FINE MAPPING OF MILK YIELD QTL ON CHROMOSOMES 6 AND 20 IN GERMAN HOLSTEIN POPULATION USING MICROSATELLITE MARKERS

Reinecke PETER, Reißmann MONIKA, Müller UWE, Abdel-Rahman SALAH*

Institute of Livestock Sciences, Faculty of Agriculture and Horticulture, Humboldt University, Berlin, Germany
*Correspondence: Abdel-Rahman SALAH, Fax: 002 034593423, Tel.: 002 034593422 and E-mail: salahmaa@yahoo.com
Current address: Genetic Engineering Institute, Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt

Manuscript received: July 18, 2005; Reviewed: September 25, 2005; Accepted for publication: September 25, 2005

ABSTRACT

In German Holstein population a very significant QTL with effects on milk yield trait (MY) was mapped on chromosome 6 and 20 to the interval BM143–ILSTS97 and AGLA29–ILSTS72, respectively. Where, three markers BM143, FBN13 and ILSTS97 on chromosome 6 and two markers AGLA29 and ILSTS72 on chromosome 20 were associated with highly significant effects ($P<0.01$) for milk yield. Findings from this study could be used in subsequent fine-mapping work and applied to marker-assisted selection (MAS) of dairy production traits.

KEY WORDS: dairy cattle, production traits, microsatellite markers, association analysis

INTRODUCTION

Most traits of economic importance in farm animals are of quantitative nature, i.e., are influenced by many genes and by environmental factors [23]. For example, milk production traits in dairy cattle is quantitative in nature. The phenotypes observed are thus the combined results of the action of large numbers of polygenes or quantitative trait loci (QTL) and environmental factors. The discovery of the genomically widespread and highly polymorphic microsatellite markers, and the subsequent development of reasonably dense microsatellite linkage maps for the bovine genome allowed a much finer exploration of animal genomes than was previously possible [22].

Linkage analyses have identified several chromosomal regions that influence livestock production traits. For example, milk production traits in dairy cattle have received considerable attention over the last 14 years and a number of quantitative trait loci (QTL) associated with these traits have been localized to particular chromosomal regions, actually. Where, segregating QTL for milk yield trait on bovine chromosomes 6 and 20 have been found in American Holsteins [7, 23], Canadian Holsteins [14], Dutch Holsteins [18,1], German Holsteins [11,10,5,6,19], British Black and White cattle [22], Israeli Holsteins [17], and Norwegian dairy cattle [8,15,16]. All these analyses, except [17], were based on granddaughter design (GDD) analyses. [17] used a daughter design for quantitative trait loci effects on chromosome 6 for five milk production traits.

The main objective of this study was to identify the significant markers which flanking the QTL for milk yield trait in a very close distance (fine-mapping), using monozygotic (MZ) female twins of the German Holstein population.

MATERIALS AND METHODS

Animals. Fifteen female monozygotic (MZ) twin pairs from German Holstein population were evaluated for marker-QTL associations. Eight twin pairs out of the fifteen came of natural birth and the other seven twin pairs constructed biotechnologically (Embryo-splitting) in the test station. After integration the animals in the test station the twin pairs were separated in two groups. One group was fed with high energy feed (high concentrated ration) and the other was fed with low energy (low concentrated ration). The female twin pairs were artificial inseminated in a weight from 390 to 410 kg. This gave a difference for the calving time with 7 months approximately, according to the kind of food for every group (high energy or low energy). After calving, the two groups were kept together without separation and feeding the same high concentrated ration as dairy cattle.

