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Genetic Characteristics of Four Microsatellite Markers Associated with Birth Weight in Bali Cattle

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Abstract: The aim of the present study was to verify associations between four microsatellite markers with birth weight in Bali cattle. A sample of 42 (including 21 dam and 21 progeny) was characterized for BMS1248, ILSTS006, TEXAN 15 and IGF-1. Associations between the genetic markers and birth weight were analyzed. The genotypes were classified according to allele size. Fragments of different sizes was different allels which named A-E and so on, respectively according to the order size of allel sequence. Four microsatellite markers were selected to analyze the genomic DNA of 42 Bali cattle The results showed that a total of 162 different alleles were found and the number of alleles in each locus was 2 to 6. The DNA fragment length was 100 bp to 444 bp and the number of mean valid alleles was 1.3069 to 4.2288. The value of heterozygosity was 0.2361 to 0.7677 and the mean polymorphism information content (*PIC*) was 0.5368. A GLM procedure was used to analyze the effects of these four microsatellites on birth weight. The BMS1248 had a significant impact on birth weight.These results suggest that microsatellite is a potential DNA marker for birth weight trait in bali cattle.

Key words: Bovine · DNA Markers · Production Efficiency

INTRODUCTION

Most genetic improvement of livestock, has been made by selection on phenotypes. Traditional breeding has been done in the absence of molecular knowledge of the genes acting on a quantitative trait locus [1]. Recent developments in DNA technologies, selection according to genotype has become an important tool in the breeding of farm animals [2]. Molecular genetics allows to study the genetic make-up of individuals at the DNA level. The discovery of genetic polymorphism at the DNA sequence level has been exploited as markers to explain the observed phenotypic variability in animals [3].

In cattle, selection of important properties that have economic value has become the goal of improving the productivity of cattle and it has been reported that microsatellite associated with gene loci that contribute to these important properties. By analizing the relationship between polymorphism of microsatellite DNA and productivity traits, some genetic markers related to productivity can be found and can be applied to animal molecular breeding [4].

Until recently, microsatellites were the markers used for mapping quantitative trait loci for production and functional traits in farm animals [5] and tightly linked markers are used for marker assisted selection in practice. They are also the prerequisite for the identification of positional and functional candidate genes responsible for quantitative traits. The major advantages of microsatellite markers are codominant transmission (the heterozygotes

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can be distinguished from homozygotes), locus-specific in nature, highly polymorphic and hypervariable, high information content and providing considerable pattern, its abundant quantity, wider distribution, higher mutation rate than standard and easy to sample preparation. Microsatellite has rapidly become the favorite method for population genetic studies as it shows advantages over othe methods. Microsatellite markers are particularly useful due to their wide variability, random distribution throughout the genome and Mendelian inheritance make them very informative markers in genome analysis [6] and possible influence on gene regulation [7, 8]. The ultimate use of microsatellite markers is for mapping quantitative trait loci (QTL), marker assisted selection (MAS) in order to practice genomic selection and improve the farm animal health [9].

In cattle, selection of important properties that improved growth properties primarily average daily have economic value has become the goal of improving weight, weaning weight and adult weight. Therefore, the objectives of this research were to evaluate polymorphism of microsatellite DNA and its possible association with birth weight of Bali cattle through for microsatellite loci, in order to use of marker-assisted selection methods to improve and accelerate breeding.

MATERIALS AND METHODS

Sample: Data from 21 offspring of 21 sires both males and females were used in this study. The sample were obtained at breeding UD Sari Laba, Bangli Bali. Blood samples (approximately 5 ml) were collected from each animal via the jugular vein. All samples were stored in EDTA-coated vacutainer tubes (BD Systems, Plymouth, UK). The samples were brought to the laboratory in an ice box and stored at 4°C till further processing.

Birth Weight Performance of Bali Cattle: All individuals of the sample possessed birth weight records and had always been maintained with the same conditions and feeding.

