Gene polymorphisms influencing on yield, composition and technological properties of milk from Czech Simmental and Holstein cows

Jindřich *Čítek*[1*], Michaela *Brzáková*[1], Lenka *Hanusová*[1], Oto *Hanuš*[2], Libor *Večerek*[1], Eva *Samková*[1], Zuzana *Křížová*[1], Irena *Hoštičková*[1], Tereza *Kávová*[1], Karolina *Strakova*[1], Lucie *Hasoňová*[1]

[*]**Corresponding author: Jindřich Čítek**

Tel: +420 38 777 2591, E-mail: citek@zf.jcu.cz

[1]University South Bohemia, Faculty of Agriculture, CZ37005 Ceske Budejovice, Czech Republic

[2]Dairy Research Institute, CZ16000 Prague, Czech Republic

ORCID

Jindřich *Čítek*

https://orcid.org/0000-0003-4305-9160

Michaela *Brzáková*

https://orcid.org/0000-0002-7510-3480

Lenka *Hanusová*

https://orcid.org/0000-0002-8180-2456

Oto *Hanuš*

http://orcid.org/0000-0002-3616-7668

Libor *Večerek*

http://orcid.org/0000-0002-2069-4615

Eva *Samková*

http://orcid.org/0000-0002-9246-4456

Zuzana *Křížová*

https://orcid.org/0000-0001-8628-8882

Irena *Hoštičková*

http://orcid.org/0000-0001-7824-5794

서식 있음: 가운데

서식 있음: 글꼴: 기울임꼴

서식 있음: 글꼴: 기울임꼴

서식 있음: 글꼴: 기울임꼴

서식 있음: 글꼴: 기울임꼴

서식 있음: 글꼴: 기울임꼴

서식 있음: 글꼴: 기울임꼴

Lucie *Hasoňová*

https://orcid.org/0000-0001-7763-8609

서식 있음: 글꼴: 기울임꼴

Running head

Factors influencing milk yield and quality

Objective: The aim of the study was to evaluate the influence of polymorphic loci and other factors on milk performance and the technological properties of milk.

Methods: The analysis was performed on Simmental and Holstein cows in field conditions. Milk yield in kg, fat and protein percentage and yield were evaluated. Technological properties were evaluated by milk fermentation ability, renneting, and an alcohol test. Polymorphisms in the *DGAT1*, *LEP*, *FASN*, *SCD1*, *CSN2*, *CSN3* and *LGB* genes were genotyped, and association analysis was performed.

Results: The *DGAT1 AA* genotype was associated with higher milk, protein and fat yields ($p<0.05$). The *MM* genotype in the *LEP* gene was associated with a lower protein percentage and the *W* allele with a higher protein percentage ($p<0.05$). In cows with the *FASN GG* genotype, the protein percentage was higher, but the *A* allele was associated with higher milk, protein and fat yields than the *G* allele. The *TT* genotype in *SCD1* was associated with the lowest milk, protein and fat yields and with the highest milk protein percentage ($p<0.01$). The *T* allele had higher values than the *C* allele ($p<0.05$) except for fat percentage. The genotype *CSN3 AA* was associated with a significantly heightened milk yield; *BB* was associated with a high protein percentage. The effect of the alleles on the technological properties was not significant. The *CSN2 BB* genotype was associated with the best alcohol test ($p<0.01$), and the renneting order was inverse. Milk from cows with the *CSN2* A^1A^1 genotype was best in the milk fermentation ability. *CSN3* significantly affected the technological properties.

Conclusion: The findings revealed the potential of some polymorphic loci for use in dairy cattle breeding and for the management of milk quality. In field research, the pivotal role of farms in milk yield, composition and technological properties was confirmed.

Keywords: Dairy Cattle; Performance; Milk fermentation ability; Renneting; Ethanol test

INTRODUCTION

Milk yield and composition substantially impact the economics of dairy farms. For milk manufacturing, in addition to the percentages of fat and protein and the microbial quality, the technological properties of milk are important, as cheese has become a very important product in the dairy industry and enzymatic coagulation of milk is a crucial step in cheese-making process [1]. The influence of diet on the cheese-making properties of milk is often analyzed [2]. Other impacts on technological properties, such as the housing system or the stage of lactation, have also been the focus of studies [3,4].

Cecchinato et al. studied milk coagulation properties and curd firmness and found heritability of up to 0.278 [5]. Part of the heritability seems to be due to the polymorphisms in major genes analyzed in their work. They confirmed some previously documented associations, e.g., those between *CSN2* and technological properties, and identified a number of novel associations. They reported that different genes are involved in the coagulation phase. An interesting study of *CSN2*, *CSN3* and *LGB* genes was performed on Czech Simmental cows [6]. The authors confirmed that first-parturition cows show a certain shift and imbalance in milk physiochemical parameters, and the effect of the composite genotype on the investigated traits mostly reflected the effects of the individual genes. Genetic polymorphism has been related to milk titratable acidity, alcohol stability, phosphorus and calcium contents in milk, yogurt pH and the number of fermenting *Lactobacilli*. Their findings support the previously accepted indirect effects of milk protein polymorphisms on milk technological quality. The authors propose to perform a detailed analysis of polymorphisms and the interaction of the genes in larger populations to confirm their results and to find differences among breeds. They suppose that further study of the milk protein composite genotypes in cattle may have future implications for the production of milk with defined characteristics.

Recently, *DGAT1* polymorphisms were studied. The lysine variant positively influenced the breeding value for fat content in milk [7]. The polymorphism of *DGAT1* in Jersey, Holstein-Friesian and Ayrshire breeds in New Zealand influenced the yield of fat, protein and milk [8]. Other authors found significant association to total not fat solid, fat and protein contents, but not milk yield [9]. *SCD1* is the other gene studied, their polymorphisms were found to be associated with fat content [10]. Animals with the *CC* genotype compare favourably with individuals with other genotypes in terms of milk yield [11]. Also, leptin (*LEP*), fatty acid synthase (*FASN*) and other gene polymorphisms were studied [12,13].

The aim of this paper was to evaluate the association of polymorphisms in the *DGAT1*, *LEP*, *FASN*, *SCD1*, *CSN2*, *CSN3* and *LGB* genes with the performance, composition and technological qualities of cow milk. Milk protein genes with the potential to affect milk quality were chosen, i.e. caseins and lactoglobulin beta. Moreover, *DGAT1*, *LEP*, *FASN* and *SCD1* genes were involved. These genes are studied in regard to the milk performance, but the influence of their polymorphisms on technological qualities of milk was not evaluated adequately so far. Analysis was performed on numerous animals in field conditions in several farms, and additional factors affecting the performance and quality of milk were also evaluated.

