



Influence of quantitative trait loci on growth traits of chromosome 1 in Sanjabi lambs during the first year of growth

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ABSTRACT

This study aimed to detect the quantitative trait loci (QTL) which affecting the growth traits in some parts of chromosomes 1 of Sanjabi lambs. The study population consisted of six groups of fathers which had an average of 39.17 lambs. Initially, the fathers were genotyped for eight microsatellite markers on chromosome 1, and the offspring of the heterozygote fathers were genotyped. The quantitative traits were the body's weight at birth, 3, 6, 9, and 12 months, which were corrected for the fixed effects of year of birth, sex, and birth type. QTL search was performed by interval mapping based on a regression model for each cM (Centimorgan) of the chromosomes. In total, one QTL in the 235 cM of chromosome 1 was identified from the beginning of the chromosome in associate with weaning weight. The sire substitution effect for both families was 2.14 kg. The confidence interval estimation of the detected QTL that was estimated using the Bootstrap method was 225–238 cM of chromosome 1. This QTL was determined between the MCM137 and MCM130 markers and at a distance of about 9 cM from the MCM137 marker. If this QTL was approved in the next research, it can be used in Marker-assisted selection programs. In this case, more accuracy should be achieved for the evaluation of superior animals.

1. Introduction

Sheep is one of the most economically important livestock used as a source of meat, milk, wool, and skin for human society. Raising sheep in Iran due to the interest of people for using sheep meat instead of cattle or chicken meat and sacrificing in religious ceremonies is very important. Determine the genetic background of quantitative traits is very important to increase the production of domestic animals (Gebreselassie et al., 2020). Sanjabi sheep is one of the most important breeds in Iran, which produce good quality of meat and wool. This animal is highly resistant to local diseases and has a good daily weight gain during fattening (220 g/day) (Mohammadi et al., 2010). Bodyweight traits at different ages are among the most important economic traits of sheep, which is directly related to the amount of production and profit of the farmer (Hadjipavlou and Bishop, 2008; McRae et al., 2005; Walling et al., 2004). Due to the growing demand for livestock products and the limitation in increasing the number of livestock, their genetic improvement to increase production is very important. Typically, in breeding

programs, the breeding value of desired traits for each animal was calculated using the animal model, then the superior father and dam were selected to produce the next generation. In recent years, efforts have been made to increase the accuracy of selection by entering marker information in the breeding value equation (Mohammad Abadi et al., 2009).

QTL is a segment of the genome that determines some part of the phenotypic variance of the quantitative traits (Geldermann, 1975). QTL detection in domestic animals can lead to the identification of genes that have a direct impact on their important economic traits. In recent decades, many studies have been conducted in many breeds to identify QTLs (Ezmailzadeh, 2010; Hadjipavlou and Bishop, 2008; McRae et al., 2005). These studies can greatly help understand the genetic structure and architecture of these traits, so will provide very useful information. In sheep, growth traits are very important, and due to their moderate heritability, the scientist has been designed various breeding programs to improve them (Walling et al., 2004). The QTL detection methods are based on detecting the relation between the simultaneous segregation of

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DNA markers and quantitative traits. Finding this relation is the first step of the marker-assisted selection (MAS) method. If QTLs and their linked markers were identified and used them in the breeding programs, these programs can be performed more accurately (Knott et al., 1996).

So far, a lot of research has been done to detect QTLs that control the quantitative traits of sheep, although the distribution of QTL in local breeds may have a different pattern. Due to the relatively large population size and a large number of microsatellite markers, 24 QTLs in the Suffolk and 9 QTLs in the Charollais sheep breed regarding with bodyweight have been detected (McRae et al., 2005; Walling et al., 2004). Relatively little research in Iran has been conducted using local sheep breeds. Ezmailizadeh (2010) using 25 microsatellite markers on chromosomes 1, 3, 6, 11, and 24 to identify three QTLs related to birth weight, growth traits of Kermani sheep. Saghi et al. (Saghi et al., 2012) with using eight microsatellite markers on chromosome 1, identify two QTLs affecting body weight in a part of the Baluchi sheep genome. Iranpour-Mobarakeh et al. (2011) also show the significant relationship between microsatellite markers used with 6-month weight trait in Lori-Bakhtiari sheep. They suggested that these QTL could be a strong candidate for use in MAS. This study aimed to confirm significant QTLs that have been reported in previous research or identify new QTLs associated with bodyweight on chromosome 1 of Sanjabi lambs breed, using microsatellite markers.

