Effect of *DGAT1* polymorphisms on the estimated breeding values of Czech Simmental sires

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ABSTRACT: The aim was to evaluate the effect of polymorphisms in the promoter and in the coding region of the *DGAT1* gene on the estimated breeding values (EBV) of Czech Simmental sires. The *K232A* polymorphism (n = 191) in the coding region was genotyped by PCR/RFLP, and the *KU* and *SA* polymorphisms in the promoter (n = 203) were identified in an automatic sequencer. In the *K232A* polymorphism, the frequency of the genotype *AA* (Alanine) was greater than that of the genotype *KA*, the homozygous genotype *KK* (Lysine) was not found. Similarly, the allele *A* predominated over the *K* allele (0.945 and 0.055). The EBV for milk performance have been assigned to the genotypes, and the associations quantified. For the *AA* genotype and *A* allele, positive association with EBV of milk yield and protein yield was found, and negative association with the breeding values of fat percentage and yield, and protein percentage, but only the value of fat content was found to be significant. The positive non-significant association of the *A* variant with the protein yield was caused by the high milk yield. In the *KU* polymorphism, the *CC* genotype was associated significantly with lower EBV for the fat percentage, both the *C* allele and the *CC* genotype were associated with higher EBV for milk yield, so both the fat and the protein yield were non-significantly increased. For the diplotypes *K232A/KU*, there was a significant association with the fat percentage. The *AACC* combination seemed to have some breeding potential. The *K232A* polymorphism explained maximum of 6.2% of EBV variability, the *KU* polymorphism of 4.4%, and the *SA* polymorphism of 4.2%. The diplotypes *K232A/KU* explained maximum of 7.4% of variability. The highest proportion of variability was explained for fat percentage. The results confirmed the important role of the BTA14 region in controlling milk performance.

Keywords: cattle; acyl-CoA diacylgycerol transferase1; *K232A;* promoter; milk performance; breeding; genotypes

INTRODUCTION

The *DGAT1* gene encodes the DGAT1 enzyme, which catalyzes the final step of triglyceride synthesis (Sanders et al. 2006).

Several studies in cattle have described a quantitative trait locus (QTL) with impact on milk production traits, and on milk fat percentage in particular, in the 3-cM region in the centromeric part of *Bos taurus* autosome 14 (BTA14) (Riquet et al. 1999; Looft et al. 2001). Grisart et al. (2002, 2004) and Winter et al. (2002, 2004) have identified a nonconservative dinucleotide substitution (*K232A*) in the acyl-CoA diacylglycerol acyltransferase1 (*DGAT1*) gene at position 10433 and 10434 in exon number 8 as the most likely mechanism underlying the QTL on this chromosome.

However, apparent differences in the effect observed between families and across populations could not be fully explained by this dialelic polymorphism alone. It was reported that genetic variation additional to the DGAT1 K232A mutation affecting milk fat

Supported by the Grant Agency of the University of South Bohemia in České Budějovice (GAJU) (Projects No. 011/2013/Z and No. 022/2013/Z).

content should be present in the same QTL (Bennewitz et al. 2004; Kuhn et al. 2004). Winter et al. (2002) considered alleles of the DGAT1 promoter region, which comprise a variable number of tandem repeats (VNTR), as likely candidates. In the German Holstein population, Kuhn et al. (2004) described 5 alleles at a VNTR polymorphism in the DGAT1 promoter, which showed an effect on fat content additional to the DGAT1 K232A mutation. The most frequent allele in the DGAT1 promoter VNTR was allele *3*. Multiple regression analysis of the DGAT1 promoter VNTR alleles in sons of genotype DGAT1 232A/232A revealed that the allele substitution effect on the milk fat content of allele *5* was higher than of all the other alleles. The regression analysis of the DGAT1 promoter VNTR alleles revealed significant effects for the allele *5* enhancing the milk fat content percentage as well as the milk protein percentage but decreasing the milk yield and milk protein yield (Kuhn et al. 2004).

