($p = 0.001$) and cow ($p = 0.001$) (Table 1). The highest SCC (transformed to a logarithmic scale) was recorded in the milk of BB cows while the lowest one-in AA cows (Table 2). The differences were confirmed statistically.

Associations between milk production traits (daily milk yield and fat, protein, lactose and dry matter content) and PRL genotype as well other factors (barn, date of study, parity, DIM) were also studied. Except for PRL genotype and HF gene share, all the factors were statistically significantly associated with all the studied traits. PRL genotype was only related to daily milk yield and fat content in milk, whilst HF gene share was statistically associated with protein and lactose content in milk (Table 1).

Cows with the BB genotype, which is least desirable due to a high SCC, were also characterized by the lowest daily milk yield (25.34 kg) and lactose content (4.71%) and the highest fat (4.45%), protein (3.31%) and dry matter (13.43%) content (Table 2) compared to other cows. However, these differences were statistically significant only for daily milk yield and fat content (Table 2).

This study proved that there are statically significant associations between genetic variants of prolactin and SCC. The thesis that prolactin may be used as a marker of udder health has also been supported by other authors. Moro-Mendez and Hayes (2005) found significant associations within families for markers of PRL (BTA 23) and ICM (incidence of clinical mastitis), OCM (Occurrence of Clinical Mastitis), CDM (Culling Due to Mastitis) and SCS (Somatic Cell Scores) in first, second and third lactations.

The vital role that prolactin plays in regulating the immune system is reflected in the fact that a large number of immune perturbations were found to be associated with prolactin deficiency (Berczi and Nagy, 1991; Nagy and Berczi, 1991). As noted in two studies (Matera, 1996; Weigent, 1996), prolactin stimulates mitogenesis in both

Table 1: Associations between milk traits and the studied factors

Factor	Somatic cell count	Daily milk yield	Fat content	Protein content	Lactose content	Dry matter content
PRL genotype	**	*	**	n.s.	n.s.	n.s.
Barn	***	***	***	*	*	***
Date of study	***	***	***	***	***	***
Parity	***	***	***	***	***	*
DIM	***	***	***	***	***	***
HF gene share	n.s	n.s	n.s	***	**	n.s
Cow	***	***	***	***	***	***

*p = 0.05; **p = 0.01; ***p = 0,001; n.s. – non-significant

Table 2: Means and Standard Deviations (SD) of the studied traits in relation to PRL genotype

			PRL genotype					
	Total 720 cows		AA 133 cows		AB 572 cows		BB 15 cows	
Trait	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SCC [pcs/mL]	5.772	1.310	5.627^{A}	1.277	5.791^{B}	1.311	6.359^{AB}	1.366
Daily mik yield [kg]	25.99	8.30	25.66	8.30	26.09^{a}	8.29	25.34^{a}	8.51
Fat content [%]	4.28	1.05	4.13^{a}	0.95	4.31^{B}	1.07	4.45^{aB}	1.11
Protein content [%]	3.30	0.36	3.29	0.35	3.30	0.36	3.31	0.41
Lactose content [%]	4.80	0.25	4.82	0.24	4.80	0.25	4.71	0.27
Dry matter [%]	13.26	1.28	13.12	1.25	13.28	1.27	13.43	1.35

The means marked with the same superscript letter differ significantly. Capital letters denote significance of difference at $p = 0.01$, whereas small letters denote significance of difference at $p = 0.05$

normal T lymphocytes and the Nb2 lymphoma cell line (Viselli *et al*., 1991). Moreover, the effects of prolactin on lymphocytes may involve Interleukin (IL)-2 since T-lymphocyte activation by IL-2 requires prolactin (Clevenger *et al*., 1990; Gushchin *et al*., 1995). Interestingly, prolactin's site of action for modifying the effects of IL-2 on lymphocytes appears to be the nucleus (Clevenger *et al*., 1991). Prolactin is also required for mitogen-stimulated proliferation of lymphocytes (Hartmann *et al*., 1989). Nb2 cells, derived from immature T lymphocytes, are dependent on the mitogenic activity of prolactin (Shiu *et al*., 1983; Tanaka *et al*., 1980).

Prolactin may influence the neutrophilic inflammation that characterizes chronic mastitis. Most of the genes encoding inflammatory proteins depend on the nuclear factor B κ(NF-κB) for their expression. Boutet *et al*. (2007) found that plasma prolactin did not differ significantly between healthy and chronic mastitis-affected cows (63.7 and 67.5 ng mL^{-1}, respectively). Milk prolactin concentration was significantly increased in chronic mastitis-affected quarters with the highest SCC and had a positive significant correlation with SCC as well as the number of neutrophils present in milk. These findings show that prolactin promotes an inflammatory response in bovine mammary epithelial cells via NF-κB activation and suggest a role for prolactin in the pathogenesis of chronic mastitis (Boutet *et al*., 2007).

The results obtained in this study did not confirm that PRL/RsaI polymorphism could be used as a marker of milk production traits, which was suggested by many other authors (Dybus, 2002; Dybus *et al*., 2004; Brym *et al*., 2005; Chung *et al*., 1996).

CONCLUSION

In this study, it was found that genotype BB, which is least desirable due to its association with a high SCC, was characteristic of cows with the lowest daily milk yield. This leads to the conclusion that PRL polymorphism can be used to improve resistance to mastitis in dairy cattle without affecting milk production traits.

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