The association of five polymorphisms with milk production traits in Czech Fleckvieh cattle

J. Boleckova, J. Matejickova, M. Stipkova, J. Kyselova, L. Barton

Institute of Animal Science, Prague-Uhříněves, Czech Republic

ABSTRACT: The aim of this study was to estimate allelic and genotypic frequencies of five DNA markers that are positional and functional candidates for milk production traits in Czech Fleckvieh cattle. In addition, we evaluated the association of these markers with milk production traits and breeding values for milk production traits and also estimated linkage disequilibrium (LD) between two markers within the prolactin (*PRL*) gene. As part of this study, 505 Czech Fleckvieh cows were genotyped. The markers in proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*), secreted phosphoprotein (*SPP1*), cytochrome P450 family 11 subfamily B hydroxylase (*CYP11B1*), and the two polymorphisms in the prolactin gene (*PRL*) showed evidence of segregation in our study. The *PPARGC1A* polymorphism was associated with milk yield, milk fat and protein traits. The polymorphism in *SPP1* was significantly associated with milk protein percentage. The *CYP11B1* polymorphism showed positive associations with milk composition traits and breeding values for milk yield, milk fat, and protein traits. Both polymorphisms within the *PRL* gene were associated with milk yield, milk fat and milk protein yield (individually and grouped). Linkage disequilibrium between the two polymorphisms in *PRL* was not observed. In conclusion, all markers examined in this study are important markers for milk production traits in Czech Fleckvieh cattle, and both markers within the *PRL* gene should be evaluated in future research.

Keywords: dairy cattle; *PPARGC1A*; *CYP11B1*; *PRL*; *SPP1*; milk yield; milk composition

The knowledge of particular genes, their polymorphisms and their effects can help in understanding the molecular pathways that lead to health problems or production variability in cattle. Candidate genes for economically important traits are selected based upon previous linkage studies in cattle as well as biological functions in cattle and other species. Such knowledge may be important for genetic selection as well as disease prevention.

In our study, we investigated the *c.1892+19C>T* polymorphism in intron 9 of the bovine proliferator-activated receptor gamma coactivator 1 alpha gene (*PPARGC1A*), the *g.8514C>T* polymorphism in intron 4 of the secreted phosphoprotein (*SPP1*), and the *p.Val30Ala* polymorphism in the first exon of cytochrome P450 family 11 subfamily B hydroxylase (*CYP11B1*). Additionally, two polymorphisms in the prolactin (*PRL*) gene were evaluated: *g.8398G>A* in exon 4 of the *PRL* gene (*bPRL*) and *c.–1043A>G* in the enhancer sequence of the *PRL* gene (*PRLE*). These markers were previously associated with production traits in other bovine breeds, however, excluding the *bPRL* polymorphism they have not been investigated in Czech Fleckvieh cattle (Čítek et al., 2001). In this study, we chose to evaluate this particular *bPRL* polymorphism due to the probable linkage with the polymorphism in the enhancer region of the *PRL* gene. Previous association analyses of this polymorphism with milk performance traits did not present consistent results (Chrenek et al., 2003; Dybus et al., 2005; Mehmannavaz et al., 2009). Though located within an exon, the *PRL* polymorphic change is silent and does not alter the

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prolactin protein. We hypothesise that this polymorphism is not causative but is in linkage with a functional polymorphism. Moreover, the *PRLE* polymorphism is known to influence transcription of the *PRL* gene (Brym et al., 2007).

Czech Fleckvieh cattle belong to the European Fleckvieh cattle family; they are primarily bred for milk and beef production with emphasis on milk production. This breed was established from traditional Czech cattle breeds but was improved using other worldwide cattle breeds. The re-evaluation of previously associated polymorphisms in separate cattle populations is important for understanding the development of milk production traits.

We hypothesise that Czech Fleckvieh cattle are polymorphic in the SNPs examined herein and that these polymorphisms influence milk production traits as well as breeding values for milk production. We also anticipate the presence of linkage disequilibrium between the polymorphisms of the *PRL* gene and their influence on the traits we have investigated. Such a linkage study was not performed in previous studies and is important for future association research in the bovine prolactin gene.