Sanders et al. (2006) reported 6 alleles found in the DGAT1 promoter VNTR in the Angeln population, which were denoted as VNTR alleles *A*, *B*, *C*, *D*, *E*, and *F*, respectively. The allele *F* was present in two unrelated daughters only. The most frequent DGAT1 allele was *E*. In their study, they observed that the VNTR allele *E* showed significant effects for some milk production indicators compared with all other alleles in the DGAT1 promoter. The same results were reported by Kuhn et al. (2004) for the DGAT1 VNTR allele *5*. However, in contrast to Kuhn et al. (2004), the VNTR allele *E* was mainly linked to the K variant at DGAT1 K232A (Sanders et al. 2006), whereas the DGAT1 VNTR allele *5* showed up with the *A* variant in the German Holstein Friesian population (Kuhn et al. 2004). The VNTR allele *E* of Sanders et al. (2006) probably corresponds to the DGAT1 VNTR allele *5* of Kuhn et al. (2004).

In the paper, we report on our analysis of the effect of the genotypes and alleles in the promoter, and in the coding region at nucleotide positions 10433 and 10434 of the *DGAT1* gene on the estimated breeding values (EBV) of Czech Simmental sires.

MATERIAL AND METHODS

The analyzed group was made of the Czech Simmental sires born in the period 2000–2004, the sires were selected randomly. The DNA was isolated from frozen sperm. The respective parts of the *DGAT1* locus carrying the polymorphisms studied were amplified in the PCR. The primer sequences and PCR conditions were as in Kuhn et al. (2004) and Sanders et al. (2006) for the polymorphisms in the promoter and as in Winter et al. (2002) for the polymorphisms at nucleotide positions 10433 and 10434 of the *DGAT1* gene (*K232A* polymorphism). In promoter, the polymorphisms *SA* and *KU* were studied (Kuhn et al. 2004; Sanders et al. 2006). The PCR was carried out on the Biometra TGradient Thermocycler (Biometra GmbH, Göttingen, Germany). The VNTR polymorphisms *KU* and *SA* in the promoter of the *DGAT1* gene were distinguished in the automatic sequencer ABI PRISM®310 Genetic Analyzer (Applied Biosystems, Foster City, USA). The polymorphism *K232A* causing *K* to *A* substitution was distinguished by a restriction fragment length polymorphism using the restriction endonuclease *Cfr*I (PCR/RFLP method). An alternative genotyping method was published by Abdolmohammadi et al. (2011). For the *K232A* polymorphism, 191 sires were genotyped, and 203 sires for the polymorphisms in the promoter.

The actual breeding values estimated in 2011 (Plemdat; www.plemdat.cz) have been assigned to the genotypes, and the associations quantified. The genotypes with low frequency were left out in the association analysis. So, for the *K232A* polymorphism, 180 sires were involved, namely purebred Simmentals (n = 54), crossbreds of Simmental with Holstein and Ayrshire with the proportion of Simmental above 75% (n = 93), crossbreds with the proportion of Simmental of 50–74% (n = 21), and purebred Montbeliardes (n = 12). For the promoter polymorphisms, the counts were the same, just the number of purebred Simmentals was 55, and so the total number of sires was 181.

We analyzed the relation between the detected genotypes and estimated breeding values for the milk production traits: milk yield (kg), relative breeding value for milk yield, fat content (%), fat yield (kg), protein content (%), relative breeding value for protein content, protein yield (kg), and relative breeding value for protein yield. The loci were analyzed independently. Generally, the using of daughter yield deviations (DYD) is preferred to EBV, because EBV contain information from other relatives than the bull's daughters. However, the large number of daughters make the difference between the DYD and EBV negligible (Viitala et al. 2006). The reliabilities of EBV were equable, which enabled their direct comparison (Table 1).

