

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 nowadays 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 investment 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 improvement 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 half-sib 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 microsatellite 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 °C for 5 minutes, denaturation at 95 °C for 45 seconds, annealing at 58 °C for 90 seconds, extension 72 °C for 60 seconds and kept at 4 °C until being used. The reagent was prepared in the laboratory with composition as follows: 10x NZ buffer 1 µl, MgCl₂ (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, ddH₂O 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 scoring 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 (BW₁) and weight at age of 360 days (BW₃₆₀) were conducted to all backcross progeny that collected from 1999 to 2002. Four reference families were established within four years in four periods and weights were measured twice (*i.e.*, BW₁ and BW₃₆₀) 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 elicitation 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 (BW₁) 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 illustrates 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 marks 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 BW₃₆₀ 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 ($p < 0.01$) for production traits of weight 360 (BW₃₆₀) at chromosome 18.

Table 1. List of markers per sheep chromosome

No	Markers	Chromosome	No	Markers	Chromosome	No	Markers	Chromosome
1	MCM46	1	48	MCM73	98	95	MCM152	13
2	EPCDV21	1	49	MCM380	99	96	HUJ616	13
3	HH51	1	50	TGLA303	100	97	BMS2319	13
4	BM6465	1	51	BMS2258	5	98	CSRD70	14
5	BM4129	1	52	BMS792	5	99	BMS2213	14
6	BMS482	1	53	TGLA137	5	100	LSCV30	14
7	BM6438	1	54	MCM527	5	101	BR3510	15
8	MAF64	1	55	BMS1247	5	102	Z27076	15
9	CSSM04	1	56	CP125	6	103	BM848	15
10	INRA011	1	57	MCM204	6	104	RM106	16
11	BM6506	1	58	HH55	6	105	BM1225	16
12	URB038	1	59	BM4621	6	106	MCM150	16
13	BMS4045	1	60	CSRD93	6	107	VH98	17
14	BMS1789	1	61	MCM214	6	108	AGLA299	17
15	ARO28	1	62	BM3033	7	109	VH116	17
16	MCM357	1	63	RNS5/BRN	7	110	BM7136	17
17	LSCV42	2	64	BMS1620	7	111	TGLA322	17
18	CSRD65	2	65	BMS2721	7	112	BM1117A	18
19	MCM147	2	66	MCM185	7	113	VH54	18
20	MCM505	2	67	BM1227	8	114	HH47	18
21	BMS1341	2	68	UWCA9	8	115	CSSM18	18
22	FCB128	2	69	KD101	8	116	TMR1	18
23	TGLA10	2	70	BMS1967	8	117	CSSM06	19
24	BM81124	2	71	ETH225	9	118	BMS1520	19
25	HH30	2	72	BM757	9	119	BMS875	19
26	BMS1126	2	73	BL1009	9	120	INRA132	20
27	MCM554	2	74	BM4513	9	121	CSRD26	20
28	FCB11	2	75	RJH1	9	122	SMHCC1	20
29	BMS1350	3	76	BM6108	9	123	VH110	21
30	ILSTS28	3	77	SRCRS25	10	124	BMC1206	21
31	BMS710	3	78	AGLA226	10	125	BMS651	22
32	TGLA67	3	79	HH41	10	126	BMS907	22
33	INRA131	3	80	ILSTS56	10	127	BM1314	22
34	BM827	3	81	BMS585	10	128	MAF36	22
35	ILSTS42	3	82	TGLA441	10	129	BL6	23
36	BMS1617	3	83	HEL10	11	130	CSSM31	23
37	VH130	3	84	CSSME70	11	131	MCM136	23
38	BM8230	3	85	BM17132	11	132	URB031	23
39	BMS1248	3	86	MCM120	11	133	JMP29	24
40	BMS772	3	87	ETH3	11	134	BMS744	24
41	BMS1788	4	88	HUJ614	12	135	BM737	24
42	BMS1237	4	89	TGLA53	12	136	MCM200	25
43	MCM218	4	90	CSSM03	12	137	MCMA7	25
44	MCM2	4	91	BM8225	12	138	RBP3	25
45	BMS1237	4	92	MCMA52	12	139	BMS2168	26
46	MCM144	4	93	LSCV38	12	140	BMS629	26
47	HH35	4	94	IL2RA	13	141	JMP23	26