MATERIAL AND METHODS

Data

A total of 1299 lactations from 505 Czech Fleckvieh cows were examined in this study. The cows were sampled from 4 herds and 64 sires. Lactation data were collected in the years 2001–2010. Data on cow identity, sire, year of calving, breed, milk production traits and breeding values were extracted from the official progeny testing database of the Czech-Moravian Breeders' Corporation. Cows were divided into three groups according to the genetic representation of the Czech Fleckvieh breed in the pedigree. The first group was composed of purebred Czech Fleckvieh cows, the second group contained the 76–99% pedigree cows, and the third group contained the 50–75% pedigree Czech Fleckvieh cows.

Genotyping

Genomic DNA for molecular analysis was extracted from peripheral blood using Genomic DNA Purification Tray II (Applied Biosystems, Foster City, USA). Genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism method. The method for detecting the *c.1892+19T>C* polymorphism in the *PPARGC1A* gene was previously reported by Khatib et al. (2007). The *g.8514C>T* polymorphism of the *SPP1* gene in intron 4 was detected as reported recently by Leonard et al. (2005). The valine (*Val*) to alanine (*Ala*) polymorphism (*p.Val30Ala*) in the *CYP11B1* gene product was detected by the method designed by Kaupe et al. (2007). Two polymorphisms were examined in the *PRL* gene. The first polymorphism examined was *g.8398G>A* previously demonstrated by Mitra et al. (1995); however, we used the method designed by Chrenek et al. (1998) in our evaluation. The second polymorphism (*c.–1043A>G*), located within the enhancer region, was detected according to Brym et al. (2007). A total of 505 cows were genotyped for polymorphisms in the *CYP11B1*, *SPP1* and *PRL* genes, while 500 cows were genotyped for the polymorphism in the *PPARGC1A* gene.

Statistical analyses

Genotypic frequencies and deviations from the Hardy-Weinberg equilibrium were tested by the χ^2-test. Statistical analyses were performed for milk yield (MY), fat yield (FY), fat percentage (FP), protein yield (PY), and protein percentage (PP); additionally, cows' estimated breeding values for milk yield (EBVMY), fat percentage (EBVFP), fat yield (EBVFY), protein percentage (EBVPP), and protein yield (EBVFY) were also estimated. For statistical analysis, a fixed-effects linear model was used as follows:

$$y_{ijklmno} = m + HYS_{ijk} + B_l + N_m + G_n + \beta_x + e_{ijklmno}$$

where:

$y_{ijklmno}$ = trait observed
m = population mean
HYS_{ijk} = combined effect of herd, year and season of the first calving
B_l = effect of breed group
N_m = effect of lactation number (the first lactation; the second lactation; the third and further lactation)
G_n = effect of genotype
β_x = regression of cow age at the first calving
$e_{ijklmno}$ = residual effect

The analyses were done separately for each marker *PPARGC1A* (*CC*, *CT*, or *TT*), *SPP1* (*CC*, *CT*, or *TT*), *CYP11B1* (*Ala/Ala*, *Ala/Val*, or *Val/Val*), *bPRL* (*AA*, *AG*, or *GG*), and *PRLE* (*AA*, *AG*, or *GG*). The genotypic impact was analysed by ANOVA, followed by Tukey's test.

Further associations were tested using the combination of the two polymorphisms within the *PRL* gene. This combination was denoted *bPRLE*, where the genotypes represent *bPRL* and *PRLE*, respectively (e.g., *bPRL*/*PRLE* genotypes are presented as *AA/AA*, *AA/AG*, *AA/GG*, *AG/AA*, *AG/AG*, *AG/GG*, *GG/AA*, *GG/AG*, or *GG/GG*).

Additionally, allele substitution effects of analysed traits were estimated using the following model:

$$y_{ijklmno} = m + HYS_{ijk} + B_l + N_m + A_n + \beta_x + e_{ijklmno}$$

where:

A_n = regression of allelic number ($PPARGC1A^T$, $SPP1^T$, $CYP11B1^{Val}$, $bPRL^G$ and $PRLE^G$). The remaining terms are as defined in the previous model

The analyses were implemented using the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, USA).

