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Association between *CSN3* and *BCO2* gene polymorphisms and milk performance traits in the Czech Fleckvieh cattle breed

P. Bartonova, I. Vrtkova, K. Kaplanova and T. Urban

Department of Animal Morphology, Physiology and Genetics,
Mendel University, Brno, Czech Republic

Corresponding author: P. Bartonova
E-mail: s.petra@volny.cz

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ABSTRACT. Daily milk, fat and protein yield and amount of somatic cells in cow milk are very important factors that influence milk performance traits. An association between polymorphisms in the kappa casein (*CSN3*) gene and milk production, composition and technical properties has been previously reported; however, this type of information is not available for the bovine β-carotene oxygenase 2 (*BCO2*) gene - the *BCO2* gene has relationship with milk color and meat fat color, which is dependent on content of β-carotene. We analyzed these two genes and their relationship with milk performance traits (daily milk, fat and protein yield, somatic cell count, SCC) in one cattle population, Czech Fleckvieh (N = 152). All animals were milked twice a day and kept in the same environmental conditions. The Fleckvieh is a typical Czech cattle breed farming for milk and meat production. It is the most common breed in the Czech Republic. DNA was isolated from milk or from hairs. Genes were analyzed using PCR-RFLP, frequencies of alleles and genotypes were calculated and association analysis was performed using a GLM Procedure in SAS. Statistical analysis established that the *CSN3* gene has no statistically significant influence on daily milk, fat and protein yield and SCC. Compared to other references this result can

be explained by, e.g., small group of animals and different cattle breed. The *BCO2* gene (genotypes *AA* and *AG*) shows a statistically significant relationship (P = 0.05) with daily milk, protein yield and SCC.

Key words: Milk performance traits; *CSN3*; *BCO2*

INTRODUCTION

Cattle raising for milk is the most time-consuming and difficult part of animal husbandry, especially because of long doubling time and investment costliness. Since 2004, when the Czech Republic became a member of European Union, the number of milk producers decreased by about 20% and no new producers registered (Motycka, 2010). Milk is a very important part of human nourishment. It is a source of many proteins, vitamins and minerals. Milk properties are very important for processing of milk, and that is why the kappa-casein (*CSN3*) gene was chosen for this analysis. The second gene, β,β-carotene-9',10'-dioxygenase; β-carotene oxygenase 2 (*BCO2*), has an influence on milk color, which is an important esthetic property of milk.

The *CSN3* gene is located on BTA6. *CSN3* is a gene that codes for the protein influencing the amount, composition and technical properties of milk. Grosclaude et al. (1972) described two main allelic variants of this gene, allele *A* and allele *B*. Allele *E* origins in allele *A*. Polymorphism in the *CSN3* gene has an influence on production traits. Allele *E* has a negative effect on technological milk quality (Futerova, 1997). According to Hamza et al. (2010), polymorphism of the *CSN3* gene does not have any significant effect on milk yield and reproductive traits.

The *BCO2* gene plays an important role in cleavage of β-carotene, and that is why it can control the color of cow's milk and meat. It is located on BTA15 (Tian et al., 2010). The *BCO2* gene is described in two allelic variants - allele *A* and allele *G*. Allele *A* is associated with higher concentration of β-carotene in bovine milk (Berry et al., 2009; Tian et al., 2010). No data about an association between the *BCO2* gene polymorphism and milk performance traits have been published.

The aim of this study was to detect genotypes of the *CSN3* gene and the *BCO2* gene and to associate polymorphic variants with chosen milk performance traits. All analysis was done in one cattle population, Czech Fleckvieh.

MATERIAL AND METHODS

Animals

The study included a herd of 152 Czech Fleckvieh dairy cows. All animals were milked twice a day and kept in the same environmental conditions. The data concerning daily milk (kg), milk fat yield (%), milk protein yield (%), and SCC (somatic cell count; thousand/mL) were collected in May 2010 on the basis of monthly milking tests.

DNA analysis

DNA was isolated from milk or from hairs using the JetQuick blood and cell culture

DNA spin kit (GenomedSM, USA) or the QIAamp DNA Extraction kit (QIAGEN, Germany) for milk, and the JetQuick tissue DNA spin kit (GenomedSM) for hair bulbs according to the attached protocol.

Isolated DNA was used for polymerase chain reaction (PCR) amplification. Primers are shown in Table 1, and the length of PCR fragments and annealing temperatures are shown in Table 2. PCR for the *CSN3* gene detection was performed according to Soria et al. (2003) and for the *BCO2* gene according to Tian et al. (2010). Amplification of genomic DNA was performed in a Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA).

Table 1. Primers used for polymerase chain reaction.

Gene	Primers
CSN3	Forward: (5'-CAC GTC ACC CAC ACC CAC ATT TAT C-3') Reverse: (5'-TAA TTA GCC CAT TTC GCC TTC TCT GT-3')
BCO2	Forward: (5'-AAC CCA TCC CAC TTC CTT ATC T-3') Reverse: (5'-GCT GAA ATC AAA CCC CAA AG-3')

Restriction analysis was done by restriction fragment length polymorphism (RFLP) using the enzymes shown in Table 2. The products were visualized on a 4% agarose gel (TBE buffer, 100 V).

