

Effect of *DGAT1*, *BTN1A1*, *OLR1*, and *STAT1* genes on milk production and reproduction traits in the Czech Fleckvieh breed

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ABSTRACT: The impact of polymorphism of the diacylglycerol acyltransferase (*DGAT1*), butyrophilin (*BTN1A1*), oxidized low-density lipoprotein receptor (*OLR1*), and signal transducer and activator of transcription 1 (*STAT1*) genes on milk production and reproduction traits in 419 Czech Fleckvieh cows was examined using polymerase chain reaction and restriction fragment length polymorphism. The loci *DGAT1* and *BTN1A1* were observed simultaneously to affect milk production, estimated breeding value of milk production traits, as well as reproduction parameters. Significant differences were found also between genotypes of the *STAT1* loci in relation to estimated breeding value of milk production traits. Similar findings in pure dairy breeds suggest that heterogeneous effects of the observed loci can be explained by different genetic backgrounds in various breed populations selected to achieve different commercial goals. Thus, it is necessary to determine variability and influence of a molecular marker in a specific population when considering its inclusion into a breeding programme.

Keywords: cattle; genetic polymorphism; fertility

Dairy cattle breeding programmes are directed at using genetics for improving economically important traits. Consequently, breeding dairy cows for profitability must consider not only high milk production but also high quality of their functional characteristics (e.g. fertility, health, and functional body conformation). A major focus of dairy cattle genomics is to identify genes underlying the genetic variability of selected economically important traits that could be improved by breeding. Genetic polymorphisms at the diacylglycerol acyltransferase (*DGAT1*), oxidized low-density lipoprotein receptor (*OLR1*), butyrophilin (*BTN1A1*), and signal transducer and activator of transcription 1 (*STAT1*) genes could be related to such traits.

Diacylglycerol acyltransferase (*DGAT1*) gene encodes the DGAT1 enzyme, which catalyzes the final step in triglyceride synthesis (Thaller et al.,

2003a). A non-conservative lysine-to-alanine substitution (p.Lys232Ala) in this gene has been proven to have a major influence on milk production traits, and particularly on milk fat content (Gautier et al., 2007). Polymorphism at the *DGAT1* gene has a strongly significant effect on intramuscular fat content (Thaller et al., 2003b). There is sparse (and contradictory) information available as to the effects of *DGAT1* variants on fertility traits. Kaupe et al. (2007) found that the *K* allele (lysine variant) had a negative effect on maternal non-return rate, but Oikonomou et al. (2009) showed that the *A* allele (alanine variant) increased the number of inseminations per conception and the presence of reproductive problems while decreasing the conception rate in the first 305 days of lactation.

Butyrophilin (*BTN1A1*) is a trans-membrane glycoprotein especially expressed on the apical

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surface of mammary epithelial cells in the final stage of pregnancy and during lactation. *BTN1A1* is also the primary protein in the membrane surrounding fat droplets in milk (Mather, 2000). Recent studies have shown that the functional protein *BTN1A1* is necessary for the proper secretion of milk components, especially fat (Ogg et al., 2004).

Oxidized low-density lipoprotein receptor (OLR1) is the major protein that binds, internalizes, and degrades oxidized low-density lipoprotein (oxLDL) (Khatib et al., 2006). The oxidized lipids have also been found to impair glucose metabolism and influence lipid metabolism in the liver and mammary glands (Liao et al., 2008). As an important protein for oxLDL metabolism, OLR1 may contribute to these effects (Komisarek and Dorynek, 2009).

Signal transducers and activators of transcription (STATs) consist of seven structurally and functionally related latent cytoplasmic transcription factors (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) (Darnell, 1997). The STAT proteins are transcription factors that play important roles in cytokine signalling pathways (Kisseleva et al., 2002) and regulate diverse cellular functions including proliferation, differentiation, apoptosis, and embryonic development (Bromberg, 2001). *STAT1* is involved in the development and differentiation of the mammary gland. Cobanoglu et al. (2006) proved a significant association of a single-nucleotide polymorphism (C/T) in the 3' untranslated region with improved milk yield and composition traits at the position 3141.