To account for multiple testing, we considered the false discovery rate (FDR) and calculated *q*-values with the package *q*-value in R 2.12.1 (R Development Core Team, 2010) using the method of Benjamini and Hochberg (1995). The FDR procedure was run separately for genotypic effects (60 tests) and allelic substitution effects (50 tests). The *q*-value of 0.05 was used as a threshold value.

Table 1. Genotype and allele frequencies

SNP	Genotype frequencies		Allele frequencies	
PPARGC1A	*CC*	0.64	*C*	0.80
	CT	0.33	*T*	0.20
	TT	0.03		
SPP1	*CC*	0.04	*C*	0.18
	CT	0.28	*T*	0.82
	TT	0.68		
CYP11B1	*Ala/Ala*	0.05	*Ala*	0.25
	Ala/Val	0.40	*Val*	0.75
	Val/Val	0.55		
bPRL	*AA*	0.01	*A*	0.12
	AG	0.22	*G*	0.88
	GG	0.77		
PRLE	*AA*	0.65	*A*	0.81
	AG	0.32	*G*	0.19
	GG	0.02		
bPRLE[1,2]	*GG/AA*	0.475		
	GG/AG	0.275		
	GG/GG	0.024		
	AG/AA	0.168		
	AG/AG	0.050		
	AA/AA	0.008		

[1]combined genotype – the first genotype represents *bPRL* and the second genotype represents *PRLE*
[2]combined genotypes *AA/AG*, *AA/GG*, and *AG/GG* were not present in the population

Linkage disequilibrium

Haplotype frequencies were estimated using the expectation-maximisation algorithm. Within the *PRL* gene, pair-wise linkage disequilibrium among SNPs was inferred using the EMLD program (Huang, 2003). Calculations in the EMLD program are based on genotyping results without pedigree consideration.

RESULTS AND DISCUSSION

Genotypes in all analysed loci were distributed according to the Hardy-Weinberg equilibrium.

Allelic and genotypic frequencies of the examined population are presented in Table 1. Results of the association study of *PPARGC1A*, *SPP1*, *CYP11B1*, *bPRL* and *PRLE* polymorphisms with milk production traits and with breeding values for milk production traits are shown in Tables 2 and 3, respectively. Association analyses between *bPRLE* genotypes, milk production traits, and breeding values are presented in Table 4.

The *q*-value measures the proportion of results that are declared significant (at the nominal *P*-value) but are actually false (Storey and Tibshirani, 2003). All associations that were declared significant ($P < 0.05$) in our study also withstood correction for multiple testing (FDR *q*-value < 0.05), data not shown.

Table 2. Estimates of genotype and allele substitution effects on milk production traits associated with *PPARGC1A*, *SPP1*, *bPRL*, *PRLE*, and *CYP11B1* polymorphism in Czech Fleckvieh cows

