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Quantitative trait loci affecting lactose and total solids on chromosome 6 in Brazilian Gir dairy cattle

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ABSTRACT. Fourteen Brazilian Gir sire families with 657 daughters were analyzed for quantitative trait loci (QTL) on chromosome 6 affecting lactose and total solids. Cows and sires were genotyped with 27 microsatellites with a mean spacing between markers of 4.9 cM. We used a 1% chromosome-wide threshold for QTL qualification. A QTL for lactose yield was found close to marker MNB66 in three families. A QTL for total solid yield was identified close to marker BMS2508 in three families. A QTL for lactose percentage, close to marker DIK1182, was identified in two families. A QTL for total solid percentage, close to marker MNB208, was identified in four families. These QTLs could be used for selection of animals in dairy production systems.

Key words: *Bos indicus*; Dairy cattle; Daughter design; Cattle genome

INTRODUCTION

The main objective of quantitative trait loci (QTL) mapping is to find genes/markers that can be implemented in breeding programs by marker-assisted selection (MAS). In dairy cattle, MAS could be used to pre-select young candidate bulls prior to progeny testing, thus increasing selection differentials, shortening generation interval and increasing genetic gain. In dairy cattle a number of studies have shown that quantitative trait loci (QTL) can be detected and mapped in commercial dairy cattle populations using the daughter design strategy (Lipkin et al., 1998; Ron et al., 2001; Chen et al., 2006). Different studies have found a large number of QTL affecting milk production, and all 29 bovine autosomes have been suggested as harboring QTL for these traits (Khatkar et al., 2004).

Lactose, the major carbohydrate of milk, controls milk volume by maintaining its osmolarity. Therefore, the rate of lactose synthesis in the epithelial cells of the mammary gland serves as a major factor influencing milk volume (Neville et al., 1983; Cant et al., 2002; Zhao and Keating, 2007). Because lactose is the major osmotic molecule in milk it is tempting to suggest that many QTL detected for milk yield are due to the genetic factors related to lactose synthesis and secretion. However, it should not be forgotten that lactose is not the only osmotic component of milk. Some minerals, especially calcium and phosphorus, affect osmotic potential, too (Viitala et al., 2003). The effect of this process is to leave the total amount of other milk constituents such as proteins and solids unchanged. Therefore, although milk yield is increased, the concentration of its constituents is decreased (Shahbazkia et al., 2010).

Despite the fact that a large amount of information focuses on mapping QTL for milk production traits (i.e., milk yield, protein yield and percentage, and fat yield and percentage) on BTA6 (*Bos taurus* autosome 6), the vast majority of QTL were detected in *Bos taurus* breeds. Further studies are needed to map QTL for milk production traits in *Bos indicus* breeds such as dairy Gir, since this breed is very important to tropical countries due to its heat and parasite tolerance use in the formation of Gir x Holstein crossbreeds that are extensively used for milk production in Brazil and throughout the tropics.

The Brazilian Gir was imported from India in the 1930s and introduced in herds aimed at meat production, but later in the 60s some Gir breeders started a selection for milk production and although the number of imported animals was relatively small, these animals adapted very well in the Brazilian environment and multiplication developed rapidly (Santiago, 1985). Breeding programs for economically important traits of Zebu dairy cattle have been recently introduced in Brazil and Gir was the first Zebu breed in the world with a program of genetic evaluation. The first genetic evaluation of Gir sires in the progeny test was performed in 1993, and a total of nineteen early evaluations have been conducted so far (Verneque et al., 2011).

The aim of this work was to map QTL for milk composition traits (lactose yield and percentage and total solids yield and percentage) on chromosome 6 in Brazilian Gir breed through a daughter design strategy.

MATERIAL AND METHODS

Population sample

From the Brazilian National Program for Improvement of the Dairy Gir Cattle database, a

total of 14 half-sib families with a minimum of 20 daughters containing records for milk composition traits were selected. Blood samples were collected from 657 Brazilian Gir dairy cattle cows and semen samples were collected from 14 sires. The half-sib families and the informative number of daughters per sire family used in the analysis are given in Table 1. Daughters were considered informative if the daughter genotype was different from her sire's genotype (Ron et al., 1996).

Table 1. Half-sib families and informative number of daughters per sire family used to map QTL for milk composition traits in Dairy Gir cattle.

