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MAPPING OF QUANTITATIVE TRAIT LOCI INFLUENCING DAILY BODY WEIGHT GAIN (DBWG) ON CHROMOSOME 6 IN GERMAN HOLSTEIN POPULATION[1]

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Abstract: Twelve microsatellite markers on chromosome 6 were analyzed in German Holstein population to detect and locate QTL affecting daily body weight gain (DBWG). The results indicate promising location for QTL controlling daily body weight gain trait on chromosome 6. Where, three markers BMS2508, BM3026 and TGLA37 at three different positions in a distance 15.2 cM on BTA6 were associated with significant effects for daily body weight gain trait (DBWG). Comparison between this finding and previously identified QTL support the location of a QTL for growth traits on chromosome 6, where a significant QTL for birth and yearling weight was previously identified on chromosome 6 tightly close to marker BM3026. Finding from this study could be used in subsequent fine-mapping work and applied to marker-assisted selection (MAS) of production traits.

Key words: cattle, body weight, microsatellite markers, association analysis

Introduction and literature review

Everything a living organism does throughout its life is ultimately determined by its genes. The genes do not act in total isolation, of course, but in response to triggers in the organism's internal and external environment (*Kearsey and Pooni, 1996*). Most traits of economic importance in farm animals are of quantitative nature, i.e., are influenced by many genes and by environmental factors (*Zhang et al., 1998*). For example, growth traits (birth weight, weaning weight, yearling weight and live weight) are quantitative in nature. The phenotypes observed are thus the combined results of the action of large numbers of polygenes or

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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 (*Wiener et al., 2000*).

With the rapid advancement of molecular technology, available and ordered genetic markers into genetic linkage maps, and increasing popularity of QTL mapping in economically important animals (e.g. cattle), fine mapping of the QTL region became the way to localize and characterize the gene (s) underlying the QTL of interest (i.e., growth traits). In order to move forward in the identification of the genes involved in growth traits, confirmation of the associations between traits and chromosomal regions is required, in conjunction with finer-scale mapping of these regions to better localize the genes.

The objective of the present study was to locate and identify the significant markers which flanking the QTL affecting daily body weight gain trait (DBWG) in a very close distance, using microsatellite markers in 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. 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. 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***.** Twelve microsatellite markers were selected from chromosome 6 according to linkage maps of Barendse 1997, Fisher 1997, IBRP97 (International Bovine Reference Population, Roslin Institute, Scotland, UK), Kühn 1999, Kappes 1997, MARC97 (U.S. Meat Animal Research Center), USDA 98 (United States Department of Agriculture), Velmala 1999, and Weikard 1997. These microsatellite markers were selected based on their location in the region of interest, and with their primer sequences and annealing temperatures are listed in the Table 1.

Table 1. Microsatellite markers, primer sequences and annealing temperatures
Tabela 1. Mikrosatelitski markeri, sekvence prajmera i temperature njihove hibridizacije

Microsatellite marker Mikrosat. marker	*Forward primer sequence/ Sekvenca prednjih prajmera (5′→3′) A*	*Reverse primer sequence/ sekvenca obrnutih prajmera (5′→3′) B*	*Ann. temp. °C*
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

Where; 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) / Gde je: Ann. Temp. – temperature hibridizacije. Primedba: svi obrnuti prajmeri (B's) su fluorescentno označeni za sve mikrosatelitske markere osim FBN12 i FBN13, prednji prajmeri (A's) su fluorescentno označeni tokom sinteze (MWG-Biotech AG, Co., Ebersberg, Germany)

*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 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***.** Data for daily body weight gain (DBWG) were obtained from fifteen female monozygotic (MZ) twin pairs of German Holstein population. Where, samples of body weight were collected according to the age of the animals, consequently, daily body weight gain data were calculated as follows:

$$DBWG = \frac{BW_{\text{time } m+1} - BW_{\text{time } m}}{A_{m+1} - A_m}$$

where, BW is the body weight, m is sample m, m + 1 is the following sample, and A is the age of the animal. 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 daily body weight gain (DBWG) 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 (12 markers, separately) on DBWG trait were estimated, using least-square differences (LSD) with significance level 0.05. The statistical model was:

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

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), Proal is the age of the animal (covariate) and e is the residual error.

Results

*Marker genotyping***.** Thirteen microsatellite markers have been carefully selected from chromosome 6 (see Table 1) based on their location in the region of interest, where locate QTL controlling growth traits for genotyping in 15 female monozygotic (MZ) twin pairs of German Holstein population. All the thirteen microsatellite markers, except one (marker FBN9), were successfully PCR amplified and sufficiently genotyped (2 to 8 alleles) to be included in the analysis.

