Relationship between the insertion/deletion polymorphism within the promoter and the intron 1 sequence of the *PRNP* gene and milk performance traits in cattle

J. Strychalski[1], U. Czarnik[1], M. Pierzchała[2], C.S. Pareek[3]

[1]Department of Animal Genetics, Faculty of Animal Bioengineering, University of Warmia and Mazury, Olsztyn, Poland
[2] Institute of Animal Genetics and Breeding, Polish Academy of Sciences in Jastrzębiec, Poland
[3]Laboratory of Functional Genomics, Institute of General and Molecular Biology, Faculty of Biology and Earth Science, Nicolaus Copernicus University, Torun, Poland

ABSTRACT: Current studies on the prion protein (*PRNP*) gene polymorphism focus primarily on the causative mutations that influence BSE susceptibility in cattle. The specific genetic structure determined by the insertion/deletion (*indel*) polymorphism within the 23 bp promoter sequence and the 12 bp intron 1 sequence of the *PRNP* gene, and its genomic location suggest that this polymorphism can be a potential QTL marker. The objective of the present study was to determine whether the *indel* polymorphism within the promoter sequence (23 bp) and the intron 1 sequence (12 bp) of the *PRNP* gene can be used as a factor differentiating the values of milk performance traits. The experimental materials comprised 285 primiparous Polish Holstein-Friesian cows, daughters of two dihybrid (23*ins*-12*ins*/23*del*-12*del*) sires (progeny of sire 1 – 149 cows, progeny of sire 2 – 136 cows). The following milk performance traits were analysed: milk yield, milk fat yield, milk protein yield, fat and protein contents of milk during the first 305-day lactation. The polymorphism in the promoter region (23 bp) was found to have a significant (P = 0.040) effect on protein yield and a highly significant (P = 0.007) effect on the protein content of milk. The highest values of these traits were noted in 23 *ins/del* heterozygotes and the lowest in 23 *del*/*del* homozygotes. There was an interrelation between diplotype variants and the concentrations of milk components. The protein content of milk was highly significantly (P = 0.007) higher in 23*ins*-12*ins*/23*del*-12*del* heterozygotes and significantly (P = 0.028) higher in 23*ins*-12*ins*/23*ins*-12*ins* homozygotes, compared to 23*del*-12*ins*/23*del*-12*del* cows. 23*ins*-12*ins*/23*del*-12*del* heterozygotes were also characterized by a significantly (P = 0.046) higher fat content of milk, in comparison with 23*del*-12*del*/23*del*-12*del* homozygotes.

Keywords: Polish Holstein-Friesian cattle; *PRNP* polymorphism; milk performance traits; QTL marker

The prion protein (*PRNP*) gene has been localized on bovine chromosome 13 (BTA13) band q17 (Schläpfer et al., 2000). The *PRNP* gene extends over 20.2 kbp, and it consists of three exons and two introns. Exon 3 is the longest and it contains an open reading frame (ORF) of 795 bp (Hills et al., 2001). Polymorphic loci within the *PRNP* gene ORF are correlated with susceptibility to Creutzfeldt-Jakob disease (CJD) in humans (Jeong et al., 2005) and to scrapie in sheep (Goldmann et al., 1994), but none of the polymorphisms found in this region has been reported to be associated with BSE susceptibility in cattle (Xue et al., 2008). However, it is believed that the insertion/deletion (*indel*) polymorphism within the 23 bp promoter sequence and the 12 bp intron 1 sequence of the *PRNP* gene might influence BSE

incubation time and/or BSE susceptibility in cattle both in Germany (Sander et al., 2004) and in the United Kingdom (Juling et al., 2006). It has been demonstrated that the polymorphism within the promoter sequence (23-bp *indel*) contains a binding site for the RP58 protein – a strong transcription repressor, while the nucleotide sequence in intron 1, where a 12-bp *indel* mutation was found to occur, contains a potential binding site for the SP1 transcription factor. A model has been proposed in which the 23*del*-12*del* haplotype affects higher promoter activity and the 23*ins*-12*ins* haplotype determines lower gene expression (Sander et al., 2005). The 23*del*-12*del* haplotype is associated with an increased risk of BSE. This haplotype is more frequent in healthy Holstein-Friesian cattle (Juling et al., 2006; Nakamitsu et al., 2006; Haase et al., 2007; Brunelle et al., 2008) selected for increased milk production, and less frequent in breeds characterized by lower performance but known for their good adaptive traits (Jeong et al., 2006; Juling et al., 2006; Haase et al., 2007; Czarnik et al., 2009).

