The Relation between Genetic Polymorphism Markers and Milk Yield in Brown Swiss Cattle Imported to Slovakia

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ABSTRACT : The aim of this study was to determine genotypes of four genetic markers and to investigate their association with milk production traits in Brown Swiss cattle imported to Slovakia. The bovine κ-casein, β-lactoglobulin, growth hormone and prolactin genotypes of 107 cows were identified by polymerase chain reaction. Effects all four genetic markers on milk, fat, protein and lactose yields and fat, protein and lactose percentage were estimated from a data set of 249 lactations. The frequency of desirable B allele of κ-casein gene to milk production was 0.46, alleles A of β-lactoglobulin gene was 0.55, allele and L of growth hormone gene was 0.45 and allele A and B of bovine prolactin gene were 0.61 and 0.39. The results of milk production obtained in our work showed that BB genotypes of κ-CN gene, AA genotypes of β-LG gene, LL genotypes of bGH gene were significantly associated with better milk production traits, mainly about the fat content. Association of a bovine prolactin genotypes with milk production were not found. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1397-1401)***

Key Words : Brown Swiss, Gene Marker, Milk Production

INTRODUCTION

One of the posibility to progress in dairy cattle is selection of animal with favorable genotypes of genetic markers. Molecular genetic techniques allow direct genotyping of genetic markers using DNA isolated from blood or hair samples, regardless of age or sex of the animal (Chrenek. 1997). The techniques allow selection of desirable genotypes to milk or meat production at an early age in both sexes.

However, ambiguous evidence has been reported regarding the association of κ-casein (κ-CN) and β-lactoglobulin (β-LG) genotypes with milk yield and composition (Ng Kwai Hang et al., 1990, Mukhopadhyaya and Mehta, 2002). Despite these contradictory results, several reports (Lin et al., 1986, Cerbulis and Farrell, 1974) suggest that κ-CN B allele and β-LG A allele have desirable effects on milk yield in the first lactation in Holstein cattle. The allele B of κ-CN gene is more desirable than allele A because the former is associated with higher casein content in milk, higher cheese-yielding capacity, more favorable cheese composition and better coagulating properties in terms of rennet clotting time and curd firmness (Schaar 1984, Ng Kwai Hang 1987).

It is well established that growth hormone and prolactin are important regulators of mammary growth and lactogenesis (Collier et al., 1984). Evidence also exists that bovine growth hormone (bGH) and bovine prolactin (bPRL) markers play a major role in lactation (Woolliams et al., 1993, Cowan et al., 1990). In case of bGH, the results indicated an effect of bGH gene polymorphism Taq I restriction fragment on 305 day production of milk, fat and protein production in Holstein-Friesian cattle (Falaki et al., 1996). In studies of British Friesian calves, Lovendahl et al., (1991) found a positive association between GH release induced by growth-hormone releasing hormone and predicted breeding value for milk yield. The PRL insertion/deletion is associated with differences in predicted milk production in offspring of Holstein bulls (Cowan et al., 1990).

The used of such detectable markers (κ-CN, β-LG, bGH and bPRL gene) in breeding programs could render the selection of animals more accurate and efficient for milk production in cattle (Schlee et al., 1994, Chung et al., 1998).

The goal of this work was to determine genotypes of four genetic markers and to investigate their association to milk production in Brown Swiss cattle imported to Slovakia.

MATERIAL AND METHODS

A total of 107 cows of the Brown Swiss cattle from 4 herds were genotyped. Genomic DNA was isolated from blood (Chrenek et al., 1997). The PCR mix (30 μl) contained: 200 μM dNTPs, PCR reaction buffer, 0.5 μM each of primers (Table 1), 1 U Taq DNA polymerase (AGS, Germany) and approximately 100 ng DNA.

Samples were amplified for 35 cycles in the following conditions: denaturation step at 94°C for 20 s, annealing at (Table 1) for 20 s and extension step at 72°C for 20 s with additional 5 min prolongation of the extension step in the last cycle. PCR products (10 μl) were digested with restriction endonuclease (Table 2) and following electrophoresed on 2.5% agarose gel.