The main goals of this study were to investigate the effects of selected genes which participate in fat metabolism on milk production characteristics and reproductive parameters in the dual-purpose Czech Fleckvieh breed and to compare these results with similar findings for pure dairy breeds for which different breeding strategies are pursued.

MATERIAL AND METHODS

Data. Phenotype data available for 419 Czech Fleckvieh (a dual-purpose breed) cows from four commercial herds in the Czech Republic were evaluated. The animals were divided into three groups: Group 1 had 100% Czech Fleckvieh parentage ($n = 115$; 256 observations), Group 2 had 76–99% Czech Fleckvieh parentage ($n = 216$; 458 observations), and Group 3 had 50–75% Czech Fleckvieh parentage ($n = 88$; 170 observations).

The three-generation pedigree file included 2503 animals. All phenotypic data and estimated breeding values for milk production traits used in this study were obtained from the official progeny testing and estimated breeding value databases kept by the Czech-Moravian Breeders' Corporation. The data on reproduction parameters are from the Cattle Breeding Department of the Institute of Animal Science (Prague, Czech Republic). The methodology for estimating breeding values for milk production traits is available at http://plem-dat.cz/cz/pages/Popis_mleko.pdf.

Genotyping. Genomic DNA was extracted from blood using ABI PRISM 6100 Nucleic Acid PrepStation instrument (Applied Biosystems Inc., Foster City, USA) by standard protocol.

To detect genetic polymorphism of the selected candidate genes – *DGAT1* (*K232A*), *STAT1* (*g.3141C>T*), and *BTN1A1* (*K468R*), we used the polymerase chain reaction and restriction fragment length polymorphism methods (PCR-RFLP) according to Winter et al. (2002), Cobanoglu et al. (2006), and Sadr et al. (2008), respectively. A 582 bp fragment containing exon 5 and the 3' untranslated region of the bovine *OLR1* gene (*OLR1_{g.8232A>C}*) was amplified by PCR using a new set of primers designed on the basis of the GenBank bovine sequence (NM_174132.2) and Primer3Plus software, version of 2007: forward 5'-TAT CCT TCA GGG ACC TGT GC-3; reverse 5'-CAG CAA ATG TTG CAA AAA CAA-3'. The PCR was performed in 18 µl reaction mixture consisting of 3 µl genomic DNA (10–100 ng), 0.5 U of Taq DNA polymerase, 200 µM of each dNTP (Top Bio Ltd., Prague, Czech Republic), 0.5 µM of each primer (TIB Molbiol GmbH, Berlin, Germany), 2.5 mM MgCl₂, 1× PCR buffer, and 1× PCR red loading buffer (Top Bio, Ltd., Prague, Czech Republic). Thermal cycling conditions consisted of an initial denaturation step at 95°C for 5 min; followed by 35 cycles of 95°C for 30 s, 53°C for 45 s, and 72°C for 30 s; then a final elongation step at 72°C for 5 min (TGradient 96 Thermocycler; Biometra GmbH, Göttingen, Germany). The fragments of the PCR product (582 bp) were digested by the *PstI* endonuclease (Fermentas, Vilnius, Lithuania) and separated on 2% agarose gel. The *A* allele (uncut) was indicated by a band of 582 bp and the *C* allele (cut) by two bands, 337 and 245 bp (Figure 1). The amplification products and restriction patterns were visualized on agarose gel (PCR-agarose; Top-Bio, Ltd.) in TBE buffer stained with ethidium bromide.