MATERIALS AND METHODS

Animals

All experiments were performed in accordance with relevant guidelines and regulations recommended by the Ministry of Agriculture of the Czech Republic. All animal experiments were under supervision of the Institutional Animal Care and Use Committee of the Faculty of Agriculture of the South Bohemia University, where the experiment was carried out, approval number 22036/2019-MZE-18134. DNA was extracted noninvasively from milk samples.

The analysis was performed in cows of the Czech Simmental (part of the Simmental group) and Holstein breeds and their crosses; the cows were kept in the Czech Republic. In all, 401 Simmental cows and crosses and 347 Holstein cows and crosses were included. As the crossbreds of Holstein and Simmental with small proportions of Ayrshire are common in herds in the Czech Republic, they were included into our field research as well. The numbers of purebred and crossbred cows are given in Table S1. Cows were kept by five companies in free housing and fed with maize silage, grass silage, hay and feed concentrates year-round. The ratios differed among companies in terms of the share of constituents and their quality. The cows calved from 2015 – 2017. The 1^{st} lactation was recorded for 748 cows, and the 2^{nd} was also recorded for 660 of those cows. The mean milk yield was 8036 kg in the 1^{st} lactation and 8722 kg in the 2^{nd} lactation. The fat percentages were 4.12 and 4.12, the crude protein percentages were 3.46 and 3.48, the fat yields were 329.8 kg and 358.0 kg, and the crude protein yields were 274.8 kg and 301.3 kg for the first and second lactations, respectively.

The technological properties (milk fermentation ability, renneting measured by classical procedure and by nephelometry, ethanol test) of milk samples from 242 cows were examined. Of these cows, 81 were sampled once, 86 twice, 53 three times, 16 four times, and 6 five times. The cows were sampled throughout the course of the year.

Genotyping

Milk samples were individually collected, and DNA was isolated using the DNA/RNA extractor MagCore HF16 Plus (RBC Bioscience). Isolation was performed according to the manufacturer's instructions using the MagCore DNA Whole Blood Kit and MagCore Genomic DNA Tissue Kit (RBC Bioscience). The quality and quantity of the isolated DNA were verified by electrophoresis and spectrophotometry.

149 Genotyping of all loci was performed by the PCR/RFLP method. Acyl-CoA diacylgycerol transferase 1
150 gene (*DGAT1*) alleles *A* (alanine) and *K* (lysine) were genotyped according to the methods of Kuhn et al. (2004);
151 leptin gene (*LEP*) alleles *M* and *W* according to Buchanan et al. (2002); fatty acid synthase gene (*FASN*) alleles
152 *A* and *G* according to Roy et al. (2006); casein beta gene (*CSN2*) alleles *A* and *B* according to Medrano and
153 Sharrow (1991); and alleles A^1 and A^2 according to Miluchová et al. (2013). Casein kappa gene (*CSN3*) alleles *A*,
154 *B*, *C* and *E* were analyzed according to the methodology of Barroso et al. (1998); lactoglobulin beta gene (*LGB*)
155 alleles *A* and *B* according to Strzalkowska et al. (2002); and stearoyl CoA desaturase 1 gene (*SCD1*) alleles *C*
156 and *T* according to Inostroza et al. (2013) [14-21]. The primer sequences are given in Table S2.
157 The resulting genotypes were electrophoretically determined, and genotype and allelic frequencies were
158 calculated. To evaluate the Hardy-Weinberg Equilibrium (HWE), the differences between the observed and
159 expected frequencies of the genotypes were tested using a $\chi 2$ test with the significance level $p < 0.05$ and $p < 0.01$.
160 Table S3 gives the frequencies of genotypes and alleles of all genotyped Simmental, Holstein and crossbred
161 cows.
162
163 **Milk performance, composition and analysis of technological qualities**
164 Data on milk performance were collected from the milk recording breeder ś database. Milk yield in kg, fat
165 and crude protein percentage, and fat and crude protein yield in kg were evaluated. Milk composition (fat and
166 crude protein contents) was determined in breeder milk laboratories of the Czech-Moravia Breeders Association
167 using infrared spectroscopy (Foss Electric Denmark and Bentley Instruments USA) instrumentation. These
168 laboratories are accredited to the ISO standard (CSN EN ISO/IEC 17025) for official milk performance analysis
169 in the Czech Republic and are working under the ICAR (International Committee for Animal Recording)
170 umbrella (ICAR certificate to Czech Moravian Breeders' Corp. for identification of dairy cattle, production
171 recording in dairy cattle, genetic evaluation, milk laboratory operation, linear classification/scoring, and data
172 processing), regularly taking part in relevant proficiency testing. Analytical instruments were regularly monthly
173 calibrated according to the reference method results (extraction by the Röse–Gottlieb method for fat content [22]
174 and distillation and titration according to the Kjeldahl method for crude protein content (total nitrogen content ×
175 6.38) [23]). Technological properties were evaluated by a milk fermentation ability test, renneting was measured
176 subjectively and instrumentally, and an ethanol test was performed.

The milk ethanol stability was determined by milk titration (5 ml) with 96% ethanol until the first precipitation flakes of milk protein were visible and is reported as ml of alcohol. This procedure was modified according to Horne (2016) [24].

The milk fermentation ability of the yogurt test was carried out according to the Czech milk industry standards. A sample of raw milk (50 ml) was heated at 85 ℃ for 5 min and cooled at 43 ℃ ±2 ℃. Subsequently, the sample was inoculated with 2 ml of the thermophilic lactic culture YC-180-40-FLEX (Chr. Hansen, Denmark; *Streptococcus thermophilus*, *Lactobacillus delbrűeckii* subsp. *lactis*, and *L. d.* subsp. *bulgaricus*). The inoculated sample was incubated at 43 ℃ for 3.5 hours. The result was expressed as the titration acidity of the yogurt in ml of 0.25 mol $\times L^{-1}$ NaOH $\times 100$ ml^{-1} (or the so-called Soxhlet-Henkel degree) [25,26].

Rennetability (classical procedure) was determined during the tempering (35 ℃) of a defined milk volume after the addition of rennet (1% vol.) by measuring the time (rennet coagulation time RCT) until the first flakes of lactoproteins formed (beginning of coagulation). Rennetability was also determined by using nephelometry (turbidimetry measurement) to assess the milk coagulation time (ML – 2 analyzer). This is the use of the optical method (NEF) to evaluate the intensity of the so-called diffusely scattered Tyndall light on dispersed particles (coagulating lactoprotein flakes) [26,27].

