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Technological properties of cow's milk: correlations with milk composition, effect of interactions of genes and other factors

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Abstract: We analysed the correlations of milk fermentability (yogurt test acidity), renneting (time to rennet coagulation of milk protein) and results of ethanol tests (indirect indicator of milk thermostability) with the percentages of the milk components. The correlations of the milk component percentages with renneting were mostly weak, while the correlations with the ethanol test were even poorer. The *CSN3* and *LGB* genotypes did not show a significant interaction in their influence on milk fermentability, renneting or ethanol test results. For the ethanol test, many significant interactions were observed between the *DGAT1*, *LEP*, *FASN*, *SCD1*, *CSN2*, *CSN3* and *LGB* genotypes. *DGAT1* interacted significantly with all genes for milk fermentability. For renneting that was measured instrumentally, *CSN3* interacted significantly with *DGAT1*, *FASN*, *SCD1*, *CSN2 A/B*, *CSN2 A1/A2*; *SCD1* interacted significantly with *LEP* and *FASN*. The breed and genotypes did not show any interactions. Farm and genotypes interacted significantly for all the technological properties studied; similar results were observed for the protein percentage and the genotypes, except that no interactions influenced the ethanol test results.

Keywords: casein; ethanol test; gene polymorphisms; milk fermentation; renneting

Technological properties of cow milk considerably influence its value for the dairy industry. Environmental and genetic factors that affect the technological properties are of interest. Different feed rations have been tested; for example Migliorati et al. (2017) and Cattani et al. (2017) tested silages made of different green fodders and did not find any changes in the milk coagulation properties. Ghelichkhan et al. (2019) added soybean meal into the feed ration and found higher milk yield and lower fat content, but the milk protein content and yield and fat yield were not significantly different. Krizova et al. (2014) revealed some differences depending on the breed, feeding system or season, mainly related to fat and protein content, which may slightly affect cheese properties but without having considerable effect on the quality of retail dairy products. Additionally, Beux et al. (2018) found a significant influence of the season on somatic cell count, rennet coagulation time and

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curd firmness, with worse results in summer. The health aspects of milk production performance and milk quality are often studied (e.g., Kasna et al. 2018; Krpalkova et al. 2019). Joudu et al. (2008) studied rennet coagulation properties in relation to milk protein contents and stated that an increase of milk protein, casein, all protein fractions, and casein number reduced the rennet coagulation time and formed a firmer curd. Dual-purpose breeds have better milk composition with higher protein and casein percentage than dairy breeds (Manuelian et al. 2019).

Enormous attention has been paid to the effect of genetic variants of milk proteins on milk technological properties. *CSN3* genotypes are very often studied, and Alipanah and Kalashnikova (2007) reported that CSN3 BB milk had significantly superior rennet properties than AA and AB milks. BB milk had a higher content of protein and lower fat content, and higher protein and fat recoveries in cheese. Better quality is commonly determined in CSN3 BB milk both for protein content and technological properties (Djedovic et al. 2015; Tyulkin et al. 2018). Additionally, other casein fractions have been investigated, and Gustavsson et al. (2014) reported the poor gelation properties of composite *CSN2-CSN3* genotypes A^1A^2/AE. Ketto et al. (2018) studied physical properties and observed a significant effect of beta-lactoglobulin polymorphisms and of *CSN1S1/CSN3* composite genotypes as well. Kyselova et al. (2019) analysed the genetic polymorphisms of *CSN2*, *CSN3* and *LGB*. The *CSN3* genotype was significantly associated with milk alcohol stability, with the composite genotype influencing titratable acidity, active acidity, and the log of the *Lactobacilli* count in yogurt test.

Analyses of factors determining milk technological properties are of great importance. Therefore, the aim of this research was to evaluate the relations of milk component contents to milk technological properties. Furthermore, we aimed to evaluate the interactions of genotypes of polymorphic genes as well as the interactions of other factors and their importance in relation to milk technological properties and repeatability of quality parameters.

MATERIAL AND METHODS

Animals and genotyping. All experiments were performed in accordance with relevant guidelines and regulations recommended by Ministry of Agriculture of the Czech Republic. Non-invasive DNA extraction from milk samples was used.

For this analysis, 242 genotyped cows were used; of these cows, 160 were Czech Simmental and crosses with a prevailing proportion of the breed, and 82 were Holstein and crosses. The cows calved in 2015–2017, milk samples were obtained repeatedly in the 1st and 2nd lactation, and milk technological properties were analysed.

