

Joint effects of *CSN3* and *LGB* genotypes and their relation to breeding values of milk production parameters in Czech Fleckvieh

A. MATĚJÍČEK¹, J. MATĚJÍČKOVÁ², E. NĚMCOVÁ², O.M. JANDUROVÁ³,
M. ŠTÍPKOVÁ², J. BOUŠKA², J. FRELICH¹

¹University of South Bohemia, Ceske Budejovice, Czech Republic

²Institute of Animal Science, Prague-Uhrineves, Czech Republic

³Crop Research Institute, Prague, Czech Republic

ABSTRACT: The objective of this study was to estimate the joint effects of *CSN3* and *LGB* genotypes on breeding values of milk production parameters. *CSN3* (kappa-casein) and *LGB* (beta-lactoglobulin) genotypes of 120 Czech Fleckvieh sires were detected using the PCR-RFLP method. Breeding values of sires were obtained from the Official Database of Progeny Testing. Ten genotype combinations were detected. Genotypes *ABAB* (25.0%), *ABAA* (13.3%) and *ABBB* (13.3%) were the most frequent. Significant effects of genotype combinations on breeding values for fat and protein content were found. The highest breeding values for milk (+621 kg) and protein (+15.8 kg) yields were associated with genotype combination *ABAA*, while the highest breeding values for content parameters (+0.15% for protein content and +0.55% for fat content) were associated with genotype combination *BBAB*.

Keywords: cattle; kappa-casein; beta-lactoglobulin; milk production parameters; breeding values

Kappa-casein (*CSN3*) and beta-lactoglobulin (*LGB*) loci affect the milk production parameters and quality of milk protein. Their polymorphisms explain a part of the genetic variance and improve the estimation of breeding value. Such loci can be taken into account as a suitable supplement to conventional breeding procedures (Příbyl, 1995).

The kappa-casein gene is situated on bovine chromosome 6 and encodes milk protein important for the structure and stability of casein micelles (Alexander et al., 1988). Polymorphism of the kappa-casein gene has been known since 1964 (Neelin, 1964), and nine alleles are known in all. The most common alleles are *A*, *B* and *E* (Neubauerová, 2001). Allele *A* is associated with higher milk yield but lower protein content, while allele *B* is linked with higher protein content (Neubauerová, 2001) and higher milk quality (Strzalkowska et al., 2002)

but lower milk yield (Boettcher et al., 2004; Caroli et al., 2004). The negative effect of allele *E* on milk protein quality was reported by Ikonen et al. (1997).

The beta-lactoglobulin (*LGB*) gene is situated on bovine chromosome 11 and encodes the main protein of whey (Eggen and Fries, 1995). Polymorphism of this gene was discovered in 1955 (Aschaffenburg and Drewry, 1955), and a total of 15 alleles are known. Common alleles are *A*, *B*, *C* and *D*, with alleles *A* and *B* being the most frequent (Panicke et al., 1996). The *LGB* locus affects mainly milk composition and milk quality and especially *B* allele was recognized as superior for milk quality in European cattle breeds (Strzalkowska et al., 2002). Allele *A* is associated rather with yield parameters (Neubauerová, 2001).

Supported by the Ministry of Agriculture of the Czech Republic (Project MZe No. 0002701402) and the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6007665806).

According to Strzalkowska et al. (2002) there are significant interactions between *CSN3* and *LGB* loci, therefore their effects should not be investigated separately but as joint effect. Some studies on milk protein genes and their relation to milk production parameters and milk protein quality focused on single marker effects (Ojala et al., 1997; Ng-Kwai-Hang, 1998; Ikonen et al., 1999; Choi and Ng-Kwai-Hang, 2002). Other studies focused on the effects of haplotypes (Ikonen et al., 2001; Boettcher et al., 2004), or effects of sire genotypes (Ron et al., 1994; Sabour et al., 1996; Kaminski et al., 2002; Kučerová et al., 2006).

The objective of this study was to determine genotype frequencies of *CSN3* and *LGB* loci and find the joint effects of their genotypes on milk production parameters expressed by the breeding values of sires. The results will help to explain the role of the joint effect of *CSN3* and *LGB* genotypes in the Czech Fleckvieh breed.