Trait	Genotype effects					Allele substitution effects	
PPARGC1A	*CC* (*n* = 805)	*CT* (*n* = 430)	*TT* (*n* = 49)	*F*-value	*P*-value	α/2^{+} ± SE	*P*-value
MY	7423.21^{a} ± 97.42	7460.46 ± 106.03	8057.81^{a} ± 258.18	3.22	*	139.05 ± 80.86	
FP	3.93^{A} ± 0.02	3.94^{b} ± 0.02	4.11^{A,b} ± 0.06	5.29	**	0.04 ± 0.02	*
FY	289.36^{A} ± 3.90	293.33^{B} ± 4.25	328.88^{A,B} ± 10.34	7.94	***	9.71 ± 3.24	**
PP	3.44 ± 0.01	3.45 ± 0.01	3.44 ± 0.03	0.33		0.01 ± 0.01	
PY	253.74^{a} ± 3.26	256.06 ± 3.55	275.33^{a} ± 8.63	3.42	*	5.40 ± 2.70	*
SPP1	*CC* (*n* = 46)	*CT* (*n* = 362)	*TT* (*n* = 891)				
MY	7456.80 ± 267.20	7346.61 ± 116.76	7493.26 ± 99.05	0.93		101.94 ± 91.61	
FP	4.00 ± 0.06	3.93 ± 0.03	3.94 ± 0.02	0.62		–0.01 ± 0.02	
FY	297.77 ± 10.76	286.20 ± 4.70	293.23 ± 3.99	1.53		3.79 ± 3.69	
PP	3.46 ± 0.03	3.45 ± 0.01	3.43 ± 0.01	2.18		–0.02 ± 0.01	*
PY	256.96 ± 8.94	252.37 ± 3.91	255.89 ± 3.32	0.51		2.11 ± 3.07	
CYP11B1	*Ala/Ala* (*n* = 70)	*Ala/Val* (*n* = 521)	*Val/Val* (*n* = 708)				
MY	7599.88 ± 220.98	7533.90 ± 103.15	7360.97 ± 98.87	1.96		–149.97 ± 77.47	
FP	3.94 ± 0.05	3.90^{A} ± 0.02	3.97^{A} ± 0.02	5.23	**	0.05 ± 0.02	**
FY	298.63 ± 8.91	292.54 ± 4.16	290.17 ± 3.99	0.58		–3.17 ± 3.12	
PP	3.47 ± 0.02	3.43 ± 0.01	3.45 ± 0.01	2.03		0.01 ± 0.01	
PY	262.92 ± 7.39	257.29 ± 3.45	252.36 ± 3.31	1.93		–5.08 ± 2.59	*
bPRL	*AA* (*n* = 12)	*AG* (*n* = 270)	*GG* (*n* = 1012)				
MY	6660.71 ± 459.25	7244.10^{a} ± 126.58	7511.88^{a} ± 91.38	4.34	*	296.39 ± 102.96	**
FP	3.97 ± 0.10	3.97 ± 0.03	3.93 ± 0.02	0.85		–0.03 ± 0.02	
FY	262.70 ± 18.52	285.06 ± 5.11	293.74 ± 3.69	3.08	*	9.92 ± 4.15	*
PP	3.48 ± 0.05	3.45 ± 0.01	3.44 ± 0.01	1.42		–0.02 ± 0.01	
PY	229.33 ± 15.37	248.78 ± 4.24	256.98 ± 3.06	3.80	*	9.22 ± 3.45	**
PRLE	*AA* (*n* = 842)	*AG* (*n* = 418)	*GG* (*n* = 39)				
MY	7384.32 ± 93.15	7549.03 ± 112.25	7976.12 ± 271.78	3.43	*	207.41 ± 83.71	*
FP	3.93 ± 0.02	3.97 ± 0.03	4.02 ± 0.06	2.71		0.04 ± 0.02	*
FY	287.99^{A,b} ± 3.74	297.71^{b} ± 4.51	319.38^{A} ± 10.92	6.48	**	11.67 ± 3.36	***
PP	3.44 ± 0.01	3.45 ± 0.01	3.47 ± 0.03	0.86		0.01 ± 0.01	
PY	252.53^{a} ± 3.11	258.96 ± 3.75	275.57^{a} ± 9.08	4.67	**	8.09 ± 2.80	**

Values for genotype effects are given as least square means (± SE) of milk production traits
Within rows, means with the same superscript differ at $P < 0.05$ (lowercase letters) or $P < 0.01$ (uppercase letters)
$^{+}$regression coefficients for the number of copies of the $PPARGC1A^{T}$, $SPP1^{T}$, $CYP11B1^{Val}$, $bPRL^{G}$ and $PRLE^{G}$ alleles representing half of the allele substitution effects
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 3. Estimates of genotype and allele substitution effects on breeding values associated with *PPARGC1A*, *SPP1*, *bPRL*, *PRLE* and *CYP11B1* polymorphism in Czech Fleckvieh cows