DNA was isolated from the milk samples using a DNA/RNA MagCore HF16 Plus extractor (RBC Bioscience, Taiwan). Genotyping was performed by the PCR/RFLP method. Acyl-CoA diacylglycerol transferase 1 gene (*DGAT1*) alleles *A* (alanine) and *K* (lysine) were genotyped according to the methods of Kuhn et al. (2004); leptin gene (*LEP*) alleles *M* and *W,* as in Buchanan et al. (2002); fatty acid synthase gene (*FASN*) alleles *A* and *G,* according to Roy et al. (2006); casein beta gene (*CSN2*) alleles *A* and *B,* as in Medrano and Sharrow (1991); alleles A^1 and A^2, according to Miluchova et al. (2013); casein kappa gene (*CSN3*) alleles *A*, *B*, *C* and *E* were analysed according to the methodology of Barroso et al. (1998); lactoglobulin beta gene (*LGB*) alleles *A* and *B,* according to the methods of Strzalkowska et al. (2002); and stearoyl CoA desaturase 1 gene (*SCD1*) alleles *C* and *T*, according to the methods of Inostroza et al. (2013). The genotype and allelic frequencies were calculated.

Milk performance, composition and analysis of technological properties. Data on milk performance were collected from the dairy producers' databases of milk records. Milk yield in kg, fat and crude protein percentage were evaluated. The milk composition, i.e. the contents of fat, crude protein, casein, lactose monohydrate, non-fat solids (NFS), urea, citric acid, acetone, ketone as beta-hydroxybutyrate (BHB), and somatic cells as somatic cell count were determined in milk laboratories of the Czech-Moravian Breeders Association using infrared spectroscopy by filter technology and by technology with Fourier data transformation (FT), Foss Electric (Denmark) and Bentley Instruments (USA) instrumentation (Combi Foss 6000 and Bentley 2500 and Somacount). The milk instruments used for milk composition determination were calibrated monthly for main milk components (fat, crude protein, and lactose monohydrate) according to the results of the relevant reference methods (Kjeldahl mineralization, distillation and titration method for crude protein as milk total

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nitrogen × 6.38 and the Roese-Gottlieb extraction and gravimetric method for milk fat). The laboratories are accredited for the official milk recording analysis in the Czech Republic according to International Committee for Animal Recording (ICAR). Technological properties were evaluated by the milk fermentability (titration acidity of yogurt test), renneting was measured subjectively and instrumentally, and the heat stability of milk (ethanol or alcohol test) was determined. The measurement of technological properties was done repeatedly during the 1st and 2nd lactation. The numbers for the sample measures according to the respective genotypes are given in Table 1.

Table 1. Number of samples according to the respective genotypes measured for cow's milk technological properties

Gene	Genotype	Milk fermentability	Renneting measured subjectively	Renneting measured instrumentally	Ethanol test
DGAT1	*AA*	435	470	438	445
	KA	25	31	23	25
LEP	*MM*	288	315	289	293
	MW	81	92	84	83
	WW	14	15	13	14
FASN	*AG*	118	130	117	115
	GG	338	367	340	351
SCD1	*CC*	135	148	131	136
	TC	284	305	287	288
	TT	41	48	43	46
CSN2	*AA*	22	22	21	22
	AB	171	187	172	176
	BB	267	292	268	272
CSN2	A^1A^1	42	45	38	43
	A^1A^2	148	161	150	150
	A^2A^2	224	249	227	230
CSN3	*AA*	215	228	212	220
	AB	191	216	196	197
	BB	24	25	24	23
	BC	4	4	4	4
	EE	1	1	1	1
	AE	13	15	13	13
	BE	12	12	11	12
LGB	*AA*	12	15	8	12
	AB	390	416	393	394
	BB	58	70	60	64

The heat stability of milk was measured indirectly using milk ethanol stability, determined by milk titration (5 mL) with 96% ethanol to the point of the creation of the first visible milk protein precipitated flakes and is reported as mL of alcohol.

The milk fermentability or the yogurt test was carried out according to the Czech dairy industry standard ON 57 0534:1986. The sample of raw milk (50 mL) was heated at 85 °C for 5 min and cooled to 43 ± 2 °C. Subsequently, the sample was inoculated with 2 mL of the thermophilic lactic culture YC-180-40-FLEX (Chr. Hansen, Denmark; *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus*). The inoculated sample was incubated at 43 °C for 3.5 h. The result was expressed as the titration acidity of yogurt in mL of 0.25 mol/L NaOH × 100 m/L (or the so-called Soxhlet-Henkel degree).