DNA samples. DNA was extracted from 15 monozygotic (MZ) female twins of German Holstein dairy cattle. Where, the whole blood with EDTA was collected and with TE-buffer (1 M Tris, 0.5 M EDTA, pH 8.0) was washed. To digest the protein, digesting buffer (1 M Tris, pH 7.4 and 0.5 M EDTA, pH 8.0) in addition to proteinase K (20 mg/ml) and 20% SDS were used, then the samples at 56 °C over night were incubated. TE-solution was added and with equal volume of phenol-chloroform-isoamylalcohol (25:24:1) the sample was extracted, followed by chloroform-isoamylalcohol (24:1) extraction. The DNA pellet was precipitated in 3 M sodium acetate in ratio 10-1, in addition to equal volume of the isopropanol, dried, and resuspended in double distilled water (ddH_2O) until needed.

Microsatellite markers. Nineteen microsatellite markers were selected from chromosomes 6 and 20 according to linkage maps of [2,4], IBRP97 (International Bovine Reference Population, Roslin Institute, Scotland, UK), [10,9], MARC97 (U.S. Meat Animal Research Center), USDA 98 (United States Department of Agriculture) and [20,21]. These microsatellite markers were selected based on their location between the two microsatellite markers which flanking the region of interest, and with their primer sequences and annealing temperatures are listed in the Table 1.

Genotyping. PCR was performed in a reaction volume of 25 µl using 100 ng of genomic DNA from each animal, 5 pmol of each primer, 1X reaction buffer (160 mM $(NH_4)_2SO_4$, 500 mM Tris–HCl pH 8.8 at 25 °C, 0.1% Tween 20), 1.5–3.0 mM $MgCl_2$, 200 µM dNTP and 0.5 U Taq polymerase (Invitek Co., Berlin, Germany). Thermal cycling (UNO–Thermoblock Biometra) was carried out by initial denaturation at 92–95 °C for 2–6 min, followed by 25–40 cycles each at 92–95 °C for 15–60 sec, annealing temperature at 52–59 °C for 30–60 sec (Table 1), and polymerization temperature at 72 °C for 15–180 sec, followed by a final extension step for 4–10 min at 72 °C. After thermocycling, PCR products were checked electrophoretically on 1% agarose gel for the presence or absence. Electrophoresis was performed in 1X Tris 90 mM , Boric acid 90 mM and EDTA 2 mM (pH 7.5) at 80 V for 30 min at room temperature. PCR products (DNA) were detected by ethidium bromide under UV light and photographed by Polaroid Camera.

After thermocycling, PCR products were diluted with 40–80 µl double distilled water (ddH_2O), approximately. 2 µl PCR product was mixed with 2 µl molecular weight marker (138/142, 177/185 or 188/194-bp) plus 3 µl loading buffer (10 ml formamide and 5 mg Dextran Blue). The mixture was then denaturated at 100 °C for 5 min

Table 1: Microsatellite markers, primer sequences and annealing temperatures.