The milk ethanol stability, milk fermentation ability and milk rennetability are not introduced by an official standard as technological property in world literature references, but they are known according to citations in the scientific literature. These procedures were modified according to literature sources cited.

Statistical analysis

Statistical analyses were performed using SAS (SAS 9.3, SAS Institute, Cary, NC, USA). Descriptive statistics for milk yield in kg, protein and fat percentages and protein and fat yield in kg during the first and second lactations are given in Table S4. Descriptive statistics for the indicators of the technological quality of milk, i.e., the milk fermentation ability, rennetability assessed subjectively and instrumentally, and alcohol test are given in Table S5. For the descriptive statistics and genotype frequencies in Table S4, each record was assessed as a separate entry; when two lactations were recorded for the same cow, it was included twice. Similarly, for Table S5, when a cow was measured repeatedly, the genotype was included repeatedly as well.

The data set contained repeated measurements per cow. Repeated measurements were obtained for the first and second lactation for milk performance traits. For technological quality, measurements were obtained several times over the course of two consecutive lactations. To analyze the influence of polymorphisms on milk yield

and technological quality, the MIXED procedure of the SAS system with repeated measurements and the LSM method were used to compare genotypes. The models were developed as follows.

For milk performance traits, the following mathematical model was used:

$$Y_{ijk} = \mu + gen_i + lac_j + anim_k + e_{ijk}$$

where Y_{ijk} = milk performance trait; μ = population mean; gen_i = fixed effect of the genotype (class effect i=1, 2, 3); lac_j = fixed effect of the lactation order (class effect j=1, 2); $anim_k$ = random effect of the animal; and e_{ijk} = random residual effect.

Different mathematical models were used to determine the technological quality of milk:

$$Yogurt_{ijklmn} = \mu + gen_i + farm_j + protein_k + casein_l + lacs_m + anim_n + e_{ijklmn}$$

where $Yogurt_{ijklmn}$ = yogurt test values; μ = population mean; gen_i = fixed effect of genotype (class effect i=1, 2, 3); $farm_j$ = fixed effect of farm (class effect j=1, 2, 3, 4, 5); $protein_k$ = fixed effect of protein percentage content in milk; $casein_l$ = fixed effect of casein content in milk; $lacs_m$ = fixed effect of lactation stage in days; $anim_n$ = random effect of the animal; and e_{ijklmn} = random residual effect.

$$Rennetability_{ijklmn} = \mu + gen_i + farm_j + protein_k + NFS_l + season_m + anim_n + e_{ijklmn}$$

where $Rennetability_{ijklmn}$ = rennetability assessed subjectively or instrumentally; μ = population mean; gen_i = fixed effect of genotype (class effect i=1, 2, 3); $farm_j$ = fixed effect of farm (class effect j=1, 2, 3, 4, 5); $protein_k$ = fixed effect of protein percentage content in milk; NFS_l = fixed effect of not fat solids content in milk; $season_m$ = fixed effect of season (class effect m=1, 2, 3, 4)*; $anim_n$ = random effect of the animal; and e_{ijklmn} =random residual effect. *The fixed effect of season was created as a combination of three months according to natural weather conditions, temperature, pasture quality, etc. A year was divided into four seasons: 1 = December, January, February; 2 = March, April, May; 3 = June, July, August; and 4 = September, October, November.

$$Ethanol_{ijk} = \mu + gen_i + farm_j + anim_k + e_{ijk}$$

where $Ethanol_{ijk}$ = ethanol stability; μ = population mean; gen_i = fixed effect of genotype (class effect i=1, 2, 3); $farm_j$ = fixed effect of farm (class effect j=1, 2, 3, 4, 5); $anim_k$ = random effect of the animal; and e_{ijk} = random residual effect.

The effect of alleles on milk production traits and the technological quality of milk was computed using the following mathematical model:

$Y_{ij} = \mu + allele_i + anim_j + e_{ij}$

where Y_{ij} = observed trait; μ = population mean; $allele_i$ = fixed effect of allele (class effect i=1,2); $anim_j$ = random effect of the animal; and e_{ij} =random residual effect.

For post hoc comparisons, the Tukey-Kramer test was used [28].

RESULTS AND DISCUSSION

Milk yield and composition

In the *DGAT1* gene, the genotype *AA* and allele *A*, which codes for alanine, had a higher frequency than the genotype *KA* and the *K* allele, which codes for lysine (Table S3); the homozygous genotype *KK* was not found at all. Other researchers found similarly unbalanced frequencies. In Israeli Holstein cows, the frequency of the *K* allele was reported to be 0.09 overall and 0.16 in sires [29]; in another study, the *A* allele had the highest frequency in dairy breeds, with the exception of Jersey [30]. Similarly, low frequencies of the *K* allele and *KK* homozygous genotype were found in Simmentals [31]. The *A* allele was confirmed repeatedly to be associated with higher milk, fat and protein yields, and its frequency in intensively selected populations increases due to indirect selection [14,32]. In our analysis, cows with the *AA* genotype outperform the heterozygous ones significantly in milk, protein and fat yields (Table 1). Additionally, when the effects of alleles are evaluated, *A* is advantageous but not significantly so (Table 2). This result is generally in agreement with previous findings and confirms our previous finding in German Holsteins regarding the trend of increasing frequency of the alanine variant [32,33].

Additionally, for the *LEP* gene, the *MM* genotype dominated (Table S3). In Holstein cows, a reverse order of genotypes was also published [34]. *MM* homozygous cows had a lower protein percentage, and the difference between *MM* and *MW* was significant (Table 1). Allele *W* positively and significantly influenced the protein percentage (Table 2); differences in other indicators of milk performance were nonsignificant.

For the *FASN* gene, the protein content was slightly but significantly higher in *GG* homozygous cows. The *A* allele had significantly higher milk yield than the *G* allele, which resulted in significantly higher protein and fat yield. The differences in fat and protein percentages between alleles were negligible and nonsignificant (Table 2). The frequencies of allele *G* were markedly higher than those of *A*, which does not correspond fully with the differences between alleles in terms of performance. However, considering both genotypes and alleles, the performance differences were low, which may explain the differences in frequency.

The *TT* homozygous genotype in the *SCD1* gene was significantly associated with the lowest milk, protein and fat yields and with the highest protein contents (Table 1). The analysis of allele associations showed superiority of the *T* allele in all characteristics except fat content. The differences were not high but significant (Table 2). The differences among genotypes hint at intermediate heredity.