Polymorphism of the *BCO2* gene was determined using direct PCR. This method is PCR without DNA isolation; it means that hair bulbs were added directly to the PCR master mix. The Phire Animal Tissue Direct PCR kit (Finnzymes, Finland) was used and PCR fragments were amplified according to the attached protocol in a Piko Thermal Cycler (Finnzymes). Annealing temperature was 60°C. RFLP was done using the restriction enzymes and restriction temperatures mentioned in Table 2. Restriction enzyme was added directly to amplified PCR fragments, and the products were visualized on a 4% agarose gel (TBE buffer, 100V).

Table 2. Length of polymerase chain reaction (PCR) fragments and annealing temperatures and restriction enzymes and temperature required.

Gene	PCR fragment (bp)	Annealing temperature (°C)	Restriction enzyme	Restriction temperature (°C)
CSN3	379	60	*Hin*fI (Fermentas, Germany) A/B *Hae*II (Fermentas, Germany) A/E	37
BCO2	525	60	*Bsr*I (BioLabs, USA)	65

Polymorphisms of the *CSN3* gene and the *BCO2* gene were found in Czech Fleckvieh. Alleles detected by PCR and their sizes are shown in Table 3.

Table 3. Alleles detected by polymerase chain reaction and their sizes.

Gene	Alleles	Size of alleles (bp)
CSN3	*A*	156, 132, 91
	B	288, 91
	E	201, 145, 33
BCO2	*A*	391, 134
	G	391, 113, 21

Statistical analysis

The frequencies of alleles and genotypes were determined. Genotype deviation from Hardy-Weinberg equilibrium was evaluated by the exact chi-square test (R version 2.12.1). The statistical analysis was also performed to determine the association between gene polymorphisms (*CSN3*, *BCO2*) and daily milk, milk fat yield, milk protein yield, and SCC. SCC was transformed to a logarithmic scale (log2) in order to balance the distribution.

Association analysis was performed using a general linear model (GLM Procedure) in SAS for Windows 9.1.4 using the following equation:

$$y_{ijkl} = \mu + CSN3_i + BCO2_j + lactation_k + e_{ijkl}$$

where y_{ijkl} = the phenotypic value of the trait analyzed; μ = the population mean; $CSN3_i$ = the fixed effect of the i^{th} genotype of the *CSN3* gene; $BCO2_j$ = the fixed effect of the j^{th} genotype of the *BCO2* gene; $lactation_k$ = the fixed effect of the k^{th} lactation; e_{ijkl} = random error effect of each observation.

RESULTS

The frequencies of alleles and genotypes were calculated for the Czech Fleckvieh cattle population (Table 4). In the *CSN3* gene, substantial differences were found in allele and genotype frequencies. In the *BCO2* gene polymorphism, frequencies of alleles were almost equal. All polymorphisms were in Hardy-Weinberg equilibrium.

Table 4. Frequencies of alleles and genotypes.

Gene	Allele	Allele frequency	Genotype (N)	Genotype frequency
CSN3	*A*	0.552	*AA* (45)	0.296
	B	0.418	*AB* (74)	0.487
	E	0.030	*AE* (4)	0.026
			BB (24)	0.158
			EB (5)	0.033
BCO2	*A*	0.563	*AA* (42)	0.277
	G	0.437	*AG* (87)	0.572
			GG (23)	0.151

The associations between genotypes of the *CSN3* gene and the *BCO2* gene and daily milk, fat and protein yield and SCC were studied. An association between the *CSN3* gene and daily milk was significant. Association analysis performed with the *CSN3* gene revealed that there was a highly significant difference between genotypes *AA* and *AE*, *AB* and *AE*, and *AE* and *BB*, and a significant difference was seen between genotypes *AE* and *EB* regarding daily milk. No relationship was found between the *CSN3* gene and fat and protein yield. The difference between genotypes *AA* and *AB* was statistically significant regarding log SCC (Table 5). The relationship between the *BCO2* gene and daily milk was statistically significant. Association analysis performed with *BCO2* revealed that there was a highly significant difference between genotypes *AA* and *GG*, *AG* and *GG*, and *AA* and *AG* regarding daily milk. There was no significant difference regarding milk fat yield. There was a statistically significant association

between the *BCO2* gene and protein yield and log SCC. In regard to protein yield, a significant difference was seen between *AA* and *GG* and *AG* and *GG*. A highly significant difference was found between *AA* and *GG* and *AG* and *GG* regarding log SCC (Table 5).

Table 5. Association between polymorphisms of genes and milk performance traits.