The biological effects of the insertion/deletion polymorphism within the *PRNP* gene are an important consideration in cattle breeding and production. Most studies conducted to date have focused on the interrelation between the *indel* polymorphism within the *PRNP* gene and BSE susceptibility to cattle, while the possibility to use mutations in the *PRNP* gene as QTL markers has been disregarded. Several regions carrying QTLs for milk production traits have been identified within the structure of bovine chromosome 13 (BTA13). Their location was confirmed with the use of microsatellite markers (STR). Linkage disequilibrium (LD) was found out between variants at polymorphic loci BMS1742 (22.9 cM), BMC1222 (27.6 cM), HUJ616 (51.7 cM) (Mosig et al., 2001), BMS1352 (38.6 cM) (Olsen et al., 2002) and QTLs affecting the protein content of milk. Implementing a three-generation design, Ashwell et al. (2004) determined the location of QTL for milk protein content between markers BMC1222 (27.6 cM) and ILSTS59 (41.7 cM), and confirmed the presence of QTLs for the yields of milk and milk protein between markers BMS1226 (73.3 cM) and BMS995 (96.0 cM). STR markers, influencing somatic cell count (SCC) in milk, were identified in the same region of BTA 13 (Rupp and Boichard, 2003). It has been suggested that a QTL determining milk fat yield is located in the region flanked by BMS1145 (42.5 cM) and BLA42 (58.9 cM), and a causative mutation was detected at position 47.5 cM (Plante et al., 2001).

Independently of QTL detection, efforts should be made to search for mutations with a high phenotypic effect, since no candidate genes associated with milk production traits have been recognized within the structure of BTA13 to date. As demonstrated by Schläpfer et al. (2000), the *PRNP* gene is located between the loci of microsatellites UWCA25 (59.2 cM) and BM4509 (60.0 cM), which means that its approximate location is 60 cM.

Further studies are needed to investigate interbreed differences in genotype frequencies in particular *indel*s within a sequence, and to test the hypothesis regarding functional differences between haplotypes which assumes the existence of a mechanism promoting the occurrence of certain *indel* combinations. Theoretical studies suggest that the insertion/deletion polymorphism could be a potential marker of milk performance traits, since the *PRNP* gene is located in the region containing QTLs for the percentage protein content of milk (Ashwell et al., 2004) and milk fat yield (Plante et al., 2001).

The objective of this study was to verify the hypothesis that there exists a relationship between the insertion/deletion (*indel*) polymorphism and the values of milk performance traits in Polish Holstein-Friesian cattle.

MATERIAL AND METHODS

The experimental materials comprised 285 primiparous Polish Holstein-Friesian cows, half-sib daughters of two dihybrid (23*ins*-12*ins*/23*del*-12*del*) sires (progeny of sire 1 – 149 half-sib heifers, progeny of sire 2 – 136 half-sib heifers). Two sires included in the experimental design were not related with each other.

Genomic DNA was isolated from peripheral blood leukocytes using the Master Pure^{TM} Purification Kit (EPICENTRE Biotechnologies, Madison, WI, USA). Insertion/deletion polymorphisms at the *PRNP* gene were determined by PCR. The amplified sequences included two fragments of the *PRNP* gene (GenBank AJ298878), located within its promoter (position 47784 to 47883 bp) and within intron 1 (position 49686 to 49777 bp). PCR primers were used for amplification, as described by Sander et al. (2004). PCR products were electrophoresed in 1.5% AmpliSize agarose gel (Bio-Rad Laboratories, CA, USA). Polymorphism was visualized by Fluor S^{TM} Multimager (Bio-Rad Laboratories, CA, USA).

The database contained the results obtained for the following milk performance traits: milk yield, milk fat yield, milk protein yield, percentage fat content of milk and percentage protein content of milk during the first 305-day lactation of cows at the same age. The animals were kept under uniform housing conditions in two herds, and were calved evenly during the same season to reduce the impact of environmental factors on the variation of the investigated traits.