For the *CSN2* gene, the differences among genotypes were not significant. The *B* allele had significantly higher milk yield and therefore protein yield than *A*. In fat yield, the *P* value was near the significance threshold. The differences in contents were low and nonsignificant (Table 1, 2). Similarly, for the A^2 and A^1 genotypes, the effect was nonsignificant. Allele A^2 was significantly better in terms of milk, protein and fat yields. The results of Ozdemir et al. indicated that none of the *CSN2* variants provide an advantage [35].

Genotype *AA* in the *CSN3* gene was significantly associated with high milk yield. *BB* homozygous cows had milk with a significantly higher protein percentage. Although the highest value was associated with the *EE* genotype, there were only two cows with a total of three lactations with this genotype, making it a minor consideration; similarly, for the *BC* genotype, there were two cows with four lactations. However, there were 21 cows of the *BE* genotype with 40 lactations, and they had significantly higher protein percentages comparing with the *AA* genotype (Table 1). The lowest protein content was found in *AA* homozygous cows, and the difference was significant. Thus, the positive influence of the *B* variant on the protein content was repeatedly shown. This was confirmed when evaluating the effect of alleles, specifically, the following significant effect was observed: *E*>*B* and *B*>*A*. However, the *CSN3* genotypes were not significantly associated with protein yield. Additionally, *BB* and *B* performed significantly better than *AA* and *A* in fat percentage. The differences in fat yield were not significant.

Our findings on the prevalence of the *AA* genotype and its effects on milk yield agree with other results found in Simmentals [36]. The authors also found the *BB* genotype to be associated with the highest protein percentage, but the fat percentage and yield were highest in milk from *AA* cows. In Czech Simmental cows, significant differences were reported among genotypes in daily milk yield, but the differences in protein and fat

296 percentages were nonsignificant [37]. Ozdemir et al. conclude their review and meta-analysis by stating that the
297 *CSN3* genotypes are ranked *BB>AB>AA* in terms of protein content and that the *B* allele could be considered a
298 marker to improve milk protein content. The prevalence of the *BB* genotype in relation to protein yield was not
299 always obvious. Additionally, for fat content, the *BB* genotype was better. They report that the associations of
300 genotypes and alleles with milk yields were not significant [35]. These findings are in general agreement with
301 our results.

302 The *AA* genotype of the *CSN3* gene is usually the most frequent in both Black-and-White and Simmental
303 cattle [20,36]. The frequencies of the *BB* genotype and *B* allele in our cows, both Holstein and Simmental, were
304 rather low, which is also consistent with the frequencies found by other authors in Czech Simmental [6].
305 However, one other group of authors also found that, in Czech Simmental, the most frequent genotype was *AB*
306 (0.487), and the frequency of the *B* allele was high (0.418) [37]. The frequency of the *E* allele (0.030) was the
307 same as in our Simmental group (0.036). Apparently, there is leeway for breeding, and many Czech breeding
308 companies report the genotype of the *CSN3* gene for the sires in their catalogues. However, changing genotype
309 frequencies is a long-distance run.

310 For the *LGB* gene, the *AB* genotype was significantly associated with higher milk yield than in both
311 homozygous genotypes, resulting in higher protein and fat yields. The rank of genotypes may indicate the effect
312 of heterosis. Allele *B* outperformed *A* in milk, protein and fat yields, but the differences in the contents were not
313 significant (Table 1, 2). Additionally, other authors found significantly higher milk, protein yield and fat
314 contents in Simmental cattle and higher fat yields (which were nonsignificant) in *AB* heterozygous cows [36].

315 The other factors potentially affecting milk performance were evaluated. Farm, breed and lactation order
316 were tested in a general linear mixed model. The milk yield, fat yield and protein percentage were significantly
317 influenced by all the factors. Fat content was influenced by farm, and protein yield was influenced by farm and
318 lactation order. Thus, the importance of the effect of farms, i.e., specific stable, management, nutrition,
319 veterinary care, milking, etc. was emphasized, even if some polymorphisms showed a significant association
320 with milk performance.

321

322 **Milk technological characteristics**

323 The testing of milk fermentation ability, renneting and ethanol stability was the final goal of our analysis.
324 The topic is relevant because genetic background exerts a strong influence on the cheese-making properties of
325 milk, largely due to genetic polymorphisms in the major milk protein genes [38]. In our analysis, the effect of

alleles was not significant with the exception of *CSN2*, with the *B* allele outperforming the *A* allele ($p < 0.01$) in terms of milk fermentation ability (yogurt test) (Table 2). The milk of *KA* heterozygous cows in the *DGAT1* gene significantly exceeded that of homozygous *AA* cows in milk fermentation ability (Table 3). The leptin gene had no significant effect, but the differences among genotypes in the alcohol test showed rising values in the order *MM>MW>WW* with significance differences of *MM* and *MW* vs. *WW* at $p < 0.05$. The differences among *FASN* genotypes were not significant. For the *SCD1* gene, the milk of *TT* cows was associated with significantly poorer performance in terms of renneting.

Certainly, the effects of polymorphous variants of milk protein genes are in focus. For the *CSN2* gene, the *BB* genotype had the best heat stability of milk as measured by the ethanol test ($p < 0.01$), which is important for ultrahigh temperature (UHT) milk production. However, with regard to renneting, the order was reversed. The A^1A^1 genotype was significantly associated with the best milk fermentation ability and was not significantly associated with renneting. Poulsen et al. refers to the negative association of A^2 with coagulation [39]. According to our results, it is difficult to describe the preferable genotype or allele in the *CSN2* gene.

Kappa-casein is the gene most often examined, as its influence on technological properties has been confirmed repeatedly. In our analysis, the rennetability measured instrumentally was significantly affected ($p < 0.05$). Genotype *BC* was associated with the best milk, but only four measurements were performed; because of the high standard errors, this association is of limited importance. The *BB* genotype was associated with significantly better renneting than the *AA* genotype but not the *AB* genotype (Table 3). Genotypes with the *A* and *E* alleles (*AE*, *AA*, *BE*) were associated with the poorest rennetability. A probable explanation for the differences in milk protein coagulation is the changes in the primary amino acid sequence between the A and B variants of the kappa casein as a protective factor for raw milk casein micelles. The B variant of kappa casein differs from the A variant by amino acid substitutions at two positions: 136^{th}, replacing threonine with isoleucine, and 148^{th}, replacing asparagine with alanine. These changes in the amino acid sequence of the B variant may interact positively with the action of the rennet enzyme that starts cleavage of the casein molecule between the 105^{th} and 106^{th} amino acids of the peptide chain, i.e., not far from the changed amino acids.

