Joint effects of *CSN3* and *LGB* genes on milk quality and coagulation properties in Czech Fleckvieh

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ABSTRACT: The aim of this study was to determine the joint effects of *CSN3* and *LGB* genotypes on parameters of production, quality and coagulation of milk in Czech Fleckvieh cows. Three hundred and twenty-eight Czech Fleckvieh cows were determined for *CSN3* (kappa-casein) and *LGB* (beta-lactoglobulin) genotypes using the PCR-RFLP method, milk quality parameters and coagulation properties. Milk production parameters were obtained from the Official Database of Progeny Testing. Fifteen genotype combinations were detected, with *ABAB* (21.0%) and *AAAB* (18.3%) occurring as the most frequent. The observed genes significantly affected the contents of milk protein (crude protein, true protein, casein and whey protein) as well as solid non-fat in milk, casein number and curd quality. *BBAA* was found to be the genotype with the highest positive impact on most of the milk characteristics evaluated. Whereas *ABBB*, *BBBB*, *BBAB* and *ABAB* had a positive influence on milk quality and milk coagulation properties, genotypes containing *CSN3* allele *E* had a negative effect. Results presented in this study are applicable in the selection of Czech Fleckvieh cattle.

Keywords: cattle; milk protein genes; kappa-casein; beta-lactoglobulin; milk production parameters; coagulation properties

Milk protein genes, especially kappa-casein (*CSN3*) and beta-lactoglobulin (*LGB*), are important determinants of milk quality and milk coagulation properties. According to Strzalkowska et al. (2002) there are significant interactions between *CSN3* and *LGB* genes, and their influence on milk quality should be evaluated as a joint effect.

Kappa-casein protein, the only casein fraction that contains the sulphur amino acids cysteine and methionine, constitutes approximately 13% of milk casein (Farrell et al., 2004). The kappa-casein gene (*CSN3*) is situated on bovine chromosome 6, and its polymorphism has been known since 1964 (Neelin, 1964). Eleven alleles have been identified (Farrell et al., 2004) with *A*, *B* and *E* as the most common (Neubauerová, 2001). Whereas the amino acids threonine and aspartic acid are encoded by allele *A* at positions 136 and 148, the corresponding amino acids for allele *B* are isoleucine and alanine (Eigel et al., 1984). At position 155, allele *E* encodes the amino acid glycine, while the amino acid serine commonly occurs at this position (Erhardt, 1989).

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Allele *A* has been reported as the most frequent, with the effect of increasing milk yield but decreasing protein content (Neubauerová, 2001; Kučerová et al., 2005). Allele *B* is often referred to as a "key allele", increasing milk protein quality and coagulation properties (Hanuš et al., 1995; Amigo et al., 2001; Comin et al., 2006), whereas a negative effect of allele *E* on milk coagulation properties was reported by Ikonen et al. (1999a) and Comin et al. (2006).

Beta-lactoglobulin is the main protein of whey. The gene for beta-lactoglobulin (*LGB*) is situated on bovine chromosome 11, with its polymorphism discovered in 1955 (Aschaffenburg and Drewry, 1955). A total of 11 alleles are known (Farrell et al., 2004), with alleles *A* and *B* being found the most frequent (Panicke et al., 1996) and alleles *C* and *D* rarely occurring in European cattle breeds. Allele *A* encodes the amino acids aspartic acid and valine at positions 64 and 118. At corresponding positions, allele *B* encodes glycine and alanine (Eigel et al., 1984). Allele *B* was reported to increase milk protein and milk fat contents, while allele *A* increases milk and protein yields (Neubauerová, 2001; Kaminski et al., 2002; Kučerová et al., 2006). In Czech Fleckvieh cows, Hanuš et al. (1995) found a positive effect of the *BB* genotype on milk coagulation properties, whereas higher curd production due to allele *B* was reported by Choi and Ng-Kwai-Hang (2002).

Studies evaluating milk protein genes usually focus either on phenotypic characteristics of cows (Ikonen et al., 2001; Choi and Ng-Kwai-Hang, 2002; Strzalkowska et al., 2002; Comin et al., 2006) or on breeding values of sires (Ron et al., 1994; Sabour et al., 1996; Kaminski et al., 2002; Kučerová et al., 2006). In a previous study (Matějíček et al., 2007) the joint effects of *CSN3* and *LGB* genotypes on breeding values of sires were evaluated. The aim of the present study was to investigate the joint effects of *CSN3* and *LGB* genotypes on parameters of milk production, milk quality and milk coagulation properties of Czech Fleckvieh cows.