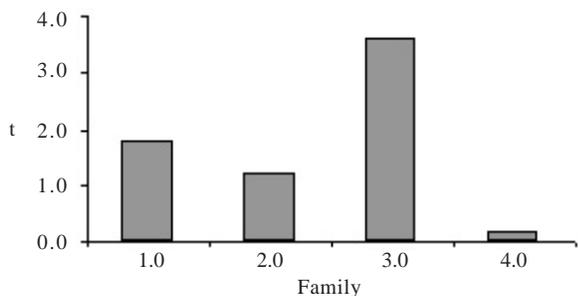


Figure 1. Absolute t-values of bootstrap for birth weight at chromosome 5 (1.0 = 1261; 2.0 = 1262; 3.0 = 1263; 4.0 = 1273).

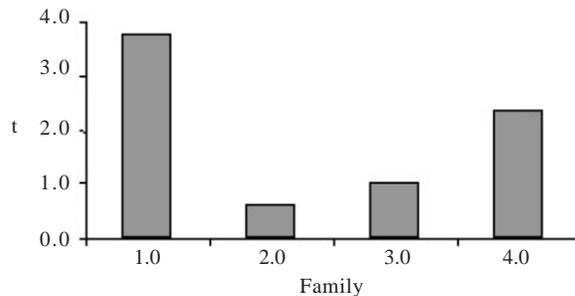


Figure 2. Absolute t-values of bootstrap for weight 360 at chromosome 18 (1.0 = 1261; 2.0 = 1262; 3.0 = 1263; 4.0 = 1273).

Table 2. Summary of permutate chromosome wide and bootstrap tests for birth weight (BW₁) of backcross lambs (ITTxMerinoxMerino)

Chr no	QTL location (cM)	Flanking markers	Bootstrap summary			
			Bootstrap sample	Av QTL Loc (cM)	95% CI of QTL Loc (cM)	Length of CI (cM)
5**	112	MCM527-BMS1247	623	98.028	0.0-128.00	128.0

**P ≤ 0.01

Table 3. Summary of permutate chromosome wide and bootstrap tests for birth weight 360 (BW₃₆₀)

Chr no	QTL location (cM)	Flanking markers	Bootstrap summary			
			Bootstrap sample	Av QTL Loc (cM)	95% CI of QTL Loc (cM)	Length of CI (cM)
7*	80	RNS5-BMS1620	634	65.873	0-132.00	132.0
8*	104	KD101-BMS1967	625	96.384	6.0-128.00	122.0
18**	104	CSSM018-TMR1	646	95.857	25.0-125.00	100.0
23*	52	CSSM31-MCM136	609	52.328	20.0-76.00	56.0

*p ≤ 0.05, **p ≤ 0.01

Table 4. Markers used on sheep chromosome 18

Length of chromosome 18	Marker	Distance (cM)
127.2 cM	BMS1117	12.7
	OARVH54	42.9
	OARHH47	77.9
	CSSM018	107.1
	TMR1	124.8

DISCUSSION

QTL for Birth Weight Traits. QTL analysis for birth weight traits showed that there was a chance of QTL for birth weight trait locating at chromosome 5. Compared to the previous finding (Margawati & Subandriyo 2004), this study on QTL analysis showed a more powerful tool in detecting the present of candidate gene(s) or markers flanking for the loci of complex traits. It may be this study involved markers and quantitative characters while the previous study was only using quantitative character data. Previous finding had not found significantly the presence of major gene for birth weight, while this study found more significant in detecting the location of candidate gene(s) that affects on birth weight trait.

