QTL mapping for production traits in Czech Fleckvieh cattle

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ABSTRACT: The objective of this study was to find QTL for milk production traits in Czech Fleckvieh cattle on chromosomes 6, 7, 11, 14, and 23 where QTL were previously identified in other dairy cattle populations. Sixteen grandsire families were genotyped for 38 microsatellite markers on the selected chromosomes. A QTL mapping model based on variance component analysis was implemented via restricted maximum likelihood (REML) to estimate QTL positions and their effects. A significant QTL affecting fat percentage was found at the beginning of chromosome 14 (0 cM), near marker ILSTS039. Suggestive QTL associated with milk production traits appeared on other studied chromosomes (BTA6, BTA7, BTA11, and BTA23). This first QTL search on five chromosomes in Czech Fleckvieh population showed several suggestive QTL that can be promising for further studies and contribute to better understanding of genetics of milk production in the Czech Fleckvieh cattle.

Keywords: chromosomes; markers; quantitative trait loci; milk traits; cattle; REML

The main goal of QTL mapping studies in livestock populations is to identify genes or markers which could be used in breeding programs through marker assisted selection (MAS). The MAS has the potential to increase genetic gain faster and is expected to be more effective than traditional selection systems (Abdel-Azim and Freeman, 2002). All the relevant chromosomes have been searched for QTL associated with milk production traits in dairy cattle. Significant QTL were often found on chromosome 6 and were associated mostly with protein percentage (PP) and fat percentage (FP), and then also with yield traits – milk yield (MY), protein yield (PY), and fat yield (FY) (Freyer et al., 2003; Ashwell et al., 2004; Olsen et al., 2004; Szyda et al., 2005; Chen et al., 2006; Kučerová et al., 2006). QTL reported on BTA7 affected FY and FP (Ron et al., 2004; Weller et al., 2008). QTL associated with PP and FP, and also with PY and FY were detected on BTA11 (Mosig et al., 2001; Ashwell et al., 2004; Kučerová et al., 2006). The association between QTL at the beginning of BTA14 for FP was found in the cited studies. Moreover, this QTL was also found affecting FY, MY, PP, and PY (Weller et al., 2003; Ashwell et al., 2004; Schrooten et al., 2004; Kučerová et al., 2006). QTL found at BTA23 were associated with PP and FP (Mosig et al., 2001; Schrooten et al., 2004).

No QTL search has been carried out in Czech Fleckvieh cattle so far. The population of this local dual-purpose breed is not large but it is a part of the European Fleckvieh population (German Fleckvieh, Austrian Fleckvieh, etc.) with the same phylogenetical origin (Bouška et al., 2006). On the basis of previous studies finding QTL on the previously identified chromosomes in Czech Fleckvieh population for milk production traits was expected.

The aim of this study was to detect QTL associated with milk production traits (FY, FP, MY, PY, and PP) in Czech Fleckvieh cattle on chromosomes 6, 7, 11, 14, and 23 where QTL were already identified in other dairy cattle populations.

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MATERIAL AND METHODS

Animals

A granddaughter design population structure (Weller et al., 1990) including families of 16 grandsires of Czech Fleckvieh cattle was used for the QTL study. Each sire \((n = 352, \text{ min. } = 7, \text{ max. } = 24)\) had at least 30 daughters with records. DNA was obtained from semen of sires. Breeding values (BV) of observed animals were obtained from the national official database of progeny testing. Breeding values for 5 milk production traits (MY, PY, FY, PP, and FP) were used for the analyses and were calculated by the Czech-Moravian Breeders Corporation Ltd. using a multi-trait test-day model.

Genetic markers

Grandsires and their sons were genotyped for 38 markers. The marker names and their position based on the linkage map are presented in Table 1. Marker positions were obtained from the USDA cattle marker map (http://www.marc.usda.gov/genome). Genotyping was carried out at the Institute of Animal Science, Prague-Uhříněves, Department of Molecular Genetics.