2. Materials and methods

2.1. Animals and quantitative traits

Six unrelated fathers of Sanjabi sheep breed from Mehrgan station on the Kermanshah state of Iran were selected. Quantitative traits were birth weight, 4 months, 6 months, 9 months, and 12 months. Blood samples were collected from all of the fathers and their offspring from the jugular vein.

2.2. DNA genotyping

DNA was extracted by the salting-out method (Salazar et al., 1998). The quantity and quality of the extracted DNA were determined using a spectrophotometer, and then their concentration was decreased to 50 ng/mL using distilled water. Microsatellite markers were selected based on previous studies and also based on genetic maps of sheep microsatellite (DeGortari et al., 1998; Maddox et al., 2001, 2007) (Table 1). Forward primers were marked using a fluorescent dye to identify amplified DNA fragments using an ABI3130 genetic analyzer. In the first stage, all fathers were genotyped for all microsatellite markers. Then, the offspring of Heterozygote's fathers were genotyped. Polymerase chain reaction (PCR) includes an initial step for 5 min (95 °C), and cycle

Table 1
Name of markers, their location on chromosome 1, and the sequence of primers.

Primer sequence	Accession number	Position (NCBI-cM)	Marker name
5- TCCATGGGGTCGCAACAGTGG-3	Pr012487094	166.9	RM095
5- ATCCCTCCATTGTGTGGAGTT-3			
5- CTTAAAATCTGTCTTTCTTCC-3	Pr012487030	179	ILSTS004
5- TAGTGTGTATTAGGTTTCTCC-3			
5-TCAGTGAAAGCAAGAGAAATATCC-3	Pr009715834	208.1	BMS527
5-TCCATTCCCTTTGAATATCCC-3			
5- AGGGGAGCCCCAGTAAGTATCA-3	Pr009715838	226.4	MCM137
5- AAACAAGTGGGGATGTTAGCTCTT-3			
5- AAACCTTGTGCTGTTGGGTGTATC-3	Pr009715843	248.8	MCM130
5- CTCACCTCTGCCTTCTATCTCTCT-3			
5- TGGTAGAGCAATATGAAGGCC-3	Pr012486882	267.3	BM864
5- GGAAATCCAAGAAAGAGGGG-3			
5- AAAGTCTTGACTCTGTGTCGG-3	Pr012487631	293.8	MAF4
5- TCCACGGGGTCTCAAAGAGTCG-3			
5- GCCTTGACCATCTACTCCA-3	Pr009715854	307.3	DIK5034
5- TTGCCATCTCTCCATCTTCC-3			

steps in 30 cycles, including denaturing (30 s in 94 °C), annealing (40 s in annealing temperature for each marker), and extension steps (60 s in 72 °C) and final extension step was performed for 5 min at 72 °C. Electrophoresis of PCR product was performed in Genetic analyzer 3130 and the size of DNA fragments was determined by GeneMapper V4.0 software using the GeneScan500LIZ standard ladder during the electrophoresis.

2.3. Statistical analysis

QTL location in half-sib is mainly based on the interval mapping of one QTL on a particular region of chromosomes. Knott et al. (1996) described a method that used regression to show the location and effect of the QTL between two adjacent markers. They suggested that the use of multiple marker mapping could increase the power of the statistical model to accurately estimate the location and effect of QTL. For each marker haplotype inherited from the father, the probability of inheriting the Q or q allele can be assessed. So, QTL location analysis can be performed based on the following regression equation (Knott et al., 1996).

$$Y_{ij} = \mu_i + \alpha_i x_{ij} + e_{ij} \tag{1}$$

Where y_{ij} is the bodyweight of offspring, μ_i is an average of each trait for fathers i , α_i is the alleles substitute effect of QTL within the family, x_{ij} is the possibility that an offspring inherit one allele from the father and e is the residual effects. Haley and Knott (1992) By re-parameterizing the previous model, presented a more general model in which QTL genotypes are dependent on marker genotypes. In this case, the probability of inheriting QTL alleles from fathers to offspring at each cM of the chromosome was calculated with GridQTL using the following equation.

$$F = \frac{(SSE_{reduced} - SSE_{full}) / (DFE_{reduced} - DFE_{full})}{SSE_{full} / DFE_{full}} \tag{2}$$

Significant thresholds were calculated using the permutation test along the chromosome with 5000 repetitions and the confidence interval of the QTL position was calculated using the 1000-repeat bootstrap method.