MATERIAL AND METHODS

Kappa-casein (*CSN3*) and beta-lactoglobulin (*LGB*) genotypes were identified in 328 Czech Fleckvieh cows using a Polymerase Chain Reaction and Restriction Fragment Length Polymorphism method (PCR-RFLP) and electrophoresis on agarose gel. DNA was obtained from blood samples of cows (Kawasaki, 1990). The identification of *CSN3* alleles *A*, *B* and *E* was done according to Lien and Rogne (1993) and of *LGB* alleles *A* and *B* 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. The protocol for *LGB* was 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.

Milk samples were collected between 50 and 140 days after first calving and analysed for contents of:

– dry matter (%);
– solid non-fat (%);
– crude protein (%; Kjeldahl total N × 6.38);
– true protein (%; Kjeldahl protein N × 6.38);
– casein (%; Kjeldahl casein N × 6.38);
– whey protein (%) = true protein – casein;
– non-protein nitrogen substances (%) = crude protein – true protein;
– casein number = % of casein from crude protein.

The parameters were analysed according to standardized methods defined by ČSN 57 0530 and ČSN 57 0536 and standard operation procedures of the accredited laboratory in the Research Institute for Cattle Breeding, Rapotín (National Reference Laboratory for Raw Milk; No. 1 340, EN ISO 17025). The fractions such as crude protein, true protein and casein contents were determined on the instrument 2200 Kjeltec Auto Distillation (Foss-Tecator AB, Sweden), contents of dry matter and solid non-fat were measured on the instrument MilkoScan 133B (Foss Electric, Denmark).

Evaluated cheese-making parameters were as follows:

– coagulation time (s) = means the interval (s) from adding Renilase (microbial enzyme) into the milk sample (50 ml, 35°C) until the first visible milk protein coagulation;
– curd quality (graded from 1 = excellent to 4 = poor) = evaluated by examination and palpation of curd cake obtained from 50 ml of milk;
– curd firmness (mm; lower mm = firmer curd) = investigated after 60 min of curd incubation (i.e. 60 min after Renilase addition) by measurement of the penetration depth of cylindrical corpuscle which falls into the curd cake under standard repeated conditions (weight and fall height of the corpuscle);
– whey amount (ml) = amount of whey obtained from coagulation of 50 ml of milk (coagulated under previously described conditions) and measured with a volumetric glass cylinder.

Milk production parameters (milk yield, protein and fat yields, protein and fat contents) were obtained from the Official Database of Progeny Testing.

Frequencies of *CSN3* + *LGB* genotypes were calculated, and the statistical analysis (GLM – general linear model – with fixed effects) was performed by SAS program. The following model was used to evaluate the relation between genotypes and milk production characteristics:

$$y_{ijklmn} = \mu + HYS_i + G_j + C_k + S_l + bA_m + e_{ijklmn} \quad \text{(Model 1)}$$

where:

y = observed characteristic
μ = average of the characteristic
HYS = effect of herd, year and season of calving
G = joint effect of *CSN3* and *LGB* genotypes
C = effect of the genetic part of Czech Fleckvieh breed in cows
S = effect of the breeding value of sire
bA_m = effect of the age at first calving of cow
e_{ijklmn} = residual effect

The model used to investigate the relation between genotypes and milk quality parameters and coagulation properties was as follows:

$$y_{ijkl} = \mu + HYS_i + G_j + bA_k + e_{ijkl} \quad \text{(Model 2)}$$

where the symbols correspond to the symbols explained in Model 1.

RESULTS AND DISCUSSION

Fifteen genotype combinations of *CSN3* and *LGB* genes were found in Czech Fleckvieh cows (Table 1). Genotype *ABAB* occurred as the most frequent, which is consistent with previous results (Matějíček et al., 2007). Whereas a high frequency of the genotype combination *AAAB* was found, a low rate (less than 2%) of genotypes containing the *CSN3* allele *E* occurred. Genotype *BEAA* was identified only in a single cow. The low frequency of allele *E* is beneficial because this allele has a negative effect on milk coagulation properties (Ikonen et al., 1999a; Comin et al., 2006).