Statistical analysis

Statistical analysis was carried out with the aid of the software package DMU (Madsen et al., 2006) using the average information REML algorithm (Jensen et al., 1997). The restricted likelihood was maximized with respect to the variance components associated with the random effects in the model. Maximizing a sequence of restricted likelihoods over a grid of specific positions yields a profile of the restricted likelihood of the QTL position (Sørensen et al., 2003). The multivariate mixed model was as follows:

\[
y = X\beta + Zu + Wq + e
\]

where:

\(y\) = vector of records (breeding value recorded for each individual on observed parameter: milk, protein or fat yield, protein or fat content)

\(X\) = matrix relating records to the fixed effects

\(\beta\) = vector of fixed effects representing overall mean of trait

\(Z\) = matrix relating the records of each individual to polygenic effects

\(u\) = vector of additive polygenic effects

\(W\) = matrix relating the record of each individual to its QTL effect

\(q\) = vector of QTL effect

\(e\) = vector of residuals

The random variables \(u, q,\) and \(e\) are assumed to be multivariate, normally distributed, and mutually uncorrelated. The details are reported in Lund et al. (2003).

**IBD matrices.** The elements in the IBD matrix are a function of the marker data and the position \((p)\) of a putative QTL on the chromosome. Here we used the most likely marker linkage phase in the sire and computed the IBD + matrix using a recursive algorithm (Wang et al., 1995; Sørensen et al., 2003). The IBD matrices were computed for every 5 cM along the chromosomes and used in the subsequent variance component estimation procedure using the average information restricted maximum likelihood algorithm (described above).

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>No. of markers</th>
<th>Markers (positions in cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>9</td>
<td><strong>MNB197</strong> (8.05) <strong>MNB66</strong> (29.37) <strong>DIK1054</strong> (44.98) <strong>BM143</strong> (53.72) <strong>BMS470</strong> (67.40) <strong>BMS360</strong> (72.88) <strong>BM415</strong> (81.96) <strong>AFR227</strong> (96.99) <strong>BM4203</strong> (119.05)</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td><strong>BM269</strong> (14.68) <strong>BM6105</strong> (36.95) <strong>BM6117</strong> (62.25) <strong>BMS2258</strong> (77.19) <strong>BM1853</strong> (85.33) <strong>ILSTS006</strong> (116.63) <strong>BMS1979</strong> (126.25)</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td><strong>BM304</strong> (33.59) <strong>INRA177</strong> (35.09) <strong>BM3169</strong> (50.31) <strong>BMS1822</strong> (65.88) <strong>DIK4892</strong> (74.59) <strong>DIK3333</strong> (89.87) <strong>TGLA436</strong> (105.21)</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td><strong>ILSTS039</strong> (0) <strong>C5SM006</strong> (5.13) <strong>DIK5092</strong> (17.85) <strong>DIK2964</strong> (37.91) <strong>RM011</strong> (43.63) <strong>BMS740</strong> (60.60) <strong>BM4305</strong> (83.30) <strong>BM6425</strong> (95.14)</td>
</tr>
<tr>
<td>23</td>
<td>7</td>
<td><strong>BM47</strong> (13.77) <strong>DIK5399</strong> (20.66) <strong>BM1258</strong> (28.30) <strong>MGTG7</strong> (47.76) <strong>RM185</strong> (52.29) <strong>BP34</strong> (64.37) <strong>DIK4203</strong> (73.78)</td>
</tr>
</tbody>
</table>
**Significance level.** Hypothesis tests for the presence of QTL were based on the asymptotic distribution of the likelihood ratio test (LRT) statistic:

\[
LRT = -2 \ln (L_{\text{reduced}} - L_{\text{full}})
\]

where:

- \(L_{\text{reduced}}\) = maximized likelihoods under the reduced model
- \(L_{\text{full}}\) = maximized likelihoods under the full model

The reduced model always excluded the QTL effect for the chromosome being analyzed:

\[
y = X\beta + Zu + e
\]

Thresholds were calculated using the method presented by Piepho (2001).

