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# A partial genome scan to identify quantitative trait loci affecting birthweight in Kermani sheep

A.K. Esmailizadeh*

*Research Division of New Technologies in Animal Production and Processing of Animal Products, Shahid Bahonar University of Kerman, Kerman, Iran*

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ABSTRACT

The aim of this study was to map quantitative trait loci (QTL) underlying variation in birthweight (BWT) in a population of Kermani sheep, an indigenous fat-tailed sheep breed in southeast of Iran. Based on the combined results from QTL analyses in different livestock species, genome homology among mammalian species and functional and positional candidate gene studies, genomic intervals located on ovine chromosomes 1, 3, 6, 11 and 14 were considered as candidate genomic regions for BWT. Progeny (276 animals) from six half-sib families were genotyped for 25 informative microsatellite markers flanking the candidate intervals. QTL analysis in the candidate intervals was conducted using the least squares regression interval mapping approach. Linkage analysis indicated significant QTL for BWT on sheep chromosomes 1, 3, and 6 (chromosome-wide significance of $P < 0.01$). No QTL was detected on OAR11, and OAR14. QTL effect ranged from 1.0 to 1.6 in unit of residual standard deviation in different families. Although growth QTL have been mapped on sheep chromosomes 1, 3 and 6, this is the first report of QTL for BWT on these chromosomes.



## 1. Introduction

Lamb size at birth and its manifestation, birthweight, are quantitative traits of major importance in sheep industry. Birthweight is a complex trait affected by both genetic and environmental factors. Environmental factors such as dam age, intrauterine malnutrition, and placental functioning may lead to low birthweight (Gardner et al., 2007).

Theoretically, in all mammalian species, there is an 'optimum' birthweight in which an uncomplicated natural delivery can occur and neonatal survival is maximized (Gardner et al., 2007). Clearly, there is a strong genetic component accounting for some of the variation in birthweight as extremes beyond this range will, over time, be selected out: low birthweight is associated with increased neonatal mortality, high birthweight with complicated labour (dystocia) and exposure of the dam to increased risk of death or injury during the delivery process (Alexander, 1974). In sheep, there is a curvilinear relationship between lamb birthweight and survival to weaning (Fogarty et al., 1992), with lamb mortality being greatest at both low and high birthweights and survival optimized between 3 and 5 kg, regardless of birth type (Hatcher et al., 2009).

Quantitative genetic studies in sheep have provided ample evidence that genetic factors also influence birthweight (see review of Safari et al., 2005). Weighted mean of direct and maternal heritability values obtained from literature for birthweight in different sheep breeds have been reported between 0.15–0.21 and 0.18–0.24, respectively (Safari et al., 2005) suggesting that genetic selection is a valuable approach for achieving improved birthweight. However, as it was pointed by Sawalha et al. (2007) selection for improved viability at birth and birthweight should consider optimal rather than maximum birthweight. Therefore, for more effective genetic selection to optimize birth size, it would be beneficial to discern the genomic variants controlling birthweight, and to utilize this information for genetic improvement.

* Tel.: +98 913 395 80 41; fax: +98 341 322 20 43.
*E-mail address:* aliesmaili@mail.uk.ac.ir.

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**Table 1**
Summary statistics for the families and birthweight (kg) data.

| Family | No. of progeny | Unadjusted mean | Mean[a] | SD[b] | RSD[c] | Min | Max | Variability (%)[d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 50 | 3.82 | 3.63 | 0.52 | 0.47 | 2.80 | 4.97 | 12.9 |
| 2 | 40 | 3.74 | 3.57 | 0.58 | 0.56 | 2.58 | 4.75 | 15.7 |
| 3 | 50 | 3.81 | 3.61 | 0.46 | 0.45 | 2.69 | 4.89 | 12.5 |
| 4 | 43 | 3.73 | 3.62 | 0.55 | 0.49 | 2.64 | 5.07 | 13.5 |
| 5 | 46 | 3.63 | 3.47 | 0.53 | 0.47 | 2.38 | 4.90 | 13.5 |
| 6 | 47 | 3.71 | 3.37 | 0.57 | 0.51 | 2.62 | 5.14 | 15.1 |
| Total | 276 | 3.75 | 3.45 | 0.53 | 0.49 | 2.38 | 5.14 | 14.2 |

[a] Adjusted birth weight mean for fixed effects and covariates included in the model.
[b] Standard deviation of birth weight data before correcting for fixed effects and covariates.
[c] Residual standard deviation after fitting the basic fixed effects and covariates (see text).
[d] Variability measured as coefficient of variation.

Identifying genes affecting quantitative traits (QTL) of economic importance in agricultural species has the potential to significantly increase the rate of genetic improvement through the use of marker-assisted selection (Spelman and Bovenhuis, 1998). In addition, genomic research and the identification of QTL help improve our understanding of the underlying biology of a specific trait.