MATERIAL AND METHODS

Genotypes of kappa-casein (*CSN3*) and beta-lactoglobulin (*LGB*) loci were detected in 120 Czech Fleckvieh sires using a Polymerase Chain Reaction and Restriction Fragment Length Polymorphism method (PCR-RFLP) and electrophoresis on agarose gel. DNA was obtained from the semen of sires (Ashwell et al., 1996).

Detection of *CSN3* alleles *A*, *B* and *E* was carried out according to Lien and Rogne (1993). *LGB* alleles *A* and *B* were identified according to Agrawala et al. (1992).

The PCR conditions for *CSN3* were 1 min. at 95°C, followed by 35 cycles of 30 s at 95°C, 25 s at 62°C, 30 s at 72°C, and for *LGB* 3 min. at 95°C, followed by 40 cycles of 60 s at 95°C, 30 s at 60°C, and 30 s at 72°C.

Breeding values of observed sires were obtained from the Official Database of Progeny Testing.

First, the frequencies of *CSN3* and *LGB* genotypes were calculated. In the second step, statistical analysis was carried out by means of the programme package SAS using GLM and the following model equation:

$$y_{ijklm} = \mu + G_i + b_1 R_j + b_2 P_k + G_i(O_l) + e_{ijklm}$$

where:

y = the breeding value of the sire

μ = the average of observed breeding value

G = the joint effect of *CSN3* and *LGB* genotypes

bR = the linear regression on the year of sire birth

bP = the linear regression on the proportion of the Czech Fleckvieh breed in sires

$G(O)$ = the effect of grandsire with the nested effect of *CSN3* and *LGB*

e = the set of residual effects

RESULTS AND DISCUSSION

The observed sires improved only the average of breeding values for yield parameters (Table 1). The average breeding values for milk and protein yields were +127 kg and +13 kg, respectively.

A total of 10 genotype combinations of *CSN3* and *LGB* genotypes were found in the observed sires (Table 2). The most frequent genotype combination was *ABAB* (25.0%). The frequency of combination *BBBB*, which is reported to increase breeding values for protein and fat contents (Kučerová et al., 2006), was detected only in 4.2%. On the other hand, the frequency of combination *AAAA*, which is reported to increase breeding values for milk and protein yields (Kaminski et al., 2002), was found in 8.3%. Equal frequencies were detected in genotypes *ABAA* and *ABBB* (13.3%) and genotypes *AAAB* and *BBAB* (10.8%).

Significant associations between genotype combinations *CSN3* and *LGB* and breeding values for fat and protein contents were found (Table 3). The highest breeding values for milk and protein yields

Table 1. Basic statistics of observed sires ($n = 120$)

Breeding value for	\bar{x}	$s_{\bar{x}}$	Min.	Max.
Milk yield (kg)	+127	423	−1 162	+1 164
Protein content (%)	+0.00	0.12	−0.45	+0.28
Protein yield (kg)	+13	15	−42	+45
Fat content (%)	−0.05	0.19	−0.55	+0.50
Fat yield (kg)	+2	18	−55	+43

Table 2. Genotype frequencies in the observed sires

<i>CSN3</i> + <i>LGB</i> genotypes	<i>n</i>	Frequency (%)
AAAA	10	8.3
AAAB	13	10.8
AABB	5	4.2
ABAA	16	13.3
ABAB	30	25
ABBB	16	13.3
BBAA	8	6.7
BBAB	13	10.8
BBBB	5	4.2
BEBB	4	3.4

Table 3. The relation between genotype combinations of *CSN3* and *LGB* loci and breeding values of milk production parameters

