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Quantitative Trait Loci (QTL) Analysis for Production Traits of Birth Weight and Weight 360 days in Backcross Sheep

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Four half-sib families (n = 382) consisting predominantly of ITT x Merino x Merino backcross progeny, including some F2 progeny were used to analyze QTL for two production traits (Birth weight = BW_1 and Body weight at 360 days = BW_{360}). The study exploited differences in weight performance between the Merino and ITT sheep. A total of 141 informative microsatellite markers were used in a genome-wide scan covering the 26 autosomal sheep chromosomes. QTL analysis was conducted online using QTL Express. This study reports the effect of QTL for birth weight on Chromosomes 5 ($p \leq 0.05$) at 112cM (0cM-128cM). Location of candidate genes for birth weight was predicted at the region of flanking markers MCM527-BMS1247. A QTL for BW_{360} days existed on Chromosome 18 ($p \leq 0.01$) at 104cM (25.0-125cM). Location of candidate genes related to production traits for body weight 360 days was predicted at the segment of flanking markers of CSSM018-TMR1. Only the QTL on Chromosome 18 retained significance ($p \leq 0.01$) under experiment-wide significance testing. This QTL region is being examined for candidate genes by investigating to the homologous human chromosomal segments.

Key words: Quantitative trait loci, production traits, birth weight, weigh 360, backcross sheep

INTRODUCTION

Currently there is much interest in the use of molecular markers to analyze the genetic basis of quantitative or complex traits. Development of large numbers of molecular markers and internal-mapping methods furnished the means of quantitative trait loci (QTL) mapping for identification of economic traits in livestock population. Current research in identification of any species has used polymorphic molecular markers. The marker has capability in looking for inheritance from genome segment of a pedigree. There is obviously correlation between the inheritance of specific marker allele and measured quantitative traits (Haley 2000). There is linkage between genetic markers to certain genes for production traits (Kinghorn & van der Werf 2000). Molecular approaches firstly have been applied in plant to determine QTL controlling the differences genetically among lines (Paterson *et al*. 1988). Last decade in animal livestock, it has been identified for milk production traits in Holstein population (Georges *et al*. 1995) and nowdays has been studied within inbred and outbred population.

As stated by Kinghorn and van der Werf (2000) that genotyping animal with a number of genetic markers is an investation in breeding to analyze accurately genetic traits of animals that have genetic merit. Application of molecular biology and biotechnology towards animal breeding to accelerate development and solve the problems for production improvement called as molecular breeding. Recent inprovement in breeding was dominated by advanced technology and science (Kinghorn & van der Werf 2000) and even conducted by large industries. Advances in computerizing have provided software for QTL analysis such as online QTL Express. Seaton *et al*. (2002) suggested that a suitable population for QTL analysis is halfsib outbred population which consists of many sires with each sire generates a number of offspring or F2 population originated from crossing between inbred or outbred. Mapping QTL in crossing between lines which different genetically has shown availability of many QTL, that also happens within animal population. QTL analysis provides information associated with a number of genes that plays a role in complex traits or polygenic genes, QTL location, and the effect of genes. QTL data provides worth knowledge in a number of interested genes affecting interest and economic traits in livestock and gene position on chromosomes and strength of each QTL.

Previous study in determining major genes for pre-weaning growth traits using segregation analysis, predicted availability of polygene effects in backcross sheep (Margawati *et al*. 2004). However, it could not identified the location of the complex traits or QTL. Those previous studies in segregation analysis relied on quantitative data without involving genetic markers. Therefore, the present study investigated the identification of QTL locations for production traits of birth weight and weight at 360 days and involved a number of microsatelllite markers in backcross progeny population of crossbred between Indonesian Thin Tail (ITT) sheep and Merino and backcrossed to Merino.

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MATERIALS AND METHODS

Reference Families and Backcross Population. Four reference families (F1 Sires of 1261, 1262, 1263, and 1273) were designed to establish a number of 382 halfsib backcross progeny population. Crossing of two differences genetically was suggested for QTL studies (Evans *et al*. 2003). Therefore this study involved ITT sheep which presented a small type while Merino as a large type in body weight. A large population of sheep is needed for QTL study in term to map a specific chromosomal region when the location of genes has not been known (Cockett *et al*. 2001; Raadsma *et al*. 2002).

Genomic DNA. Individual DNA of the population was collected based on a modified method of Montgomery and Sise (1990). The modification was performed in reagent concentrations and the dye as we used a Li-COR DNA Analyzer Gene ReadIR 4200. DNA was also collected from all F1 Sires (ITT x Merino), all their GrandSires and GrandDams.