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Table 1. Sense and antisense primers for PCR reactions

Locus	Sequence	Reference
κ-CN	5' - GCT GAG CAG GTA TCC TAG TTA T - 3'	Vasicek et al., 1995
	5' - CTT CTT TGA TGT CTC CTT AGA G – 3'	
β-LG	5' – TGT GCT GGA CAC CGA CTA CAA AAA G - 3'	Uhrin et al., 1995
	5' – GCT CCC GGT ATA TGA CCA CCC TCT – 3'	
bGH	5' – GCT GCT CCT GAG GGC CCT TCG - 3'	Schlee et al., 1994
	5' – GCG GCG GCA CTT CAT GAC CCT – 3'	
bPRL	5' – CGA GTC CTT ATG AGC TTG ATT CTT – 3'	Mitra et al., 1995
	5' – GCC TTC CAG AAG TCG TTT GTT TTC – 3'	

Table 2. PCR and RFLP conditions for genotyping of specific loci

Locus	Annealing (°C)	PCR-product (bp)	Restriciton endonuclease	PCR/RFLP (bp)	Genotype
κ-CN	53	443	HindIII	443	AA
				348, 95	BB
β-LG	55	247	HaeIII	148, 99	AA
				99, 74, 74	BB
bGH	60	223	AluI	223	VV
				171, 52	LL
bPRL	59	156	RsaI	156	AA
				82, 74	BB

Table 3. Number and frequency of genotypes and alleles

Genotype		Number	Frequency of genotype (%)	Frequency of allele (%)
κ-CN	AA	23	21.49	A-45.79
	AB	52	48.59	B-54.21
	BB	32	29.92	
β-LG	AA	37	34.58	A-0.55
	AB	43	40.19	B-0.45
	BB	27	25.23	
bGH	VV	13	12.14	V-0.55
	LV	82	76.65	L-0.45
	LL	12	11.21	
bPRL	AA	49	45.79	A-0.61
	AB	46	42.99	B-0.39
	BB	12	11.22	

Calculation of genotype frequency was based on direct gene count method by $(n_{AB}+2n_{BB})/2n$. Least square means (LSM) method was used for statistical analysis of data from milk recording total of 249 lactations. Differences between genotypes were tested by using Scheffe multiple range test for all traits studied. The model included herd, parity and genotypes of κ-CN or β-LG or bGH or bPRL as fixed effects and number of lactation days and age at first calving as a covariables.

RESULTS

Results of genotyping of four genetic markers of Brown Swiss cattle imported to Slovakia by PCR-RFLP method and their frequency are summarized in the Table 3. Representative results of PCR-RFLP analysis detected on agarose gel electrophoresis are showed in Figures 1, 2, 3 and 4.

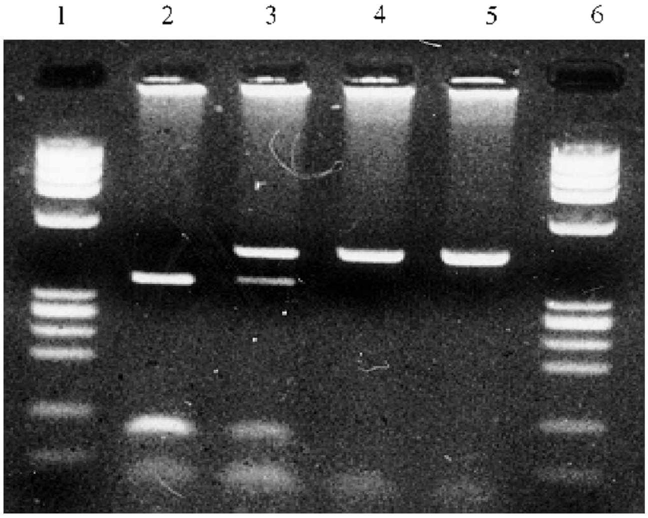

Figure 1. Representative results of PCR-RFLP analysis of κ-CN gene. Line 1, 6-marker PhiX174/HaeIII, Line 2-genotype BB, Line 3-genotype AB, Line 4, 5-genotype AA.