Table 1. Reliability of estimated breeding values (EBV) of Czech Simmental sires with different *DGAT1* genotypes

Polymorphism	Genotype	Reliability of EBV
K232A	*AA*	89 ± 3.36
	KA	89 ± 3.08
KU	*CC*	89 ± 3.69
	CD	89 ± 2.89
	CE	89 ± 4.25
	DD	90 ± 2.64
	DE	88 ± 2.14
SA	*221/221*	89 ± 3.73
	221/239	89 ± 2.87
	221/256	89 ± 4.25
	239/239	90 ± 2.65
	239/256	88 ± 2.14

The statistical evaluation was based on the model equation:

$$EBV = \mu + G_i + e_{ijk}$$

where:

EBV = estimated breeding value for partial milk production parameter

μ = overall mean

G_i = fixed effect of genotype/allele of polymorphic sites in the promoter, or in *K232A*

e_{ijk} = residual effect

Assumptions for the Analysis of Variance (ANOVA) were tested by using the Bartlett test of homogeneity of variance. The estimated breeding values were evaluated by ANOVA depending on the genotype on the *DGAT1* locus; similarly, the differences between the alleles were quantified. The differences were evaluated at the significance levels of $P < 0.05$*, and $P < 0.01$**. The software STATISTICA (Version 10, 2013) and the ANOVA/MANOVA procedure were used. The Hardy-Weinberg equilibrium was tested by the χ^2 test.

RESULTS AND DISCUSSION

Genotypes and alleles frequencies. In the *K232A* polymorphism, the genotype *AA* prevailed substantially over the *KA*, while the homozygous genotype *KK* was not found. Similarly, the allele *A* predominated over the *K* allele in the group analyzed (0.945 and 0.055, respectively) (Table 2). This is different from frequencies found in German Holsteins in our own previous study (Hradecka et al. 2008), where the frequencies of 0.660 and 0.340 were found, but the population mentioned was upgraded with the Jersey breed. Weller et al. (2003) in Israeli Holstein cows gave a frequency of *K* allele of 0.09, in sires of 0.16. By contrast, Thaller et al. (2003) gave the frequency of the allelic variant coding for *K* in the German Holstein of 0.548. As in Grisart et al. (2002), *K* is probably the ancestral allele; its frequency is indirectly influenced by selection and decreased while selecting for high milk yield. The low frequency of the *K* allele found in this paper implies indirect selection as a consequence of the preference of the protein yield in Czech Simmentals in the last decades. Moreover, the Czech Simmental was upgraded by using Ayrshire and Red Holstein cattle with the aim of bettering the milk performance, and the crossing could have influenced the

Table 2. Genotype and allele frequencies of *DGAT1* polymorphisms

Genotype	*n*	Frequency	c^2	Allele	Frequency
K232A					
AA	170	0.890		*A*	0.945
KA	21	0.110	0.662[a]	*K*	0.055
KK	0	0.00			
KU					
BD	2	0.010		*B*	0.005
CC	45	0.222		*C*	0.480
CD	65	0.320		*D*	0.318
CE	39	0.192		*E*	0.192
CF	1	0.005	3.750[a]	*F*	0.005
DD	20	0.099			
DE	21	0.103			
DF	1	0.005			
EE	9	0.044			
SA					
205/239	2	0.010		*205*	0.005
221/221	44	0.217		*221*	0.478
221/239	66	0.325		*239*	0.320
221/256	39	0.192		*256*	0.192
221/273	1	0.005	4.305[a]	*273*	0.005
239/239	20	0.099			
239/256	21	0.103			
239/273	1	0.005			
256/256	9	0.044			

[a]non significant

c^2 = test for evaluation of average variation from expected frequencies by Hardy-Weinberg equilibrium

Table 3. Estimated breeding values according to genotypes and alleles of *DGAT1 K232A* polymorphism

Genotype/allele	*n*	Trait							
		milk yield		fat (%)	fat yield (kg)	protein (%)	protein (%) RBV	protein yield	
		(kg)	RBV					(kg)	RVB
AA	161	166 ± 490	109 ± 12	−0.01 ± 0.20[a]	6.09 ± 18.81	−0.01 ± 0.12	98.66 ± 11.42	5.19 ± 15.18	109.08 ± 12.11
KA	19	−4 ± 618	105 ± 15	0.18 ± 0.30[a]	9.21 ± 20.47	0.01 ± 0.12	100.05 ± 11.55	0.16 ± 19.92	105.21 ± 15.63
F-test		1.93	1.77	4.32*	0.46	0.35	0.25	1.74	1.63
R^2		0.005	0.004	0.062	−0.003	−0.003	−0.004	0.004	0.003
A	341	157 ± 498	109 ± 13	0.00 ± 0.21[b]	6.27 ± 18.87	0.00 ± 0.12	98.74 ± 11.40	4.91 ± 15.47	108.87 ± 12.32
K	19	−4 ± 618	105 ± 15	0.18 ± 0.30[b]	9.21 ± 20.47	0.01 ± 0.12	100.05 ± 11.55	0.16 ± 19.92	105.21 ± 15.63
R^2		0.002	0.002	0.029	−0.002	−0.002	−0.002	0.002	0.001

