**Table S1.** The number of purebred and crossbred cows in the analysis

| Breed | n | % |
|---|---:|---:|
| Holstein 100% | 329 | 43.98 |
| Holstein 75% + Simmental | 17 | 2.27 |
| Holstein 50% + Simmental, Ayrshire | 12 | 1.60 |
| Simmental 100% | 259 | 34.63 |
| Simmental 75% + Holstein | 71 | 9.50 |
| Simmental 75% + Ayrshire | 40 | 5.35 |
| Simmental 75% + Holstein, Ayrshire | 20 | 2.67 |
| Sum | 748 | 100 |

**Table S2.** Sequences of primers used in the polymerase chain reactions

| Locus | Primer | Sequence |
|---|---|---|
| *DGAT1* | Forward | 5´ - GCA CCA TCC TCT TCC TCA AG – 3´ |
| | Reverse | 5´- GGA AGC GCT TTC GGA TG – 3´ |
| *LEPTIN* | Forward | 5´- ATG CGC TGT GGA CCC CTG TAT C – 3´ |
| | Reverse | 5´- TGG TGT CAT CCT GGA CCT TCC – 3´ |
| *FASN* | Forward | 5´- AGA GCT GAC GGA CTC CAC AC – 3´ |
| | Reverse | 5´- GCC GAT GCA CTC GAT GTA G – 3´ |
| *SCD1* | Forward | 5´- ACC TGG CTG GTG AAT AGT GCT – 3´ |
| | Reverse | 5´- TCT GGC ACG TAA CCT AAT ACC CT- 3´ |
| *CSN3* | Forward | 5´- TGT GCT GAG TAG GTA TCC TAG TTA TGG – 3´ |
| | Reverse | 5´- GCG TTG TCT TCT TTG ATG TCT CCT TAG – 3´ |
| *CSN2 (*$A^1$, $A^2$*)* | Forward | 5´- CCT TCT TTC CAG GAT GAA CTC CAG G – 3´ |
| | Reverse | 5´- GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT – 3´ |
| *CSN2 (A, B)* | Forward | 5´- CCA GAC ACA GTC TCT AGT CTA TCC C – 3´ |
| | Reverse | 5´- CAA CAT CAG TGA GAG TCA GGC TCC G – 3´ |
| *LGB* | Forward | 5´- TGT GCT GGA CAC CGA CTA CAA AAA G – 3´ |
| | Reverse | 5´- GCT CCC GGT ATA TGA CCA CCC TCT -3´ |