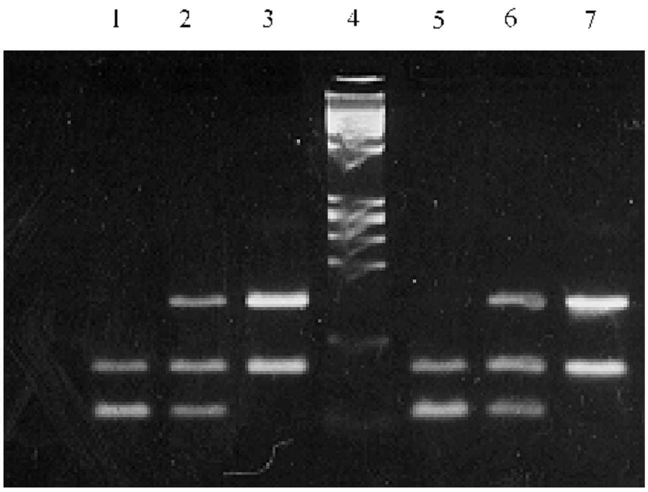

Figure 2. Representative results of PCR-RFLP analysis of κ-LG gene. Line 4-marker PhiX174/HaeIII, Line 1, 5-genotype BB, Line 2, 6-genotype AB, Line 3, 7-genotype AA.

Frequency of B allele of the κ-CN gene in our population of Brown Swiss cattle was 54.21 and allele A of the β-LG gene was 0.55. In case of bGH gene the frequency of allele L was 0.45 and in bPRL gene the frequency allele

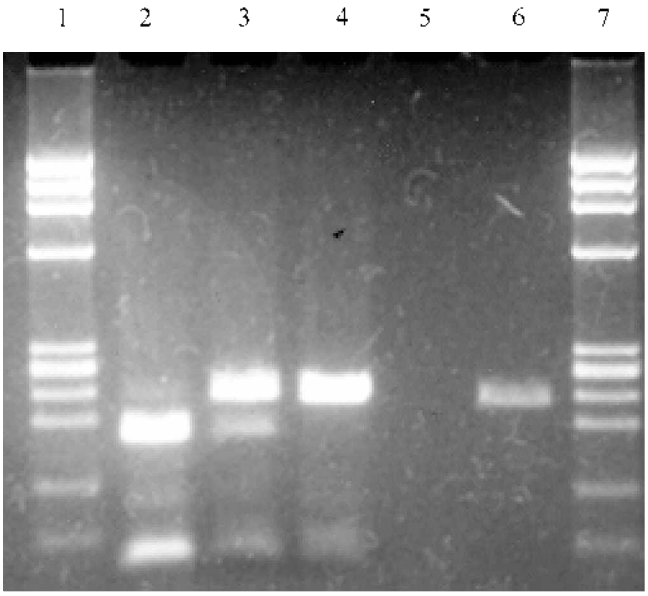

Figure 3. Representative results of PCR-RFLP analysis of bGH gene. Line 1, 7-marker PhiX174/HaeIII, Line 2-genotype LL, Line 3-genotype LV, Line 4-genotype VV, Line 5-negative control, Line 6-PCR product of bGH.

A and B were 0.61 and 0.39 respectively.

In order to evaluate the relationships between polymorphism and milk production, the 249 lactations of the cows were taken.

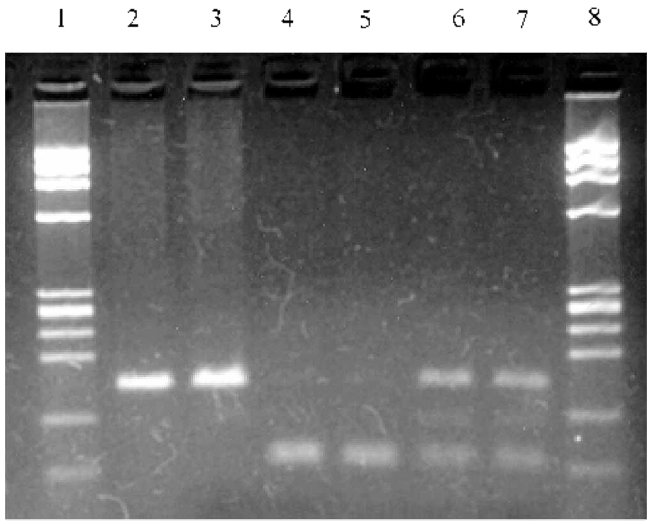

Figure 4. Representative results of PCR-RFLP analysis of bPRL gene. Line 1, 8-marker PhiX174/HaeIII, Line 2, 3-genotype AA, Line 4, 5-genotype BB, Line 6, 7-genotype AB.