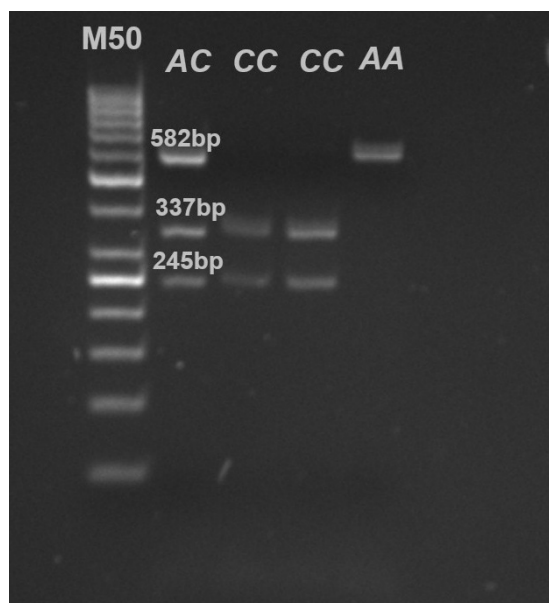


Figure 1. PCR-RFLP patterns of bovine *OLR1* gene

Statistical analyses. PowerMarker software (Version 3.25, 2005) was used to determine allele and genotype frequencies. We used two mixed linear models to determine the effects of selected genes on milk production and reproduction traits. Using the PROC MIXED and PROC INBREED procedures of SAS (Statistical Analysis System, Version 9. 2, 2008), a pedigree matrix having a three-generation structure was generated.

The optimal model was chosen by minimizing the Akaike Information Criterion as well as according to significance of the chosen effects and their reciprocal relationships. Significant effect of an investigated genotype was determined by *F*-test and subsequent possible significance differences between groups with different genotypes were investigated using multiple comparisons.

The following mathematical model was used in the analysis of fertility traits:

$$y_{ijklmno} = \mu + HYS_{ijk} + L_l + P_m + G_n + a_o + e_{ijklmno}$$

where:

$y_{ijklmno}$ = observed trait

μ = estimated mean of the investigated population

HYS_{ijk} = combined fixed effect of herd, year, and season of calving

L_l = fixed effect of lactation number

P_m = fixed effect of breed group

G_n = fixed effect of the investigated gene

a_o = random effect of animal

$e_{ijklmno}$ = random residual effect

The following mathematical model was used in the analysis of milk production traits:

$$y_{ijklmno} = \mu + HYS_{ijk} + L_l + P_m + G_n + \beta * DIM_{ijklmno} + a_o + e_{ijklmno}$$

where:

$y_{ijklmno}$ = observed trait

μ = estimated mean of the investigated population

HYS_{ijk} = combined fixed effect of herd, year, and season of calving

L_l = fixed effect of lactation number

P_m = fixed effect of breed group

G_n = fixed effect of the investigated gene

$DIM_{ijklmno}$ = regression on days in lactation

a_o = random effect of animal

$e_{ijklmno}$ = random residual effect

The following mathematical model was used in the analysis of estimated breeding values for milk production traits:

$$y_{ij} = \mu + G_i + a_j + e_{ij}$$

where:

y_{ij} = breeding value of the observed trait

μ = estimated mean of the investigated population

G_i = fixed effect of the investigated gene

a_j = random effect of animal

e_{ij} = random residual effect

RESULTS AND DISCUSSION

Allele and genotype frequencies of the examined population are presented in Table 1. Results of the association study of *DGAT1*, *BTN1A1*, *OLR1*, and *STAT1* polymorphisms with milk production traits and estimated breeding value of milk production traits are shown in Tables 2 and 3, respectively. Association analyses between the evaluated genes and reproduction parameters are presented in Table 4.

DGAT1. In the present study, only two genotypes – *KK* and *KA* – were observed (Table 1). The allele frequencies in tested Czech Fleckvieh cows were similar to those reported by Thaller et al. (2003a) in German Holsteins ($K = 54.8\%$). A quite contrasting situation seems to exist in various populations of beef cattle breeds or their crosses. For instance, Pannier et al. (2010) observed that frequency of the *K* allele ranged from 0.00 to 0.18 in beef cattle population. Thaller et al. (2003b) observed a similar

Table 1. Allele and genotype frequencies of selected genes in Czech Fleckvieh cattle