**Table S3.** Frequencies of genotypes and alleles of Holstein and Czech Simmental cows and crosses

| | | Cows of all breeds | | | | Holstein cows and crosses | | | | Czech Simmental cows and crosses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Genotype | n | Relative frequencies | $\chi^2$ | Allele frequencies | n | Relative frequencies | $\chi^2$ | Allele frequencies | n | Relative frequencies | $\chi^2$ | Allele frequencies |
| *DGAT1* | *AA* | 716 | 0.959 | 0.043 | *A* 0.979 | 337 | 0.974 | 0.020 | 0.987 | 379 | 0.945 | 0.009 | 0.973 |
| | *KA* | 31 | 0.041 | | *K* 0.021 | 9 | 0.026 | | 0.013 | 22 | 0.055 | | 0.027 |
| | *KK* | 0 | 0.000 | | | 0 | 0.000 | | | 0 | 0.000 | | |
| *LEP* | *MM* | 489 | 0.769 | 3.263 | *M* 0.865 | 236 | 0.792 | 5.355 | 0.876 | 253 | 0.749 | 1.914 | 0.855 |
| | *MW* | 122 | 0.192 | | *W* 0.135 | 50 | 0.168 | | 0.124 | 72 | 0.213 | | 0.145 |
| | *WW* | 25 | 0.039 | | | 12 | 0.040 | | | 13 | 0.038 | | |
| *FASN* | *AA* | 2 | 0.003 | 1.931 | *A* 0.140 | 0 | 0 | 4.938 | 0.167 | 2 | 0.005 | 0.659 | 0.116 |
| | *AG* | 204 | 0.274 | | *G* 0.860 | 115 | 0.333 | | 0.833 | 89 | 0.223 | | 0.884 |
| | *GG* | 539 | 0.723 | | | 230 | 0.667 | | | 309 | 0.773 | | |
| *SCD1* | *CC* | 207 | 0.279 | 3.681 | *C* 0.571 | 113 | 0.328 | $6.663^{*}$ | 0.623 | 94 | 0.236 | 2.538 | 0.525 |
| | *CT* | 434 | 0.584 | | *T* 0.429 | 204 | 0.591 | | 0.377 | 230 | 0.578 | | 0.475 |
| | *TT* | 102 | 0.137 | | | 28 | 0.081 | | | 74 | 0.186 | | |
| *CSN2* | *AA* | 18 | 0.025 | 2.257 | *A* 0.105 | 12 | 0.037 | $7.748^{*}$ | 0.103 | 6 | 0.015 | 0.191 | 0.106 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *AB* | 115 | 0.159 | | *B* | 0.895 | 43 | 0.132 | | 0.897 | 72 | 0.181 | | 0.894 |
| | *BB* | 589 | 0.816 | | | | 270 | 0.831 | | | 319 | 0.804 | | |
| *CSN2* | $A^1A^1$ | 80 | 0.114 | 0.887 | $A^1$ | 0.306 | 39 | 0.125 | 0.313 | 0.335 | 41 | 0.105 | 1.553 | 0.283 |
| | $A^1A^2$ | 270 | 0.385 | | $A^2$ | 0.694 | 132 | 0.422 | | 0.665 | 138 | 0.355 | | 0.717 |
| | $A^2A^2$ | 352 | 0.501 | | | | 142 | 0.454 | | | 210 | 0.540 | | |
| *CSN3* | *AA* | 346 | 0.471 | 3.876 | *A* | 0.694 | 179 | 0.534 | 1.529 | 0.733 | 167 | 0.419 | 9.246 | 0.662 |
| | *AB* | 308 | 0.420 | | *B* | 0.275 | 122 | 0.364 | | 0.243 | 186 | 0.466 | | 0.301 |
| | *BB* | 36 | 0.049 | | *C* | 0.001 | 18 | 0.054 | | 0.001 | 18 | 0.045 | | 0.001 |
| | *AE* | 19 | 0.026 | | *E* | 0.030 | 11 | 0.033 | | 0.022 | 8 | 0.020 | | 0.036 |
| | *BE* | 21 | 0.029 | | | | 4 | 0.012 | | | 17 | 0.043 | | |
| | *EE* | 2 | 0.003 | | | | 0 | 0.000 | | | 2 | 0.005 | | |
| | *BC* | 2 | 0.003 | | | | 1 | 0.003 | | | 1 | 0.003 | | |
| *LGB* | *AA* | 17 | 0.024 | 64.31[**] | *A* | 0.473 | 5 | 0.015 | 68.708[**] | 0.471 | 12 | 0.030 | 61.256[**] | 0.475 |
| | *AB* | 648 | 0.899 | | *B* | 0.527 | 296 | 0.911 | | 0.529 | 352 | 0.889 | | 0.525 |
| | *BB* | 56 | 0.078 | | | | 24 | 0.074 | | | 32 | 0.081 | | |

[*] Significant differences between genotype frequencies calculated on the basis of Hardy-Weinberg equilibrium and empirical frequencies ($p < 0.05$)

[**] Significant differences between genotype frequencies calculated on the basis of Hardy-Weinberg equilibrium and empirical frequencies ($p < 0.01$)