The influence of the joint effects of *CSN3* + *LGB* genotypes on milk production parameters during the first 100 and 200 days of lactation is presented in Table 2. A significant gene effect on protein content was found in both lactation periods. The genotype combinations *AEAA* and *AEBB* resulted in the highest milk and protein yields. However, it caused a low protein content. With genotype *AEAA* detected in two cows only no conclusions on this genotype are justified. High yield characteristics were also associated with genotypes *BBAA*, *AABB* and *AAAB*. This is in agreement with Ikonen et al. (1999b), who found that *CSN3* alleles *E* and *A* increase milk yield but decrease protein content. The highest protein content was found in the genotype combinations *BBAA*, *ABBB* and *ABAB*, which illustrates the effect of *CSN3* allele *B* on increasing protein content (Ikonen et al., 1999b; Kučerová et al., 2006). Similarly, Kaminski et al. (2002) reported the highest protein content as well as fat content associated with genotype combinations *ABBB* and *ABAB*.

Table 1. Genotype frequencies found in cows

CSN3 + *LGB* genotypes	*n*	Frequency (%)
AAAA	43	13.1
AAAB	60	18.3
AABB	35	10.7
ABAA	33	10.1
ABAB	69	21.0
ABBB	33	10.1
AEAA	2	0.6
AEAB	6	1.8
AEBB	6	1.8
BBAA	5	1.5
BBAB	12	3.7
BBBB	12	3.7
BEAA	1	0.3
BEAB	6	1.8
BEBB	5	1.5

A significant effect of *CSN3* + *LGB* genotypes was found in almost all milk quality parameters (Table 3), except for the contents of dry matter (DM) and non-protein nitrogen substances (NPNS). This effect was highly significant ($P < 0.001$) for the contents of true protein (TP), casein (C) and whey protein (WP), and for casein number (CN). The most favourable results were associated with genotype combinations *BBAA*, *BBBB* and *ABBB*. Genotype *BBAA* excelled in all parameters except for whey protein content and casein number. Genotypes

Table 2. Joint effects of *CSN*3 + *LGB* genotypes on milk production parameters during the first 100 and 200 days of lactation (n = 328)

Genotype	*n*	Parameter for the first 100 and 200 days of lactation									
		milk yield (kg) 100/200		protein yield (kg) 100/200		protein* content(%)100/200		fat yield (kg) 100/200		fat content (%) 100/200	
AAAA	43	2 434	4 739	80	159	3.29	3.37	95	182	3.91	3.87
AAAB	60	2 531	4 926	82	164	3.26	3.35	99	190	3.91	3.88
AABB	35	2 545	4 918	84	167	3.33	3.41	106	197	4.15	4.02
ABAA	33	2 467	4 888	82	164	3.34	3.37	100	190	4.05	3.91
ABAB	69	2 459	4 826	82	165	3.37	3.43	98	188	4.00	3.92
ABBB	33	2 498	4 703	84	163	3.38	3.47	102	183	4.08	3.92
AEAA	2	3 163	6 277	103	202	3.22	3.19	117	242	3.66	3.84
AEAB	6	2 486	4 552	82	154	3.32	3.38	97	177	3.91	3.89
AEBB	6	2 738	5 520	87	181	3.18	3.29	111	218	4.09	3.95
BBAA	5	2 418	4 699	84	169	3.46	3.59	99	189	4.08	4.02
BBAB	12	2 527	5 018	83	170	3.30	3.40	99	189	3.92	3.79
BBBB	12	2 440	4 562	81	155	3.32	3.41	95	170	3.92	3.77
BEAA	1	2 548	4 873	75	152	3.00	3.16	68	147	2.83	3.12
BEAB	6	2 301	4 456	75	148	3.26	3.34	90	169	3.95	3.85
BEBB	5	2 224	4 274	72	145	3.25	3.40	87	170	3.98	4.02

*significance at $P < 0.05$

Table 3. Joint effects of *CSN*3 + *LGB* genotypes on milk quality parameters (n = 328)

Genotype	*n*	Milk quality parameter	DM (%)	SNF** (%)	CP** (%)	TP*** (%)	NPNS (%)	C*** (%)	WP*** (%)	CN*** (%)
AAAA	43	13.03	9.05	3.38	3.17	0.21	2.64	0.53	78.05	
AAAB	60	13.02	9.04	3.36	3.17	0.19	2.69	0.48	79.87	
AABB	35	13.14	9.06	3.39	3.20	0.19	2.73	0.49	80.51	
ABAA	33	12.98	8.96	3.32	3.13	0.20	2.59	0.54	77.89	
ABAB	69	13.13	9.11	3.44	3.25	0.20	2.76	0.49	80.23	
ABBB	33	13.35	9.19	3.54	3.34	0.20	2.90	0.43	82.08	
AEAA	2	12.37	9.20	3.32	3.14	0.17	2.58	0.57	77.67	
AEAB	6	12.98	9.08	3.35	3.16	0.19	2.68	0.48	80.05	
AEBB	6	12.96	8.98	3.29	3.10	0.19	2.72	0.37	82.82	
BBAA	5	13.49	9.54	3.78	3.62	0.16	3.07	0.55	81.19	
BBAB	12	13.25	9.07	3.44	3.24	0.20	2.77	0.47	80.40	
BBBB	12	13.01	9.09	3.47	3.27	0.20	2.82	0.45	81.41	
BEAA	1	12.83	9.16	3.19	2.98	0.21	2.53	0.45	79.31	
BEAB	6	13.41	8.95	3.33	3.10	0.24	2.65	0.45	79.32	
BEBB	5	13.19	9.23	3.49	3.24	0.24	2.83	0.41	81.13	