Rennetability (conventional procedure) was determined during the tempering (35 °C) of a defined milk volume after the addition of rennet (1% vol.; bacterial rennet matter, enzyme Fromase 75TL), usually by measuring the time, while held at 35 ± 0.5 °C (rennet coagulation time RCT for milk protein) until the appearance of the first flakes of lactoproteins (beginning of coagulation).

Rennetability was also determined by nephelometry (turbidity measurement) for the assessment of the milk coagulation time (ML – 2 analysers, manual operation; Pribyla et al. 2006). This is the use of the optical method (NEF) for evaluating the intensity of the so-called diffusely scattered Tyndall light on dispersed particles (coagulating lactoprotein flakes).

Statistical analysis – correlations. Statistical analyses were performed using the SAS Version 9.4 software. Phenotype correlations between traits were computed by Pearson correlation coefficients (CORR Procedure, SAS 9.4). Partial correlations which measure the strength of the relationship between two variables while controlling the effect of another variable were also computed by the CORR Procedure.

Interactions. In this study, we tested interactions between the genotypes, genotypes and milk traits (protein %, fat %, and NFS %) and genotypes and the effect of the farm. To analyse the influence of the interactions on the technological quality of milk, the MIXED Procedure of the SAS system with repeated measurements per cow was used (the permanent effect of cow).

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Contrasts between effects were estimated by the LSM method. The tested interaction has been added as a new effect to the mathematical models described as follows:

Model 1

$Yogurt_{ijklmn} = \mu + gen_i + farm_j + protein_k + casein_l + lacs_m + Interaction_{xy} + pe_n + e_{ijklmnxy}$

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 % in milk;
$casein_l$ = fixed effect of casein content in milk;
$lacs_m$ = fixed effect of lactation stage in days;
$Interaction_{xy}$ = fixed effect of interaction between combination of two genotypes, combination of genotype and milk trait (protein %, fat %, NFS %) or farm, or combination of milk traits and farm;
pe_n = permanent effect of cow;
$e_{ijklmnxy}$ = random residual effect.

Model 2

$Rennetability_{ijklmn} = \mu + gen_i + farm_j + protein_k + NFS_l + season_m + Interaction_{xy} + pe_n + e_{ijklmnxy}$

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 % in milk;
NFS_l = fixed effect of NFS % in milk;
$season_m$ = fixed effect of season (class effect m = 1, 2, 3, 4)*;
$Interaction_{xy}$ = fixed effect of the interaction between the combination of two genotypes, combination of genotype and milk trait (protein %, fat %, NFS %) or farm, or combination of milk traits and farm;
pe_n = permanent effect of cow;
$e_{ijklmnxy}$ = 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, and February; 2 = March, April, and May; 3 = June, July, and August; 4 = September, October, and November.

Model 3

$Ethanol_{ijk} = \mu + gen_i + farm_j + Interaction_{xy} + pe_k + e_{ijkxy}$

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);
$Interaction_{xy}$ = fixed effect of the interaction between the combination of two genotypes, combination of genotype and milk trait (protein %, fat %, and NFS %) or farm, or combination of milk traits and farm;
pe_k = permanent effect of cow;
e_{ijkxy} = random residual effect.

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

As the effect of breed was not significant in the overwhelming majority of models for interactions, it was not involved.

RESULTS AND DISCUSSION

Effects of milk yield and composition on technological properties. The correlations between the milk technological properties and milk yield and the content of the components are given in Table 2. Generally, the correlation coefficients in the ethanol test were weak in most cases, both positive and negative, and nonsignificant. The only exceptions were the correlations with protein and fat without protein percentage, which were associated with the worse ethanol stability of milk, which is consequent and follows partly also from the principle of the test.