RBV = relative breeding value (%)

[a]values in one column with identical letters differ significantly ($P < 0.05$)

*$P < 0.05$

Table 4. Estimated breeding values according to genotypes and alleles of *DGAT1 KU* polymorphism

Genotype/allele	*n*	Trait							
		milk yield		fat (%)	fat yield (kg)	protein (%)	protein (%) RBV	protein yield	
		(kg)	RBV					(kg)	RBV
CC	44	306 ± 418	113 ± 11	−0.06 ± 0.20[a]	8.86 ± 18.28	−0.04 ± 0.11	95.25 ± 10.61[Aa]	8.55 ± 13.76	112.00 ± 10.87
CD	62	101 ± 584	107 ± 15	0.07 ± 0.24[a]	7.48 ± 20.74	0.02 ± 0.13	101.06 ± 11.45[A]	4.19 ± 18.02	108.02 ± 14.14
CE	36	107 ± 481	108 ± 12	0.04 ± 0.23	6.27 ± 14.77	0.02 ± 0.14	101.06 ± 12.86[a]	4.31 ± 14.93	108.53 ± 11.75
DD	19	223 ± 483	111 ± 12	−0.03 ± 0.21	7.79 ± 19.65	−0.03 ± 0.08	95.89 ± 7.67	5.31 ± 14.57	109.63 ± 11.57
DE	20	105 ± 342	108 ± 8	−0.06 ± 0.19	0.75 ± 12.64	−0.02 ± 0.13	97.20 ± 12.28	2.35 ± 10.33	107.00 ± 8.25
F-test		1.21	1.36	2.65*	0.60	1.90	2.08	0.76	1.00
R^2		0.009	0.011	0.044	−0.006	0.027	0.032	−0.005	−0.001
C	186	199 ± 498	109 ± 13	0.00 ± 0.23	7.90 ± 18.43	−0.01 ± 0.13	98.31 ± 11.63	6.27 ± 15.56	110.00 ± 12.27
D	120	140 ± 517	108 ± 13	0.02 ± 0.22	6.46 ± 19.22	0.00 ± 0.12	98.78 ± 10.72	4.24 ± 15.77	108.36 ± 12.44
E	70	98 ± 412	107 ± 11	0.02 ± 0.22	5.10 ± 14.78	0.00 ± 0.13	98.86 ± 12.27	3.00 ± 13.32	107.16 ± 11.10
R^2		0.001	0.003	−0.004	−0.002	−0.005	−0.005	0.002	0.003

RBV = relative breeding value (%)

[a]values in one column with identical letters differ significantly ($P < 0.05$)

[A]values in one column with identical letters differ significantly ($P < 0.01$)

*$P < 0.05$

frequencies in the *DGAT1* locus. Spelman et al. (2002) recorded variable frequencies in Holstein sires based on the origin of the genetic material. The frequency was higher in the homebred (New Zealander) population, where it was similar to the Jersey breed, and it indicates changes due to indirect selection. They report a low frequency of 0.22 in Ayrshire. In Montbeliarde, the *A* variant is practically fixed (Gautier et al. 2007), and this is concordant with our frequencies in phylogenic related Simmental cattle.