**Table S4.** Descriptive statistics of the milk performance of Holstein and Czech Simmental cows

| | | Milk, kg | | Protein, % | | Protein, kg | | Fat, % | | Fat, kg | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Genotype | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *DGAT1* | *AA* n=1344 | 8392 | 2418 | 3.47 | 0.23 | 288.4 | 75.7 | 4.12 | 0.35 | 344.6 | 98.9 |
| | *KA* n=60 | 7590 | 2607 | 3.49 | 0.26 | 261.3 | 79.7 | 4.04 | 0.30 | 306.4 | 102.8 |
| *LEP* | *MM* n=925 | 8419 | 2429 | 3.45 | 0.23 | 287.9 | 75.6 | 4.11 | 0.35 | 345.0 | 98.4 |
| | *MW* n=229 | 8112 | 2320 | 3.50 | 0.23 | 282.0 | 75.0 | 4.13 | 0.37 | 334.4 | 98.6 |
| | *WW* n=45 | 7843 | 2938 | 3.50 | 0.25 | 271.4 | 96.1 | 4.13 | 0.40 | 325.1 | 121.8 |
| *FASN* | *AG* n=378 | 8535 | 2475 | 3.44 | 0.22 | 290.8 | 77.3 | 4.13 | 0.36 | 350.2 | 101.0 |
| | *GG* n=1018 | 8299 | 2417 | 3.48 | 0.23 | 286.0 | 75.6 | 4.11 | 0.34 | 340.5 | 98.8 |
| *SCD1* | *CC* n=398 | 8587 | 2570 | 3.43 | 0.24 | 291.5 | 79.1 | 4.10 | 0.37 | 349.9 | 102.2 |
| | *TC* n=811 | 8427 | 2401 | 3.47 | 0.22 | 290.2 | 76.1 | 4.13 | 0.34 | 347.0 | 99.6 |
| | *TT* n=187 | 7627 | 2062 | 3.53 | 0.22 | 266.8 | 63.5 | 4.12 | 0.33 | 312.9 | 82.5 |
| *CSN2* | *AA* n=32 | 8229 | 2663 | 3.41 | 0.29 | 279.3 | 85.9 | 4.25 | 0.35 | 349.1 | 114.1 |
| | *AB* n=220 | 8602 | 2340 | 3.50 | 0.24 | 297.8 | 70.8 | 4.14 | 0.41 | 354.7 | 97.0 |
| | *BB* n=1105 | 8334 | 2441 | 3.47 | 0.23 | 286.3 | 76.3 | 4.11 | 0.34 | 341.6 | 99.3 |
| *CSN2* | $A^1A^1$ n=143 | 7931 | 2332 | 3.48 | 0.25 | 274.8 | 75.5 | 4.15 | 0.38 | 329.4 | 96.3 |
| | $A^1A^2$ n=501 | 8418 | 2475 | 3.46 | 0.23 | 288.7 | 78.2 | 4.11 | 0.35 | 345.4 | 102.1 |
| | $A^2A^2$ n=675 | 8240 | 2437 | 3.48 | 0.23 | 283.6 | 75.6 | 4.10 | 0.34 | 336.6 | 98.5 |
| *CSN3* | *AA* n=646 | 8497 | 2431 | 3.43 | 0.23 | 288.5 | 74.9 | 4.09 | 0.34 | 345.9 | 96.6 |
| | *AB* n=586 | 8267 | 2420 | 3.50 | 0.22 | 287.1 | 76.3 | 4.14 | 0.35 | 341.3 | 101.2 |
| | *BB* n=70 | 8355 | 2658 | 3.54 | 0.23 | 293.9 | 88.2 | 4.22 | 0.37 | 352.1 | 109.7 |
| | *BC* n=4 | 8140 | 405 | 3.51 | 0.12 | 285.5 | 19.5 | 4.33 | 0.22 | 352.3 | 27.3 |
| | *EE* n=3 | 5627 | 2452 | 3.79 | 0.09 | 212.0 | 89.2 | 4.23 | 0.08 | 237.7 | 102.1 |
| | *AE* n=32 | 7594 | 2535 | 3.47 | 0.20 | 261.8 | 84.0 | 4.20 | 0.43 | 317.1 | 108.1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *BE* n=40 | 8139 | 2307 | 3.51 | 0.21 | 283.5 | 72.3 | 4.04 | 0.38 | 329.9 | 104.2 |
| *LGB* | *AA* n=30 | 7066 | 2208 | 3.55 | 0.23 | 249.6 | 73.2 | 4.00 | 0.37 | 284.2 | 86.2 |
| | *AB* n=1222 | 8484 | 2438 | 3.47 | 0.23 | 291.5 | 75.7 | 4.12 | 0.35 | 348.4 | 99.7 |
| | *BB* n=103 | 7511 | 2115 | 3.47 | 0.21 | 258.6 | 68.2 | 4.12 | 0.35 | 309.2 | 85.5 |

n, number of lactations of cows with a particular genotype; SD, standard deviation