Chr. no.	Microsatellite marker	Forward primer sequence (5′→3′)A	Reverse primer sequence (5′→3′)B	Ann. temp. °C
6	1- URB16	AGCTTTCTCTCACGGGTTTCG	CGGACAGGACTGAGCTACTGA	58
	2- BM1329	TTGTTTAGGCAAGTCCAAAGTC	AACACCGCAGCTTCATCC	58
	3- BMS2508	TTTCTGGGATTACAAAATGCTC	TTTCTTAGGGGAGTGTTGATTC	55
	4- BM143	ACCTGGGAAGCCTCCATATC	CTGCAGGCAGATTCTTTATCG	57
	5- BMS382	GGCACATATGAATAAATGCTTTG	TCTGACACAACTTAGCAACTAAACA	57
	6- BMS1242	AGTGTGATCAACAACGGCAG	AGTGACTGGTGCAGTGCTTG	57
	7- BM3026	CCTCCAGCTTAGAACACATTCTT	TACCTAAGGCCTAACTGAAATGTG	57
	8- FBN12	CCCTTATGTTCATTGCAGCACTATTTAC	GCTGTGGCAAATGGCAAAATTCC	59
	9- DIK82	CCCACTCTGTCTCCAGTTTG	TATCCTGAGAAAAGCTGCTAGA	59
	10- TGLA37	CATTCCAATCCCCTATCCTGAG	TTGAATGATTCTATGAAGACCTGTA	57
	11- FBN13	ACTTTCATTAGATTGCTGCAAATAG	AAATATGGAAACGACCTGTGG	56
	12- ILSTS97	AAGAATTCCCGCTCAAGAGC	GTCATTTCACCTCTACCTGG	56
20	1- AGLA29	AGGAAGCCGAGTGAGATATGTAAGC	TTACAGCCTGTGTGAATGTCCTCTA	57
	2- BM4107	ATAGGCTTTGCATTGTTCAGG	AGCCCCTGCTATTGTGTGAG	57
	3- BMS117	TGAAAGTAAAGAAACACGCGC	AAACCACCAATTTCTGGGG	56
	4- ILSTS72	ATGAATGTGAAAGCCAAGGG	CTTCCGTAAATAATTGTGGG	52
	5- BMS1120	CTTGAACTGTGGATGATTGATG	GAATGCTTATTAGGTGCATTTACA	57
	6- BMS703	CAATGAGCTCAGATTGTTGCA	ATACATGTAGTCAAAAGGCTCATCC	57
	7- BM5004	TCTGGAGTGAATGTTTCTGAGG	TTGTGATGAGCACCTGAAGG	57

Where; Chr. no. = Chromosome number and Ann. temp. = Annealing temperature. It should be noted that, all the reverse primers (B's) are fluorescent–labeled for all microsatellite markers except FBN12 and FBN13, the forward primers (A's) are fluorescent–labeled during synthesis (MWG-Biotech AG, Co., Ebersberg, Germany).

and cooled (shocked) on ice for 5 min. Resulting single strands were separated electrophoretically at 65 mA and 760 V at 50 °C for 190–320 min on a 0.5 mm acrylamide gel using the A.L.F. DNA Sequencer (Pharmacia). The A.L.F. DNA Sequencer is designed for the automated electrophoresis and analysis of sequencing reactions by the direct detection of fluorescently labeled DNA molecules. After electrophoresis the marker genotype data were displayed as picks and tables on the computer screen automatically.

Statistical analysis. General Linear Model (GLM, Univariate and Multivariate) of SPSS program (10.0 in English) was used to estimate the significant effects (P – values) of the microsatellite markers on milk yield (MY) with $P < 0.05$. Before the analysis, the normal distribution of the data for these traits was previously tested, using Test of Normality, SPSS program. The main effects of the microsatellite markers (19 markers, separately) on

MY trait were estimated, using least-square differences (LSD) with significance level 0.05. The statistical model was:

$y_{ijkl} = \mu + Panr_{(i)} + Inex_{(j)} + M_{(k)} + e_{ijkl}$,

where, y is the trait value (dependent variable), μ is the overall mean, Panr is the twin pair number (random factor), Inex is the low/high energy feeding (fixed factor), M is the microsatellite marker allele effect (fixed factor) and e is the residual error. It should be noted, however, that to analyze the milk yield data, we have classified the lactation season (300 days) to three sections: section one (1–100 days), section two (100–200 days) and section three (200–300 days), where these three sections were used for statistical analysis separately.

RESULTS

Marker genotyping. Twenty microsatellite markers have been carefully selected from chromosomes 6 and 20 (see Table 1) based on their location between the two microsatellite markers which flanking the region of interest, where locate QTL controlling milk yield (MY) for genotyping in 15 female monozygotic (MZ) twin pairs of German Holstein population. All the twenty microsatellite markers, except one (marker FBN9 on chromosome 6), were successfully PCR amplified and sufficiently genotyped to be included in the analysis.