The effects of *PRNP indel* genotypes as well as combination of diplotype genotypes on milk production traits were investigated using the least squares method (at significance levels $P \leq 0.05$ and $P \leq 0.01$) according to the GLM (General Linear Model) procedure. To achieve this, the following statistical model was fitted to 285 observations using SAS 9.1 software (SAS Institute, Inc., Cary, USA):

$$Y_{ijkl} = \mu + G1_i + G2_j + S_k + H_l + \beta_{(MY1 - MY)} + e_{ijkl}$$

where:

Y_{ijkl} = production trait

μ = overall mean

$G1_i$, $G2_j$ = combined effects of the 23 i^{th} and 12 j^{th} *indel PRNP* genotypes

S_k = effect of the k^{th} sire

H_l = effect of the l^{th} herd

$\beta_{(MY1 - MY)}$ = regression on milk yield

e_{ijkl} = residual error

RESULTS

The distribution of *indel PRNP* genotypes in the half-sib daughters of two heterozygous dihybrid sires is presented in Table 1. The heterozygous genotype (*ins/del*) was the most frequent at both examined polymorphic loci of the *PRNP* gene (23 *ins/del* – 54.4%, 12 *ins/del* – 56.1%), intermediate values were recorded for *del*/*del* homozygotes (23 *del*/*del* – 33.7%, 12 *del*/*del* – 25.3%), while *ins*/*ins* homozygotes were the least frequent (23 *ins*/*ins* – 11.9%, 12 *ins*/*ins* – 18.6%).

An analysis of the relationship between the *indel* polymorphism within the promoter sequence (23 bp) and the intron 1 sequence (12 bp) of the *PRNP* gene and the values of milk performance traits revealed that 23 *ins/del* heterozygotes had the highest milk yield and milk contents, while the lowest values of these traits were noted for the 23 *del*/*del* homozygotes.

Statistically significant ($P = 0.040$) differences were observed in the protein yield of milk, while highly significant ($P = 0.007$) differences were noted in the protein content of milk. The milk protein concentration was higher on average by 0.08% in *ins/del* heterozygotes than in *del*/*del* homozygotes. A high protein content of milk, similar to that reported in *ins/del* heterozygotes, was also noted in *ins*/*ins* homozygotes, but the difference between these two groups was non-significant. Polymorphism had no effect on milk yield, fat yield and fat content of milk. There was no interrelation between the *indel* polymorphism in intron 1 (12 bp) and the values of the analysed milk production traits (Table 1).

Table 2 presents the results of the genotypic effect of combined polymorphic variants of diplotypes (*cis* vs *trans*) on milk performance traits. The analysis included the combination of polymorphisms within both the promoter sequence (23 bp) and the intron 1 sequence (12 bp). The diplotypes of sires were determined based on the progeny genotypes, at both examined polymorphic loci. The *cis* linkage was observed in both sires, as 23-bp insertion within the promoter sequence was accompanied by 12-bp insertion within the intron 1 sequence, while 23-bp deletion was accompanied by 12-bp deletion.

Diplotype frequencies (*cis* linkage) among the examined cows, in descending order, were as follows: 23*ins*-12*ins*/23*del*-12*del* – 42.5%, 23*del*-12*del*/23*del*-12*del* – 21.8%, 23*del*-12*ins*/23*del*-12*del* – 11.9%, 23*ins*-12*ins*/23*ins*-12*ins* – 10.2%, 23*ins*-12*ins*/23*del*-12*ins* – 8.4%, 23*ins*-12*del*/23*del*-12*del* – 3.5%, 23*ins*-12*ins*/23*ins*-12*del* – 1.7%. The presence of cows with the following diplotypes: 23*ins*-12*del*/23*del*-12*del*, 23*ins*-12*ins* per 23*ins*-12*del*, 23*ins*-12*ins*/23*del*-12*ins*, and 23*del*-12*ins*/23*del*-12*del*, and of daughters originating from 23*ins*-12*ins*/23*del*-12*del* sires indicates that the 23*ins*-12*del* and 23*del*-12*ins* haplotypes (*trans* linkage) were inherited from mothers.

A group of five cows with the 23*ins*-12*ins*/23*ins*-12*del* diplotype was eliminated from the study, because it was found to be too small to serve as a reliable statistical sample of reference.