Gene	Genotype	Daily milk (kg)	Milk fat yield (%)	Protein yield (%)	Log SCC
CSN3	*AA*	22.68 ± 2.03^{A}	4.06 ± 0.14	3.45 ± 0.06	5.02 ± 0.51^{a}
	AB	23.33 ± 1.87^{B}	4.09 ± 0.13	3.53 ± 0.05	5.95 ± 0.47^{a}
	AE	$36.21 \pm 4.52^{A,B,C,a}$	3.85 ± 0.31	3.32 ± 0.13	4.71 ± 1.13
	BB	22.92 ± 2.38^{C}	4.10 ± 0.16	3.47 ± 0.07	5.67 ± 0.60
	EB	23.90 ± 4.08^{a}	4.22 ± 0.28	3.41 ± 0.12	6.00 ± 1.02
BCO2	*AA*	$19.72 \pm 2.39^{A,B}$	4.16 ± 0.17	3.51 ± 0.07^{a}	6.37 ± 0.60^{A}
	AG	$24.35 \pm 2.07^{A,C}$	4.09 ± 0.14	3.46 ± 0.06^{b}	5.95 ± 0.52^{B}
	GG	$33.34 \pm 2.68^{B,C}$	3.95 ± 0.19	$3.33 \pm 0.08^{a,b}$	$4.09 \pm 0.67^{A,B}$

Data are reported as least squares mean ± standard error. Numbers followed by the same superscript letters indicate significant differences between the genotypes: ^{a} = significant difference ($P \leq 0.05$); $^{A, B, C}$ = highly significant difference ($P \leq 0.01$). SCC = somatic cell count.

DISCUSSION

In the *CSN3* gene, alleles *A*, *B* and *E* were also detected by Matejickova et al. (2010) in Czech Fleckvieh. Frequencies of alleles *A*, *B* and *E* were in agreement with results of Czech Fleckvieh reported by Kucerova et al. (2006) and Manga et al. (2006). Frequencies of genotypes *AA*, *AB*, *AE*, *EB*, and *BB* were in agreement with the results of Kucerova et al. (2006). Matejickova et al. (2010) reported that the *CSN3* gene has a main influence on protein content in milk of Czech Fleckvieh. In this research, no relationship between *CSN3* and fat and protein yield was found. These results are in agreement with Neubauerová (2001).

According to Tian et al. (2010) and Berry et al. (2009), the *BCO2* gene has an effect on milk and meat fat color in cattle, but there was no mention about allele and genotype frequencies and about a relationship between gene polymorphism and performance traits of milk. According to our results, the *BCO2* gene has an influence on milk yield, protein content and SCC. No association was found between the *BCO2* gene and milk fat content.

According to this study, the *CSN3* gene and the *BCO2* gene can be used as genetic markers of daily milk and SCC but not of milk fat content. Additional studies on this problem are necessary to confirm these associations before this criterion is used in large-scale selection.

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REFERENCES

Berry SD, Davis SR, Beattie EM, Thomas NL, et al. (2009). Mutation in bovine beta-carotene oxygenase 2 affects milk

color. *Genetics* 182: 923-926.
Futerova J (1997). Genetic polymorphism of kappa-casein gene and beta-lactoglobulin gene in relation to production characteristics of cattle (in Czech). Doctoral thesis, CZU, Praha.
Grosclaude F, Mahé MF, Mercier JC and Ribadeau-Dumas B (1972). Localisation des substitutions d´acides amines differenciant les variants a et b de la caseine x bovine. *Genet. Sel. Evol.* 4: 515-521.
Hamza AE, Yang ZP, Wang XL, Chen RJ, et al. (2010). Kappa casein gene polymorphism and its impact on milk yield and reproductive performance traits of Chinese Holstein cattle. *Agric. J.* 5: 283-285.
Kucerova J, Matejicek A, Jandurova OM, Soerensen P, et al. (2006). Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parametres in Czech Fleckvieh. *Czech J. Anim. Sci.* 51: 241-247.
Manga I, Riha J and Dvorak J (2006). Comparison of influence markers CSN3 and CSN2 on milk performance traits in Czech Spotted and Holstein cattle tested at first, fifth and higher lactation. *Acta Fytotech.* 13: 13-15.
Matejickova J, Štípková M, Kyselová J, Rychtárová J, et al. (2010). Milk protein genes and quality of milk of Czech Spotted Cattle (in Czech). *Náš chov*. 2: 72-74.
Motycka J (2010). Utility and efficiency of milk production. Svaz chovatelu holstynskeho skotu CR (in Czech). Available at [www.holstein.cz]. Accessed January 20, 2011.
Neubauerová V (2001). Detection of genetic markers and possibility of its use in cattle and another ungulate (in Czech). Doctoral thesis, ZF JU, Ceske Budejovice.
Soria LA, Iglesias GM, Huguet MJ and Mirande SL (2003). A PCR-RFLP test to detect allelic variants of the bovine kappa-casein gene. *Anim. Biotechnol.* 14: 1-5.
Tian R, Pitchford WS, Morris CA, Cullen NG, et al. (2010). Genetic variation in the beta, beta-carotene-9', 10'-dioxygenase gene and association with fat colour in bovine adipose tissue and milk. *Anim. Genet.* 41: 253-259.