Significant markers. Significant effects of twelve microsatellite markers on chromosome 6 were estimated to identify or to locate quantitative trait loci (QTL) influencing milk yield trait (MY). Table 2 shows the significant effect values of these 12 markers, where only three markers have significant effects, while the other nine markers have no effects. However, in the first 100 days, all the 12 markers have no effects on milk yield during this lactating period. In the second 100 days, only marker ILSTS97 is highly significant effect ($P<0.01$) for milk yield, while the others (11 markers) are not significant. Lastly, in the third 100 days, two markers BM143 and FBN13 are also highly significant effects ($P<0.01$) on milk yield trait, while the other 10 markers are not significant.

On the other hand, on chromosome 20, seven microsatellite markers were analyzed to locate quantitative trait loci (QTL) affecting milk yield trait (MY). Where three markers AGLA29, ILSTS72 and BM5004 are significant, while the other four markers are not significant (Table 2). No significant markers on milk yield in the first 100 days, while markers AGLA29 and ILSTS72 are highly significant ($P<0.01$) for milk yield in the second and third 100 days, respectively.

DISCUSSION

In the present study, there is a highly significant segment controlling milk yield trait (MY) on chromosome 6 to the interval BM143-ILSTS97. As can be seen from Figure 1, we are highly motivated to expect that there are three different loci (in a distance 24 cM, approximately) with stronge influence on milk yield at that segment, one locus located in the region of or close to marker BM143, the second locus located tightly close to marker FBN13 and the third locus located close to or in the region of marker ILSTS97. However, in this study we report of a QTL with effects on milk yield at three different positions on chromosome 6 in German Holstein population.

Several of the previous studies have also indicated the presence of a QTL variously affecting milk yield on cattle chromosome 6. Where, significant QTL for milk yield trait on chromosome 6 between markers BM1329 and TGLA37 have been detected in American Holsteins [7], Dutch Holsteins [18], German Holsteins [11,10,5,6] and Norwegian cattle [8], using granddaughter design (GDD). In another study on American Holsteins, [23] found a significant QTL effects on milk yield on chromosome 6 between markers BM143 and RM29 (telomere's direction after marker ILSTS97), while in British dairy cattle, [22] reported a QTL for milk yield on chromosome 6 between markers BM143 and ILSTS90 (centromere's direction before marker URB16). In the region of marker BM143 on chromosome 6, a QTL affecting milk yield was also identified in both Canadian [14] and Israeli Holsteins [17]. In two separate studies on Norwegian dairy cattle, [15,16] could identify a QTL with alleles that cause an increase in milk yield on chromosome 6 close to marker FBN9 and surrounded by the markers BMS2508 and FBN12, respectively. In a recent study in German Holstein dairy cattle population, [19] found a QTL affects on milk yield on chromosome 6 at three positions in distances 18, 31, and 17 cM from the leftmost marker DIK82.

The previous MY-QTL findings are confirmed here in our study. However, we agree with all the author's QTL-location on chromosome 6, except [22], where our QTL finding is flanked with markers BM143 and ILSTS97. It should be noted, however, that from our and all previous studies, it has been observed that there is a highly significant QTL effects on milk yield trait positioned near marker BM143.

After having discussed MY-QTL position on chromosome 6, a significant QTL effects on milk yield trait (MY) was also found on chromosome 20. This QTL located between markers AGLA29 and ILSTS72 in a distance 2.1 cM as shown in Figure 1. In a previous study to [1], granddaughter design (GDD) in Dutch Holsteins was used to map a QTL affecting milk yield trait. The author

Table 2: The *P- values* of the genotyped markers on chromosome 6 for milk yield trait