**Table S5.** Descriptive statistics of milk technological qualities of Holstein and Czech Simmental cows

| Gene | Genotype | Milk fermentation ability, ml NaOH | | | Renneting assessed subjectively, seconds | | | Renneting assessed instrumentally, seconds | | | Ethanol test, ml of ethanol | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | Mean | SD | n | Mean | SD | n | Mean | SD | n | Mean | SD |
| *DGAT1* | *AA* | 435 | 14.85 | 3.90 | 470 | 504.01 | 240.79 | 438 | 312.19 | 134.25 | 445 | 0.988 | 0.997 |
| | *KA* | 25 | 16.92 | 4.73 | 31 | 438.00 | 156.24 | 23 | 266.22 | 118.09 | 25 | 0.904 | 1.011 |
| *LEP* | *MM* | 288 | 15.38 | 4.03 | 315 | 495.02 | 216.87 | 289 | 310.95 | 136.11 | 293 | 0.902 | 0.831 |
| | *MW* | 81 | 14.66 | 3.90 | 92 | 503.55 | 273.26 | 84 | 308.83 | 141.95 | 83 | 0.992 | 1.058 |
| | *WW* | 14 | 15.34 | 4.15 | 15 | 478.80 | 351.77 | 13 | 264.92 | 122.52 | 14 | 1.436 | 1.746 |
| *FASN* | *AG* | 118 | 15.42 | 3.91 | 130 | 500.99 | 240.29 | 117 | 312.08 | 141.91 | 115 | 1.036 | 1.050 |
| | *GG* | 338 | 14.78 | 3.97 | 367 | 500.76 | 236.97 | 340 | 310.16 | 130.85 | 351 | 0.970 | 0.985 |
| *SCD1* | *CC* | 135 | 14.77 | 4.10 | 148 | 489.42 | 239.55 | 131 | 311.48 | 134.66 | 136 | 1.025 | 1.115 |
| | *TC* | 284 | 14.92 | 3.93 | 305 | 495.77 | 232.94 | 287 | 303.10 | 127.72 | 288 | 0.988 | 1.009 |
| | *TT* | 41 | 15.88 | 3.86 | 48 | 558.69 | 249.82 | 43 | 350.44 | 163.46 | 46 | 0.835 | 0.341 |
| *CSN2* | *AA* | 22 | 14.54 | 3.62 | 22 | 561.00 | 345.83 | 21 | 300.24 | 132.23 | 22 | 0.800 | 0.506 |
| | *AB* | 171 | 13.93 | 3.79 | 187 | 457.60 | 215.03 | 172 | 296.63 | 118.20 | 176 | 0.923 | 0.896 |
| | *BB* | 267 | 15.66 | 3.98 | 292 | 522.42 | 237.22 | 268 | 319.17 | 142.67 | 272 | 1.038 | 1.084 |
| *CSN2* | $A^1A^1$ | 42 | 15.53 | 4.53 | 45 | 554.56 | 337.56 | 38 | 344.32 | 152.31 | 43 | 0.800 | 0.276 |
| | $A^1A^2$ | 148 | 14.49 | 3.87 | 161 | 494.07 | 199.76 | 150 | 319.75 | 124.05 | 150 | 0.994 | 0.987 |
| | $A^2A^2$ | 224 | 15.12 | 3.98 | 249 | 514.01 | 244.57 | 227 | 309.87 | 136.74 | 230 | 1.009 | 1.075 |
| *CSN3* | *AA* | 215 | 15.03 | 4.14 | 228 | 540.74 | 249.31 | 212 | 330.25 | 147.92 | 220 | 0.961 | 0.995 |
| | *AB* | 191 | 14.84 | 3.92 | 216 | 469.88 | 235.72 | 196 | 288.19 | 116.86 | 197 | 0.944 | 0.899 |
| | *BB* | 24 | 16.22 | 3.00 | 25 | 443.12 | 143.47 | 24 | 290.17 | 103.40 | 23 | 1.576 | 1.774 |
| | *BC* | 4 | 16.20 | 3.74 | 4 | 337.00 | 74.13 | 4 | 216.00 | 136.45 | 4 | 0.850 | 0.252 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *EE* | 1 | 11.60 | - | 1 | 473.00 | - | 1 | 352.00 | - | 1 | 0.900 | - |
| | *AE* | 13 | 15.12 | 3.47 | 15 | 423.87 | 166.76 | 13 | 318.38 | 136.59 | 13 | 0.750 | 0.284 |
| | *BE* | 12 | 12.88 | 3.83 | 12 | 535.25 | 145.28 | 11 | 367.64 | 126.23 | 12 | 1.217 | 0.980 |
| *LGB* | *AA* | 12 | 15.71 | 4.79 | 15 | 426.60 | 184.67 | 8 | 233.88 | 80.23 | 12 | 0.650 | 0.294 |
| | *AB* | 390 | 14.89 | 3.95 | 416 | 492.02 | 232.22 | 393 | 307.16 | 134.32 | 394 | 0.950 | 0.960 |
| | *BB* | 58 | 15.28 | 4.03 | 70 | 562.60 | 264.13 | 60 | 338.00 | 131.15 | 64 | 1.252 | 1.239 |

n, number of samples from cows with a particular genotype; SD, standard deviation