For the milk fermentation ability, the *P* value was close to the significance threshold. The ethanol test resistance was the best in milk from the cows with *CSN3 BB* genotypes. The differences in these scores between the *BB* genotype on one hand and *AA* and *AB* genotypes on the other hand were significant at $p < 0.01$. The difference *BB>AE* was significant at $p < 0.05$. Overall, the advantage of the *CSN3 BB* genotype was confirmed, but the *B* allele was not significantly better than the others (Table 2). Better properties for the *BB* genotype have

also been reported by other authors [39,40], who stated a positive effect of *CSN2 B* and *CSN3 B*. They hinted at the additive genetic variation of milk coagulation and the possibility of selective breeding for variants associated with superior milk coagulation.

Lactoglobulin beta is the most important whey protein. In our analysis, the effect of genotype on ethanol number was significant at $p < 0.05$, and *BB* was associated with a better value than *AB* at $p < 0.01$.

When evaluating other factors, the farm was found to significantly affect all parameters of technological quality. Protein percentage was associated with the milk fermentation ability and rennetability scores, while fat percentage was associated with ethanol test performance. Nonfat solid content was associated with renneting, while somatic cell count was associated with renneting measured subjectively. The content of casein, month and season were associated with milk fermentation ability. The lactation order, day in milk and breed did not show significant effects. Thus, the key effect of the farm must be emphasized again. Interestingly, the farm with the best milk fermentation ability and rennetability had the worst ethanol test.

CONCLUSIONS

According to our results, genotype *AA* and allele *A* of the *DGAT1* gene were associated with higher milk, protein and fat yields in kg, similar to allele *A* of the *FASN* gene, the *B* and A^2 alleles of the *CSN2* gene, and the *AB* genotype and *B* allele of the *LGB* gene. Alleles *B* and *E* of the *CSN3* gene were associated with a higher protein percentage, allele *B* was also associated with the fat percentage, and genotype *AA* was associated with the milk yield. For the *SCD1* gene, allele *T* seemed to be associated with better scores in all characteristics of milk performance. Regarding the technological properties, the *BB* genotype of *CSN3* proved repeatedly to have merit, while the other genes did not show unequivocal influences in our field study. The importance of the effect of the farm on milk performance and milk quality was confirmed, so the effect of gene polymorphisms in the field conditions seemed to be somewhat blurred. Nevertheless, the polymorphisms in *DGAT1* and *CSN3* are ready for practical use in breeding. The polymorphisms in other genes should be a subject of further research.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Agriculture, project MZe NAZV KUS QJ1510339, and by the Grant Agency of University of South Bohemia, project No. 028/2019/Z.

REFERENCES

1. Garcia-Gomez B, Vazquez-Oderiz ML, Munoz-Ferreiro N, Romero-Rodriguez MA, Vazquez M. Interaction between rennet source and transglutaminase in white fresh cheese production: Effect on physicochemical and textural properties. LWT-Food Sci Technol 2019;113:Article Number UNSP 108279. https://doi.org/10.1016/j.lwt.2019.108279
2. Migliorati L, Boselli L, Pirlo G, Moschini M, Masoero F. Corn silage replacement with barley silage in dairy cows' diet does not change milk quality, cheese quality and yield. J Sci Food Agric 2017;97:3396-340. https://doi.org/10.1002/jsfa.8190
3. Radkowska I, Herbut E. The effect of housing system of Simmental cows on processing suitability of milk and quality of dairy products. Anim Sci Pap Rep 2017;35:147-58.
4. Gulati A, Galvin N, Kennedy E, et al. Effect of reducing daily herbage allowance during early lactation on composition and processing characteristics of milk from spring-calved herds. Int Dairy J 2019;92:69-76. https://doi.org/10.1016/j.idairyj.2019.01.008
5. Cecchinato A, Chessa S, Ribeca C, et al. Genetic variation and effects of candidate-gene polymorphisms on coagulation properties, curd firmness modeling and acidity in milk from Brown Swiss cows. Animal 2015;9:1104–12. https://doi.org/10.1017/S1751731115000440
6. Kyselová J, Ječmínková K, Matějíčková J, et al. Physiochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of ß-casein, κ-casein, and ß-lactoglobulin. Asian-Australas J Anim Sci 2019;32:14-22. https://doi.org/10.5713/ajas.17.0924
7. Winter A, Kramer W, Werner FAO, et al. Association of lysine232 alanine polymorphism in bovine gene encoding acylCoA:diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. PNAS 2002;99:9300 – 9305. https://doi.org/10.1073/pnas.142293799

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: Times New Roman

서식 있음: 글꼴: Times New Roman, 10 pt

8. Spelman RJ, Ford CA. McElhinney P, Gregory GC, Snell RG. Characterization of the *DGAT1* gene in New Zealand dairy population. J Dairy Sci 2002;85:3514-3517. https://doi.org/10.3168/jds.S0022-0302(02)74440-8
9. Carvajal AM, Huircan P, Dezamour JM, et al. Milk fatty acid profile is modulated by *DGAT1* and *SCD1* genotypes in dairy cattle on pasture and strategic supplementation. Genet Mol Res 2016;15:gmr.15027057 https://doi.org/10.4238/gmr.15027057
10. Cecchinato A, Ribeca C, Chessa S., et al. Candidate gene association analysis for milk yield, composition, urea nitrogen and somatic cell scores in Brown Swiss cows. Animal 2014;8:1062-1070. https://doi.org/10.1017/S1751731114001098
11. Safina NY, Shakirov SK, Zinnatova FF, Fattakhova ZF, Gaynutdinova ER, Shayakhmetova LN, et al. Dynamics Of Dairy Production Of Heifers Of Different Genotypes Of Stearoyl-CoA Desaturase (SCD1). Res J Pharm Biol Chem Sci 2018;9:2028-2031.
12. Sanchez M-P, Ramayo-Caldas Y, Wolf V, et al. Sequence-based GWAS, network and pathway analyses reveal genes co-associated with milk cheese-making properties and milk composition in Montbéliarde cows. Genet Sel Evol 2019;51:34. https://doi.org/10.1186/s12711-019-0473-7
13. Mauric M, Masek T, Ljoljic DB, Grbavac J, Starcevic K. Effects of different variants of the *FASN* gene on production performance and milk fatty acid composition in Holstein x Simmental dairy cows. Veterinarni medicína 2019;64:101-108. https://doi.org/10.17221/73/2018-VETMED
14. Kuhn C, Thaller G, Winter A, et al. Evidence for multiple alleles at the *DGAT1* locus better explains a quantitative trait locus with major effect on milk fat content in cattle. Genetics 2004;167:1873–81. https://doi.org/10.1534/genetics.103.022749
15. Buchanan FC, Fitzsimmons CJ, van Kessel AG, Thue TD, Winkelman-Sim DC, Schmutz SM. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet Sel Evol 2002;34:105-16. https://doi.org/10.1186/1297-9686-34-1-105
16. Roy R, Ordovas L, Zaragosa P, et al. Association of polymorphisms in the bovine *FASN* gene with the milk fat content. Anim Genet 2006;37:215-8. https://doi.org/10.1111/j.1365-2052.2006.01434.x
17. Medrano JF, Sharrow L. Genotyping of bovine beta-casein loci by restriction site modification of polymerase chain reaction (PCR) amplified DNA. J Dairy Sci 1991;74:282.