Chr. no.	***Marker***	***P - values***		
		The first 100 days	The second 100 days	The third 100 days
6	1- URB16	0.19	0.141	0.098
	2- BM1329	0.759	0.392	0.566
	3- BMS2508	0.267	0.046	0.052
	4- BM143	0.129	0.085	**0.008**
	5- BMS382	0.184	0.407	0.235
	6- BMS1242	0.47	0.786	0.729
	7- BM3026	0.979	0.095	0.207
	8- FBN12	0.651	0.128	0.296
	9- DIK82	0.133	0.34	0.084
	10- TGLA37	0.68	0.8	0.931
	11- FBN13	0.059	0.056	**0.004**
	12- ILSTS97	0.046	**0.008**	0.129
20	1-AGLA29	0.106	**0.005**	0.031
	2-BM4107	0.589	0.751	0.949
	3-BMS117	0.204	0.201	0.069
	4-ILSTS72	0.343	0.051	**0.008**
	5-BMS1120	0.152	0.717	0.92
	6-BMS703	0.275	0.876	0.638
	7-BM5004	0.053	0.025	0.029

could identify a significant QTL affecting milk yield on chromosome 20 between markers AGLA29 and BM5004, which confirmed and fine mapped in our study.

Admittedly, in dairy cattle, implementations of marker-assisted selection (MAS) for selection of young sires before progeny testing and for selection in nucleus breeding schemes have been shown to potentially produce additional genetic and economic gains [13,3,12]. Application of MAS would be more efficient if essentially nonrecombining marker haplotypes bracketing the QTL could be identified. Consequently, the quantitative trait loci (QTL) identified in this study may be useful for marker-assisted selection to increase and accelerate the rate of genetic improvement on traits such as growth and milk production traits.

ACKNOWLEDGEMENTS

The authors thank Dr. Zeller K., Mrs. Ackermann A., and Mrs. Hoffmann C., Department of Breeding Biology and Molecular Animal Breeding, Institute of Livestock Sciences, Faculty of Agriculture and Horticulture, Humboldt University, Berlin, Germany, for giving their kind help.

REFERENCES

[1] Arranz J., Coppieters W., Berzi P., Cambisano N., Grisart B., Karim L., Marcq F., Moreau L., Mezer C., Riquet J., Simon P., Vanmanshoven P., Wagenaar D., Georges M., A QTL affecting milk yield and composition maps to bovine chromosome 20: a confirmation, Animal