In the promoter region, two possible polymorphisms, *SA* and *KU,* were studied. In the *SA* polymorphism, the most frequent allele *221* had a frequency of 0.478 and the least frequent alleles *205* and *273* of 0.005. Using the same allele designation as Sanders et al. (2006), the most frequent allele in our study was allele *B* and the least frequent were alleles *A* and *E*. These results are in contrast to results of the authors mentioned, who found allele *E* to be the most frequent. The allele with the lowest frequency was allele *A* in both studies. In the *KU* polymorphism, we studied 5 alleles. The most frequent was allele *C* (0.480) and the least frequent were alleles *B* and *F* (0.005). Our results are also not completely concordant with Kuhn et al. (2004). They described allele *3* (*D*) as the most frequent, and allele *1* (*B*) as the least frequent. In our paper, the frequencies as compared among the purebred Simmentals, purebred Montbeliarde, and crosses were not significantly different both in *K232A* and promoter polymorphisms.

Association analysis of the K232A polymorphism. For the coding region of the *DGAT1* gene (Table 3), significant differences in estimated breeding values of fat content both for genotypes and alleles were found; the *K* variant was associated with higher values. The results are in accordance with those previously found in German Holsteins (Citek et al. 2007). However, also in Holsteins, the estimated breeding values of milk yield, fat yield, protein yield, and protein content were significant. In this paper, the trend of estimated breeding values for genotypes and alleles was the same, i.e. the homozygous *AA* genotype and *A* allele were associated with higher milk yield, protein yield, and with lower fat percentage, fat yield, and protein percentage, but significance was found only in fat content. The positive non-significant link of the *A* variant on protein yield was caused by high milk yield. The highest portion of variability was explained in EBV for fat content (6.20% by genotype; 2.90% by allele), the other values did not exceed 1%.

Because there was a high breeding value for milk yield and a negative value for fat content in the homozygotes for the Alanine variant, the *K232A* polymorphism may contribute to the negative correlations between the traits. The group has been in Hardy-Weinberg equilibrium, that implies that the selection on breeding value for protein yield did not importantly affect frequencies on the *K232A* polymorphism. The possible reasons may be seen in the rational selection on the fat and protein yield in the breeding programme, which could steady the frequencies; and also in the fact that the *DGAT1* itself does not directly influence the protein synthesis (compare Citek et al. 2007). Our results correspond well with many other authors (Grisart et al. 2002; Weller et al. 2003; Sanders et al. 2006), and are applicable in breeding practice.

Association analysis of the promoter polymorphisms. The *CC* genotype in the *KU* polymorphism was associated significantly with lower EBV for fat percentage compared with *CD*, and with the protein percentage compared with *CD* and *CE* genotypes, but non-significantly with the others (Table 4). But both *C* allele and *CC* genotype were linked to higher EBV for milk yield, so the fat and protein yield were increased non-significantly. For the *EE* genotype, the lowest EBV for milk yield of 65 kg was stated, the highest increase of fat percentage of 0.09%, and low protein yield of 0.57 kg; this was similar to the allele *E* effect, although the number of animals was very low (n = 7), and thus the data are not shown in Table 4. Again, the highest portion of variability was explained in EBV for fat content, 4.40% for genotype. Evidently, the *SA* polymorphism (Table 5) is the same as that of *KU*, as noted by other authors (Kuhn et al. 2004, Sanders et al. 2006).

The relationship between the combined genotypes of *K232A* and *KU* polymorphisms and the estimated breeding values was quantified (Table 6). The significant association with the fat percentage was noted. At the *KU* polymorphism, the combination of the *AA* genotype in *K232A* and the *CC*, *DE,* and *DD* in *KU* had the negative link, whereas *LA/CD* had the positive. For fat content, the combined genotype explained 7.4% of variability. The *AA/CC* combination seems to have some potential, as it was related with the higher estimated breeding values of the milk, fat and protein yield, but the differences were not significant for the traits mentioned.

