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Correlation Analysis of Microsatellite DNA Markers with Body Size, Lenght and Height of Bali Cattle

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Abstract: Forty one Bali cattle and 3 microsatellite DNA markers(BMS1248, ILSTS066 and IGF-1) were used to study the population genetic variation Bali cattle and the relationships between the gene loci and their body size, lenght and height traits.A GLM procedure was used to analyze the effects of these 3 microsatellites on body weight, length and height. The results showed that a total of 10 different alleles were found and the number of alleles in each locus was 2 to 6. The DNA fragment length for BMS1248 loci, ILSTS066 and IGF-1 were 120-180bp, 120-170 bp and 210-230 bp respectively.Mean values for observed heterozygosity for ILSTS066, BMS1248 and IGF-1 were 0.927, 0,561 and 1 respectively. The PIC value at the BMS1248 loci demonstratedhigh polymorphism in this population with PICvalues 0.6090. The microsatellite loci BMS1248 had a positive correlation with body weight and body lenght. While for ILSTS066 and IGF-1 loci had a negative correlation with body weight, length and height.

Key words: Microsatellite · Body size · Body height · Body lenght · Bali cattle

INTRODUCTION

Traditionally, the selection of calves superior to the properties that have high economic value based on individual performance. Since the 1990s the availability of molecular biology techniques has resulted in a new way in the selection of a number of economically important genetic traits. Selection is initially done conventionally now have switched to selection based on the identification of genes that may affect the nature of the economically valuable [1]. Selection is based on genomic data provide an advantage in order to improve the results of selection such as productivity, health and fertility [2]. Right now, the selection based on genotype has become a very important tool in the selection of superior breeding stock [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. Now this, is a DNA microsatellite genetic markers that provide information about the diversity of alleles at a gene locus. This diversity allows us to analyze and evaluate in detail and also detect genes that affect the nature of high economic value [4,5].

Until now, microsatellites are used to map genetic loci that influence the trait plays a role in animal production [6,7] and is a prerequisite for the identification of candidate genes responsible for a particular trait. Microsatellite markers can be used to identify genetic variation at each locus genes and chromosomal regions containing QTL important role in the expression of economically important traits in cattle [8,9,4]. Mizoshita *et al*. [10] has been used microsatellite DNA markers for the detection of chromosomal segments containing QTL that contribute to the growth and carcass composition in Japanese Black cattle.

In animals, it is known that QTL associated with improved growth properties primarily average daily weight, weaning weight and adult weight [11]. Malau-Aduli *et al*. [12] has been reported that the QTL on chromosome no.5 contribute to birth weight in Japanese Black beef cattle. Kim *et al*. [13] reported that a QTL on chromosome no.5effectonweight gain in cattle.No.5 bovine chromosome (BTA 5) has been known to contain

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QTL affecting growth and carcass [14-16]. In addition,many gene located adjacent to the QTL was also considered as a potential candidate genes that contribute to growth.QTL on chromosome no. 5 in cattle has been known to significantly affect the growth and body conformation.Information about the correlation between microsatelite DNA markers with body size, height and lenght of Bali cattle had never been shown. Therefore, the objectives of this research were to evaluate polymorphism of microsatellite DNAand its possible association with body weight, height and lenght of Bali cattle, in order to use of marker-assisted selection methods to improve breeding and to provide a basis for further progress to accelerate breeding in Bali cattle.

MATERIALS AND METHODS

Sample: The study was carried out on a total of 41 Bali cattle at breeding instalation Pulukan, Bali. Blood samples (approximately 5 ml) was collected from each animal via the jugular vein. All samples were stored in EDTA-coated vacutainer tubes (BD Vacutainer Systems, Plymouth, UK). The samples were brought to the laboratory in an ice box and stored at 4°C till further processing.

DNA Isolation and PCR Amplification: Genomic DNA was isolated from peripheral blood lymphocytes by the DNAzol method (Invitrogen, Carlsbad, CA). The 3 microsatellite loci related to some body size were selected from chromosome no.5. Genomic DNA were amplified at the following 3 microsatellite loci BMS1824, ILSTS006 and IGF-1 with Polymerase Chain Reaction. The primers PCR shown in Table 1. PCR was performed in a 25 µl. A master mix for minimum of 10 samples was prepared and aliquated 22 µl in each PCR tube. Three µl 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 system.

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 (HE)[17] and PIC were calculated using MICROSATELLITE TOOLKIT V. 3.1[18]. Association between genotypes of DNA microsatellite andbody weigh, lenght and height was calculated by general linear model (GLM)(SPSS 16.0).