We obtained the best milk production (4,503±160.8), highest fat percentage (4.63±0.11) and protein production (157.7±5.4) in cows with BB genotypes of κ-CN gene ($p<0.05$). Genotype of κ-CN gene was not associated with protein and lactose percentage (Table 4). This result showed that BB genotype of κ-CN to milk production was the best.

Significant difference of fat content was obtained

Table 4. LSM estimates and standard errors according to κ-CN genotypes

Trait \ Genotype	AA N=60	AB N=107	BB N=82	Scheffe multi range test
Milk production (kg)	4,168.9±168.4	4,489.2±156.4	4,503±160.8	AA:AB+ AA:BB+
Fat (%)	4.49±0.11	4.40±0.11	4.63±0.11	AB:BB+
Fat (kg)	184.1±8.1	197.3±7.5	206.9±7.7	AA:AB+ AA:BB+
Protein (%)	3.49±0.05	3.45±0.05	3.50±0.05	-
Protein (kg)	145.6±5.7	155.4±5.3	157.7±5.4	AA:AB+ AA:BB+
Lactose (%)	4.81±0.04	4.85±0.04	4.80±0.04	-
Lactose (kg)	201.5±8.5	218.1±7.9	216.7±8.2	AA:AB+ AA:BB+

− (p<0.05). P-value of the test.

Table 5. LSM estimates and standard errors according to β-LG genotypes

Trait \ Genotype	AA N=88	AB N=52	BB N=109	Scheffe multi range test
Milk production (kg)	4,631.9±139.1	4,620.9±132.5	4,526.3±157.9	-
Fat (%)	4.64±0.08	4.50±0.08	4.59±0.09	AA:AB+
Fat (kg)	211.6±6.80	207.7±6.48	206.4±6.48	-
Protein (%)	3.48±0.04	3.42±0.04	3.44±0.04	-
Protein (kg)	160.8±4.79	158.46±4.57	157.04±5.44	-
Lactose (%)	4.83±0.03	4.83±0.03	4.82±0.03	-
Lactose (kg)	224.5±7.25	223.9±6.67	218.6±7.95	-

− (p<0.05). P-value of the test.

between AA (4.64±0.08) and AB (4.59±0.09) genotypes ($p<0.05$) of the β-LG gene. Genotypes of β-LG gene in the other traits were not significantly difference (Table 5).

The highest fat (4.77%) and protein (3.55%) content were obtained in cows with LL genotype of bGH gene ($p<0.05$). Dairy cows with VV genotype achieved only 4.41% fat and 3.37% protein percentage in milk. Genotype of bGH gene was not associated with milk, fat, protein and lactose production (Table 6). The LL genotypes of bGH gene was the best to milk production in comparing the VV or heterozygote LV genotype.

No significant differences in bPRL genotypes associated to milk production were found (Table 7).

DISCUSSION

The Brown Swiss cattle is a breed with many advantages (Averdunk, 1997) characterized by good milk production in mountain areas. The dairy cows of Brown Swiss breed achieved average 6,011 kg with the 3.99% fat and 3.35% protein content in Switzerland in 2000 (ICAR, 2002). Brown Swiss cattle would be increase milk production in Slovak mountain areas with preferable composition of alleles of milk proteins. The import of Brown Swiss pregnant heifers to Slovakia has started after 1990 from Germany (Huba et al., 1997).

Frequency of B allele of κ-CN gene in Slovak population of Brown Swiss cattle is smaller in comparing with population in Germany (Averdunk, 1997) and in Switzerland (Zogg, 1997), but is higher than frequency of this allele in other cattle breeds in Slovakia (Vasicek et al., 1995, Zitny et al., 1996, Chrenek, 1997).