3. Results

In this study, the lowest number of lambs was related to sire 2 (30 lambs) and the highest number of lambs was related to sire 1 (49 lambs) and the average number of offspring per all sire was 39.17 lambs. The phenotypic data included 0, 3, 6, 9, and 12 months of bodyweights were corrected for fixed effects including the year of birth, sex, and type of birth. The lowest birth weight was related to family 6 (3.81 kg) and the highest birth weight was related to family 3 (4.28 kg) (Table 2). The lowest and highest three-month weights were observed in families 5

Table 2
Number of progeny, average weight and their standard deviation.

12 MW (kg)	9 MW (kg)	6 MW (kg)	3 MW (kg)	Birth weight	No. progeny	Family
33.24 ± 6.81	29.64 ± 7.07	26.95 ± 6.74	23.13 ± 6.03	3.87 ± 0.58	49	743 (1)
45.26 ± 5.64	38.86 ± 4.81	30.98 ± 5.11	23.19 ± 4.99	4.12 ± 0.66	30	3043 (2)
39.72 ± 8.48	32.94 ± 7.08	30.38 ± 6.66	25.84 ± 6.57	4.28 ± 0.52	45	3049 (3)
32.62 ± 5.12	28.21 ± 10.05	28.27 ± 7.38	23.12 ± 5.33	4.01 ± 0.57	44	3104 (4)
26.06 ± 6.29	23.73 ± 6.09	23.48 ± 6.09	20.83 ± 5.68	4.10 ± 0.79	34	3206 (5)
28.37 ± 8.43	23.56 ± 7.89	23.68 ± 7.66	21.11 ± 5.89	3.81 ± 0.61	33	3261 (6)
34.21 ± 7.16	29.49 ± 5.83	27.29 ± 3.22	22.87 ± 1.81	4.03 ± 0.17	39.17 ± 7.78	Mean

3 MW- three-month weight.

6 MW- Six-month weight.

9 MW- Nine-month weight.

12 MW- Twelve-month weight.

(20.83 kg) and 3 (25.84 kg), respectively, so family 3 showed more average daily gain weight in comparison with other families.

The ILSTS004 marker showed the highest H_{EXP} (Nei = 0.74) while the MAF4 marker showed the lowest H_{EXP} (Nei = 0.22), so ILSTS004 and MAF4 marker provide the highest and lowest levels of information respectively. In the present study, one QTL was significantly associated with the 3-month old (weaning weight) at 235 cM of chromosome 1. The above positions were significant based on the significance threshold (F statistic) obtained from the interval mapping analysis at the chromosome level (Table 3). In the same way, one QTL affecting 3-month bodyweight (weaning weight) was significant in families 1 and 3 ($P < 0.05$). Unlike pure populations, due to the nature of livestock populations, QTL may not be separable in all families, so in the first step, each family was analyzed separately. The MCM137 marker was a closet marker at 9 cM distance (Figs. 1 and 2). The sire substitution effect for both families was 2.14 kg. The confidence interval estimation of the detected QTL that was estimated using the Bootstrap method was 225–238 cM of chromosome 1, Which is relatively high (13 cM).

4. Discussion

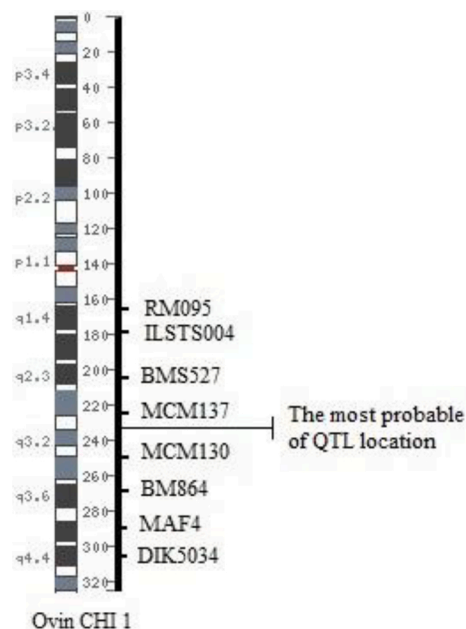
One of the most important factors influencing the accuracy of QTL location is the number of within each sire. Various studies have used a variety of offspring for this purpose. MacRae et al. (2005) for identifying the QTLs affecting the growth and carcass traits of the Charollais breed, used five sires and the average number of offspring per sire was 81 lambs. Comparing the population size in previous studies, it's possible to use different population sizes for QTL detection. It can directly affect the statistical power of QTL design. A large number of population size will lead to more statistical power (Van der Werf et al., 2007). In addition, the results of simulation data have shown that the more offspring will result that the smaller QTLs be identified (Esmailzadeh, 2010), but the cost of genotyping and construction of half-sib family are restricting factors.