Table 5. Estimated breeding values according to genotypes and alleles of *DGAT1 SA* polymorphism

Genotype/ allele	*n*	Trait							
		milk yield		fat (%)	fat yield (kg)	protein (%)	protein (%) RBV	protein yield	
		(kg)	RBV					(kg)	RBV
221/221	43	308 ± 423	113 ± 11	–0.06 ± 0.20[a]	9.19 ± 18.37	–0.04 ± 0.11	95.53 ± 10.56	8.77 ± 13.84	112.12 ± 10.92
221/239	63	103 ± 579	107 ± 15	0.06 ± 0.23[a]	7.29 ± 20.63	0.02 ± 0.13	100.78 ± 11.58	4.11 ± 17.89	107.95 ± 14.03
221/256	36	107 ± 481	108 ± 12	0.04 ± 0.23	6.28 ± 14.77	0.02 ± 0.14	101.06 ± 12.86	4.31 ± 14.93	108.53 ± 11.75
239/239	19	224 ± 483	111 ± 12	–0.03 ± 0.21	7.79 ± 19.65	–0.03 ± 0.08	95.89 ± 7.67	5.32 ± 14.57	109.63 ± 11.57
239/256	20	105 ± 342	108 ± 8	–0.06 ± 0.19	0.75 ± 12.64	–0.02 ± 0.13	97.20 ± 12.28	2.35 ± 10.33	107.00 ± 8.25
F-test		1.21	1.36	2.57*	0.63	1.65	1.81	0.82	1.07
R^2		0.009	0.011	0.042	–0.005	0.020	0.024	–0.003	0.000
221	185	199 ± 499	110 ± 13	0.00 ± 0.23	7.97 ± 18.45	–0.01 ± 0.13	98.39 ± 11.61	6.31 ± 15.59	110.03 ± 12.30
239	121	141 ± 515	108 ± 13	0.01 ± 0.22	6.36 ± 19.17	–0.01 ± 0.12	98.65 ± 10.77	4.20 ± 15.71	108.32 ± 12.39
256	70	98 ± 412	107 ± 11	0.02 ± 0.22	5.10 ± 14.78	0.00 ± 0.13	98.86 ± 12.27	3.00 ± 13.32	107.16 ± 11.10
R^2		0.001	0.003	–0.004	–0.001	–0.005	–0.005	0.002	0.004

RBV = relative breeding value (%)

[a]values in one column with identical letters differ significantly ($P < 0.05$); *$P < 0.05$

Table 6. Estimated breeding values according to combined genotypes of *K232A* and *KU* polymorphisms

Genotype	*n*	Trait							
		milk yield		fat (%)	fat yield (kg)	protein (%)	protein (%) RBV	protein yield	
		(kg)	RBV					(kg)	RBV
AA/CC	44	306 ± 418	113 ± 11	–0.06 ± 0.20[A]	8.86 ± 18.28	–0.04 ± 0.11	95.25 ± 10.61	8.55 ± 13.76	112.00 ± 10.87
AA/CE	32	140 ± 474	108 ± 12	0.02 ± 0.21	6.63 ± 15.52	0.01 ± 0.14	100.28 ± 12.98	5.03 ± 14.89	109.06 ± 11.72
AA/CD	53	125 ± 552	108 ± 14	0.04 ± 0.22	6.87 ± 19.64	0.02 ± 0.13	101.04 ± 11.56	4.96 ± 16.56	108.58 ± 13.01
AA/DE	18	74 ± 346	107 ± 8	–0.05 ± 0.20[b]	0.11 ± 13.20	–0.01 ± 0.13	98.50 ± 12.00	1.94 ± 10.84	106.67 ± 8.66
AA/DD	16	263 ± 461	112 ± 12	–0.06 ± 0.15[c]	8.19 ± 20.45	–0.03 ± 0.09	96.18 ± 8.10	6.69 ± 13.50	110.81 ± 10.73
AA/EE	6	63 ± 372	105 ± 12	0.03 ± 0.17	4.50 ± 16.21	–0.06 ± 0.11	93.67 ± 10.25	–0.33 ± 14.80	102.83 ± 14.39
KA/CE	4	–153 ± 524	101 ± 13	0.19 ± 0.30	3.50 ± 6.76	0.09 ± 0.13	107.25 ± 11.44	–1.50 ± 16.13	104.25 ± 12.84
KA/CD	9	–38 ± 771	104 ± 19	0.24 ± 0.27[Abc]	11.11 ± 27.51	0.02 ± 0.12	101.22 ± 11.40	–0.33 ± 25.83	104.67 ± 20.24
KA/DD	3	17 ± 658	105 ± 16	0.10 ± 0.45	5.67 ± 18.15	–0.05 ± 0.06	94.33 ± 5.78	–2.00 ± 21.17	103.33 ± 16.44
F-test		1.126	1.196	2.845*	0.472	1.417	1.521	0.788	0.496
R^2		0.005	0.008	0.074	–0.024	0.018	0.022	–0.009	–0.002