**RESULTS AND DISCUSSION**

**Chromosome 14**

Significant QTL with effect on fat percentage was detected on chromosome 14 at position 0 cM near marker *ILSTS039* (Table 2, Figure 4). QTL peaks for PP and FY were also shown at the same position but did not exceed the significant threshold. Variance component estimates for the effect of this QTL on FP are presented in Table 3. The QTL explained about 15% of total variance for FP. As reviewed by Khatkar et al. (2004), many studies detected QTL at 0 cM position associated in most of the cases with FP, then also with FY, and in some cases with other milk production traits. The same findings (QTL at 0 cM position affecting FP) were made by Rodriguez-Zas et al. (2002) in the US Holstein population and by Druet et al. (2006) in the French Holstein cattle. Ashwell et al. (2004) reported QTL at similar position (46 cM) but affecting PP and FY in Danish Holstein cattle. Two QTL associated with PP were reported by Ashwell et al. (2004) at positions 51 and 106 cM in Holstein breed. Moreover, they reported QTL for PP at 49 cM and for FY at 24 cM. However, Olsen et al. (2004) detected QTL with effect on PP and FP at approximate position 13 cM within

Two suggestive QTL associated with protein percentage were also observed at positions 20 and 55 cM on BTA14 (Figure 3). At a similar position (56 cM), Moisio et al. (2000) reported a QTL for PP in Finnish Ayrshire cattle. However, Mosig et al. (2001) detected QTL affecting PP at 79 cM position in the Israeli Holstein population. Bagnato et al. (2008) found QTL affecting PP and MY in the region 0–11 cM in the Brown Swiss population.

**Chromosome 6**

No significant QTL was found on chromosome 6 (Table 2). At position 120 cM (marker *BM6117*) a suggestive QTL with effect on PY, FY, and MY was observed (Figure 1). Schrooten et al. (2004) reported QTL at similar position (113 cM) but with effect on PP and PY in Holstein-Friesian cattle. Szyda et al. (2005) also detected QTL with effects on PP, FY, and MY between 9 and 30 cM in German Holstein cattle.

Two non-significant QTL peaks for PP appeared at positions 40 and 70 cM (Figure 1). Kučerová et al. (2006) found QTL at similar (46 cM) position affecting PP and FP in Danish Holstein cattle. Two QTL associated with PP were reported by Ashwell et al. (2004) at positions 51 and 106 cM in Holstein breed. Moreover, they reported QTL for FP at 49 cM and for FY at 24 cM. However, Olsen et al. (2004) detected QTL with effect on PP and FP at approximate position 13 cM within

![Figure 1](image-url)
BMS2508 and FBN12 marker bracket in dual-purpose Norwegian Dairy cattle. Druet et al. (2006) reported QTL effect on all observed milk production traits except for MY at position 50 cM in the French Holstein population. Near this position (marker BM143) Kowalewska-Luczak et al. (2010) examined the PPARGC1A gene associated with milk yield and milk protein content. Cohen-Zinder et al. (2005) identified the ABCG2 gene located at 38 Mb on BTA6 associated with milk fat and protein concentration in dairy cattle. The suggestive peak we observed at 40 cM is probably due to ABCG2 gene segregating in Czech Fleckvieh cattle population.

Chromosome 7

Suggestive QTL with the effect on all analyzed milk traits was found between 60 cM (BM6117) and 70 cM (BMS2258) on chromosome 7 (Table 2). The QTL peak closest to the significance threshold was for MY at 70 cM (Figure 2). As reviewed by Khatkar et al. (2004), QTL associated with all milk production traits were reported by several studies in this region on BTA7. Similarly, Ron et al. (2004) detected QTL with effects on yield traits at approximate position 60 cM in the Israeli Holstein population. Another QTL affecting all milk production traits except for MY was found near 15 cM. Mosig et al. (2001) searched for QTL associated with protein percentage in the Israeli Holstein cattle and found one at 60 cM and another at 91 cM. Rodriguez-Zas et al. (2002) detected a QTL with the effect on PP in the US Holstein population close to the marker BMS2258 at position 81 cM.