Family	Sire	Daughters	Percent
1	001	127	19.3
2	129	94	14.3
3	224	68	10.4
4	293	62	9.4
5	356	59	8.9
6	416	46	7.0
7	463	34	5.2
8	498	28	4.3
9	527	28	4.3
10	556	24	3.6
11	581	23	3.5
12	605	22	3.3
13	628	22	3.3
14	651	20	3.0
Total	14	657	100

Marker data

A total of 27 microsatellite markers were selected from bovine chromosome 6, according to the Meat Animal Research Center (MARC) map (Ihara et al., 2004). The average spacing among markers was 4.9 cM. Markers spanned from 0 to 130.8 cM of chromosome 6. Markers were chosen based on their positions (cM), number of alleles and Polymorphic Information Content (PIC). Marker order and map distances among markers were estimated with the "fixed" option of the CRIMAP 2.4 program (Green et al., 1990), with map distances based on Kosambi's mapping function. The order of markers achieved was generally consistent with the MARC map, although the generated map covered 134.5 cM. The MARC consensus map was the map of choice for the association studies since it was generated with various breeds: Angus, Australian Friesian, Boran, Brahman, Brangus, Charolais, Gelbvieh, Gir, Hereford, Holstein, Indubrasil, Nelore, N'Dame, Normande, Piedmontese, Sahiwal and Simmental (Bishop et al., 1994; Barendse et al., 1994; Ihara et al., 2004). Moreover, it was shown that the differences in estimated recombination frequency did not bias the test for QTL or estimates of QTL effects (Haley and Knott, 1992).

DNA samples were extracted from semen and blood with modifications from the Sambrook and Russel (2001) protocol. PCR reactions were performed using 45 ng template DNA, 0.2 mM dNTPs, 20 mM Tris, pH 8.3, 50 mM KCl, and 0.1 μM of each primer. An $MgCl_2$ concentration was determined for each marker. Forward primers were labeled with fluorescent dye (FAM, HEX or TAMRA). Annealing temperatures of PCR ranged from 50° to 58°C. Cycling parameters consisted of 35 cycles at 94°C for 1 min, annealing temperature for 1 min, 72°C for 1 min, and one extension step of 45 min at 72°C.

PCR reactions were loaded on a MegaBACE 1000 DNA sequencer (GE Healthcare,

Buckinghamshire, United Kingdom). Fragment analysis, size calling, and binning were analyzed using Fragment Profiler software. The markers genotyped on chromosome 6, their map location, numbers of alleles and number of heterozygous sires for each marker are given in Table 2. Marker heterozygosity and Polymorphic Information Content (PIC) was calculated with the program CERVUS 2.0 (Marshall et al., 1998).

Table 2. Map location, number of alleles and number of heterozygous sires for each genotyped marker on chromosome 6.

No.	Marker	Map location (cM)[1]	Alleles (MARC)[1]	Alleles (Embrapa)[3]	Heterozygous sires
1	ILST093	0.0	21	9	11
2	DI4408	9.02	9	12	11
3	DIK5285	15.3	7	17	13
4	DIK4498	20.1	8	8	8
5	MNB66	29.3	11	12	12
6	BM1329	35.39	9	11	8
7	DIK1058	38.16	7	8	12
8	BMS2508	43.93	9	8	14
9	DIK4382	50.09	10	14	13
10	DIK4482	54.5	7	8	10
11	MNB-208	60.2	7	9	11
12	BM4322	63.86	6	12	8
13	BMS470	67.4	9	9	10
14	DIK3026	71.5	9	9	11
15	DIK2294	75.27	10	17	13
16	DIK4867	81.96	7	8	7
17	ILSTS035	87.26	19	18	10
18	DIK4574	90.5	10	6	8
19	BMS5021	93.8	8	11	10
20	AFR227	96.98	11	11	10
21	DIK2174	101.4	5	8	12
22	DIK4827	107.12	7	10	13
23	DIK2995	109.9	5	6	12
24	DIK1182	115.32	14	9	10
25	DIK2690	121.49	4	8	11
26	BM2320	127.49	10	21	13
27	DIK4992	130.78	4	7	7

[1]MARC/USDA (Meat Animal Research Center/United States Department of Agriculture) (Ihara et al., 2004). [2]Embrapa - Brazilian National Program for Improvement of the Dairy Gir Cattle.