Dna Extraction and Pcr Amplification: Genomic DNA was extracted from peripheral blood lymphocytes using DNAzol method (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Genomic DNA was amplified by polymerase chain reaction (PCR). All animals (both sire and offspring) were genotyped using four microsatellite markers (BMS1824, ILSTS006, TEXAN 15 and IGF-1). The primers PCR shown in Table 1. PCR was performed in a 25 ìl. A master mix for minimum of 10 samples was prepared and aliquated 22 ìl in each PCR tube. Three ìl sample DNA was added in each tube to make the final volume. The amplified PCR products were electrophoresed in 6% nondenaturing polyacrylamide gel containing acrylamide and bis- acrylamideand stained with 0.1% silver nitrate following the standard protocol. The gel was visualized and documented under a white light gel documentation.

Genotype Determination: All the alleles of microsatellite locus were analysed. Fragments of different sizes was different allels which named A-E and so on, respectively according to the order size of allel sequence.

Statistical Analysis: The exact allele sizes were determined by direct comparison with adjacent PCR bands and 100 bp ladder (Invitrogen, USA). Allele frequencies were estimated from the genotypes. The polymorphism information content (PIC) was calculated using the individual frequencies in which the alleles occur at each locus. Allele frequencies, number of alleles and both observed (HO) and unbiased expected heterozygosities

Table 1: Primer microsatellite BMS1248, ILSTS066, IGF-1 and TEXAN-15 locus.

Primer sequence (5'-3')	Annealing temp	Alelle size	Locus
F: TGGAGGTTTGGGATTGGAGG	58^{0}C	122-164 bp	BMS1248
R: GAGTATGGAGTGACTGAGGG			
F: TGGAGGTTTGGGATTGGAGG	58^{0}C	149-153 bp	ILSTS066
R.:GAGTATGGAGTGACTGAGGG			
F: GCTTGGATGGACCATGTTG	58^{0}C	225-231 bp	IGF-1
R: CACTTGAGGGGCAAATGATT			
F:TCGCAAACAGTCAGAGACCACTC	58^{0}C	202-220 bp	TEXAN-15
R:TGGATGAGAAAGAAGAGCAGAGTTG			

(HE and PIC were calculated using MICROSATELLITE TOOLKIT V. 3.1. Association between genotypes of DNA microsatellite and birth weight was calculated by general linear model (GLM)(SPSS 16.0).

RESULT AND DISCUSSION

Birth Weight: It was found that the mean birth weight of sire was 17.38 ± 2.13 kg and dam birth weight was 17.33 ± 1.87 kg. Statistical analysis showed that there was no significant difference between the birth weight of the dam with the child birth weight (P> 0.05).

Genetic Diversity of DNA Microsatelit ILSTS066, IGF-1, Texan15 and BMS1248: Four microsatellite markers were selected to analyze the genomic DNA of 42 Bali cattle, the three markers are ILSTS066, IGF-1 and BMS1248 amplified well in Bali cattle, while Texan15 not amplified. The results showed that a total of 14 different alleles were found and the number of alleles in each locus was 3 to 6. ILSTS066 locus had 5 alleles with fragment lenght was by range of 130-170 bp. BMS1248 locus had 6 alleles with fragment lenght was 120-170 bp. IGF-1 locus had 3 alleles by range 210-230 bp (Table 2.). The result showed that these 3 microsatellite loci had polymorphism in Bali cattle. The PIC values ranged from 0.524 (locus IGF-1) to 0.767 (locus BMS1248). Heterozygosity values ranged 0.00 (IGF-1 and BMS1248) to 0.214 (ILST066).

Based on the value of Ho, three microsatellite loci showed values below 0.5. The highest PIC value is microsatellite BMS1248 (Table 3). The number of alleles at markers BMS1248 and IGF-1 on Bali cattle was different when compared with cows Canchim [10]. Canchim cows have 4 allele on BMS1248 and 4 of IGF-1. Polymorphism information conten is a index to indicate the polymorphism of segments [11] and were used to evaluate the degree of gene variance. Microsatellite loci has high polymorphism if PIC = 0.5 and medium polymorphism if 0.5 <PIC<0.25. In this study locus BMS1248 and ILSTS066 has high polymorphism. It could be verified that the high coefficiencies of polymorphism was apparently that there was a relationship between genotypes of DNA microsatellite with body weight [12]. The abundant polymorphism conten could provide the references for MAS study which is to accelerate the breeding of Bali cattle.