Trait	Genotype effects					Allele substitution effects	
PPARGC1A	*CC* (*n* = 805)	*CT* (*n* = 430)	*TT* (*n* = 49)	*F*-value	*P*-value	$\alpha/2^{+}$ ± SE	*P*-value
EBVMY	245.99 ± 24.74	224.44 ± 26.92	373.57 ± 65.56	2.60		9.48 ± 20.55	
EBVFP	-0.02^{A} ± 0.01	0.00^{b} ± 0.01	$0.07^{A,b}$ ± 0.03	6.00	**	0.02 ± 0.01	**
EBVFY	9.91^{A} ± 1.05	9.78^{B} ± 1.14	$20.86^{A,B}$ ± 2.77	8.43	***	1.91 ± 0.87	*
EBVPP	-0.02^{a} ± 0.01	-0.01^{a} ± 0.01	0.00 ± 0.02	3.51	*	0.01 ± 0.00	**
EBVPY	8.15^{a} ± 0.87	8.19^{b} ± 0.94	$13.60^{a,b}$ ± 2.30	3.01	*	1.01 ± 0.72	
SPP1	*CC* (*n* = 46)	*CT* (*n* = 362)	*TT* (*n* = 891)				
EBVMY	277.06 ± 68.26	221.98 ± 29.83	235.97 ± 25.30	0.35		1.96 ± 23.40	
EBVFP	−0.02 ± 0.03	0.00 ± 0.01	−0.01 ± 0.01	0.63		−0.01 ± 0.01	
EBVFY	11.62 ± 2.90	9.76 ± 1.27	9.73 ± 1.08	0.19		−0.34 ± 1.00	
EBVPP	−0.02 ± 0.02	0.00^{A} ± 0.01	-0.02^{A} ± 0.01	6.31	**	−0.01 ± 0.01	*
EBVPY	9.35 ± 2.39	8.46 ± 1.04	7.82 ± 0.88	0.35		−0.68 ± 0.82	
CYP11B1	*Ala/Ala* (*n* = 70)	*Ala/Val* (*n* = 521)	*Val/Val* (*n* = 708)				
EBVMY	396.50^{A} ± 56.03	272.73^{B} ± 26.15	$192.09^{A,B}$ ± 25.07	10.68	***	−89.90 ± 19.64	***
EBVFP	−0.02 ± 0.02	−0.02 ± 0.01	0.00 ± 0.01	1.97		0.02 ± 0.01	
EBVFY	16.50^{A} ± 2.39	10.99^{b} ± 1.12	$8.50^{A,b}$ ± 1.07	7.59	***	−3.14 ± 0.84	***
EBVPP	−0.01 ± 0.01	-0.02^{a} ± 0.01	-0.01^{a} ± 0.01	3.63	*	0.01 ± 0.00	
EBVPY	$14.23^{A,b}$ ± 1.96	8.96^{b} ± 0.92	7.00^{A} ± 0.88	8.57	***	−2.67 ± 0.69	***
bPRL	*AA* (*n* = 12)	*AG* (*n* = 270)	*GG* (*n* = 1012)				
EBVMY	57.36 ± 117.26	179.80^{a} ± 32.32	252.95^{a} ± 23.33	4.43	*	77.62 ± 26.29	**
EBVFP	0.09 ± 0.05	0.01 ± 0.01	−0.02 ± 0.01	4.68	**	−0.03 ± 0.01	**
EBVFY	7.48 ± 5.00	8.40 ± 1.38	10.34 ± 1.00	1.37		1.85 ± 1.12	
EBVPP	0.02 ± 0.03	0.01^{A} ± 0.01	-0.02^{A} ± 0.01	7.95	***	−0.02 ± 0.01	***
EBVPY	3.80 ± 4.11	7.18 ± 1.13	8.48 ± 0.82	1.42		1.49 ± 0.92	
PRLE	*AA* (*n* = 842)	*AG* (*n* = 418)	*GG* (*n* = 39)				
EBVMY	$201.94^{A,b}$ ± 23.65	305.23^{A} ± 28.50	372.57^{b} ± 69.00	10.62	***	97.44 ± 21.25	***
EBVFP	$-0.02^{A,B}$ ± 0.01	$0.01^{A,c}$ ± 0.01	$0.08^{B,c}$ ± 0.03	9.60	***	0.04 ± 0.01	***
EBVFY	$7.69^{A,B}$ ± 0.99	$14.29^{A,C}$ ± 1.20	$21.60^{B,C}$ ± 2.89	28.43	***	6.72 ± 0.89	***
EBVPP	−0.02 ± 0.01	−0.01 ± 0.01	0.01 ± 0.02	1.84		0.01 ± 0.01	
EBVPY	$6.88^{A,B}$ ± 0.83	10.68^{A} ± 0.99	14.38^{B} ± 2.41	13.04	***	3.79 ± 0.74	***