Phenotypic records

Genetic improvement of milk traits in Dairy Gir cattle is based on the use of genetically superior sires in the herds belonging to the Brazilian National Program for Improvement of Dairy Gir Cattle. These sires are often evaluated in progeny test programs using the best linear unbiased prediction (BLUP) and animal model (Arnold et al., 1992). This method has also been used for the genetic evaluation of Zebu dairy cattle (Martinez et al., 2005). Phenotypic data of milk composition traits, lactose yield (LY), total solids yield (SY), lactose percentage (LP) and total solids percentage (SP) were obtained for 657 cows. These traits over 305 days, preadjusted for calving age and month, were analyzed by the repeatability animal model (Weller et al., 1990):

$$y = Xb + Za + Wpe + e \qquad \text{(Equation 1)}$$

where y = vector of observations; b = vector of fixed effects; a = vector of random animal

effects; *pe* = vector of random residual effect, and *X*, *Z* and *W* are incidence matrices relating records to fixed, animal and permanent environmental effects, respectively. The genetic base for all traits was the mean breeding value of cows born in 2000. The EBV (Estimated Breeding Values) from November 2008 evaluations were used as phenotypic data for this study. Means, standard deviations, minimum and maximum values for EBV of the cows are given in Table 3.

Table 3. Mean, standard deviation, minimum and maximum values of estimated breeding values of the Gir cows for milk composition traits.

Trait	Mean	SD	Minimum	Maximum
Lactose yield (kg)	15.407	13.186	-12.2	61.5
Total solids yield (kg)	45.837	33.08	-44.3	182.1
Lactose (%)	0.026	0.0677	-0.190	0.264
Total solids (%)	0.095	0.1923	-0.456	0.864

Statistical analysis for QTL mapping

Weller et al. (1990) proposed the use of the granddaughter design (GDD) and daughter design (DD) as methods for QTL detection in dairy cattle. For a DD, genotypic information is recorded for sires and their daughters, with phenotypic observations made on daughters. The QTL analysis for 27 markers was accomplished using the linear model:

$$BV_{ijkl} = S_{ij} + M_{ijk} + e_{ijk} \quad \text{(Equation 2)}$$

where BV_{ijkl} is the estimated breeding value for trait *i* of cow *l*, daughter of sire *j*, that received paternal allele k; S_{ij} is the effect of sire *j* on trait *i*; M_{ijk} is the effect of paternal allele k of sire *j* on trait *i*, and e_{ijkl} is the random residual associated with each record. A significant paternal allele effect is indicative of a segregating QTL linked to the genetic marker (Ron et al., 2001). The linkage analysis was performed using the regression approach described by Knott et al. (1996), and using the web-based software GridQTL (http://www.gridqtl.org.uk) (Seaton et al., 2006). The analysis was carried out across all families and the significant families were identified based on the absolute *t* value with degrees of freedom equal to the number of informative daughters (*n*) in the family, and when ABS(*t*) was higher than the critical value of 5% ($t_{0.025,n}$). Chromosome-wide significance thresholds of 5 and 1% were established in the GridQTL along with ten thousand permutations (Churchill and Doerge, 1994). The 95% confidence intervals of QTL locations were determined by bootstrapping.

RESULTS

Information content

The mean number of heterozygous sires per marker was 10.6 (75.7%) and the marker with all 14 heterozygous sires corresponded to BMS2508, located in the position 43.93 cM. The information content across 14 families, family 3 and family 8 on chromosome 6 is plotted in Figure 1. This is calculated from variance of the conditional probabilities of inheriting a chromosomal region at each centiMorgan as a proportion of the variance when true descent is known (Chen et al., 2006).

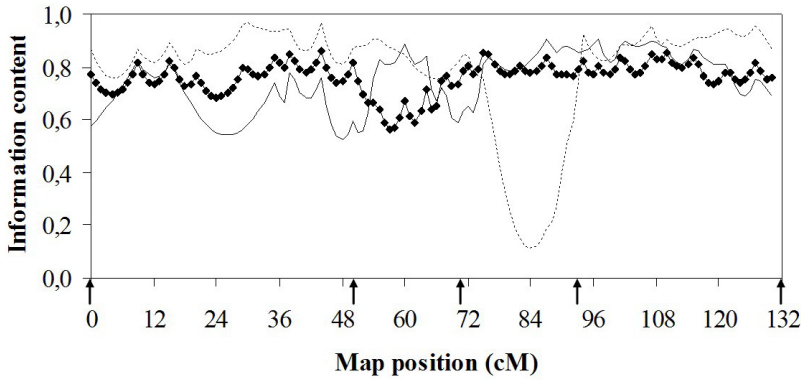

Figure 1. Information content across 14 families (♦), within family 3 (—), and within family 8 (---). Location of 5 out of 27 markers are indicated by arrows.