,*significance at $P < 0.01$ and $P < 0.001$

DM = dry matter; SNF = solid non-fat; CP = crude protein; TP = true protein; NPNS = non-protein nitrogen substances; C = casein; WP = whey protein; CN = casein number

Table 4. Joint effects of *CSN*3 + *LGB* genotypes on cheese-making parameters (n = 328)

Genotype	*n*	Cheese-making parameter			
		coagulation time (s)	curd quality* (grade)	curd firmness (mm)	whey amount (ml)
AAAA	43	126	2.70	1.82	33
AAAB	60	127	2.40	1.78	33
AABB	35	121	2.24	1.82	33
ABAA	33	105	2.24	1.82	34
ABAB	69	110	2.16	1.80	34
ABBB	33	123	1.98	1.77	33
AEAA	2	95	2.16	1.66	35
AEAB	6	181	2.83	1.72	31
AEBB	6	131	2.65	1.88	34
BBAA	5	90	1.55	1.79	32
BBAB	12	100	1.61	1.72	34
BBBB	12	135	2.17	1.68	33
BEAA	1	131	1.06	1.83	35
BEAB	6	100	2.62	1.83	35
BEBB	5	110	2.77	1.88	30

*significance at $P < 0.05$

BBBB and *ABBB* resulted in a high casein content and low whey protein content. Genotypes *AAAA*, *ABAA*, *AEAA*, *AAAB* and *BEAB* were linked with the least favourable results. The results of genotype *BEAA* are low informative due to its presence in only one cow. Hanuš at al. (2000) reported that genotypes *BB* of *CSN3* and *LGB* genes significantly increased casein content and casein number, and genotype *AA* of *LGB* gene increased whey protein content, in milk from Czech Fleckvieh cows.

Regarding cheese-making characteristics, the joint effects of *CSN3* and *LGB* genes were significant only on curd quality (Table 4). As for milk quality and milk production characteristics, the genotype combination *BBAA* had the most favourable influence. Genotype *BBAA* was associated with the shortest coagulation time, and relatively high curd quality and firmness. However, it resulted in a lower amount of eliminated whey relative to other genotypes. Also genotypes *BBAB* and *ABBB* had a positive impact on cheese-making characteristics. Genotype *BBBB* was associated with relatively high curd firmness and good curd quality, but with a long coagulation time. A negative influence on cheese-making characteristics was caused by genotypes *AEAB*, *AEBB*, *BEBB*, *BEAB* and *AAAA*. Although genotype *BEAA* was associated with a relatively high curd quality, it was identified in one cow only. Results are in agreement with Choi and Ng-Kwai Hang (2002), who found that the genotype combinations *BBBB*, *BBAB*, *BBAA*, *ABBB* and *AABB* positively affected cheese-making parameters, whereas *AAAA* and *AAAB* had a negative influence. Unfortunately, they did not identify *CSN3* allele *E* in their study. On the contrary, Comin et al. (2006) reported that the genotypes containing *CSN3* allele *E* were associated with poor coagulating milk, and genotypes with *CSN3* allele *B* resulted in the best coagulation properties of milk.

CONCLUSION

A strong influence of the *CSN3* gene in combination with the *LGB* gene on milk protein composition and milk coagulation properties was found in the present study. Whereas *CSN3* allele *B* had a positive influence on most characteristics evaluated, allele *E* had a negative impact. The genotype combination *BBAA* was found to be the most favourable. Genotypes *ABBB*, *BBBB*, *BBAB* and *ABAB* had a positive effect on milk quality and coagulation properties, while genotypes containing *CSN3* allele *E* caused a negative response. Findings presented in this study are applicable in the selection of Czech Fleckvieh cattle.

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