The average of total heterozygosity for all markers was 0.66. Proximity to one indicates that the markers used in this study were highly polymorphic and they were good markers for this study. In QTL studies, fathers and offspring should be heterozygote both in marker and QTL location. In practice, when the father is not heterozygous, QTLs couldn't be traced, and it is not necessary to determine the genotype of the offspring. If more fathers are heterozygote, more offspring will be available to determine the genotype. In the present study, 214

Table 3
QTLs detected for 3 MW on CHI1 of Sanjabi sheep.

F statistic	QTL position	Segregated family
3.84*	230	1
4.17*	233	3
5.24*	235	1 and 3

* $P < 0.05$ Chromosome wide significant.



A Snapshot of STR markers chased on Sheep Chromosome 1

Fig. 1. Arrangement and position of microsatellite markers and approximate QTL location affecting the milking weight on chromosome 1 of Sanjabi sheep.

individuals (91 %) containing useful information for QTL analyzes, so they provided good information for locating QTLs. Although in some parts, the F statistic was very close to the significant level but didn't reach the minimum required threshold ($P < 0.05$).

So far, many genes have had a major impact on sheep economic traits. The performance of these genes is not the same, and some of them have synergistic effects and some of them have opposite effects on the phenotype expression. Although there is extensive research in the world on the detection and application of QTL using microsatellite markers, there is limited study in this field in Iran.

Given the relatively high confidence interval for the detected QTL position, the candidate gene suggestion is not very reliable, but it has been shown that chromosome 1 contains several genes that directly affect the body weight traits (Gebreselassie et al., 2020). Therefore, more research is needed to determine its exact location before using this information in MAS.

The QTL identified in this study was somewhat consistent with the findings of other researchers in other sheep breeds. Walling et al. (2004) concluded that on chromosome 1 of Suffolk and Texel sheep at position 227 cM MorganQ was significant for muscle depth. McRae et al. (2005) showed that there were two significant QTLs on chromosome 1 for 2 and 5-month old weight traits. Saghi et al. (2012) showed three significant

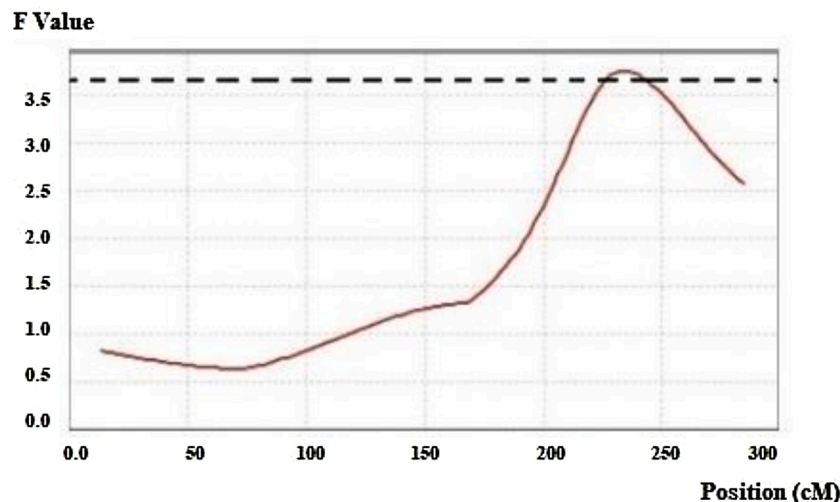


Fig. 2. Statistical F curve in different parts of chromosome 1 for 3 MW of Sanjabi sheep. The dotted line represents the QTL significance threshold at the 5% probability level ($F_{0.05} = 3.56$).

QTLs for birth, 9, and 12-month weight. It proposed that the QTL detected in this study is different from the QTLs that have been reported for chromosome 1 previously. There is usually more connection between genotypes and phenotypes for traits with high heritability. Low heritability usually indicates a lower choice than conventional selection programs, and the use of marker selection methods can lead to faster genetic progress. After confirming and validating the QTL identified in this study, this information can be used to more accurately select ewes and rams with high genetic potential.

5. Conclusion

Growth traits are important and economic traits in sheep. The development of molecular genetics and genomic tools has provided a good opportunity to identify functional genes associated with economic traits. However, in several studies in Iran, using a limited number of markers, several genomic locations associated with quantitative traits have been identified and some of them can be used in the MAS equation. However, to more accurately estimate the genomic location of more QTL in Iranian sheep breeds, it is necessary to design a national plan with a larger experimental population. In this case, the genetic progress of Iranian sheep is expected to increase significantly with the use of this information in breeding programs.

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