Between the markers IST093 (0.0 cM) and DIK4382 (50.09 cM) the information content was higher for family 8 and lower for family 3. The information content between makers DIK4382 and DIK3026 (71.5 cM) was lower across 14 families in comparison to families 3 and 8. For family 8, the information content between markers DIK3026 and BMS5021 (93.8 cM) was even lower, and between markers BMS5021 and DIK4992 (130.78), all values were above 0.78 for family 3, family 8 and across all families.

Analysis across families

For each family and trait, families 8, 11 and 13 were identified as significant for lactose yield (LY); families 3, 8 and 9 for total solids yield (TSY); families 4 and 7 for lactose percentage (LP); and families 1, 2, 3 and 4 for total solids percentage (TSP). The F-value, 1% chromosome-wide threshold, significant family for the 4 milk production traits, substitution effect, standard deviation (SE) and absolute *t*-value are shown in Table 4.

Table 4. Quantitative trait loci location, nearest marker, F-value, 1% chromosome-wide threshold, significance families, substitution effect, standard error (SE), and *t*-value.

Trait	Location (cM)	Nearest marker	F-value[1]	Threshold	Family	Effect[2]	SE	ABS (*t*)[3]
LY	29	MNB66	6.22	5.95	8	-14.26	4.37	3.26
					11	-10.32	5.45	1.89
					13	12.30	5.83	2.10
TSY	43	BMS2508	7.69	7.66	3	16.11	8.05	2.00
					8	-38.69	10.52	3.67
					9	-27.55	11.69	2.35
LP	114	DIK1182	9.02	7.75	4	0.056	0.016	3.35
					7	-0.077	0.029	2.60
TSP	58	MNB208	5.27	4.64	1	-0.101	0.052	1.93
					2	-0.115	0.058	1.98
					3	-0.136	0.054	2.49
					4	0.300	0.112	2.66

LY = lactose yield; TSY = total solids yield; LP = lactose percentage; TSP = total solids percentage. [1]Significance of the effect paternal marker allele computed all heterozygous sires. [2]Marker allele substitution effect for LY, SY, LP and SP. [3]Significance of the within-family effect of paternal marker allele.

The 95% confidence interval was estimated by using 5,000 bootstrap samples. The length of the 95% confidence interval ranged from 5 to 87 cM for LY in the significant families; for TSY the length of the 95% confidence interval ranged from 3 to 83 cM; for LP the lengths of the 95% confidence interval ranged from 21 to 116 cM; for TSP it ranged from 23 to 94 cM (data not shown).

In Figure 2, the statistical test (F-value) is shown for the traits LY, TSY, LP and TSP in the significant families. The highest peak in all four traits was detected for LP, located near position 114 cM, close to marker DIK1182 and the lowest peak was detected for TSP, located near position 58 cM, close to marker MNB208. The peak for LY was located near position 29 cM, close to marker MNB66 and the peak for TSY was located at position 43 cM, close to marker BMS2508.

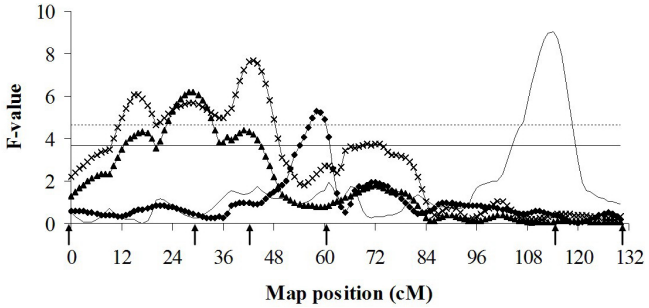

Figure 2. F-values for the analysis of families significant for lactose yield (- -), total solids yield (-x-), lactose percentage (—) and total solids percentage (-♦-). The positions of the most significant markers are indicated by arrows. Thresholds at the 5% (—) and 1% (---) chromosome-wide level for the trait total solids percentage.