<i>CSN3</i> + <i>LGB</i> genotypes	<i>n</i>	Average breeding values for				
		milk yield (kg)	protein yield (kg)	protein content (%)	fat yield (kg)	fat content (%)
AAAA	10	+313	+15.2	−0.08 ^{ab}	+14.1	+0.04 ^{af}
AAAB	13	+400	+16.7	−0.13 ^c	−6.8	−0.41 ^{bd}
AABB	5	+229	+12.1	−0.08 ^d	+12.9	+0.08 ^c
ABAA	16	+621	+15.8	−0.25	−36.3	−1.20 ^f
ABAB	30	+24	+10.1	+0.03 ^{ac}	+2.7	+0.04 ^d
ABBB	16	+361	+14.5	−0.11	−22.0	−0.72 ^e
BBAA	8	+427	+7.6	−0.27	−51.9	−1.34 ^a
BBAB	13	−317	+4.7	+0.15 ^{bd}	+14.9	+0.55 ^{bd}
BBBB	5	+533	+15.1	−0.27	−63.9	−1.67 ^c
BEBB	4	+144	+8.7	−0.06	−27.4	−0.66

a, b, c, d, e or f means the significant difference $P < 0.05$ between two *CSN3* + *LGB* genotypes marked with the identical character within one parameter

were associated with genotype combination *ABAA*, while Kaminski et al. (2002) reported the highest breeding values for milk and protein yields in relation to genotype combination *AAAA*. A similar tendency was found in genotype combination *BBBB*, which is not fully consistent with the results of Kučerová et al. (2006). The authors reported the association of *B* alleles with higher breeding values for content parameters, especially for protein content. Our result could be due to only 5 sires carrying the *BBBB* genotype combination, which is insufficient for drawing reliable conclusions. On the other hand, the highest breeding values for content parameters were associated with the similar genotype combination *BBAB*. Compared to our findings, Kaminski et al. (2002) found the

highest breeding values for content parameters associated with genotype combinations *ABAB* and *ABBB*. Positive breeding values of all observed parameters were linked only with genotype combination *ABAB*. Contrary to our results, Neubauerová (2001) did not find any significant association between *CSN3* and *LGB* genotypes and breeding values of milk production parameters.

In comparison with the results of previous studies focused on the separate testing of *CSN3* and *LGB* genotypes, the results of the present study refer to the advantage of comparing the effects of all genotype combinations due to separating the animals with identical genotype of one locus (e.g. *BB*) according to different genotypes of the second locus (e.g., *BBAA*, *BBAB* and *BBBB*). This finding

is consistent with the finding of Strzalkowska et al. (2002), brings a clearer view on loci effects and helps to simplify decisions useful for breeding.

CONCLUSION

The occurrence of more *A* alleles in genotype combinations of *CSN3* and *LGB* loci tends to increase yield parameters, while the occurrence of more *B* alleles tends to increase content parameters. The highest breeding values for milk and protein yields were associated with genotype combination *ABAA*, whereas the highest breeding values for content parameters were associated with genotype combination *BBAB*. The results of genotypes *BBBB*, *AABB* and *BEBB* cannot be taken into consideration for reliable conclusions due to the small number of animals carrying these combinations.

Acknowledgements

The authors thank the breeding companies for providing semen samples of sires and also the Czech-Moravian Breeders' Corporation Ltd. for providing the breeding values of sires.