The milk fermentability (yogurt test) was correlated significantly with twelve characteristics, and moreover, protein without fat and acetone were near the significance threshold. The higher daily milk yield in kilograms was associated with the inferior milk fermentability, and the correlation of daily milk yield with renneting properties was negative; that is, the higher the milk yield, the better the coagulation, with the subjective

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Table 2. Pearson correlations for milk technological property and milk performance phenotypes

	Milk fermentability (mL NaOH)	Renneting measured subjectively (s)	Renneting measured instrumentally (s)	Ethanol test (mL ethanol)
Days in milk	0.0833	0.0456	0.1020	0.0342
Milk yield (daily) (kg)[1]	–0.1761*	–0.1037*	–0.0631	0.0323
Milk yield (305-day lactation) (kg)	–0.0596	0.0170	0.0734	0.1417
Fat (%)[1]	0.1785*	–0.0406	–0.0462	–0.0418
Fat (305-day lactation) (%)	0.1001**	–0.0206	–0.0225	–0.0405
Protein (%)[1]	0.0423	–0.0599	–0.0092	–0.1521*
Protein (305-day lactation) (%)	0.0976**	–0.0839**	–0.0354	–0.0561
Casein (%)[1]	0.3980*	0.0043	–0.0049	0.0289
Not fat solid (%)[1]	0.1698*	–0.0650	0.0255	–0.0537
Lactose (%)[1]	–0.1056*	–0.4022*	–0.2954	0.0377
Fat without protein (%)[1]	0.1640*	–0.0067	–0.1184*	–0.1233*
Protein without fat (%)[1]	0.0839	0.1381*	0.1888*	–0.0220
Casein without fat (%)[1]	0.3819*	0.0020	0.0284	0.0440
Somatic cell count (thousand/mL)[1]	0.1303*	0.2757*	0.1847*	–0.0540
Urea (mg/100 mL)[1]	0.1404*	–0.0623	–0.1168*	0.0503
Citric acid (%)[1]	–0.0151	–0.0375	–0.0793	–0.0703
Acetone (mmol/L)[1]	0.0869	–0.0731	–0.0636	–0.0314
Ketones BHB (mmol/L)[1]	0.1307*	0.0296	0.0519	–0.0241
Milk fermentability (mL NaOH)	x	–0.0494	–0.0485	0.0195
Renneting subj. (s)	x	x	0.6915**	0.0764
Renneting instr. (s)	x	x	x	–0.0122

[1]at the day of testing of technological properties; **($P < 0.01$), *($P < 0.05$)

measures being significantly different ($P < 0.05$). A positive and significant association with the milk fermentability was revealed for the content of fat, casein, NFS, fat without protein, casein without fat, urea and ketones on the day of testing, and average fat and protein lactation content. The percentage of lactose was negatively correlated with fermentability but it showed significantly better renneting properties.

Renneting that was measured subjectively as well as instrumentally had only a few significant associations with the contents of the milk components (Table 2). Joudu et al. (2008) reported significant negative correlations between rennet coagulation time and contents of fat, protein, caseins, beta lactoglobulin and lactose. In our analysis, only the lactose content on the day of testing to renneting, which was measured subjectively, and the fat without protein content on the day of testing to renneting, which was measured instrumentally, showed significant negative correlations. Interestingly, the protein percentage in the whole lactation also improved the renneting subjectively ($P < 0.01$), even though the degree of the relationship was very low (–0.0839). Protein without fat even showed a positive significant correlation. Fat, protein, casein, and NFS percentages on the day of testing had negative, but nonsignificant and low correlations with renneting. Similar results were reported by Beux et al. (2018), who found negative nonsignificant and very low correlations of rennet coagulation time with fat, protein, casein and lactose contents. Bittante et al. (2012), in their meta-analysis, summarized the results to show that the contents of fat, protein and casein have unrelated or contradictory relationships with the rennet coagulation time. Our results agree with this statement.

In this field research, mutual correlations between indicators of milk technological properties were nonsignificant and weak, with the exception of rennetability which was measured subjectively and instrumentally.

Somatic cell counts were significantly correlated with worse rennetability in concordance with Beux

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et al. (2018), and Joudu et al. (2008) found the same trend, but their correlations were nonsignificant.

Interactions of factors influencing milk technological properties. The *P*-values of interactions between genotypes are given in Tables 3 and 4, the detailed results for those interactions which were significant are in Supplementary Tables S1–S4 in Electronic Supplementary Material (ESM). For milk fermentability (yogurt test), *DGAT1* interacted with all other genes in the analysis, i.e. *LEP*, *FASN*, *SCD1*, *CNS2*, *CSN3* and *LGB,* and *LEP* interacted with the *CSN2* A^1/A^2 genotypes. The other genes did not show any interactions. For the ethanol test, which reflects the heat stability of milk, a significant interaction was observed in ten cases, most frequently for the *CSN2 A/B* genotypes and *LEP*. Ketto et al. (2018) described the importance of having the knowledge how different genetic variants of *CSN3* and *LGB* interact when studying the effects of milk protein genetic polymorphism on the technological properties. The authors noted that until then limited research was undertaken to relate the interaction of the noted genes during heat treatments. In our analysis, *CSN3* and *LGB* interacted in neither ethanol nor yogurt tests, nor in rennetability. This result can be affected by the structure of the tested population or the used statistical method and therefore the topic needs further research.