Analysis within family

Within-family analyses were carried out for each of the nine significant families listed in Table 4, but only results for families 3, 4 and 8 are presented here, which are the most significant among these nine families. In family 3, the F-values did not reach the 1% chromosome-wide threshold for all traits (Table 5) and only the F-value for TSY was above the 5% chromosome-wide threshold showing a peak at the position 48 cM (Figure 3). The lengths of the 95% confidence intervals ranged from 116 to 125 cM.

Table 5. Quantitative trait loci location, nearest marker, F-value, 1% chromosome-wide threshold, and 95% confidence intervals of QTL positions after analyses within family 3, 4 and 8 for the lactose and total solids production traits.

Family	Trait	Location (cM)	Nearest marker	F-value	Threshold	95%CI (cM)
3	LY	12	DIK4408	7.66	11.49	1-126
	TSY	48	DIK4382	9.63	11.96	3-125
	LP	18	DIK4498	3.38	11.44	13-130
	TSP	58	MNB208	7.51	11.87	14-130
4	LY	65	BM4322	5.70	13.62	17.5-127
	TSY	65	BM4322	3.84	13.31	6-128
	LP	114	DIK1182	10.96	13.45	13-115
	TSP	57	DIK4482	7.76	13.70	0.0-113
8	LY	41	BMS2508	10	13.82	4-91
	TSY	16	DIK5285	9.88	14.61	2-89
	LP	16	DIK5285	2.67	13.06	0.0-120
	TSP	128	BM2320	1.79	13.51	0.0-129

LY = lactose yield; TSY = total solids yield; LP = lactose percentage; TSP = total solids percentage; CI = confidence interval.

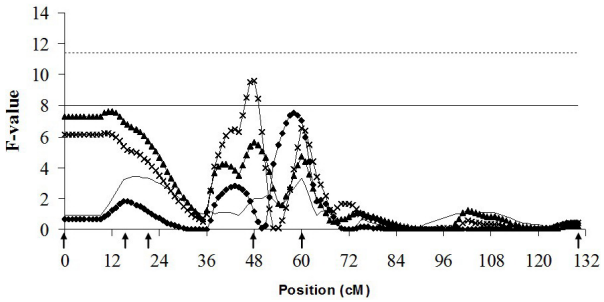

Figure 3. F-values for lactose yield (-▲-), total solids yield (-x-), lactose percentage (—), and total solids percentage (-♦-), from the analysis within family 3. The positions of the most significant markers are indicated by arrows. Thresholds at 5% (—) and 1% (---) chromosome-wide for the trait lactose percentage.

Analysis within family 4 showed only one significant peak for LP, located at position 114 cM, with F-value above the 5% chromosome-wide level (Figure 4). The lengths of the 95% confidence intervals ranged from 102 to 122 cM.

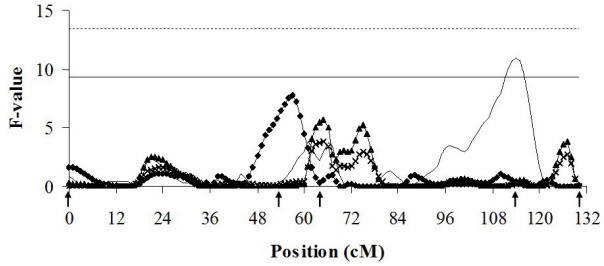

Figure 4. F-values for lactose yield (-▲-), total solids yield (-x-), lactose percentage (—), and total solids percentage (-♦-), from the analysis within family 4. The positions of the most significant markers are indicated by arrows. Thresholds at the 5% (—) and 1% (---) chromosome-wide level for the trait lactose percentage.

Analysis within the family 8 showed two defined peaks for LY and TSY, located at positions 16 and 41 cM with F-values above the 5% chromosome-wide threshold (Figure 5).