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

18. Miluchová M, Gábor M, Trakovická A. Analysis of Slovak Spotted breed for bovine beta casein A1 variant as risk factor for human health. Acta Bioch Pol 2013;60:799-801. https://doi.org/10.18388/abp.2013_2061
19. Barroso A, Dunner S, Cañón, J. Technical Note: Detection of Bovine Kappa-Casein Variants A, B, C, and E by Means of Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP). J Anim Sci 1998;76:1535-8. https://doi.org/10.2527/1998.7661535x
20. Strzalkowska N, Krzyzewski J, Zwierzchowski L, Ryniewicz Z. 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. Anim Sci Pap Rep 2002;20:21–35.
21. Inostroza KB, Scheuermann ES, Sepulveda NA. Stearoyl CoA desaturase and fatty acid synthase gene polymorphisms and milk fatty acid composition in Chilean Black Friesian cows. Rev Colomb Cienc Pec 2013;26:263-9.
22. CSN EN ISO 1211 (570534). Milk – Fat content determination – Gravimetric method (Reference method). Czech Normalization Institute, Prague; 2011.
23. CSN EN ISO 8968-1 (570528). Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl method and calculation of crude protein. Czech Normalization Institute, Prague; 2014.
24. Horne DS. Ethanol Stability and Milk Composition. In: Mc Sweeny PLH, O Mahony JA, editors. Advanced Dairy Chemistry, Volume 1B, Proteins, Applied Aspects. New York: Springer-Verlag; 2016.
25. ON 57 0534. Milk fermentation ability determination. Standard Milk Industry Prague; 1986 [in Czech].
26. Hanuš O, Kučera J, Yong T, et al. Effect of sires on wide scale of milk indicators in first calving Czech Fleckvieh cows. Arch Anim Breed 2011;54:36-50. https://doi.org/10.5194/aab-54-36-2011
27. Samková E, Cempírková R, Hanuš O, et al. Milk: production and quality. Scientific monograph. Ceske Budejovice: University of South Bohemia, Agricultural Faculty; 2012 (in Czech).
28. Haynes W. Tukey's Test. In: Dubitzky W, Wolkenhauer O, Cho KH, Yokota H, editors. Encyclopedia of Systems Biology. New York: Springer-Verlag; 2013.
29. Weller JI, Golik M, Serousi E, Ezra E, Ron M. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. J Dairy Sci 2003;86:2219-27. https://doi.org/10.3168/jds.S0022-0302(03)73812-0

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

30. Carvajal AM, Huircan P, Dezamour JM, et al. Milk fatty acid profile is modulated by DGAT1 and SCD1 genotypes in dairy cattle on pasture and strategic supplementation. Genet Mol Res 2016;15:gmr.15027057. https://doi.org/10.4238/gmr.15027057

31. Barton L, Bures D, Kott T, Rehak D. Association of polymorphisms in bovine *DGAT1*, *FABP4*, *FASN* and *PPARGC1A* genes with intramuscular fat content and the fatty acid composition of muscle and subcutaneous fat in Fleckvieh bulls. Meat Sci 2016;114:18-23. https://doi.org/10.1016/j.meatsci.2015.12.004

32. Citek J, Rehout V, Hradecka E, Vecerek L, Panicke L. The Breeding values of German Holstein Sires and the *DGAT1* Polymorphism. Arch Anim Breed 2007;50:136-46. https://doi.org/10.5194/aab-50-136-2007

33. Čítek J, Hanusová L, Brzáková M, Večerek L, Panicke L, Lískovcová L. Associations between Gene Polymorphisms, Breeding Values, and Glucose Tolerance Test Parameters in German Holstein Sires. Czech J Anim Sci 2018;63:167–73. https://doi.org/10.17221/8/2017-CJAS

34. Chebel RC, Susca F, Santos JEP. Leptin genotype is associated with lactation performance and health of Holstein cows. J Dairy Sci 2008;91:2893-2900. https://doi.org/10.3168/jds.2007-0891

35. Ozdemir O, Kopuzlu S, Topal M, Bilgin OM. Relationships between milk protein polymorphisms and production traits in cattle: a systematic review and meta-analysis. Archi Anim Breed 2018;61:197–206. https://doi.org/10.5194/aab-61-197-2018

36. Neamt RI, Saplacan G, Acanticai S, Cziszter LT, Gavojdian D, Ilie D. The influence of *CSN3* and *LGB* polymorphism on milk production and chemical composition in Romanian Simmental cattle. Acta Bioch Pol 2017;64:493-7. https://doi.org/10.18388/abp.2016_1454

37. Bartonova P, Vrtkova I, Kaplanova K, Urban T. Association between *CSN3* and *BCO2* gene polymorphisms and milk performance traits in the Czech Fleckvieh cattle breed. Genet Mol Res 2012;11:1058-63. https://doi.org/10.4238/2012.April.27.4

38. Cecchinato A, Bobbo T, Ruegg PL, Gallo L, Bittante G, Pegolo, S. Genetic variation in serum protein pattern and blood beta-hydroxybutyrate and their relationships with udder health traits, protein profile, and cheese-making properties in Holstein cows. J Dairy Sci 2018;101:11108-19. https://doi.org/10.3168/jds.2018-14907

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

39. Poulsen NA, Bertelsen HP, Jensen HB, et al. The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds. J Dairy Sci 2013;96:4830–42. https://doi.org/10.3168/jds.2012-6422