The obtained results validated the trends observed for individual *indels*. A correlation was found out between the polymorphic variants of diplotypes and the concentrations of milk components. The protein content of milk was highly significantly ($P = 0.008$) higher in 23*ins*-12*ins*/23*del*-12*del* heterozygotes and significantly ($P = 0.028$) higher in

Table 1. The effects of *indel PRNP* genotypes determined in the promoter region (23 bp) and in intron 1 (12 bp) on milk performance traits in two half-sib families of Polish Holstein Friesian cattle

Region of *PRNP*	Genotype	Number of animals	Milk yield (kg)		Fat				Protein			
					yield (kg)		content (%)		yield (kg)		content (%)	
			LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Promoter 23-bp indel	*ins*/*ins*	34	6824	229	293.4	6.2	4.37	0.09	220.3	2.4	3.30	0.04
	ins/*del*	155	6800	126	298.2	3.4	4.46	0.05	**221.7**^{a}	1.3	**3.31**^{A}	0.02
	del/*del*	96	6632	135	293.6	3.6	4.38	0.05	**217.6**^{a}	1.4	**3.23**^{A}	0.02
Intron 12-bp indel	*ins*/*ins*	53	6782	161	295.6	4.3	4.41	0.06	219.4	1.7	3.28	0.03
	ins/*del*	160	6728	131	296.0	3.6	4.42	0.05	219.0	1.4	3.26	0.02
	del/*del*	72	6746	182	293.5	4.9	4.38	0.07	221.2	1.9	3.31	0.03

^{a}means within columns followed by small letters are significantly different at $P \leq 0.05$

^{A}means within columns followed by capital letters are significantly different at $P \leq 0.01$

23*ins*-12*ins*/23*ins*-12*ins* homozygotes, compared to 23*del*-12*ins*/23*del*-12*del* cows. A high sub-threshold value, $P = 0.0511$, was noted for protein yield, which was higher in cows with the 23*ins*-12*ins*/23*del*-12*del* diplotype, and the lowest in 23*del*-12*ins*/23*del*-12*del* cows. 23*ins*-12*ins*/23*del*-12*del* heterozygotes were also characterized by a significantly ($P = 0.046$) higher fat content of milk, in comparison with 23*del*-12*del*/23*del*-12*del* homozygotes.

The polymorphic variants of diplotypes had no effect on milk yield. However, an increase in milk fat yield ($P = 0.069$) was noted in the group of 23*ins*-12*ins*/23*del*-12*del* heterozygotes, and the lowest fat concentration in the group of 23*del*-12*del*/23*del*-12*del* homozygotes.

DISCUSSION

The present study attempted to determine the relationship between the insertion/deletion polymorphism within the promoter sequence (23 bp) and the intron 1 sequence (12 bp) of the *PRNP* gene and the values of milk performance traits in half-sib daughters of two dihybrid sires. The polymorphism within the promoter sequence (23 bp) was found to affect milk protein yield and milk protein content, whereas the polymorphism within intron 1 (12 bp) had no effect on the values of the analysed traits. Both protein yield and protein content of milk were the highest in 23*ins/del* heterozygotes and the lowest in 23*del*/*del* homozygotes (protein yield $P = 0.040$,

Table 2. The effects of *indel PRNP* diplotype genotype combinations determined in the promoter region (23 bp) and in intron 1 (12 bp) on milk performance traits in two half-sib families of Polish Holstein Friesian cattle

Genotype groups	Number of animals	Milk yield (kg)		Fat				Protein			
				yield (kg)		content (%)		yield (kg)		content (%)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
23*ins*-12*ins*/23*ins*-12*ins*	29	6753	231	293.9	6.1	4.38	0.09	220.0	2.4	**3.31**^{b}	0.04
23*ins*-12*ins*/23*del*-12*ins*	24	7028	256	294.0	6.8	4.42	0.10	219.3	2.6	3.29	0.04
23*ins*-12*ins*/23*del*-12*del*	121	6728	110	299.9	2.9	**4.49**^{a}	0.04	220.8	1.1	**3.30**^{A}	0.02
23*ins*-12*del*/23*del*-12*del*	10	6951	426	287.7	11.3	4.35	0.16	219.6	4.4	3.27	0.07
23*del*-12*ins*/23*del*-12*del*	34	6715	212	293.0	5.6	4.39	0.08	216.0	2.2	**3.20**^{Ab}	0.03
23*del*-12*del*/23*del*-12*del*	62	6589	158	290.5	4.2	**4.35**^{a}	0.06	218.5	1.6	3.26	0.02

^{a,b}means within columns followed by small letters are significantly different at $P \leq 0.05$