Frequency of an A allele of β-LG gene in our population of Brown Swiss cattle is also lower than in Brown Swiss in foreign countries (Taha and Puhan, 1993), but is higher than frequency of this allele in other cattle breed in Slovakia as Slovak Pied or Slovak Pinzgauer (Uhrín et al., 1995, Trakovická and Kúbek, 1996).

Lower frequencies of the V allele, which is preferred for increased milk production traits, particularly protein (Sabour and Lin, 1996) was found in Holstein (0.09) or 0.27 in Simmental breed (Zhang et al., 1993) or 0.10 in Braunvieh cattle (Schlee et al., 1994).

The frequency of alleles A and B of the bPRL gene in Brown Swiss cows imported in Slovakia were the same as was reported in Braunvieh cattle (Mitra et al., 1995).

We obtained the best milk production and the highest fat percentage in cows with BB genotypes κ-CN gene. In studied population it was not confirmed results of some authors (Bovenhuis et al., 1992, Michalcová et al., 1996) about higher protein percentage in milk of cows with BB genotypes. Higher protein percentage (about 0.13%) in milk of Brown Swiss cows with BB genotypes presented Ng-Kwai-Hang et al. (1984).

Our results showed the significant difference only in fat content between AA and AB genotypes of the β-LG gene. Therefore we did not confirmed reports where whey protein and β-lactoglobulin contents in Brown Swiss cattle were influenced by the β-lactoglobulin genotypes with β-LG

Table 6. LSM estimates and standard errors according to bGH genotypes

Genotype / Trait	LL N=109	LV N=114	VV N=26	Scheffe multi range test
Milk production (kg)	4,400.5±180.56	4,696.67±130.36	4,548.9±174.13	-
Fat (%)	4.77±0.11	4.54±0.08	4.41±0.10	LL:VV+
Fat (kg)	208.8±8.85	211.8±6.39	198.2±8.53	-
Protein (%)	3.55±0.05	3.44±0.04	3.37±0.05	LL:VV+ LL:LV+
Protein (kg)	156.8±6.24	161.4±4.51	154.1±6.02	-
Lactose (%)	4.83±0.04	4.83±0.03	4.82±0.04	-
Lactose (kg)	213.1±9.09	227.6±6.57	219.9±8.77	-

+ ($p<0.05$), P-value of the test.

Table 7. LSM estimates and standard errors according to bPRL genotypes

Genotype / Trait	AA N=109	AB N=114	BB N=26	Scheffe multi range test
Milk production (kg)	4,551.7±136.47	4,644.1±133.00	4,640.8±179.96	-
Fat (%)	4.56±0.08	4.59±0.08	4.49±0.11	-
Fat (kg)	205.2±6.67	211.4±6.50	208.4±8.80	-
Protein (%)	3.46±0.38	3.45±0.04	3.45±0.04	-
Protein (kg)	157.7±4.71	160.3±6.20	158.5±6.20	-
Lactose (%)	4.83±0.03	4.82±0.03	4.87±0.04	-
Lactose (kg)	220.4±6.87	224.52±6.70	226.1±9.02	-

A>AB>B (Cerbulis and Farrell, 1974). Similarly, milk with AA genotype of β-LG gene contained 0.05% more protein than BB genotype of β-LG gene (Ng-Kwai-Hang et al., 1984).

The highest fat and protein content were obtained in cows with LL genotype of bGH gene in our studied Brown Swiss population. These results did not confirmed, that V allele is preferred for increased milk production traits, particularly protein (Sabour and Lin, 1996).

The results of milk production obtained in our work showed that BB genotypes of κ-CN gene, AA genotypes of β-LG gene, LL genotypes of bGH gene were significantly associated with better milk production traits, mainly with the fat content. The same favorable BB genotype of κ-CN gene and AA genotype of β-LG gene on milk production in Holstein dairy herds were also reported (Ng-Kwai-Hang et al., 1983, Lin et al., 1985, 1986).

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