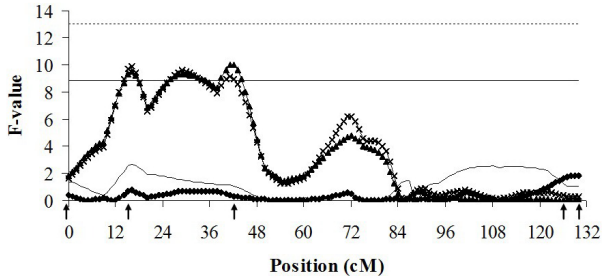

Figure 5. F-values for lactose yield (-▲-), total solids yield (-x-), lactose percentage (—), and total solids percentage (-♦-), from the analysis within family 8. The positions of most significant markers are indicated by arrows. Thresholds at the 5% (—) and 1% (---) chromosome-wide level for the trait lactose percentage.

DISCUSSION

In dairy cattle, most emphasis has been placed on detecting QTL affecting milk production. It is believed that QTL for milk production can be found in nearly all autosomal chromosomes (Khatkar et al., 2004). A large number of studies found QTL for milk production traits on chromosome 6 in *Bos taurus*, nevertheless QTL mapping studies in *Bos indicus* are very rare and therefore needed. The appropriate population structure for the daughter design strategy (several sires, each with many daughters with milk-recorded) can also be found in moderately sized populations, such as the Brazilian Dairy Gir breed, whose progeny testing program was initiated in 1985.

In daughter design procedures, only a fraction of the sires will be heterozygous for any particular marker, and not all genotypes are informative (Ron et al., 2001). The mean number of heterozygous sires per marker was 10.6 (75.7%) and the marker with all 14 heterozygous sires corresponded to BMS2508, located in the position 43.93 cM.

It should also be noted that information content between markers DIK3026 and BMS5021 for the family 8 was too low. This would result in a decreased test statistic if a QTL is segregating in this chromosomal region.

As pointed out by Darvasi et al., (1993), the confidence interval of the QTL position on the chromosome is very important information. In this study, most of the 95% confidence interval found was large and may be due to the relatively small family sizes (Table 1). For most breeds, family sizes are lower than optimum for QTL detection, thus detection power is low in most studies. As a consequence, many QTL are missed, and the effect of those detected is often overestimated (Olsen et al., 2002). Confirmation of results in independent studies is therefore needed to reveal which effects are authentic. Analysis of data from different breeds or populations might also provide additional insight into the genetics controlling the trait of interest.

In theory, the analysis of individual families should reveal QTL not detected in a joint analysis of all families because heterozygosity at QTL may be low because of selection (Georges et al., 1995). However, significance thresholds become extremely high for the analysis of individual families because it is necessary to account for the additional comparisons. This problem is one of the paradoxes in QTL mapping (Heyen et al*.,* 1999). In the present study only tree QTL were detected in individual families with F-value above the 5% chromosome-wide level.

With respect to the traits related to milk production, because of the physiological correlation between milk quantity and milk composition and the correlation among the various milk components, a single QTL can be expected to effect more than one milk production trait. There is a high genetic correlation between milk yield and lactose yield, 0.92 in Holstein according to Welper and Freeman (1992). The increase or decrease of lactose synthesis results in a change in milk volume but without a change in protein or fat yield.

It is also possible that milk production QTL are clustered, so that different studies are identifying different QTL located in the same general chromosomal region, but affecting different aspects of milk production. Cross-study comparisons are complicated by the fact that the different studies used different designs and levels of significance and examined different traits. The effects on milk production traits on BTA6 have stood out from several genome-scan studies (Georges et al., 1995; Zhang et al., 1998; Ashwell et al., 2001; Mosig et al., 2001), and a set of QTL were mapped to a large region along BTA6.

The number of individuals genotyped in this study was much smaller than many pre-

vious milk production QTL results of chromosome 6, since the progeny test program in the Brazilian Dairy Gir is relatively new. Despite that, in the analysis across 14 families and within the most significant families, QTL affecting lactose yield, total solids yield, lactose percentage and total solids percentage were detected at the 1% chromosome-wide threshold. These QTL for milk composition traits are the first results reported in the literature in the Gir breed and maybe for other *Bos indicus* dairy breeds also.

CONCLUSIONS

These results provide another example of the power of the daughter design for the detection of QTL in dairy cattle and demonstrate that QTL with considerable effects on milk production traits can be found in dairy cattle populations selected for increased milk production. The mapped QTL contribute to the genetic variance of milk production traits, which is exploited by artificial selection programs. The QTL detected in this work can be further investigated and implemented in marker-assisted selection in dairy production systems. Fine mapping or multitrait QTL mapping would improve mapping resolution and the estimation accuracy of the QTL.

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