^{A}means within columns followed by capital letters are significantly different at $P \leq 0.01$

protein content $P = 0.007$). An analysis of the influence of polymorphisms in both regions of the *PRNP* gene on milk production traits revealed the cumulative effect of both *indels*. The protein content of milk was the highest in 23*ins*-12*ins*/23*del*-12*del* heterozygotes ($P = 0.008$) and in 23*ins*-12*ins*/23*ins*-12*ins* homozygotes ($P = 0.028$), and the lowest in 23*del*-12*ins*/23*del*-12*del* cows. The combination of both *indels* also influenced the fat content of milk, which was higher in 23*ins*-12*ins*/23*del*-12*del* heterozygotes and lower in 23*del*-12*del*/23*del*-12*del* homozygotes ($P = 0.046$). However, the *indel* combinations had no effect on milk protein yield.

A few attempts have been made to verify the role of the insertion/deletion polymorphism within the *PRNP* gene as a QTL marker of milk production in cattle (Czarnik et al., 2006, 2007). At first the studies were focused on investigating correlations between the insertion/deletion polymorphism (24 bp) within exon 3 of the *PRNP* gene and the productivity of Polish Holstein-Friesian cows during their first 305-day lactation. Research results showed that homozygous *PRNP* 6/6 cows, daughters of sires with the *PRNP* 6/6 and *PRNP* 6/5 genotypes, and a group of randomly selected cows with the *PRNP* 6/6 genotype were characterized by a significantly higher protein content of milk, compared to heterozygous *PRNP* 6/5 cows. An opposite trend was noted with respect to milk fat yield, which was significantly higher in cows with the *PRNP* 6/5 genotype than in those with the *PRNP* 6/6 genotype. In a follow-up study, Czarnik et al. (2007) examined whether the *indel* polymorphism within the promoter sequence (23 bp *indel*) and intron 1 (12 bp *indel*) of the *PRNP* gene could be used as a factor differentiating milk composition and somatic cell count (SCC) in the first three months of lactation in randomly selected Polish Holstein-Friesian cows naturally infected with the bovine leukemia virus (BLV). These authors noted significantly higher concentrations of protein and solids non-fat as well as an increased SCC in milk from cows with the *ins*/*ins* genotype in the promoter region (23 bp).

The results of the present study suggest that cattle selection for improved milk production has no effect on an increase in the percentage share of animals with the *del*/*del* genotype at the polymorphic loci of promoter (23 bp) and intron 1 (12 bp) in the population of Holstein-Friesian cattle. Therefore, it may be assumed that the insertion/deletion polymorphism within the *PRNP* gene is not responsible for differences in milk protein yield, milk protein content and milk fat content, but it is a marker of an unknown causative mutation. Further investigations should be undertaken to determine the correlation between the above polymorphism and functional traits, to conduct a scan of bovine chromosome 13 (BTA13), and to analyse the effect of mutations located within coding genes associated with lactation and synthesis of milk components.

REFERENCES

Ashwell M.S., Heyen D.W., Sonstegard T.S., Van Tassell C.P., Da Y., Vanraden P.M., Ron M., Weller J.I., Lewin H.A. (2004): Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. Journal of Dairy Science, 87, 468–475.

Brunelle B.W., Kehrli M.E. Jr., Stabel J.R., Spurlock D.M., Hansen L.B., Nicholson E.M. (2008): Allele, genotype, and haplotype data for bovine spongiform encephalopathy-resistance polymorphisms from healthy US Holstein cattle. Journal of Dairy Science, 91, 338–342.

Czarnik U., Zabolewicz T., Pareek C.S., Ziemiński R., Walawski K. (2006): Evaluation of putative relationship between *PRNP* octapeptide repeat polymorphism and variability of milk production traits in cattle. Annals of Animal Science, 6, 29–36.

Czarnik U., Strychalski J., Bojarojć-Nosowicz B., Kaczmarczyk E. (2007): Prion protein gene (*PRNP*) polymorphism and indicators of secretion disorders of the mammary gland in cows naturally infected with *Bovine leukaemia virus* (BLV). Bulletin of the Veterinary Institute in Pulawy, 51, 459–464.

Czarnik U., Grzybowski G., Zabolewicz T., Strychalski J., Kamiński S. (2009): Deletion/insertion polymorphism of the prion protein gene (*PRNP*) in Polish Red Cattle, Polish White-backed Cattle and European Bison (*Bisos bonasus* L. 1785). Russian Journal of Genetics, 45, 453–459.