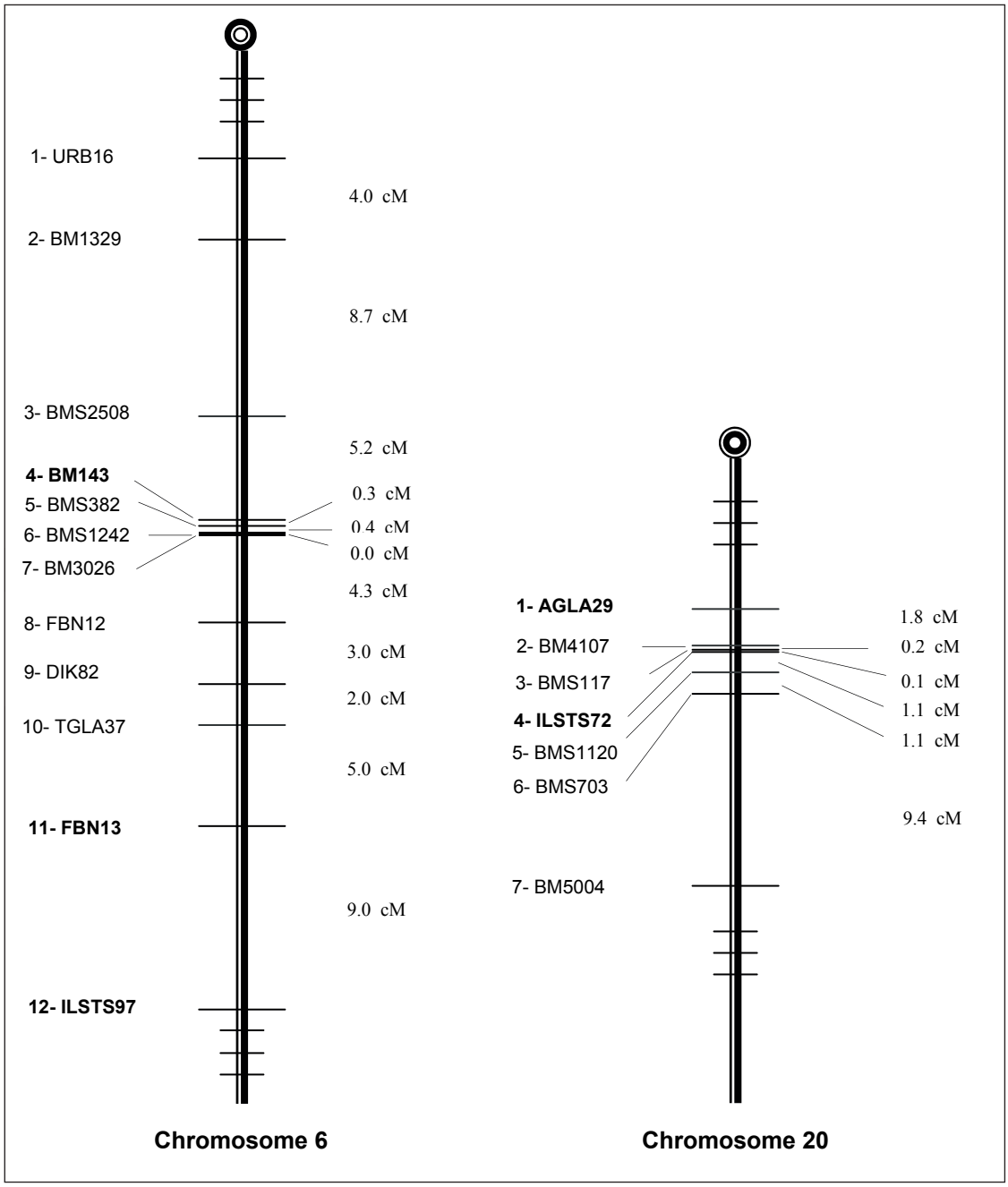

Figure 1: Map of cattle chromosomes 6 and 20 showing; the order of the markers (1–12 & 1-7, respectively) that were genotyped and the distance (in cM, relative positions) between them. It should be noted that; the significant markers on MY are in bold and the centromere is denoted with a circle on the top of the chromosome.

Genetics (1998) 29: 107-115.

[2] Barendse W., Vaiman D., Kemp S.J., et al., A medium – density genetic linkage map of the bovine genome, Mammalian Genome (1997) 8: 21–28.

[3] Brascamp E.W., van Arendonk J.A.M., Groen A.F., Economic appraisal of the utilization of genetic markers in dairy cattle breeding, J. Dairy Sci. (1993) 76: 1204–1213.

[4] Fisher S.R., Beever J.E., Lewin H.A., Genetic mapping of five human chromosome 4 orthologues to bovine chromosome 6 and 17, Animal Genetics (1997) 28: 253–257.

[5] Freyer G., Kühn C., Weikard R., Zhang Q., Mayer M., Hoeschele I., Multiple QTL on Chromosome six in Dairy Cattle affecting Yield and Content Traits, J. Anim. Breed. Genet. (2002) 119: 69–82.

[6] Freyer G., Sørensen P., Kühn C., Weikard R., Hoeschele I., Search for Pleiotropic QTL on Chromosome BTA6 Affecting Yield Traits of Milk Production, J. Dairy Sci. (2003) 86: 999–1008.

[7] Georges M., Nielson D., Mackinnon M., Mishra A., Okimoto R., et al., Mapping Quantitative Trait Loci Controlling Milk Production in Dairy Cattle by Exploiting Progeny Testing, Genetics (1995) 139: 907-920.