*Significant markers***.** Significant effects of twelve microsatellite markers on chromosome 6 were estimated for daily body weight gain trait (DBWG). Three markers BMS2508, BM3026 and TGLA37 are associated with significant effect values 0.006, 0.020 and 0.010, respectively, while the other nine markers have no effects on daily body weight gain trait (Table 2). Conspicuously, markers BMS2508 and TGLA37 could be found highly significant effects for daily body weight gain (DBWG) trait, while marker BM3026 has a low significant effect slightly . The result suggests that, markers BMS2508 and TGLA37 on chromosome 6 are associated with highly significant effects on daily body weight gain trait (DBWG). These two markers are located at two different positions in a distance 15.2 cM (Figure 1). Consequently, we expect that a significant QTL affecting or controlling daily body weight gain on chromosome 6 could be positioned to a 15.2-cM interval surrounded by the markers BMS2508 and TGLA37.

Table 2. The P- values of the genotyped markers on chromosome 6 for daily body weight gain trait (DBWG)
Tabela 2. P-vrednosti za genotipizirane markere na hromozomu 6 za osobinu dnevnog prirasta telesne mase

No./Br.	*Marker/Marker*	*P- value/P-vrednost*
1	URB16	0.274
2	BM1329	0.399
3	BMS2508	**0.006**
4	BM143	0.712
5	BMS382	0.329
6	BMS1242	0.345
7	BM3026	**0.020**
8	FBN12	0.621
9	DIK82	0.069
10	TGLA37	**0.010**
11	FBN13	0.180
12	ILSTS97	0.152

Discussion

Admittedly, daily body weight gain trait is one of the growth traits (i.e. birth weight, weaning weight, yearling weight and live weight). These growth traits are quantitative in nature and genetically, the variation in these traits is due to multiple genes or quantitative trait loci (QTL). From previous studies, *Casas et al.* (2000) analyzed 150 informative microsatellite markers in 92 individuals developed from Belgian Blue x MARC III and Piedmontese x Angus sires. The author identified a significant QTL for birth weight (BWT) and yearling weight (YW) on chromosome 6 tightly close to microsatellite marker BM3026, confirming our finding in this study, where a significant QTL effects on daily body weight gain trait was found on the same chromosome 6 restricted by markers BMS2508 and TGLA37, included marker BM3026. On another different chromosomes, *Kari T. Elo et al.,* (1999) analyzed six microsatellite loci in Finnish Ayrshire dairy cattle to search for QTL affecting live weight trait. The author found a QTL for live weight maps between markers BM1258 and BoLA DRBP on chromosome 23.

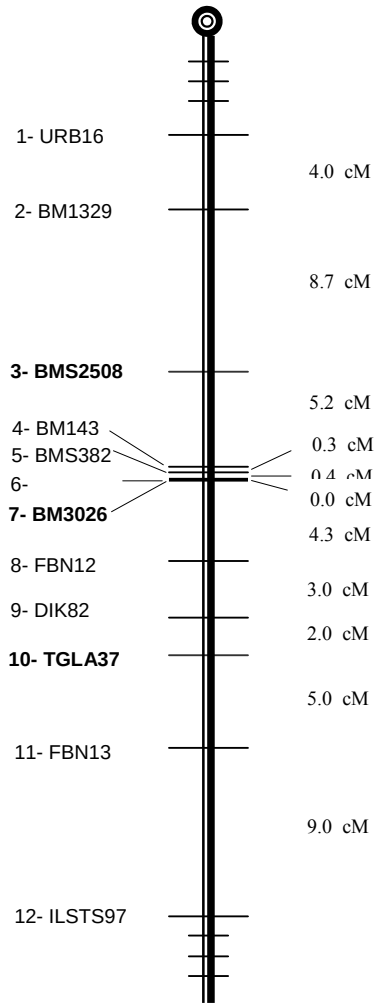

Figure 1. Map of cattle chromosome 6 showing; the order of the genotyped markers (1–12) and the distance (in cM, relative positions) between them. BMS2508, BM3026 and TGLA37 (in bold) are the significant effect markers on daily body weight gain trait (DBWG). It should be noted that the centromere is denoted with a circle on the top of the chromosome
Slika 1. Mapa hromozoma 6 kod goveda koja pokazuje redosled genotipskih markera (1-12) i razmak (u cM, relativne pozicije) između njih. BMS2508, BM3026 i TGLA37 (bold) su značajni markeri koji utiču na osobinu dnevni prirast telesne mase. Obratiti pažnju na centromeru na vrhu hrmozoma