Goldmann W., Hunter N., Smith G., Foster J., Hope J. (1994): PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. Journal of General Virology, 75, 989–995.

Haase B., Doherr M.G., Seuberlich T., Drögemüller C., Dolf G., Nicken P., Schiebel K., Ziegler U., Groschup M.H., Zurbriggen A., Leeb T. (2007): *PRNP* promoter polymorphisms are associated with BSE susceptibility in Swiss and German cattle. BMC Genetics, 8, 15.

Hills D., Comincini S., Schlaepfer J., Dolf G., Ferretti L., Williams J.L. (2001): Complete genomic sequence of

the bovine prion gene (*PRNP*) and polymorphism in its promoter region. Animal Genetics, 32, 231–233.

Jeong B.H., Lee K.H., Kim N.H., Jin J.K., Kim J.I., Carp R.I., Kim Y.S. (2005): Association of sporadic Creutzfeldt-Jakob disease with homozygous genotypes at *PRNP* codons 129 and 219 in the Korean population. Neurogenetics, 6, 229–232.

Jeong B.H., Lee Y.J., Kim N.H., Carp R.I., Kim Y.S. (2006): Genotype distribution of the prion protein gene (*PRNP*) promoter polymorphisms in Korean cattle. Genome, 49, 1539–1544.

Juling K., Schwarzenbacher H., Williams J.L., Fries R. (2006): A major genetic component of BSE susceptibility. BMC Biology, 4, 33.

Mosig M.O., Lipkin E., Khutoreskaya G., Tchourzina E., Soller M., Friedmann A. (2001): A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false Discovery Rate Criterion. Genetics, 157, 1683–1698.

Nakamitsu S., Miyazawa T., Horiuchi M., Onoe S., Ohoba Y., Kitagawa H., Ishiguro N. (2006): Sequence variation of bovine protein gene in Japanese cattle (Holstein and Japanese Black). The Journal of Veterinary Medical Science, 68, 27–33.

Olsen H.G., Gomez-Raya L., Våge D.I., Olsaker I., Klungland H., Svendsen M., Ådnøy T., Habry A., Klemetsdal G., Schulman N., Krämer W., Thaller G., Rønningen K., Lien S. (2002): A genome scan for quantitative trait loci affecting milk production in Norwegian dairy cattle. Journal of Dairy Science, 85, 3124–3130.

Plante Y., Gibson J.P., Nadesalingam J., Mehrabani-Yeganeh H., Lefebvre S., Vandervoort G., Jansen G.B. (2001): Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. Journal of Dairy Science, 84, 1516–1524.

Rupp R., Boichard D. (2003): Genetics of resistance to mastitis in dairy cattle. Veterinary Research, 34, 671–688.

Sander P., Hamann H., Pfeiffer I., Wemheuer W., Brenig B., Groschup M.H., Ziegler U., Distl O., Leeb T. (2004): Analysis of sequence variability of the bovine prion protein gene (*PRNP*) in German cattle breeds. Neurogenetics, 5, 19–25.

Sander P., Hamann H., Drögemüller C., Kashkevich K., Schiebel K., Leeb T. (2005): Bovine prion protein gene (*PRNP*) promoter polymorphisms modulate *PRNP* expression and may be responsible for differences in bovine spongiform encephalopathy susceptibility. The Journal of Biological Chemistry, 280, 37408–37414.

Schläpfer J., Stahlberger-Saitbekova N., Küffer J., Dolf G. (2000): Genetic mapping of the prion protein gene (*PRNP*) on bovine chromosome 13. Journal of Animal Breeding and Genetics, 117, 211–216.

Xue G., Sabudo A., Kim C.K., Onodera T. (2008): Coordinate regulation of bovine prion protein gene promoter activity by two Sp1 binding site polymorphisms. Biochemical and Biophysics Research Communications, 372, 530–535.

Received: 2010–01–12
Accepted after corrections: 2010–11–11

Corresponding Author

Prof. Dr. hab. Urszula Czarnik, University of Warmia and Mazury, Faculty of Animal Bioengineering, Department of Animal Genetics, M. Oczapowskiego 5, 10-957 Olsztyn, Poland
Tel. +48 89 5233 776, e-mail: czar@uwm.edu.pl