[8] Gomez-Raya L., Klungland H., Vage D.I., Olsaker I., Fimland E., Klemetsdal G., Ronningen K., Lien S., Mapping QTL for milk production traits in Norwegian cattle, Proc. 6th World Congr. Genet. Appl. Livest. Prod. (1998) 26: 429-432.

[9] Kappes S.M., Keele J.W., Stone R.T., Mc Graw R.A., Sonstegard T.S., Smith T.P.L., Lopez-Corrales N.L., Beattie C.W., A second generation linkage map of the bovine genome, Genome Research (1997) 7: 235–249.

[10] Kühn Ch., Freyer G., Weikard R., Goldammer T., Schwerin M., Detection of QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6, Animal Genetics (1999) 30: 333-340.

[11] Kühn Ch., Weikard R., Goldammer T., Gruppe S., Olsaker I., Schwerin M., Isolation and application of chromosome 6 specific microsatellite markers for detection of QTL for milk-production traits in cattle, J. Anim. Breed. Genet. (1996) 113: 355-362.

[12] Mackinnon M., Georges M., Marker-assisted preselection of young dairy sires prior to progeny-testing, Livestock Production Science (1998) 54: 227–248.

[13] Meuwissen T.H.E., van Arendock J.A.M., Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes, J. Dairy Sci. (1992) 75: 1651–1659.

[14] Nadesalingam J., Plante Y., Gibson J.P., Detection of QTL for milk production on Chromosome 1 and 6 of Holstein cattle, Mammalian Genome (2001) 12: 27–31.

[15] Olsen H.G., Gomez-Raya L., Vage D.I., Olsaker I., Klungland H., Svendsen M., Adnoy T., Sabry A., Klemetsdal G., Schulman N., Kramer W., Thaller G., Ronningen K., Lien S., A genome scan for quantitative trait loci affecting milk production in Norwegian dairy cattle, J. Dairy Sci. (2002) 85: 3124–3130.

[16] Olsen H.G., Lien S., Svendsen M., Nilsen H., Roseth A., Aasland Opsal M., Meuwissen T.H., Fine mapping of milk production QTL on BTA6 by combined linkage and linkage disequilibrium analysis, J. Dairy Sci. (2004) 87: 690-8.

[17] Ron M., Klinger D., Feldmesser E., Seroussi E., Ezra E., Weller J.I., Multiple Quantitative Trait Locus Analysis of Bovine Chromosome 6 in the Israeli Holstein Population by a Daughter Design, Genetics (2001) 159: 727–735.

[18] Spelman R.J., Coppieters W., Karim L., van Arendonk J.M., Bovenhuis H., Quantitative Trait Loci Analysis for Five Milk Production Traits on Chromosome Six in the Dutch Holstein-Friesian Population, Genetics (1996) 144: 1799-1808.

[19] Szyda J., Liu Z., Reinhardt F., Reents R., Estimation of quantitative trait loci parameters for milk production traits in German Holstein dairy cattle population, J. Dairy Sci. (2005) 88: 356-67.

[20] Velmala R.J., Vilkki H.J., Elo K.T., de Koning D.J., Mäki–Tanila A.V., 1999: A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle, Animal Genetics (1999) 30: 136–143.

[21] Weikard R., Golammer T., Kühn C., Barendse W., Schwerin M., Targeted development of microsatellite markers from the defined region of bovine chromosome 6 q 21–31, Mammalian Genome (1997) 8: 836–840.

[22] Wiener P., Maclean I., Williams J.L., Wooliams J.A., Testing for the presence of previously identified QTL for milk production traits in new populations, Animal Genetics (2000) 31: 385–395.

[23] Zhang Q., Boichard D., Hoeschele I., Ernst C., Eggen A., Murkve B., Pfister-Genskow M., Witte L.A., Grignola F., Uimari P., Thaller G., Bishop M.D., Mapping Quantitative Trait Loci for Milk Production and Health of Dairy Cattle in a Large Outbred Pedigree, Genetics (1998) 149: 1959-1973.