40. Tyulkin SV, Vafin RR, Zagidullin LR, Akhmetov TM, Petrov AN, Diel F. Technological Properties of Milk of Cows with Different Genotypes of Kappa-Casein and Beta-Lactoglobulin. Foods Raw Mat 2018;6:154–62. https://doi.org/10.21603/2308-4057-2018-1-154-162

서식 있음: 글꼴: Times New Roman, 10 pt

서식 있음: 글꼴: Times New Roman, 10 pt

504 **Table 1.** Milk yield and composition according to genotype of Holstein and Czech Simmental cows

Gene	genotype	n	Milk kg		Crude protein %		Protein kg		Fat %		Fat kg	
			LSM ±SE	p	LSM ±SE	p	LSM ±SE	p	LSM ±SE	p	LSM ±SE	p
DGAT1	*AA*	1344	8376 ± 84^{a}	0.045^{*}	3.46 ±0.01	0.548	$287.9^{a} \pm 2.6$	0.027^{*}	4.12 ±0.01	0.141	$344.0^{a} \pm 3.4$	0.019^{*}
	KA	60	7555 ± 401^{b}		3.49 ±0.04		$260.1^{b} \pm 12.3$		4.04 ±0.06		$304.8^{b} \pm 16.3$	
LEP	*MM*	925	8412 ±101	0.156	$3.45^{a} \pm 0.01$	0.038^{*}	287.7 ±3.1	0.281	4.11 ±0.01	0.909	344.7 ±4.1	0.235
	MW	229	8138 ±203		$3.50^{b} \pm 0.02$		282.7 ±6.3		4.13 ±0.03		335.1 ±8.3	
	WW	45	7667 ±450		3.51 ±0.04		266.4 ±13.9		4.11 ±0.06		317.5 ±18.4	
FASN	*AG*	378	8527 ±158	0.180	$3.43^{a} \pm 0.02$	0.017^{*}	290.5 ±4.9	0.371	4.13 ±0.02	0.562	349.8 ±6.4	0.177
	GG	1018	8277 ±97		$3.48^{b} \pm 0.01$		285.4 ±3.0		4.11 ±0.01		339.6 ±3.9	
SCD1	*CC*	398	$8549^{A} \pm 154$	0.001^{**}	$3.43^{Aa} \pm 0.01$	$<0.001^{**}$	$290.5^{A} \pm 4.7$	0.005^{**}	4.10 ±0.02	0.606	$348.4^{A} \pm 6.2$	0.001^{**}
	TC	811	$8426^{A} \pm 107$		$3.47^{Ab} \pm 0.01$		$290.0^{A} \pm 3.3$		4.12 ±0.02		$346.9^{A} \pm 4.3$	
	TT	187	$7608^{B} \pm 222$		$3.53^{B} \pm 0.02$		$266.4^{B} \pm 6.8$		4.12 ±0.03		$312.1^{B} \pm 9.0$	
CSN2	*AA*	32	8143 ±535	0.541	3.41 ±0.05	0.133	276.6 ±16.4	0.254	4.23 ±0.08	0.201	344.0 ±21.8	0.442
	AB	220	8562 ±210		3.50 ±0.02		296.6 ±6.4		4.14 ±0.03		353.2 ±8.5	
	BB	1105	8326 ±93		3.46 ±0.01		286.0 ±2.8		4.11 ±0.01		341.2 ±3.8	
CSN2	A^1A^1	143	7883 ±254	0.163	3.48 ±0.02	0.539	273.0 ±7.8	0.190	4.15 ±0.04	0.491	327.7 ±10.3	0.253
	A^1A^2	501	8416 ±137		3.46 ±0.01		288.7 ±4.2		4.11 ±0.02		345.0 ±5.6	
	A^2A^2	675	8217 ±120		3.48 ±0.01		283.0 ±3.7		4.10 ±0.02		335.7 ±4.9	
CSN3	*AA*	646	$8497^{a} \pm 96$	0.112	$3.43^{A,3} \pm 0.01$	$<0.001^{**}$	288.1 ±3.7	0.364	$4.09^{a} \pm 0.02$	0.133	345.4 ±4.9	0.377
	AB	586	8267 ±101		$3.50^{B,4} \pm 0.01$		287.0 ±3.9		4.13 ±0.02		341.1 ±5.2	
	BB	70	8355 ±291		$3.54^{B} \pm 0.03$		292.3 ±11.5		$4.20^{b} \pm 0.05$		349.8 ±15.2	
	BC	4	8140 ±1217		3.51 ±0.11		285.5 ±48.5		4.33 ±0.22		352.3 ±64.2	

	EE	3	5627[b] ±1406		3.79[B,4] ±0.13		199.2 ±50.8		4.24 ±0.23		221.8 ±67.1	
	AE	32	7594[b] ±430		3.47[4] ±0.04		258.0 ±16.2		4.20 ±0.06		311.8 ±21.4	
	BE	40	8139 ±385		3.51[3,4] ±0.04		282.4 ±15.1		4.06 ±0.07		328.8 ±20.0	
	AA	30	6991[A] ±546	<0.001**	3.52 ±0.05	0.611	245.9[A] ±16.8	<0.001**	3.97 ±0.08	0.161	281.2[A] ±22.2	<0.001**
LGB	*AB*	1222	8471[B] ±88		3.47 ±0.01		291.1[B] ±2.7		4.12 ±0.01		347.9[B] ±3.6	
	BB	103	7515[A] ±299		3.47 ±0.03		258.9[A] ±9.2		4.11 ±0.04		309.1[A] ±12.2	

n, number of lactations of cows with a particular genotype; LSM, least squared mean; SE, standard error; * significant at $p < 0.05$; ** significant at $p < 0.01$; [a,b] different letters between genotypes in the same column represent significant differences at $p < 0.05$; [A,B] different letters between genotypes in the same column represent significant differences at $p < 0.01$; [3] differences between *CSN3* genotypes *AA* and *BE* in the protein percentage are significant at $p < 0.05$; [4] differences between *CSN3* genotypes *EE* on the one hand and *AB*, *AE*, *BE* on the other hand in the protein percentage are significant at $p < 0.05$.