Locus	Genotype	Number of animals	Genotype frequency (%)	Allele	Allele frequency (%)
<i>DGAT1</i>	<i>KA</i>	381	91.15	<i>K</i>	54.43
	<i>KK</i>	37	8.85	<i>A</i>	45.57
<i>STAT1</i>	<i>CC</i>	300	71.60	<i>C</i>	84.73
	<i>CT</i>	110	26.25		
	<i>TT</i>	9	2.15	<i>T</i>	15.27
<i>OLRI</i>	<i>AA</i>	11	2.66	<i>A</i>	49.27
	<i>AC</i>	385	93.22		
	<i>CC</i>	17	4.12	<i>C</i>	50.73
<i>BTN1A1</i>	<i>KK</i>	367	87.59	<i>A</i>	93.79
	<i>KR</i>	52	12.41	<i>B</i>	6.21

result, as only 11% frequency of the *K* allele was found in their Charolais population.

In previous studies, the lysine variant (*K* allele) of the *DGAT1* gene was associated with high fat yield while the alanine variant (*A* allele) of that gene was associated with high milk yield (Thaller et al., 2003a; Pareek et al., 2005). In our study, the lysine variant increases the estimated breeding value for protein percentage ($P \leq 0.1$) as well as for fat percentage ($P \leq 0.01$) (Table 3).

Our results indicate that the *DGAT1* polymorphism had a significant effect on four fertility traits. The *KK* genotype was associated with better results for days open, calving interval, days from first to last insemination, and number of inseminations per conception (Table 4). We assume that the *A* allele is associated with a greater number of inseminations, which is comparable with results obtained by Oikonomou et al. (2009). In our study, comparable with the report of Winter

Table 2. Effect of *DGAT1*, *BTN1A1*, *OLRI*, and *STAT1* polymorphism on milk production traits in Czech Fleckvieh cows (Least Squares Means \pm standard errors)

Genotype effects	Traits				
	MY	FP	FY	PP	PY
<i>DGAT1</i>					
<i>KA</i> (381/794) ¹	7815.17 \pm 94.70	3.89 \pm 0.03	300.04 \pm 3.88	3.42 \pm 0.01	264.02 \pm 3.10
<i>KK</i> (37/87)	7750.15 \pm 182.75	3.96 \pm 0.06	302.25 \pm 7.48	3.45 \pm 0.03	263.31 \pm 6.01
<i>BTN1A1</i>					
<i>KK</i> (367/774)	7829.27 \pm 95.60	3.89 \pm 0.03	300.60 \pm 3.92	3.42 \pm 0.01	264.64 \pm 3.14
<i>KR</i> (52/110)	7681.76 \pm 161.37	3.92 \pm 0.05	297.69 \pm 6.61	3.44 \pm 0.02	259.66 \pm 5.30
<i>OLRI</i>					
<i>AA</i> (11/25)	7479.22 \pm 314.16	3.89 \pm 0.09	288.42 \pm 12.86	3.44 \pm 0.04	255.11 \pm 10.33
<i>AC</i> (385/809)	7825.36 \pm 95.70	3.89 \pm 0.03	300.77 \pm 3.92	3.42 \pm 0.01	264.50 \pm 3.14
<i>CC</i> (17/40)	7579.52 \pm 256.62	3.99 \pm 0.08	297.03 \pm 10.49	3.43 \pm 0.04	256.65 \pm 8.46
<i>STAT1</i>					
<i>CC</i> (300/644)	7793.12 \pm 97.17	3.89 \pm 0.03	299.34 \pm 3.98	3.41 \pm 0.01 ^B	263.15 \pm 3.19
<i>CT</i> (110/221)	7840.13 \pm 133.33	3.92 \pm 0.04	303.11 \pm 5.46	3.46 \pm 0.02 ^B	266.20 \pm 4.38
<i>TT</i> (9/19)	7981.29 \pm 345.80	3.80 \pm 0.10	301.18 \pm 14.15	3.40 \pm 0.05	268.10 \pm 11.36

MY = milk yield, FP = fat percentage, FY = fat yield, PP = protein percentage, PY = protein yield