The same results were reported by Di Gregorio et al. (2017), who found that *CSN3* × *LGB* interactions were nonsignificant for coagulation time, curd firmness and cheese yield. Viale et al. (2017) found only one composite genotype significantly associated with milk coagulation properties, even though there were many composite genotypes associated with milk yield and composition traits. But their composite genotypes involved four SNPs in milk protein genes (*CSN1S1*, *CSN2*, *CSN3*), and six SNPs in other genes (*DGKG*, *PPARGC1A*, *GHR*, *AGPAT6*, *STAT5A*, *LPL*). Comin et al. (2008) evaluating the composite genotypes consisting of

Table 3. *P*-values of interactions between genotypes, ethanol test over diagonal, milk fermentability (yogurt test) below diagonal

	DGAT1	*LEP*	*FASN*	*SCD1*	*CSN2 A, B*	*CSN2* A^1, A^2	*CSN3*	*LGB*
DGAT1	x	0.2862	0.4051	0.8243	0.0039**	0.8741	0.2692	0.1593
LEP	< 0.0001**	x	0.0160*	0.2881	0.0026**	0.0155*	0.0054**	< 0.0001**
FASN	0.0016**	0.7201	x	0.7750	0.0068**	0.6593	0.1123	0.1755
SCD1	0.0021**	0.6594	0.5186	x	0.0304*	0.9995	0.3469	0.4059
CSN2 A, B	0.0004**	0.9663	0.8430	0.6667	x	0.0856	0.0051**	0.0058**
CSN2 A^1, A^2	< 0.0001**	0.0064**	0.2567	0.1517	0.2715	x	0.6495	0.1568
CSN3	0.0024**	0.8429	0.7276	0.9204	0.9917	0.3394	x	0.0678
LGB	0.0017**	0.7887	0.3253	0.8658	0.6894	0.1403	0.7764	x

**($P < 0.01$), *($P < 0.05$)

Table 4. *P*-values of interactions between genotypes, renneting subjectively over diagonal, renneting instrumentally below diagonal

	DGAT1	*LEP*	*FASN*	*SCD1*	*CSN2 A, B*	*CSN2* A^1, A^2	*CSN3*	*LGB*
DGAT1	x	0.9329	0.6930	0.1881	0.7711	0.9665	0.3697	0.6970
LEP	0.4620	x	0.5285	0.1452	0.9064	0.0960	0.6202	0.7041
FASN	0.4001	0.4898	x	0.0081**	0.4774	0.1993	0.2495	0.0907
SCD1	0.3860	0.0281*	0.0477*	x	0.0092**	0.0191*	0.1105	0.2449
CSN2 A, B	0.4434	0.8539	0.4531	0.0687	x	0.6038	0.4278	0.7587
CSN2 A^1, A^2	0.4495	0.9450	0.6685	0.4147	0.3111	x	0.2593	0.8896
CSN3	0.0472*	0.4168	0.0266*	0.0087**	0.0462*	0.0387*	x	0.2282
LGB	0.9156	0.8828	0.4316	0.5504	0.7976	0.9372	0.1656	x

**($P < 0.01$),*($P < 0.05$)

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Table 5. *P*-values of interactions between genotypes and farm, protein %, fat %, and not fat solid (NFS) %