Polymerase Chain Reaction (PCR). DNA was amplified by 35 cycles of PCR. A robotic PCR (Beckman) machine and a manual MJ PCR Machine were used to accelerate the PCR works. The same PCR program was designed to all markers, the program was set up as follows: warm up the machine at 95 ^{o}C for 5 minutes, denaturation at 95 ^{o}C for 45 seconds, annealing at 58 ^{o}C for 90 seconds, extension 72 ^{o}C for 60 seconds and kept at 4 ^{o}C until being used. The reagent was prepared in the laboratory with composition as follows: 10x NZ buffer 1 µl, $MgCl_2$ (2.5 mM-25 mM) 1 µl, dNTP 200 µm 0.4 µl, IR 700 (dye) 1 pmol/µl 0.2 µl, Forward primer 0.8 pmol/reaction 0.04 µl, Reverse primer 0.8 pmol/reaction 0.04 µl, ddH_2O 7.28 µl, Taq Zung32 (5 units) 0.08 µl. Each individual DNA sample of 1 µl (DNA template) needed 10 µl PCR reagent for PCR running.

Genotyping and Microsatellite Markers. A number of 250 preselection polymorphic microsatellite markers were screened to get informative markers. A number of 141 informative markers out of 250 were obtained from the marker screening. Those informative markers were used for a genome-wide scan covering the 26 autosomal sheep chromosomes and called genotyping (to genotype allele). Allele scorring was carried out by at least two researchers (Crawford *et al*. 1995) or using a specific software. Genotyping was conducted using a semi-automatic Li-COR DNA Analyzer Gene ReadIR 4200. A number of 3 to 16 markers for each chromosome were used for marker analysis (Table 1).

Phenotyping. Quantitative measurements of birth weight (BW_1) and weight at age of 360 days (BW_{360}) were conducted to all backcross progeny that collected from 1999 to 2002. Four reference families were established within four years in four periodes and weights were measured twice (*i.e*., BW_1 and BW_{360}) for each year. Therefore, the weight measurements were conducted eight times for both traits during the study (1999 to 2002). The age of animal population was from birth up to 360 days. Weight data were not corrected since the software program would read the file inputs with fixed factors. The study of QTL analysis more concerns on the number of population rather than the sex of animals. Therefore, this study neglected the sex of animals.

Genetic and Statistic Analyses. Markers and genetic marker distances were referred from Maddox *et al*. (2001) and updating sheep genome map can be checked through http://rubbens.its.unimelb.edu.au for QTL analysis. Online QTL Express software through website http://qtl.cap.ed.ac.uk was accessed for QTL Analysis with elicidation of Seaton *et al*. (2002). Three input data of Genotype file, Marker file and Phenotype file were prepared in textfile. Time of dropped population, genotype, sex and type of birth were considered as fixed factors. Three tests of Permutate Experiment Wide, Permutate chromosome wide and Bootstrap with resampling were performed at levels of 5 dan 1%. A chromosome wide threshold for statistical significance was calculated for each chromosome based on a permutation test of 1000 iterations.

RESULTS

QTL for Birth Weight. QTL location for birth weight was predicted to exist ($p < 0.01$) at chromosome 5 after permutate-chromosome wide. Summary of QTL analysis results for birth weight (BW_1) was presented at Table 2. The effect of QTL for birth weight traits was located at 112cM (0-128cM) of chromosome 5. Confident Interval (CI) for interest chromosome 5 was predicted on 128cM which is the most likely position of the QTL. Graphs from the Bootstrap test showed that QTL location at chromosome 5 for birth weight trait was more predicted from population of 1263 (Figure 1). Bootstrap analysis showed that t-value for birth weight came out from the population of family 1263 (Figure 1). This graph ilustrates that segregation of genes associated with production traits might be occurred the highest in population of 1263. Location of candidate genes for birth weight was predicted at the region of flanking markes MCM527-BMS1247 (Table 2). Candidate genes associated with production traits of birth weight are being investigated.

QTL for Weight 360. Significant effects of QTL were detected in four different chromosomal regions for production traits of body weight 360 days (Table 3). This study reported that QTL location at chromosome 18 was strong supported ($p < 0.01$) after permutate-chromosome wide test and even pertained strongly ($p < 0.01$) after permutate-experiment wide test. The other chromosomal regions at 7, 8, and 23 showed significant effects ($p < 0.05$) after permutate-chromosome wide test. The major QTL for BW_{360} was located at 104 cM of chromosome 18 with 95% Confidence Interval of QTL location was between 25-125 cM (Table 3). Figure 2 showed graphs of t-values from Bootstrap test for chromosome 18 and predicted from the population of family 1261. The t-value indicated that population of backcross progeny from reference family of 1261 seems to be more concerned since the graph (1.0) presented higher than other reference families (2.0, 3.0, and 4.0). Therefore it needs to be more emphasized for 1261 family in the next QTL analysis since the test of chromosome wide resulted a strong support QTL ($p \leq 0.01$) for production traits of weight 360 (BW_{360}) at chromosome 18.