Values for genotype effects are given as least square means (± SE) of EBV for milk production traits
Within rows, means with the same superscript differ at $P < 0.05$ (lowercase letters) or $P < 0.01$ (uppercase letters)
$^{+}$regression coefficients for the number of copies of the $PPARGC1A^{T}$, $SPP1^{T}$, $CYP11B1^{Val}$, $bPRL^{G}$ and $PRLE^{G}$ alleles representing half of the allele substitution effects
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 4. Estimates of genotype effects on milk production traits and breeding values associated with combined genotype *bPRLE* in Czech Fleckvieh cows

Trait	*AA/AA* (*n* = 12)	*AG/AA* (*n* = 202)	*AG/AG* (*n* = 68)	*GG/AA* (*n* = 628)	*GG/AG* (*n* = 350)	*GG/GG* (*n* = 39)	*F*-value	*P*-value
MY	6643.15 ± 458.88	7167.92 ± 137.07	7551.19 ± 212.87	7475.95 ± 99.09	7542.57 ± 116.99	7963.18 ± 271.74	2.97	*
FP	3.97 ± 0.10	3.97 ± 0.03	3.98 ± 0.05	3.91 ± 0.02	3.97 ± 0.03	4.02 ± 0.06	1.82	
FY	262.18 ± 18.47	282.11^{a} ± 5.52	298.73 ± 8.57	290.65 ± 3.99	297.40 ± 4.71	319.13^{a} ± 10.94	3.50	**
PP	3.49 ± 0.02	3.46 ± 0.02	3.44 ± 0.05	3.43 ± 0.01	3.45 ± 0.01	3.47 ± 0.03	1.57	
PY	228.93 ± 4.59	246.82^{a} ± 7.12	257.85 ± 15.35	255.05 ± 3.31	259.03 ± 3.91	275.18^{a} ± 9.09	3.03	*
EBVMY	52.06 ± 116.43	$138.84^{A,B}$ ± 34.78	341.00^{A} ± 54.01	227.17 ± 25.14	296.40^{B} ± 29.68	370.69 ± 68.95	6.11	***
EBVFP	0.09 ± 0.05	0.01^{A} ± 0.01	$0.02^{A,B,C}$ ± 0.02	-0.04^{B} ± 0.01	0.01^{C} ± 0.01	0.08 ± 0.03	7.42	***
EBVFY	7.36 ± 4.90	$6.70^{A,B,C}$ ± 1.46	$16.42^{A,D}$ ± 2.27	$8.04^{D,E,F}$ ± 1.06	$13.85^{B,E}$ ± 1.25	$21.66^{C,F}$ ± 2.90	11.81	***
EBVPP	0.02 ± 0.03	0.01^{A} ± 0.01	−0.01 ± 0.01	-0.03^{A} ± 0.01	−0.01 ± 0.01	0.01 ± 0.02	6.33	***
EBVPY	3.73 ± 4.07	$6.18^{A,B}$ ± 1.22	11.76 ± 1.89	$7.21^{C,D}$ ± 0.88	$10.47^{A,C}$ ± 1.04	$14.42^{B,D}$ ± 2.41	5.58	***

Values for genotype effects are given as least square means (± SE) of milk production traits
Combined genotypes *AA/AG*, *AA/GG* and *AG/GG* were not present in the population
Within rows, means with the same superscript differ at $P < 0.05$ (lowercase letters) or $P < 0.01$ (uppercase letters)
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

PPARGC1A

PPARGC1A is a major regulator of mitochondrial DNA transcription and replication (Wu et al., 1999). It is a key factor in energy metabolism and plays a crucial role in thermogenesis, gluconeogenesis, glucose transport and β-oxidation of fatty acids (Benton et al., 2008). *PPARGC1A* is involved in mammary gland metabolism, and the expression of *PPARGC1A* is correlated with milk fat content (Bionaz and Loor, 2008).