	Interaction of genotype to				
	breed	farm	protein %	fat %	NFS %
DGAT1					
Fermentability	0.0030**	< 0.0001**	< 0.0001**	< 0.0001**	0.0014**
Renneting subj.	0.5598	0.0004**	< 0.0001**	0.7941	< 0.0001**
Renneting instr.	0.4815	< 0.0001**	< 0.0001**	0.0423*	0.0008**
Ethanol test	0.8190	0.0005**	0.7136	0.0715	0.9727
LEP					
Fermentability	0.4489	< 0.0001**	0.1709	0.5026	0.2381
Renneting subj.	0.1702	0.0015**	< 0.0001**	0.6271	< 0.0001**
Renneting instr.	0.9190	< 0.0001**	< 0.0001**	0.1433	0.0017**
Ethanol test	0.1649	< 0.0001**	0.4311	0.1156	0.6759
FASN					
Fermentability	0.4415	< 0.0001**	0.0038**	0.3167	0.0365*
Renneting subj.	0.1626	< 0.0001**	< 0.0001**	0.4504	< 0.0001**
Renneting instr.	0.3972	< 0.0001**	< 0.0001**	0.0105*	0.0002**
Ethanol test	0.9543	0.0023**	0.6217	0.0801	0.6254
SCD1					
Fermentability	0.4679	< 0.0001**	0.0089**	0.4140	0.0923
Renneting subj.	0.0570	< 0.0001**	< 0.0001**	0.0125*	< 0.0001**
Renneting instr.	0.4326	< 0.0001**	< 0.0001**	0.0263*	0.0004**
Ethanol test	0.9252	0.0030**	0.9811	0.1921	0.7909
***CSN2** A, B*					
Fermentability	0.2157	< 0.0001**	0.0095**	0.3415	0.0844
Renneting subj.	0.6327	0.0011**	< 0.0001**	0.2175	< 0.0001**
Renneting instr.	0.2217	< 0.0001**	< 0.0001**	0.0437*	0.0008**
Ethanol test	0.0526	< 0.0001**	0.0726	0.0074**	0.0556
***CSN2** A^1, A^2*					
Fermentability	0.3267	< 0.0001**	0.0075**	0.0654	0.0011**
Renneting subj.	0.7987	0.0081**	< 0.0001**	0.8754	< 0.0001**
Renneting instr.	0.0474*	< 0.0001**	< 0.0001**	0.0308*	0.0017**
Ethanol test	0.9914	0.0234**	0.6573	0.0796	0.9025
CSN3					
Fermentability	0.4982	< 0.0001**	0.0459**	0.7229	0.2975
Renneting subj.	0.1465	0.0002**	< 0.0001**	0.1982	< 0.0001**
Renneting instr.	0.0215*	< 0.0001**	< 0.0001**	0.0050**	0.0002**
Ethanol test	0.1953	0.0003**	0.0642	0.0033**	0.0401*
LGB					
Fermentability	0.0440*	< 0.0001**	0.0050**	0.2359	0.0494*
Renneting subj.	0.2727	0.0007**	< 0.0001**	0.3467	< 0.0001**
Renneting instr.	0.5876	< 0.0001**	< 0.0001**	0.0558	0.0020**
Ethanol test	0.2783	< 0.0001**	0.1150	0.0119*	0.0198*

**($P < 0.01$),*($P < 0.05$)

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CSN2 and *CSN3* genes found a strong effect on both milk coagulation traits and milk and protein yields, but not on fat and protein contents and other milk quality traits. For coagulation time, the best *CSN2–CSN3* genotypes were those with at least one *B* allele at both loci. The milk coagulation properties are quantitative traits, and milk protein gene polymorphisms contribute substantially to their variation. In their association study Penasa et al. (2010) revealed the strong effect of composite *CSN2* and *CSN3* genotypes on the variation of milk coagulation properties. The inclusion of composite genotype in their study greatly decreased the heritability estimates of MCP. However, the last three above-mentioned authors analysed composite genotypes, not the interactions. In our analysis, the milk protein genes interacted significantly only for the ethanol test, namely *CSN2* alleles *A*, *B* with *CSN3* and *LGB*, and for renneting measured instrumentally, *CSN3* with both polymorphisms in *CSN2* gene (Table 3 and Table 4).

For the renneting that was measured instrumentally (Table 4), our file showed some significant interactions, specifically *CSN3* with *DGAT1*, *FASN*, *SCD1* and both polymorphisms in *CSN2*; and *SCD1* with *LEP* and *FASN*. Amigo et al. (2001) did not find any interactions between *CNS2*, *CSN3* and *LGB* that significantly influenced the coagulation time and curd firmness.

Notably, any comparison of milk technological properties between the different studies is difficult and must be undertaken cautiously because the scale of factors affecting the traits is very broad: breed; milk proteins and their genetic variants; other major genes; polygenetic expression; gene interactions; methods of measuring technological properties; number of samples and animals; statistical method used. In addition, possible influences of inbreeding and heterosis remain unknown (Bittante et al. 2012).

As for interactions of other factors and genotypes, farm interacted significantly with genotypes of all genes for all indicators of technological quality (Table 5). These results suggest the importance of conditions in which the cows live and produce. Bittante et al. (2012) found out the strong effect of the permanent environment of cows, even though the quantitative influence of the herd was relatively low.