RBV = relative breeding value (%)

values in one column with identical letters differ significantly [A]($P < 0.01$), [b]($P < 0.05$)

*$P < 0.01$

Table 7. Estimated breeding values for different *KU* alleles in *232A/232A* homozygous sires

KU allele[1]	*n*	Trait							
		milk yield		fat (%)	fat yield (kg)	protein (%)	protein (%) RBV	protein yield	
		kg	RBV					kg	RBV
C	173	220 ± 477	110 ± 12	–0.02 ± 0.21	7.83 ± 18.11	–0.01 ± 0.13	97.95 ± 11.61	6.80 ± 14.86	110.41 ± 11.73
D	103	159 ± 493	109 ± 12	–0.01 ± 0.20	6.10 ± 18.90	–0.01 ± 0.12	99.08 ± 10.78	4.97 ± 14.68	108.94 ± 11.60
E	62	106 ± 414	107 ± 11	0.00 ± 0.20	4.32 ± 15	–0.01 ± 0.13	98.48 ± 12.21	3.10 ± 13.64	107.16 ± 11.41
R^2		0.020	0.025	0.033	0.005	0.030	0.037	0.010	0.019

RBV = relative breeding value (%)

[1]differences among alleles were not significant

Finally, the effect of the alleles at the *KU* site within the group of *232A/232A* sires was evaluated (Table 7). The differences were not significant; the allele *E* showed to be linked to lower EBV of milk, fat, and protein yield, and the relation to the fat and protein content was neutral. This is in concordance with Sanders et al. (2006) who found the interallelic differences to be insignificant but the influence of haplotypes to be significant.

Together with other markers analyzed in Czech Simmental (Boleckova et al. 2012), the *DGAT1* locus should be studied intensively, as the breeding potential is high. Then, the genomic approach (Schopen et al. 2009; Pribyl et al. 2010, 2012, 2013; Matejickova et al. 2013; Szyda et al. 2013) could be completed by the known major genes concerning milk performance and by the biometric approach (Sigl et al. 2012; Meszaros et al. 2013; Zavadilova and Stipkova 2013; Zavadilova and Zink 2013). When different effects depending on the breed are found, e.g. Suchocki et al. (2010) noted a stronger effect in Jersey than in Holstein, the gene polymorphisms should be evaluated regarding the breed. In the paper, we have analyzed the dual-purpose Czech Simmental cattle, as in most cases the dairy breeds, predominantly Holstein, are in the spotlight.

CONCLUSION

Concludingly, in the *K232A* polymorphism the *K* allele was associated significantly with higher estimated breeding values of fat content and *vice versa* for the *AA* genotype and *A* allele. The *CC* genotype in the *KU* polymorphism was associated significantly with lower EBV for fat and protein percentage. Both *C* allele and *CC* genotype were linked to higher EBV for milk yield, so the fat and protein yield were linked positively, but non-significantly. The combined genotypes of *K232A* and *KU* polymorphisms and the EBV for the fat percentage were linked significantly, and the combination *AA*/*CC* may have some breeding potential. The *K232A* polymorphism explained at the most of 6.2% of variability of estimated breeding value, the *KU* polymorphism of 4.4%, and the *SA* polymorphism of 4.2%. The combined genotypes *K232A* and *KU* explained at the most of 7.4% of variability. In all polymorphisms, the highest proportion of EBV variability was explained for fat percentage. Definitely, there is promising that repeated analyses in different breeds show the important role of the BTA14 region in controlling milk performance.

Acknowledgement. The authors thank John P. McAvoy for the language revision.

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Received: 2013–05–14
Accepted after corrections: 2014–03–04

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