^B $P \leq 0.05$

¹ n of animals/ n of observations

Table 3. Effect of *DGATI*, *BTN1A1*, *OLRI*, and *STAT1* polymorphism on estimated breeding value (EBV) of milk production traits in Czech Fleckvieh cows (Least Squares Means \pm standard errors)

Genotype effects	Traits				
	EBV MY	EBV PY	EBV FY	EBV PP	EBV FP
<i>DGATI</i>					
<i>KA</i> (381/794) ¹	176.57 \pm 20.62	5.49 \pm 0.62	6.16 \pm 0.83	-0.01 \pm 0.004 ^C	-0.03 \pm 0.008 ^A
<i>KK</i> (37/87)	111.25 \pm 58.80	5.00 \pm 1.83	7.51 \pm 2.44	0.01 \pm 0.01 ^C	0.04 \pm 0.02 ^A
<i>BTN1A1</i>					
<i>KK</i> (367/774)	160.23 \pm 21.14	4.92 \pm 0.63 ^A	5.53 \pm 0.85 ^A	-0.01 \pm 0.005 ^C	-0.03 \pm 0.008
<i>KR</i> (52/110)	242.98 \pm 56.39	9.04 \pm 1.69 ^A	11.27 \pm 2.26 ^A	0.01 \pm 0.01 ^C	0.003 \pm 0.02
<i>OLRI</i>					
<i>AA</i> (11/25)	172.97 \pm 95.47	7.59 \pm 3.10	7.03 \pm 4.13	0.02 \pm 0.02	-0.00008 \pm 0.04
<i>AC</i> (385/809)	172.97 \pm 20.58	5.45 \pm 0.62	6.28 \pm 0.83	-0.01 \pm 0.004	-0.03 \pm 0.008
<i>CC</i> (17/40)	148.92 \pm 95.95	4.98 \pm 2.89	6.21 \pm 3.88	-0.003 \pm 0.02	-0.06 \pm 0.04
<i>STAT1</i>					
<i>CC</i> (300/644)	191.08 \pm 23.35	5.79 \pm 0.70	6.25 \pm 0.94	-0.02 \pm 0.005 ^B	-0.04 \pm 0.009 ^A
<i>CT</i> (110/221)	112.75 \pm 38.95	4.42 \pm 1.17	6.27 \pm 1.58	-0.006 \pm 0.03 ^B	0.02 \pm 0.01 ^A
<i>TT</i> (9/19)	172.87 \pm 128.61	5.25 \pm 3.87	5.47 \pm 5.20	-0.01 \pm 0.03	-0.04 \pm 0.05

MY = milk yield, PY = protein yield, FY = fat yield, PP = protein percentage, FP = fat percentage

^A $P \leq 0.01$, ^B $P \leq 0.05$, ^C $P \leq 0.1$

¹ n of animals/ n of observations

et al. (2002), the lysine allele (*K*) had a positive effect on reproductive performance as well as on the breeding value for protein percentage and fat percentage. The same tendency has been shown to hold for intramuscular fat content. Based on the results of this study and the literature, the lysine allele seems to be the more efficient version of the enzyme with regard to triglyceride synthesis (Thaller et al., 2003b). The frequency of alanine allele (*A*) is noticeably decreasing in the Czech Fleckvieh cows, and we believe that it is related to the selection of animals with better fertility traits and higher daily gain. The *DGATI* gene appears to be a strong candidate gene for determining milk production and reproductive traits.

OLRI. Several quantitative trait loci (QTL) related to milk production parameters were mapped on the bovine chromosome 5, near the location of the *OLRI* gene (de Koning et al., 2001; Olsen et al., 2002). Khatib et al. (2006) identified an association of a single-nucleotide polymorphism at the 3' untranslated region of *OLRI* (*OLRI*_{g.8232A>C}) with milk fat percentage and milk fat yield in a population of the North American Holstein cattle. The most frequent genotype was *AC* in the dairy cattle population that we observed (Table 1). Wang

et al. (2012) had recorded a similar genotype frequency in Israeli population of Holsteins. Genotype frequencies were 13, 58, and 29% for *AA*, *AC*, *CC*, respectively, and the allele frequency for *C* was 58%. Komisarek and Dorynek (2009) also had confirmed the most abundant heterozygous variant to be *AC* (51.66%) and the frequency of the *C* allele to be 57% in the Polish population of Holsteins. In other studies high representation of the *C* allele has been confirmed, too. For example, in the Dutch Holstein population, the *C* allele frequency was found to be 71%, although the most prevalent genotype was *CC* (Schennink et al., 2009). Khatib et al. (2007) even observed allele frequency of *C* = 95% in the Italian Brown Swiss population.