The protein percentage interacted significantly with all genes but not with the ethanol test. The fat content did not interact with gene polymorphism in most cases. The breed and genotypes interacted exceptionally, so the polymorphisms had the same effect both in Holstein and Czech Simmental breeds. The ethanol test was influenced by interactions just scarcely with the exception of farm × genotypes interaction, as mentioned above. Also correlations of the ethanol test with the contents of the milk components (Table 2) were mostly nonsignificant, suggesting the independence and robustness of the test.

Table 6. *P*-values of interactions between farm, protein %, fat % and not fat solid (NFS) %

	Farm	Protein %	Fat %	NFS %
Farm				
Fermentability	x	< 0.0001**	0.0009**	0.0021**
Renneting subj.	x	< 0.0001**	0.0003**	< 0.0001**
Renneting instr.	x	0.0003**	< 0.0001**	< 0.0001**
Ethanol test	x	0.0042**	0.2001	0.0043**
Protein %				
Fermentability	x	x	0.3263	0.0030**
Renneting subj.	x	x	0.1875	< 0.0001**
Renneting instr.	x	x	0.1178	0.0488*
Ethanol test	x	x	0.0781	0.6486
Fat %				
Fermentability	x	x	x	0.0064**
Renneting subj.	x	x	x	0.3662
Renneting instr.	x	x	x	0.0056**
Ethanol test	x	x	x	0.0610

**($P < 0.01$), *($P < 0.05$)

The interactions of farm, protein, fat and NFS contents (Table 6) again confirmed the role of the farm. Protein and fat did not interact; protein and NFS did.

Repeatability was not calculated because the data set was too small for a reliable variance component estimation (Vostry et al. 2007).

CONCLUSION

Milk technological properties are influenced by multiple effects. The study of the network of factors, including the interactions, is of great importance and economic impact. In this study, we observed mostly nonsignificant and weak correlations of the milk component percentages with renneting (rennet coagulation time). Correlations

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of the components with results of the ethanol test (indirect milk thermostability indicator) were significant only scarcely, and correlations with milk fermentability (yogurt test titration acidity) were somewhat closer. The *CSN3* and *LGB* genotypes did not show a significant interaction effect on milk fermentability, renneting and result of the ethanol test. Approximately one-third of the interactions between the gene polymorphisms significantly influenced the ethanol test. The influence of interactions between the genotypes on fermentability and renneting was even smaller. Breed and genotypes did not interact; therefore, the polymorphisms have the same effect on Holstein and Simmental breeds. The factor most often showing significance by interaction was farm, namely, with all genotypes for all technological properties and with the protein, fat and NFS percentages. These results could contribute to the elucidation and improvement of the milk technological quality. Here, the use of gene polymorphisms is believed to be promising.

REFERENCES

Alipanah M, Kalashnikova LA. Influence of κ-casein genetic variant on cheese making ability. J. Anim. Vet. Adv., 2007;6:855-857.

Amigo L, Martin-Alvarez PJ, Garcia-Muro E, Zarazaga I. Effect of milk protein haplotypes on the composition and technological properties of Fleckvieh bovine milk. Milchwissenschaft, 2001;56:488-491.

Barroso A, Dunner S, Canon 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;76:1535-1538.

Beux S, Cassandro M, de Lima VA, Zielinski AAF, Nogueira A, Waszczynskyj N. Seasonal effect on milk composition, somatic cell content and milk coagulation properties of Italian Holstein-Friesian cows. Emir. J. Food Agr., 2018;30:998-1005.

Bittante G, Penasa M, Cecchinato A. Genetics and modeling of milk coagulation properties. J. Dairy Sci., 2012;95: 6843-6870.

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;34:105-116.

Cattani M, Guzzo N, Mantovani R, Bailoni L. Effects of total replacement of corn silage with sorghum silage on milk yield, composition, and quality. J. Anim. Sci. Biotechnol., 2017;8:15.

Comin A, Cassandro M, Chessa S, Ojala M, Dal Zotto R, De Marchi M, Carnier P, Gallo L, Pagnacco G, Bittante G. Effects of composite β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. J. Dairy Sci., 2008;91:4022-4027.

Di Gregorio P, Di Grigoli A, Di Trana A, Alabiso M, Maniaci G, Rando A, Valluzzi C, Finizio D, Bonanno A. Effects of different genotypes at the *CSN3* and *LGB* loci on milk and cheese-making characteristics of the bovine Cinisara breed. Int. Dairy J., 2017;71:1-5.