This finding agreed with Haley (2000), Kinghorn and van der Werf (2000) in livestock, and Evans *et al.* (2003) in pig that high costs associated with collection of samples, phenotypes and genotyping the large number of animal showed more powerful tool by using QTL mapping. There is an indication that segregation analysis without involving DNA markers showed less powerful results. The segregation analysis using a mixed-model calculation is impractical and computationally difficult when the data set under analysis contains many animals (Davis & DeNise 1998). Therefore, this QTL study showed better improvement in findings that could state the effect of QTL for birth weight traits of sheep was located at chromosome 5. This finding supported previous reports (Davis & DeNise 1998; Dekkers 2004) that involving the genetic markers in combining with the phenotypic data could improve on selection in term to state a specific chromosomal region. The study also supported the previous studies of Lande and Thompson (1990) that proposed to use a selection index to combine phenotypic and marker data.

This study indicated the effect of candidate genes for birth weight at the region of flanking markers MCM527-BMS1247 on chromosome 5 (Table 2). Candidate gene(s) associated with production traits of birth weight at sheep chromosome 5 was *Calpastatin (cast)* gene that associated with meat toughness (Margawati 2005). The *cast* gene is predicted at 125.5-157cM of sheep chromosome 5.

Previous study in Hereford cattle identified the estimated QTL for birth weight traits on chromosome 2 (Grosz & MacNeil 2001). The finding is genetic antagonist as higher birth weight will affect on birth difficulty or dystocia. On the other hand, a higher birth is needed in animal selection to obtain a higher yearling weight.

QTL for Body Weight 360 Traits. The effect of QTL for production trait was detected at the chromosomes of 7, 8, 18, and 23 in the backcross population of Indonesian Thin Tail (ITT) sheep crossed to Merino and backcrossed to Merino. The strong finding at the chromosome 18 supported the recent report of Walling *et al.* (2004) that QTL location for growth and carcass was identified on chromosome 18 in commercial crossbred of Suffolk and Texel. In addition, they predicted that the QTL location is close to *Callipyge (clpg)* and *Carwell* genes. *Callipyge* gene is associated with meat production traits in Dorset (Cockett *et al.* 1996) while *Carwell* gene has function on development of Rib Eye Muscle (REM) in Australian Poll Dorset (Broad *et al.* 1998). Cockett *et al.* 2005 reviewed that Australian Poll Dorset ram possess unusual large rib-eye (longissimus dorsi) areas. McEwan *et al.* (1998) detailed that *carwell* gene in sheep has been localized to the distal end of OAR18 near *clpg*.

In this study, there were 5 markers used for genotyping on chromosome 18. Those five markers stretched at 127.2cM of the chromosome 18 with distance between the markers was presented at Table 4. This table showed a number of microsatellites with distance among them which stretched on 127.2cM length of the chromosome 18. Those markers were used for genotyping in this study. Location of candidate genes related to production traits of body weight 360 days was predicted at the segment of flanking markers of CSSM018-TMR1 (Table 3) and the flanking markers located at the end of chromosome 18 (Table 4). This finding agreed with the investigation of Cockett *et al.* (1994) that reported CSSM018

marker linked to loci relating to *clpg* gene. This location of gene lies at the end of sheep chromosome 18. Freking *et al.* (1998) studied on position of the *clpg* gene and to test gene action, their finding was consistent with previous assignment of the *clpg* locus to the telomeric region of chromosome 18. Based on the gene investigation of Margawati (2005), it was reported that there were some QTL relating with the production traits in sheep. These QTL located on chromosome 7, 18, and 23 for growth traits. The candidate genes were predicted as *Calpain3* (*cap3*) and *Somatostatin receptor1* (*sstr1*) on chromosome 7, predicted genes of *clpg* and *Carwell* located in chromosome 18 and did not find the candidate genes on chromosome 23. The strong existence of putative genes relating with body weight at 360 days was indicated on the population of family 1261 (Figure 2).

When the markers or the genes can be identified, genetic merit of superior breeding stock therefore can be known more accurate and efficient. The approaches of using markers relating to certain complex traits in animal selection for breeding programs is called a marker assisted selection (MAS). When gene is used as approaches it is called as a gene assisted selection (GAS). Since the QTL was a strong evident obtained on chromosome 18, this study suggests to put more markers on chromosome 18 to obtain markers that can be used as MAS for economic traits in livestock production.

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