Several studies in livestock have reported QTL associated with birthweight (e.g. Davis et al., 1998; Stone et al., 1999; Esmailizadeh et al., 2006 for cattle; Stearns et al., 2005; Munoz et al., 2009 for pigs). Surprisingly few QTL have been published for lamb birthweight which is of direct relevance to dystocia, lamb survival and early growth of the lamb; thus, this study aimed to identify genomic regions associated with QTL affecting birth weight in Kermani sheep using data from half-sib families and homology among mammalian species and positional candidate gene studies.

## 2. Materials and methods

### 2.1. Animals and trait measurement

The research was conducted at the livestock research center of Shahid Bahonar University of Kerman, southeast of Iran. It is about 1755 m above sea level with a longitude of 56°58′E and latitude of 30°15′N. Average annual rainfall is approximately 135 mm. The region has a semi-moderate and dry climate, with a maximum and minimum temperature of 39.6 °C, and −7 °C, respectively. The experimental flock comprises 200 sheep from the dual-purpose (meat and wool) Kermani breed. Kermani Sheep are fat-tailed, medium-sized (mature weight range is 45–50 kg) with white-coat color, indigenous to the south-eastern region of Iran which has a dry and hot climate. No genetic selection has been imposed in this experimental flock and culling in the station has only been practiced for old age, health problems and wool color so that sheep with white-coat color have been kept in the herd. Ewes were flushed before mating and kept indoors during the mating period (Mid July through late August). Dams and lambs were confined for 5–10 days after birth and then only the dams were let out to pasture daily.

The population studied herein consisted of 276 Kermani lambs from six half-sib families, with progeny per family ranging from 40 to 50 individuals (Table 1). The animals were bred over a 2-year period (2007–2008). Lambs were ear-tagged soon after birth and standard records such as parentage, date of birth, gender, birth type and age of dam were collected. Birthweight (BWT) was recorded (±0.01 kg) within the 12 h following birth.

### 2.2. DNA extraction and genotyping strategy

Blood samples of the six sires and their lambs were obtained from jugular aseptic venipuncuture into vacutainers with EDTA as anticoagulant. The blood samples were frozen at −20 °C until required. DNA was extracted from blood samples using DNA purification kit according to the instruction by the supplier (CinnaGen Co., Iran). Purity of all extracted DNA was assessed by calculating the 260/280 nm ratios determined with an Eppendorf spectrophotometer.

Based on the combined results from QTL analyses in different livestock species, genome homology among mammalian species and functional and positional candidate gene studies, the genomic intervals containing, transferrin and growth hormone factor 1 (*POU1F1* gene, previously named *PIT1*) genes located on OAR1, the insulin like growth factor-1 gene (*IGF-1*) on OAR3, growth QTL (Raadsma et al., 2009) and epidermal growth factor gene (*EGF*) located on OAR6, *GH1* gene (located on OAR11) and sheep birthweight QTL on BTA14 (Hadjipavlou and Bishop, 2009) were considered as candidate intervals for birthweight in this research.

The latest version of the ovine linkage map available on the Australian Sheep Gene Mapping website (http://rubens.its.unimelb.edu.au/~jillm/jill.htm) (Maddox and Cockett, 2007) was used in the present study. Initially, markers were chosen based on the position and easy of use (i.e., how easy is the process of optimization of PCR conditions and scoring the products of PCR following gel electrophoresis) as was reported by Maddox and Cockett (2007). In the first step, informative marker panels were developed separately for each ram, in up to five of the selected candidate regions of the sheep genome. This was achieved by initially genotyping each ram for all the selected microsatellite markers across each candidate region and then choosing heterozygous markers at approximately 15–20 cM intervals wherever possible. In the second step, taking into account marker position, polymorphic information content (PIC; >0.7 if possible) (Botstein et al., 1980) and easy of use, a panel of markers was chosen to type the progeny in the candidate autosomal regions. None of the rams were heterozygote for all the selected markers. Subsequently, the offspring were genotyped for the selected markers that were heterozygous in their sire (Table 1). Blood samples were not collected from dams; hence, dams were not genotyped.

The primers, amplification conditions and other marker information were obtained from the Australian sheep gene mapping website (http://rubens.its.unimelb.edu.au/~jillm/jill.htm). Microsatellite loci were amplified from 100 ng of DNA in a final reaction volume of 25 μL using CinnaGen PCR Master Kit following the instructions by the supplier (CinnaGen Co., Iran). The Microsatellite PCR products were separated by electrophoresis on 8% polyacrylamide gels. The gels were silver stained, dried and autoradiographied. Allele sizes were calculated using 50 bp DNA ladder. The films were scored independently by two researchers and the results incorporated in a database containing marker genotype and phenotypic data. Characterization of the candidate regions studied in the present work is given in Table 2.

### 2.3. QTL analysis

A statistical model accounting for known fixed effects including sex of the lamb, age of the dam, birth type, birth year and day of birth within year (as covariate) was fitted to the birthweight records using ASReml (Gilmour et al., 2006). The residuals of the model were stored as corrected birthweight records for the subsequent QTL analysis.

QTL analyses were conducted using a univariate multimarker approach for interval mapping in half-sib families, as described by Knott et al. (1996). The probability of inheriting a particular sire allele was calculated at 1-cM intervals for each offspring, conditional on the marker