In Brahman dairy cattle a QTL affecting both; birth weight (BWT) and correlated traits yearling weight (YW) and weaning weight (WW) located between markers BMS1789 and BMS4014 on chromosome 1 (*Stone et al.,1999*). In a study on Dutch Holstein–Friesian population, *Schrooten et al., (2000*) analyzed 277 microsatellite markers on different chromosomes, using granddaughter design (GDD) to locate QTL for different growth traits. The author identified a significant QTL for the following growth traits: chest width on chromosome 2 at marker BM2113, stature and size on chromosome 5 at location 122 cM between IGF1 and marker BM315 and lastly, angularity on chromosome 12 between markers TGLA9 and AGLA226. A Putative quantitative trait locus affecting birth weight (BWT) was identified at the telomeric end of bovine chromosome 2 (maximum effect at 114 cM) using 151 progeny of a single Hereford x composite bull and 170 microsatellite markers (*Grosz and MacNeil, 2001*). For searching QTL affecting carcass traits in Hereford x composite double backcross populations using 229 microsatellite markers, *MacNeil and Grosz (2002*) could identify a QTL affecting live weight on BTA 17.

Quantitative trait loci for growth and carcass composition was detected in a family from a Bos indicus (Brahman) x Bos taurus (Hereford) sire, using 312 microsatellite markers (*Casas et al., 2003*). Where, putative QTL for birth weight were detected on chromosomes 1, 2, and 3, and for weaning weight on chromosome 29. For hot carcass weight, QTL were detected on chromosomes 10, 18, and 29. In another study to *Casas et al., (2004*), in a half-sib family from a Bos indicus (Brahman) x Bos taurus (Hereford) sire, a putative QTL on chromosome 29 (at cM 52) for body weight at castration (BYW). Finally, *Mizoshita et al., (2004*) used 342 microsatellite markers in half-sib family of purebred Japanese Black (Wagyu) cattle to locate economically important quantitative trait loci. The author could confirm QTL for carcass yield estimate on BTA 5 in the region of 45 to 54 cM and growth-related QTL on BTA 14 (29-51 cM), including slaughter and carcass weights.

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 (*Meuwissen and van Arendonk 1992, Brascamp et al., 1993 and Mackinnon and Georges 1998*). 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 production traits.

Conclusion

Putative QTL for daily body weight gain (DBWG) was detected in German Holstein population at three different positions on chromosome 6. Where, three markers BMS2508, BM3026 and TGLA37 were associated with significant effects for daily body weight gain trait (DBWG) on BTA6. The results provided a useful reference for fine-mapping work and applied to marker-assisted selection (MAS) of production traits.

MAPIRANJE LOKUSA KVANTITATIVNIH OSOBINA KOJI UTIČU NA DNEVNI PRIRAST TELSEN MASE NA HROMOZOMU 6 KOD NEMAČKE HOLŠTAJN POPULACIJE

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Rezime

Dvanaest mikrosatelitskih markera odabranih iz različitih mapa veza na hromozomu 6 su analizirani u 15 monozitgotnih (MZ) blizanačkih parova nemačke holštajn populacije kako bi se otkrili i locirali QTL/ lokusi kvalitativnih osobina koji utiču na osobine dnevnog prirasta telsne mase (DBWG), korišćenjem opšteg linearnog modela u SPSS programu. Signifikantni efekti dvanaest markera mikrosatelita na hrmozomu 6 su genotipizarani i ocenjeni u odnosu na dnevni prirast telesne mase (DBWG). Tri markera BMS2508, BM3026 i TGLA37 na tri različite pozicije na hromozomu 6 su bili povezani sa signifikantnim uticajima na dnevni prirast telesne mase. Poređenje ovog rezultata i prethodno identifikovanog QTL govori u prilog tvrdnji da je lokacija QTL-a za osobine porasta na hromozomu 6, dok je signifikantni QTL za masu pri rođenju prethodno identifikovana na hromozomu 6 u bliskoj vezi sa markerom BM3026. Rezultati dobijeni ovim istraživanjem ukazuju nna postojanje putativnog QTL-a za DBWG u populaciji nemačkog holštajna na hromozomu 6 u razmaku od 15.2 cM koji je okružen markerima BMS2508 i TGLA37. Rezultati ovog istraživanja se mogu koristiti u radu na finom mapiranju koji će uslediti i mogu se primeniti u selekciji

proizovdnih osobina koja se bazira na markerima - marker-assisted selection (MAS).

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