In *PPARGC1A,* the minor allele (*T*) was observed at a frequency of 0.20. The frequency of the rare genotype (*TT*) was 0.03. These results are comparable with those of previous studies in dairy cattle as well as beef cattle. The frequency of the *T* allele in Holstein cattle ranged from 0.16 to 0.34 (Weikard et al., 2005; Khatib et al., 2007; Komisarek and Dorynek, 2009; Schennink et al., 2009). According to White et al. (2007), the *T* allele frequencies were 0.15 and 0.17, respectively, in two beef crossbred populations in the USA. Only in Jersey cattle does the *T* allele frequency seem to be higher with an observed frequency of 0.63 (Kowalewska-Luczak et al., 2010).

This polymorphism previously exhibited an effect on milk fat composition (Schennink et al., 2009). In our study, the *PPARGC1A* polymorphism was significantly associated with milk yield, milk fat, and protein yield. Additionally, the *T* allele was significantly and positively associated with both milk fat percent and the breeding value for this trait, through both genotypic and allelic substitution effect (Tables 1 and 2). These results are in agreement with the results of Weikard et al. (2005) and Komisarek and Dorynek (2009).

SPP1

SPP1 is primarily involved in the processes of calcification and immune reaction alteration. It is also highly expressed in the mammary gland (Sørensen and Petersen, 1993) and influences mammary gland development (Nemir et al., 2000) and milk protein expression (Sheehy et al., 2009).

The *C* allele and the *CC* genotype had a lower occurrence in the studied population (Table 1) than typically observed in cattle populations with characteristically high milk production (Leonard et al., 2005; Khatib et al., 2007). Low genotypic and allelic frequencies may be correlated with cattle breeds with low milk production (Oztabak et al., 2008).

In previous studies, the *C* allele was positively associated with increasing milk components (protein and fat) (Leonard et al., 2005; Khatib et al., 2007). However, in our study, this trend was statistically significant in a positive manner only for the additive effect of the *C* allele on protein percentage and breeding value for this trait.

CYP11B1

There are several indications that *CYP11B1* is a positional and functional candidate gene for milk production traits. *CYP11B1* influences the production of cortisol, androgen function and ultimately the proliferation of milk gland cells (Brettes and Mathelin, 2008). The bovine *CYP11B1* gene is positioned in chromosomal region *BTA14q12* (Kaupe et al., 2004) near marker *ILSTS039*. This marker is associated with milk yield as well as with milk component yields (Wibowo et al., 2008). Although there is a *DGAT1* gene, this cannot explain the QTL found in this region completely; Kaupe et al. (2007) concluded that this variation is partially affected by the *CYP11B1* gene polymorphism. On the other hand, the associations found with the *CYP11B1* polymorphisms could be influenced by the linkage with the *DGAT1* gene.

The frequencies of the minor allele (*Ala*) and the rare genotype (*Ala/Ala*) of the *CYP11B1* polymorphism were 0.25 and 0.05, respectively. The allele frequencies in our Czech Fleckvieh population were similar to the results observed by Kaupe et al. (2007) in German Holstein (*Ala* 0.22) and Simmental cattle (*Ala* 0.27). Our association tests of the *Ala/Val* polymorphism in the Czech Fleckvieh population revealed a positive effect of the *Val* allele on milk fat content (Tables 2 and 3). Conversely, the gene variant *Ala* was correlated with higher milk yield, thus influencing breeding values for protein and fat yields. Similar results were reported by Kaupe et al. (2007).

PRL

PRL is a polypeptide produced not only by the pituitary gland but also by the mammary gland. This hormone has over 300 functions. It is involved in water and electrolyte balance, growth and development, endocrinology and metabolism, behaviour, reproduction and immunoregulation. It plays a crucial role in mammary gland development and lactogenesis (Bole-Feysot et al., 1998).

The *bPRL* polymorphism causes a non-synonymous amino acid change in codon 103 of the prolactin protein (Sasavage et al., 1982) and it is the most investigated polymorphism of the bovine prolactin gene. The allelic and genotypic frequencies observed in our study (Table 1) were similar to other studies of dairy cattle and combined breeds including Czech Fleckvieh (Čítek et al., 2001; Brym et al., 2005). Lower frequencies of the *G* allele were pronounced in the Jersey breed (Brym et al., 2005; Dybus et al., 2005).