Djedovic R, Bogdanovic V, Perisic P, Stanojevic D, Popovic J, Brka M. Relationship between genetic polymorphism of k-casein and quantitative milk yield traits in cattle breeds and crossbreds in Serbia. Genetika, 2015;47:23-32.

Ghelichkhan M, Amanlou H, Mahjoubi E, Patton RA. Solvent-extracted soybean meal top-dressed on a fresh cow diet increased milk production, but not milk components, and decreased plasma non-esterified fatty acids. Czech J. Anim. Sci., 2019;64:26-40.

Gustavsson F, Buitenhuis AJ, Glantz M, Stalhammar H, Lindmark-Mansson H, Poulsen NA, Larsen LB, Andren A, Paulsson M. Impact of genetic variants of milk proteins on chymosin-induced gelation properties of milk from individual cows of Swedish Red dairy cattle. Int. Dairy J., 2014;39:102-107.

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. Pecu., 2013;26:263-269.

Joudu I, Henno M, Kaart T, Pussa T, Kart O. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. Int. Dairy J., 2008;18:964–967.

Kasna E, Zavadilova L, Stipkova M. Genetic evaluation of clinical mastitis traits in Holstein cattle. Czech J. Anim. Sci., 2018;63:443-451.

Ketto IA, Oeyaas J, Adney T, Johansen A-G, Schuller RB, Narvhus J, Skeie SB. The influence of milk protein genetic polymorphism on the physical properties of cultured milk. Int. Dairy J., 2018;78:130-137.

Krizova L, Hanus O, Hadrova S, Kucera J, Samkova E, Roubal P, Vesely A. Composition, physical and technological properties of raw milk as affected by cattle breed, season and type of diet. Ann. Anim. Sci., 2014;14:721-736.

Krpalkova L, Cabrera VE, Zavadilova L, Stipkova M. The importance of hoof health in dairy production. Czech J. Anim. Sci., 2019;64:107-117.

Kuhn C, Thaller G, Winter A, Bininda-Edmonds ORP, Kaupe B, Erhardt G, Bennewitz J, Schwerin M, Fries R. 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;167:1873-1881.

https://doi.org/10.17221/150/2019-CJAS

Kyselova J, Jecminkova K, Matejickova J, Hanus O, Kott T, Stipkova M, Krejcova M. Physiochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of β-casein, κ-casein, and β-lactoglobulin. Asian-Australas. J. Anim. Sci., 2019;32:14-22.

Manuelian CL, Penasa M, Visentin G, Benedet A, Cassandro M, de Marchi M. Multi-breed herd approach to detect breed differences in composition and fatty acid profile of cow milk. Czech J. Anim. Sci., 2019;64:11-16.

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.

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;97:3396-3401.

Miluchova M, Gabor M, Trakovicka A. Analysis of Slovak Spotted breed for bovine beta casein A1 variant as risk factor for human health. Acta Biochim. Pol., 2013;60:799-801.

Penasa M, Cassandro M, Pretto D, De Marchi M, Comin A, Chessa S, Dal Zotto R, Bittante G. Short communication: Influence of composite casein genotypes on additive genetic variation of milk production traits and coagulation properties in Holstein-Friesian cows. J. Dairy Sci., 2010;93:3346-3349.

Pribyla L, Cerny V, Havova R, Prochazkova J. [Description of Curling Capacity of Milk, Nephelo-Turbidimetric Sensor for Milk Coagulation]. Prague, Czech Republic: Institute of Analytical Chemistry AS CR, Technology Centre of AS CR; 2006. Available at: www.iach.cz, www.tc.cz, www.cett.cz, www.circ.cz. Czech.

Roy R, Ordovas L, Zaragosa P, Romero A, Moreno C, Altarriba J, Rodellar C. Association of polymorphisms in the bovine *FASN* gene with the milk fat content. Anim. Genet., 2006;37:215-218.

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.

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 mater., 2018;6:154-162.

Viale E, Tiezzi F, Maretto F, De Marchi M, Penasa M, Cassandro M. Association of candidate gene polymorphisms with milk technological traits, yield, composition, and somatic cell score in Italian Holstein-Friesian sires. J. Dairy Sci., 2017;100:7271–7281.

Vostry L, Pribyl J, Vesela Z, Jakubec V. Selection of a suitable data set and model for the estimation of genetic parameters of the weaning weight in beef cattle. Arch. Tierz., 2007;50:562-574.

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