Table 2. Results of QTL search on the studied chromosomes

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL on chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>position (cM) (nearest marker)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>–</td>
</tr>
<tr>
<td>Fat yield (kg)</td>
<td>120 (BMC4203)</td>
</tr>
<tr>
<td>Milk yield (kg)</td>
<td>120 (BMC4203)</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20 (MNB66)</td>
</tr>
<tr>
<td>Protein yield (kg)</td>
<td>120 (BMC4203)</td>
</tr>
</tbody>
</table>

*P < 0.05
LRT = likelihood ratio test statistic (LRT = –2 ln (L_reduced – L_full)), where L_reduced and L_full represent the maximized likelihoods under the reduced and full models.
at 40 cM near marker INRA177 (Table 2, Figure 3). Two nearby low QTL peaks for PP and FP at 45 cM were also observed. Bagnato et al. (2008) searched for QTL associated with MY and PP in Brown Swiss population and detected QTL affecting MY at similar position (55 cM). Other two QTL with effect on both traits were reported at 19 cM and 82–117 cM, respectively. Mosig et al. (2001) mapped QTL associated with PP in the Israeli Holstein population and found two QTL, one at position 48 cM and the second one at position 85 cM. Ashwell et al. (2004) also reported two QTL with the effect on PY and FY at positions 83 and 90 cM in Holstein breed. However, Kučerová et al. (2006) found a QTL associated with PP at 70 cM.
Chromosome 23

Two suggestive QTL affecting protein percentage were found at positions 27 cM (BM1258) and 55 cM (RM185) (Table 2, Figure 5). Mosig et al. (2001) detected QTL associated with PP at positions 7 cM (CSSM5) and 17 cM (RM33) in the Israeli Holstein population. Bagnato et al. (2008) reported QTL affecting PP and MY in a similar region 43–56 cM in Brown Swiss cattle. Khatkar et al. (2004) reviewed the QTL effect on PP as the most reported effect on chromosome 23 in QTL studies.

A QTL for fat percentage was shown at 60 cM position (BP34) and a QTL for milk yield appeared at 35 cM position (BM1258) on BTA23 (Table 2, Figure 5). However, Schrooten et al. (2004) found QTL with effect on MY and PY at 8 cM in Holstein-Friesian cattle. Bolečková et al. (2012) studied at 50 cM position PRL polymorphisms which were associated with MY, PY, and FY in Czech Fleckvieh cattle.

Summary

The present QTL study was able to confirm the segregation of DGAT1 on BTA14 in Czech Fleckvieh cattle and also gave suggestive evidence for segregation of ABCG2 gene on BTA6. Though there was indication of QTL for milk yield traits segregating on all the five studied chromosomes, only the QTL for FP on BTA14 reached the significant threshold. QTL affecting multiple milk yield traits were located in the same genomic regions on several chromosomes (Table 2). This could be due to pleiotropic QTL. However, the phenotypes (BVs) were obtained from a multi-trait model. As the genetic correlations among milk yield traits are high – close to 1 (also previously reported by Dědková and Wolf, 2001), it is also possible that QTL is affecting only one trait and the effects observed on the other traits are due to genetic correlation among them. There were ample evidences of gene/QTL segregating in chromosomal regions from various other cattle populations where suggestive evidence for QTL segregation was found. These give confidence that the QTL we have found being real. However, closer look into the QTL will be necessary by analyzing with denser marker map in a bigger sample size.

CONCLUSION

First QTL search on five chromosomes in Czech Fleckvieh cattle showed promising results. Significant QTL affecting fat percentage was detected on chromosome 14 at 0 cM position (near marker ILSTS039). Several suggestive QTL associated with milk production parameters appeared on other studied chromosomes (BTA6, BTA7, BTA11, and BTA23). The results provide information for further studies and contribute to better understanding of genetics of milk production in Czech Fleckvieh cattle.

Acknowledgement

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REFERENCES


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**Table 3. Variance component estimates of significant QTL found on BTA14**

<table>
<thead>
<tr>
<th>BTA</th>
<th>QTL position (cM)</th>
<th>Trait</th>
<th>QTL variance</th>
<th>Sire variance</th>
<th>Residual variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0</td>
<td>FP</td>
<td>0.0044 ± 0.000045</td>
<td>0.0051 ± 0.000081</td>
<td>0.0199 ± 0.000034</td>
</tr>
</tbody>
</table>

*P < 0.05, FP = fat percentage (%)


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