In this study, the polymorphic *G* allele of *bPRL* was positively associated with milk yield. Consequently, it also positively influenced protein and fat yields, but it influenced protein and fat percentages negatively (EBVPP and EBVFP). Previous studies also observed associations of this polymorphism with milk production and fat yield (Dybus et al., 2005; Alipanah et al., 2007). The negative effect of the *AA* genotype on fat production was shown by Brym et al. (2005) and Khatami et al. (2005), and the *A* allele was positively associated with milk and protein yield (Mehmannavaz et al., 2009). Conversely, the association of this polymorphism with milk production traits was not confirmed in other studies (Chrenek et al., 2003).

Herein, the polymorphic minor allele of *PRLE* (*G*) was observed at a frequency of 0.19 and the rare genotype (*GG*) was observed at a frequency of 0.02. The genotypic and allelic frequencies of the *PRLE* polymorphism were similar to results reported for the Holstein–Friesian breed in Poland (Brym et al., 2007) and China (Lü et al., 2010). In the pituitary gland, the prolactin gene expression level is higher in animals with the *AA* genotype than in those with the *GG* genotype; this is presumably due to variation in the affinity of the enhancer site to transcription factors (Brym et al., 2007). In our study, the genotype *GG* was associated with higher MY, which also influenced the FY and PY and breeding values for these traits. It is obvious that the *G* allele also positively influenced milk fat content (EBVFP), contrary to the previous study (Lü et al., 2010).

Genotypic frequencies for *bPRLE* are presented in Table 1. The combined genotypes *GG/GG*, *GG/AG*, and *AG/GG* were not present in our current population. The haplotypic probabilities for *bPRLE* were calculated by the EMLD program. At a frequency of 0.69, the most common haplotype was *GA* (the first and second alleles represent *bPRL* and *PRLE*, respectively). On the contrary, the *AG* haplotype occurs infrequently (3.04×10^{-6}). The calculated probabilities of *AA* and *GG* haplotype were 0.12 and 0.19, respectively.

The combined genotype *bPRLE* was significantly associated with MY ($P < 0.05$), FY ($P < 0.01$), PY ($P < 0.05$) and all breeding values ($P < 0.001$). Between the *bPRLE* genotypic groups, there were significant differences in the association with milk performance traits (Table 4) that were influenced by both polymorphisms (*bPRL* and *PRLE*). The influ-

ence of each allele on a particular trait is in agreement with independent results for each of the *bPRL* and *PRLE* polymorphisms (Tables 2 and 3). In this study, the parameters of linkage disequilibrium between the two polymorphisms in the *PRL* gene were D' = 0.9999 and r^2 = 0.0302. The difference between D' and r^2 can be explained by two factors. First, D' may have been biased by a low representation of minor alleles in our population, and second, r^2 was influenced by differing frequencies of linked alleles. The low r^2 in our study indicates that one SNP explains only a small proportion of the variation held by the second SNP. This suggests that there will be a need to genotype both SNPs in further research because we cannot substitute one for the other.

CONCLUSION

All allele variants observed in this study were polymorphic. Each polymorphism examined in this study influenced some of the associated milk production traits and breeding values. The present results confirmed previous investigations of *PPARGC1A*, *SPP1*, *CYP11B1*, *bPRL*, and *PRLE* polymorphisms regarding allele and genotype frequencies as well as the influence on milk production traits. Moreover, we performed a linkage analysis and an association study with two polymorphisms in the *PRL* gene. We concluded that these polymorphisms influence milk production traits separately; hence both polymorphisms should be evaluated in future studies. These results revealed important information with possible application in Czech Fleckvieh breeding. Further analyses are necessary to confirm these findings before widespread application.

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Corresponding Author

Ing. Mgr. Jana Bolečková, Ph.D., Department of Cattle Breeding, Institute of Animal Science, Přátelství 815, 104 00 Prague-Uhříněves, Czech Republic
Tel. +420 267 009 540, e-mail: boleckova.jana@seznam.cz