Association Between the Genotypes of Dna Microsatellite with Body Weight: Recently, study of polymorphism of microsatellite loci on economic traits of some cattle are well documented [10, 13, 14] also some studies describes the polymorphism only [15]. The information on the association analyses on birth weight in Bali cattle were not available. The significant tests of birth weight difference among genotypes of 3 micosatellite loci were carried out. There are total 16 genotypes were found in 3 microsatelitte loci, of those 7 genotypes in ILSTS066, 6 genotypes in BMS1248 and 3 genotypes in IGF-1 respectively.

Table 2: Number,size and frequency of alleles

Locus	Allele number	Allele size (bp)	Allele frequency
ILST066	5	130	23,81
		140	33,33
		150	30,95
		160	4,76
		170	7,14
BMS1248	6	120	16,67
		130	21,43
		140	7,14
		150	14,29
		160	30,95
		170	9,52
IGF-1	3	210	52,63
		220	31,58
		230	15,79

Table 3: Expected heterozygosity, observed heterozygositas and PIC in Bali cattle population.

Locus	Expected Heterozygosities	Observed Heterozygosities	PIC values
ILSTS066	0,766	0,214	0,715
BMS1248	0,806	0	0,767
IG-1	0,606	0	0,524

Table 4: Least square and standard errors of the birth weight obtained for the genotypes of 3 microsatellite loci in Bali cattle.

Loci (no.of genotypes	Genotypes (N)	Birth Weight
ILSTS006 (7)	130/130 bp (10)	17,70 ± 2,58
	140/140 bp (10)	17,80 ± 2.57
	140/150 bp (4)	15.75 ± 0.95
	150/150 bp (8)	17.12 ± 2.16
	150/160 bp (5)	17.00 ± 2.00
	160/160 bp (2)	16.50 ± 0.70
	170/170 bp (3)	17.33 ± 2.51
BMS1248 (6)	120/120 bp (7)	15.85 ± 2.79
	130/130 bp (9)	15.66 ± 0.70
	140/140 bp (3)	16.66 ± 2.88
	150/150 bp (6)	18.83 ± 0.98
	160/160 bp (13)	18.00 ± 2.16
	170/170 bp (4)	18.75 ± 1.50
IGF-1	210/210 bp (20)	16.80 ± 2.62
	220/220 bp (12)	17.66 ± 1.87
	230/230 bp (6)	18.33 ± 1.86

The results of statistical analysis showed that BMS1248 microsatellite locus have positive influence on birth weight of Bali cattle. In birth weight evaluation for the BMS1248 locus, 150/150 genotype showed a trend a largest birth weight and significantly higher then the genotype 130/130 and 160/160 ($p < 0.05$) (Table 4), which showed that allel 150 bp has an effect on birth weight in Bali cattle. The genotype 150/150 at BMS1248 loci was favorable genotype for birth weight in Bali cattle. In a commercial population of *Bos taurus* breeds, the haplotype segments on BTA 5 were found associations between birth weight [16].

According to previus study [14], the microsatellite loci BMS1248 also had a positive correlation with body weight and body lenght. However, the genetic basis for these effects is not clear. There may be a single pleiotropic underlying gene, or several closely linked genes with identical directional dominance but alternating breed additive effects.

CONCLUSION

Analysis of microsatellite marker polymorphism at BMS1248 locus revealed that allele 150/150 bp was associated with birth weight. It was also observed that the highest average birth weight was observed for animals possessing 150 bp allele. The associations observed in the present study indicate the possible utilization of favorable genotypes of the BMS1248 microsatellites in this population to increase production efficiency.

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