RESULTS AND DISCUSSION

In this study, polymorphisms in 41 individuals belonging to Bali cattle breeds was analyzed using 3 microsatellite loci (BMS1248, ILSTS066 and IGF-1). The three loci located on chromosome no.5. A total of 10 alleles were recorded in Bali cattle and the number of alleles in each locus was 2 to 6. The BMS1248 had 6 alleles with the DNA fragment lenght was 120-180 bp. The ILSTS066 had 2 alleles with fragment lenght was 120-170 bp and IGF-1 locus had 2 alleles with fragment lenght was 210-230 bp (Table 2). The number of alleles at BMS1248 loci and IGF-1 loci differed from reported by Machado *et al*. [16] on Canchim beef cattle. The Canchim beef cattle had 4 alleles at BMS1248 and 4 alleles at IGF-1 loci. Polymorphism Information Content (PIC), expected heterozigosity and observed heterozigosity values for all 3 microsatellite markers analyzed in Bali cattle breeds are shown in Table 2. PIC is a index to indicate the degree of gene variance [19]. The microsatellite locus indicate high polymorphism if PIC = 0.5. In this reasearch the PIC value at the BMS1248 loci demonstrated high polymorphism in this population with PIC values 0.6090.

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

Locus	Primer sequence (5`-3`)	Annealing temp	Alelle size
BMS1248	F: TGGAGGTTTGGGATTGGAGG		
	R: GAGTATGGAGTGACTGAGGG	58°C	122-164 bp
ILSTS066	F: TGGAGGTTTGGGATTGGAGG		
	R.:GAGTATGGAGTGACTGAGGG	58°C	149-153 bp
IGF-1	F: GCTTGGATGGACCATGTTG		
	R: CACTTGAGGGGCAAATGATT	58°C	225-231 bp

Table 2: Number of allele, size and frequency in Bali cattle

Locus	Number of Allele	Allele Size	Allele Frequency
ILSTS066	2	150	53.66
		170	46.34
BMS1248	6	120	1.22
		130	8.54
		140	3.66
		1540	29.27
		160	48.78
		170	8.54
IGF-1	2	210	50.00
		230	50.00

Tabel 3: Expected, observed heterozygositiesand PIC in Bali cattle

Locus	Expected Heterozygosities	Observed Heterozygosities	PIC values
ILSTS066	0.503	0.927	0.3737
BMS1248	0.668	0.561	0.6090
IGF-1	0.506	1	0.375

Table 4: Association between microsatellite genotype, in ILSTS066, BMS1248 and IGF-1 locus with body weight, height and lenght of Bali cattle

Locus	Genotype	Number Allele	Body Weight	Body Height	Body Lenght
ILSTS066	150/150	3	297.00 ± 61.87	126.33 ± 5.5	121.66 ±1.52
	150/170	38	300.94 ± 109.76	122.97±10.69	120.12± 6.54
BMS1248	120/130	1	249.00 ± 0.00	117.00±0.00	123.5±0.00
	130/130	1	202.00 ± 0.00	116.00± 0.00	111.00±0.00
	130/140	2	405.00 +77.78	130.00±0.00	125.50±3.53
	130/150	1	790.00 ± 0.00	160.00 ± 0.00	145.00 ±0.00
	130/160	1	241.00 ± 0.00	121.00±0.00	116.00± 0.00
	140/160	1	204.00 ± 0.00	116.00 ± 0.00	114.00±0.00
	150/150	5	367.20 ± 136.98	129.00± 4.39	124.20±7.75
	150/160	12	278.23 ±37.63	120.83 ±6.89	119.08 ±3.44
	150/170	1	240.00 ± 0.00	120.00 ± 0.00	112.00±0.00
	160/160	11	265.81 ± 46.00	120.41±8.97	118.45±4.32
	160/170	4	287.00 ± 25.85	122.75 ±7.80	121.25±1.,25
	170/170	1	317.00 ± 0.00	127.00± 0.00	120.00±0.00
IGF-1	210/230	41	300.5±106.47	123.21±10.40	120.24± 6.31

This loci can be used to determine an imformative marker. Mean values for observed heterozygosity for ILSTS066, BMS1248 and IGF-1 were 0.927, 0.561 and 1 respectively (Table 3). Heterozygosities is generally used to evaluate genetic diversitywithin populations. The results of this research indicated that microsatellite loci IGF-1 showed greatest variability, the second was ILSTS066 and the lowest was BMS1248. The observed average heterozygosities for ILSTS066 and IGF-1 were greater than those reported by Machado *et al*. [16] for Canchim beef cattle, thus reflecting greater genetic variability in Bali cattle compared to Canchim beef cattle.

The correlation between the genotypes of DNA microsatellitewith body weight, height and lenght of the Bali cattle population was shown on Table4. Research on association between genotypes with body weight, height and lenght showed that the cattle possessing genotype of 130/150 on BMS1248 loci had the highest body weight, height and lenght compared to other genotype with 790 kg, 160 cmand 145 cm, respectively. While the genotype 130/130 had lower body weight, height and lenght compared to other genotype with 202 kg, 116 cm and 111 cm,respectively. The results showed that for microsatellite loci BMS1248 had a positive correlation with body weight and body lenght and for ILSTS066 and IGF-1 had negative correlation with body size, height and lenght.

CONCLUSION

Based on the research from the 3 loci (BMS1248, ILSTS066 and IGF-1), Bali cattle possessing typical genotypes of 130/160 in BMS1248 locuspossess high

body weight, height and lenght.While Bali cattle possessingtypical genotypes of 130/130 in BMS1248 loci had lowest body size, height and lenght. The microsatellite loci BMS1248 had a positive correlation with body weight and body lenght. While for ILSTS066 and IGF-1 loci had a negative correlation with body weight, length and height.