In the present study, no association was observed in the Czech Fleckvieh population between polymorphism in the *OLRI* gene and either milk production traits or reproduction traits (Tables 2–4).

STAT1. In *STAT1*, the minor allele *T* was observed at a frequency of 15.27%. The frequency of the rare genotype *TT* was only 2.15%. These results are comparable with those of previous studies in dairy cattle. The frequency of the genotype *TT* in Holsteins has been reported in the range of 7.92–10.63% (Cobanoglu et al., 2006; Khatib et al., 2009).

Table 4. Effect of polymorphism of *DGATI*, *BTN1A1*, *OLRI*, and *STAT1* genes on reproduction parameters in Czech Fleckvieh population (Least Squares Means \pm standard errors)

Genotype effects	Traits				
	CF	DO	CI	FL	NI
<i>DGATI</i>					
<i>KA</i> (356/583) ¹	69.73 \pm 1.42	104.72 \pm 4.00 ^B	392.73 \pm 4.03 ^B	34.98 \pm 3.80 ^B	1.76 \pm 0.09 ^C
<i>KK</i> (34/60)	70.94 \pm 2.43	90.90 \pm 6.85 ^B	377.63 \pm 6.91 ^B	19.95 \pm 6.51 ^B	1.48 \pm 0.16 ^C
<i>BTN1A1</i>					
<i>KK</i> (343/570)	69.49 \pm 1.41	102.23 \pm 4.02 ^C	390.05 \pm 4.06 ^C	32.88 \pm 3.85	1.73 \pm 0.09
<i>KR</i> (48/76)	72.22 \pm 2.24	111.80 \pm 6.38 ^C	399.94 \pm 6.45 ^C	39.72 \pm 6.12	1.82 \pm 0.15
<i>OLRI</i>					
<i>AA</i> (11/20)	69.64 \pm 3.88	99.94 \pm 11.00	386.60 \pm 11.13	31.30 \pm 10.51	1.55 \pm 0.25
<i>AC</i> (358/586)	69.74 \pm 1.42	103.17 \pm 4.01	391.06 \pm 4.06	33.51 \pm 3.83	1.75 \pm 0.09
<i>CC</i> (17/33)	71.89 \pm 3.14	99.73 \pm 8.92	388.32 \pm 9.03	27.90 \pm 8.52	1.54 \pm 0.21
<i>STAT1</i>					
<i>CC</i> (279/474)	70.24 \pm 1.41	104.00 \pm 4.02	391.81 \pm 4.07	33.91 \pm 3.86	1.74 \pm 0.09
<i>CT</i> (103/159)	68.08 \pm 1.92	100.29 \pm 5.47	388.64 \pm 5.53	32.48 \pm 5.25	1.76 \pm 0.13
<i>TT</i> (9/13)	66.95 \pm 4.82	103.71 \pm 13.69	390.64 \pm 13.82	36.98 \pm 13.09	1.83 \pm 0.32

CF = days from calving to first insemination, DO = days open, CI = calving interval, FL = days from first to last insemination, NI = number of inseminations