REFERENCES

- Agrawala P.L., Wagner V.A., Geldermann H. (1992): Sex determination and milk protein genotyping of preimplantation stage bovine embryos using multiplex PCR. *Theriogenology*, 38, 969–978.
- Alexander L.J., Stewart A.F., MacKinlay A.G., Kapelinskaya T.V., Tkach T.M., Gorodetsky S.I. (1988): Isolation and characterization of the bovine kappa-casein gene. *Eur. J. Biochem.*, 178, 395–401.
- Ashwell M.S., Rexroad C.E., Miller R.H., VanRaden P.M. (1996): Mapping economic trait loci for somatic cell score in Holstein cattle using microsatellite markers and selective genotyping. *Anim. Genet.*, 27, 235–243.
- Aschaffenburg R., Drewry J. (1955): Occurrence of different beta-lactoglobulins in cow's milk. *Nature*, 176, 218.
- Boettcher P.J., Caroli A., Stella A., Chessa S., Budelli E., Canavesi F., Ghiroldi S., Pagnacco G. (2004): Effects of casein haplotypes on milk production traits in Italian Holstein and Brown Swiss cattle. *J. Dairy Sci.*, 87, 4311–4317.
- Caroli A., Chessa S., Bolla P., Budelli E., Gandini G.C. (2004): Genetic structure of milk protein polymorphism and effects on milk production traits in local dairy cattle. *J. Anim. Breed. Genet.*, 121, 119–127.
- Eggen A., Fries R. (1995): An integrated cytogenetic and meiotic map of the bovine genome. *Anim. Genet.*, 26, 216–236.
- Choi J.W., Ng-Kwai-Hang K.F. (2002): Effects of genetic variants of κ -casein and β -lactoglobulin and heat treatment of milk on cheese and whey compositions. *Asian-Aust. J. Anim. Sci.*, 5, 732–739.
- Ikonen T., Ojala M., Syvaola E.L. (1997): Effects of composite casein and beta-lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model. *Agricultural and Food Science in Finland*, 6, 283–294.
- Ikonen T., Ojala M., Ruottinen O. (1999): Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. *J. Dairy Sci.*, 82, 1026–1033.
- Ikonen T., Bovenhuis H., Ojala M., Ruottinen O., Georges M. (2001): Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. *J. Dairy Sci.*, 84, 507–514.
- Kaminski S., Rymkiewicz-Schymczyk J., Wojcik E., Rusc A. (2002): Associations between bovine milk protein genotypes and haplotypes and the breeding value of Polish Black-and-White bulls. *J. Anim. Feed Sci.*, 11, 205–221.
- Kučerová J., Matějček A., Jandurová O. M., Sorensen P., Němcová E., Štípková M., Kott T., Bouška J., Frelich J. (2006): Milk protein genes *CSN1S1*, *CSN2*, *CSN3*, *LGB* and their relation to genetic values of milk production parameters in Czech Fleckvieh. *Czech J. Anim. Sci.*, 51, 241–247.
- Lien S., Rogne S. (1993): Bovine casein haplotypes: number, frequencies and applicability as genetic markers. *Anim. Genet.*, 24, 373–376.
- Neelin J.M. (1964): Variants of κ -casein revealed by improved starch gel electrophoresis. *J. Dairy Sci.*, 47, 506.
- Neubauerová V. (2001): Detection of genetic markers and possibility of their use in cattle and other subungulates. [Thesis.] University of South Bohemia, Ceske Budejovice, 211 pp. (in Czech)
- Ng-Kwai-Hang K.F. (1998): Genetic polymorphism of milk proteins: Relationships with production traits, milk composition and technological properties. *Can. J. Anim. Sci.*, 78, 131–147.
- Ojala M., Famula T.R., Medrano J.F. (1997): Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. *J. Dairy Sci.*, 80, 1776–1785.

- Panicke L., Freyer G., Erhardt G. (1996): Effekte der Milchproteinpolymorphismen auf die Leistung. In: Kolloquium Milchprotein und Proteinansatz, Graal-Müritz, FBN Dummerstorf, Universität Rostock, 20.
- Příbyl J. (1995): A way of using markers for farm animal selection. Czech J. Anim. Sci., 40, 375–382.
- Ron M., Yoffe O., Ezra E., Medrano J.F., Weller J.I. (1994): Determination of effects of milk protein genotype on production traits of Israeli Holsteins. J. Dairy Sci., 77, 1106–1113.
- Sabour M.P., Lin C.Y., Lee A.J., McAllister A.J. (1996): Association between milk protein variants and genetic values of Canadian Holstein bulls for milk yield traits. J. Dairy Sci., 79, 1050–1056.
- Strzalkowska N., Krzyzewski J., Zwierzchowski L., Ryniewicz Z. (2002): Effects of κ -casein and β -lactoglobulin loci polymorphism, cows' age, stage of lactation and somatic cell count on daily milk yield and milk composition in Polish Black-and-White cattle. Animal Science Papers and Reports, 20, 21–35.

Received: 2006–10–26

Accepted after corrections: 2007–02–16

Corresponding Author

Ing. Aleš Matějčík, Department of Specialized Livestock Breeding, Faculty of Agriculture, University of South Bohemia, Studentská 13, 370 05 České Budějovice, Czech Republic
Tel. +420 387 772 608, e-mail: matejcek.ales@seznam.cz