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REFERENCES

1. Oikonomou, G., K. Angelopoulou, G. Arsenos, D. Zygoyiannis and G. Banos, 2008. The effects of polymorphisms in the DGAT1, leptin and growth hormone receptor gene loci on body energy, blood metabolic and reproductive traits of Holstein cows.Animal Genetics, 40: 10-17.
2. Villumsen, T.M., L. Janss and M.S. Lund, 2009. The importance of haplotype length and heritability using genomic selection in dairy cattle. J. Anim. Breed, Genet., 126: 3-13.
3. Teneva, A., 2009. Molecular Markers In Animal Genome Analysis. Biotechnology in Animal Husbandry, 25(5-6): 1267-1284.
4. Erhardt, G. and C. Weimann, 2007. Use ofmolecular markers for evaluation of genetic diversity and in animal production. Arch. Latinoam. Prod. Anim., 15(Supl.1): 63-66.
5. Humblot, P., D. Le Bourhis, S. Fritz, J.J. Colleau, C. Gonzalez, G.G. Joly, A. Malafosse, T. Heyman, Y. Amigues, M. Tissier and C. Ponsart, 2010. Reproductive technologies and genomic selection in cattle.Vet. Med. Int. Article ID 192787.
6. Hiendleder, S., H. Thomsen, N. Reinsch, J. Bennewitz, B. Leyhe-Horn, C. Looft, N. Xu, I. Medjugorac, I. Russ, C. Kühn, G.A. Brockmann, J. Blümel, B. Brenig, F. Reinhardt, R. Reents, G. Averdunk, M. Schwerin, M. Förster, E. Kalm and G. Erhardt, 2003. Mapping of QTL for body conformation and behaviour in cattle. J. of Heredity, 94: 496-506.
7. Kühn, C., J. Bennewitz, N. Reinsch, N. Xu, H. Thomsen, C. Looft, C.A. Brockmann, M. Schwerin, C. Weimann, S. Hiendleder, G. Erhardt, I. Medjugorac, M. Förster, B. Brenig, F. Reinhardt, R. Reents, I. Russ, G. Averdunk, J. Blümel and E. Kalm, 2003. Quantitative trait loci mapping of functional traits in the German Holstein cattle population. J. Dairy Sci., 86: 360-368.
8. Schrooten, C., H. Bovenhuis, W. Coppieters and J.A.M. Van Arendonk, 2000. Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. J. Dairy Sci., 83: 795-806.
9. Tambasco, D.D., C.C. Paz, M. Tambasco-Studart, A.P. Pereira, M.M. Alencar, A.R. Freitas, L.L. Coutinho, I.U. Packer and L.C.A. Regitano, 2003. Candidate genes for growth traits in beef cattle crosses *Bos taurus* x *Bos indicus*. J. Anim. Breed. Genet., 120: 51-56.
10. Mizoshita, K., T. Watanabe, H. Hayashi, C. Kubota, H. Yamakuchi, J. Todoroki and Y. Sugimoto, 2004. Quantitative trait loci analysis for growth and carcass traits in a half-sib family of purebred Japanese Black (Wagyu) cattle. J. Anim. Sci., 82: 3415-3420.
11. Stone, R.T., J.W. Keele, S.D. Shackelford, S.M. Kappes and M. Koohmaraie, 1999. A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits, Journal of Animal Science, 77: 1379-1384.
12. Malau-Aduli, A.E.O., T. Niibayashi, T. Kojima, K. Oshima, Y. Mizoguchi and M. Komatsu, 2007. Detection and mapping of QTL on bovine chromosomes 2 and 5 segregating for live weight, average daily gain and body measurements in Japanese black cattle. Journal of Cell and Animal Biology, 1(3): 034-043.
13. Kim, J.J., F. Farnir and J.F. Taylor, 2003. Detection of quantitative trait loci forgrowth and beef carcass fatness traits in a cross between *Bos taurus* (Angus) and *Bos indicus* (Brahman) cattle. J. Anim. Sci., 81: 1933-1942.
14. Casas, E., S.D. Shackelford, J.W. Keele, R.T. Stone, S.M. Kappes and M. Koohmaraie, 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. J. Anim Sci., 78: 560-569.

15. Li, C., J. Basarab, W.M. Snelling, B. Benke, B. Murdoch and S.S. Moore, 2002. The identification of common haplotypes on bovine chromosome 5 within commercial lines of *Bos taurus* and their associations with growth traits. J. Anim. Sci., 80: 1187-1194.
16. Machado, M.B.B., M.M. Alencar, A.P. Pereira, H.N. Oliveira, E. Casas, L.L. Coutinho and L.C.A. Regitano, 2003. QTL affecting body weight in a candidate region of cattle chromosome 5.Genetics and Molecular Biology, 26: 259-265.
17. Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press. New York.
18. Park, S.D.E., 2001. Trypanotolerance in west African cattle and the population genetic effects of selection. PhD thesis, University of Dublin, Dublin.
19. Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in human using restrictionfragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.