^A $P \leq 0.01$, ^B $P \leq 0.05$, ^C $P \leq 0.1$

¹ n of animals/ n of observations

Whole-genome scans have shown significant associations between production traits and microsatellite markers in the vicinity of *STAT1* (Mosig et al., 2001; Ashwell et al., 2004; Ron et al., 2004). These observed associations have prompted Cobanoglu et al. (2006) to test the effect of the *STAT1* gene on milk production traits in the U.S. Holstein population. They confirmed that genetic variants of the *STAT1* gene (polymorphism *C/T* at position 3141) affect milk fat content and milk protein content at the same time. Allele *C* was associated with higher content of milk protein and milk fat in the population of the Holstein cattle. Our results describe significant differences between genotypes *CC* and *CT* for protein percentage (Table 2). Significant differences ($P \leq 0.05$) were also observed between *CC* and *CT* genotypes in estimated breeding value for protein percentage (EBV_PP; $P \leq 0.05$) and fat percentage (EBV_FP; $P \leq 0.01$) (Table 3). In our study, cattle carrying the *CT* genotype produced milk with the highest protein content. Individuals with the *TT* genotype showed the lowest values for fat percentage (FP) and protein percentage (PP), but these differences were not statistically significant.

Effects of *STAT1* on reproductive traits are shown in Table 4. In this study, no association was observed in the Czech Fleckvieh population between polymorphism in the *STAT1* gene and reproduction traits.

BTN1A1. The bovine *BTN1A1* gene has been mapped to chromosome 23 (Taylor et al., 1996). In the same genome region, several QTL for health and production traits have been identified (Ashwell et al., 1997; Bennewitz et al., 2004). Butyrophilin might serve as a possible candidate influencing the variation in the detected QTL. Komisarek et al. (2006) pointed out that the *K468R* polymorphism at the *BTN1A1* gene is possibly a causal mutation that affected properties of dairy cattle milk production traits.

In the present study, only two genotypes – *KK* and *KR* – were observed (allele *K* coding for lysine, allele *R* coding for arginine). The frequencies of the minor allele *R* and the rare genotype *KR* of the *BTN1A1* polymorphism were 6.21 and 12.41%, respectively. The allele frequencies in our Czech Fleckvieh cows were close to the results observed by Komisarek and Dorynek (2003) in Holstein cattle (12%) and Sadr et al. (2008) in the Najdi cattle population (14%).

Our association tests of the *K468R* polymorphism in the Czech Fleckvieh cows revealed a positive effect of the *KR* genotype on estimated breeding values for protein yield (EBV_PY), fat yield (EBV_FY), but also protein percentage (EBV_PP). Those association analyses performed to date have produced uncertain results. Zegeye et al. (1999) did not reveal a relationship between *K468R* polymorphism and milk production in five grand-sire Holstein families. By contrast, Komisarek et al. (2006) recorded that Jerseys with the *KK* genotype were characterized by higher milk, fat, and protein yields than cows with two other genotypes. The association was not, however, confirmed by within-family analysis. From previously published findings, but also from the results obtained in this study, it is therefore apparent that the *BTN1A1* gene polymorphism cannot be unambiguously ruled out or confirmed as a source of variability in milk production parameters.

Our results indicate that the *BTN1A1* polymorphism had a tendency ($P \leq 0.1$) to achieve significance level in two fertility traits. The *KK* genotype was associated with better results for the traits days open and calving interval. Further investigations, conducted on larger populations, are therefore needed to verify the effect of butyrophilin in cattle.

CONCLUSION

In this study, allele and genotype frequencies of selected genes were typed. Also, the influence of polymorphism of selected genes on milk production and reproductive performance in the tested population of dual-purpose Czech Fleckvieh cattle was demonstrated. The so far results have shown heterogeneous effects of the observed loci in cattle populations, which can be explained by the different genetic backgrounds in the chosen populations, as these have been bred with varying intensity and for achieving different commercial goals. Therefore, it is necessary to determine the variability and influence of the molecular markers in a specific cattle population when considering its inclusion into the breeding programmes. The loci *DGAT1* and *BTN1A1* simultaneously affect milk production traits, estimated breeding values for milk production traits, as well as reproduction parameters. Significant differences were found also between genotypes of the *STAT1* loci and the estimated breeding values for milk production

traits. We consider these results to be useful and applicable in breeding processes for improving economic and functional traits. Further analyses are needed to confirm these findings prior to their widespread application.

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