520 **Table 2.** Significance of differences in milk yield, composition and qualities among alleles (*p* values)

Gene		Milk kg	Crude protein %	Protein kg	Fat %	Fat kg	Milk fermentation ability, ml NaOH	Renneting subjectively seconds	Renneting instrumentally seconds	Ethanol test ml of ethanol
DGAT1		0.861	0.255	0.753	0.308	0.628	0.064	0.528	0.354	0.659
LEP		0.999	0.023* W>M	0.701	0.835	0.904	0.446	0.823	0.642	0.077
FASN		0.008** A>G	0.896	0.009** A>G	0.610	0.007** A>G	0.142	0.906	0.555	0.536
SCD1		0.024* T>C	0.014* T>C	0.014* T>C	0.774	0.028*T>C	0.266	0.078	0.173	0.461
CSN2 (*A, B*)		0.031* B>A	0.390	0.042* B>A	0.319	0.055	0.002** B>A	0.083	0.086	0.242
CSN2 (A^1,A^2)		0.002** A^2>A^1	0.367	0.002** A^2>A^1	0.324	0.006** A^2>A^1	0.663	0.909	0.411	0.344
CSN3	*A:B*	0.187	<0.001**B>A	0.899	0.039*B>A	0.556	0.740	0.512	0.217	0.901
	A:E	0.046*A>E	0.314	0.055	0.111	0.099	0.817	0.240	0.914	0.526
	B:C	0.943	0.846	0.929	0.630	0.961	0.948	0.733	0.717	0.476
	B:E	0.068	0.010* E>B	0.074	0.815	0.062	0.091	0.980	0.322	0.440
LGB		<0.001**B>A	0.063	<0.001**B>A	0.736	<0.001**B>A	0.562	0.067	0.148	0.220

521 *significant at $p < 0.05$; **significant at $p < 0.01$

522

523

524

525

526

527

528

529 **Table 3**. Milk technological qualities according to the genotype of Holstein and Czech Simmental cows

		Milk fermentation ability ml NaOH			Renneting assessed subjectively seconds			Renneting assessed instrumentally seconds			Ethanol test ml of ethanol		
gene	genotype	n	LSM ±SE	p	n	LSM ±SE	p	n	LSM ±SE	p	n	LSM ±SE	p
DGAT1	*AA*	435	14.93^{A} ±0.25	$<0.001^{**}$	470	523.16 ±16.70	0.781	438	318.13 ±9.48	0.538	445	0.913 ±0.053	0.518
	KA	25	18.24^{B} ±0.87		31	507.79 ±53.79		23	338.64 ±32.64		25	1.052 ±0.204	
LEP	*MM*	288	15.09 ±0.33	0.709	315	510.28 ±18.78	0.609	289	314.68 ±10.54	0.654	293	0.864^{a} ±0.057	0.070
	MW	81	15.58 ±0.64		92	544.2 ±32.57		84	330.11 ±17.64		83	0.938^{a} ±0.105	
	WW	14	14.80 ±1.21		15	502.47 ±77.41		13	300.65 ±41.89		14	1.445^{b} ±0.248	
FASN	*AG*	118	15.07 ±0.44	0.998	130	545.46 ±26.47	0.262	117	336.70 ±14.53	0.123	115	1.026 ±0.094	0.202
	GG	338	15.07 ±0.28		367	512.17 ±18.52		340	311.87 ±10.37		351	0.888 ±0.058	
SCD1	*CC*	135	15.01 ±0.41	0.553	148	502.28^{a} ±25.10	0.029^{*}	131	320.98 ±14.15	0.059	136	0.944 ±0.087	0.955
	TC	284	15.25 ±0.30		305	513.78^{a} ±19.74		287	309.75^{a} ±11.00		288	0.918 ±0.064	
	TT	41	14.54 ±0.67		48	625.41^{b} ±41.88		43	369.90^{b} ±24.21		46	0.898 ±0.151	
CSN2	*AA*	22	14.08 ±1.34	0.538	22	522.96 ±59.26	0.540	21	271.44^{a} ±30.86	0.121	22	0.448^{A} ±0.215	0.001^{**}
	AB	171	15.39 ±0.45		187	498.54 ±26.77		172	304.21 ±14.68		176	0.724^{A} ±0.082	
	BB	267	14.98 ±0.32		292	532.28 ±19.03		268	329.06^{b} ±10.73		272	1.058^{B} ±0.062	
CSN2	A^1A^1	42	16.43^{Aa} ±0.60	0.022^{*}	45	527.46 ±48.38	0.968	38	314.22 ±26.72	0.462	43	0.829 ±0.153	0.769
	A^1A^2	148	14.60^{B} ±0.37		161	535.74 ±25.11		150	336.75 ±13.70		150	0.919 ±0.084	
	A^2A^2	224	15.17^{b} ±0.33		249	528.31 ±22.05		227	317.65 ±12.1		230	0.950 ±0.069	
CSN3	*AA*	215	15.22 ±0.34	0.075	228	552.38^{a} ±21.62	0.116	212	337.29^{3} ±12.02	0.037^{*}	220	0.929^{A} ±0.070	0.109
	AB	191	15.04 ±0.37		216	504.38 ±22.32		196	299.82^{3} ±12.31		197	0.871^{A} ±0.073	

	BB	24	15.36 ±0.77		25	486.02 ±56.55		24	312.04 ±29.83		23	1.543^{Ba} ±0.206	
	BC	4	14.77 ±1.35		4	299.68^{b} ±122.34		4	229.11^{4} ±63.58		4	0.966 ±0.490	
	AE	13	14.97 ±1.00		15	454.31 ±68.79		13	331.77 ±39.04		13	0.701^{b} ±0.271	
	BE	12	12.89 ±1.93		12	616.24^{a} ±93.27		11	$414.90^{3,4}$ ±49.35		12	0.990 ±0.285	
	AA	12	-	0.556	15	517.50 ±68.35	0.281	8	335.45 ±45.82	0.608	12	0.942 ±0.310	0.036^{*}
LGB	*AB*	390	15.15 ±0.27		416	510.01 ±18.42		393	314.56 ±10.45		394	0.857^{A} ±0.058	
	BB	58	14.76 ±0.64		70	576.97 ±37.91		60	337.37 ±21.73		64	1.216^{B} ±0.123	

n, number of lactations of cows with a particular genotype; LSM, least squared mean; SE, standard error; *significant at $p < 0.05$; **significant at $p < 0.01$; ^{a,b}differences between genotypes with different letters in the same column are significant at $p < 0.05$; ^{A,B}different letters between genotypes in the same column represent significant differences at $p < 0.01$; ^{3}differences between *CSN3* genotypes *AB* on the one hand and *AA* and *BE* on the other hand in renneting assessed instrumentally are significant at $p < 0.05$; ^{4}differences between *CSN3* genotypes *BE* and *BC* in renneting assessed instrumentally are significant at $p < 0.05$.