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Research Article

Microsatellite Polymorphisms and its Relationship with Calving Interval and Gestation Period in Bali Cattle

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Abstract | The present study was conducted to detect polymorphisms of microsatellites and a possible relationship with calving interval and gestation period in bali cattle. The genotypes for ILSTS026, BMS1987, BMS2646, INRA072, and BMS1248 loci that involved in reproduction traits were asses by polymerase chain reaction in bali cattle. Blood samples were obtained from 42 bali cattle. The result describedthat all markers were amplified successfully in bali cattle microsatellite loci. For the five microsatellites loci analyzed were detectedto be polymorphic. The number of 29 alleles were foundin all samples, with the variation of alleles varied from 3 to 8. The fragment size was 105 bp to 300 bp. The PIC varied between 0.5025 to 0.859. For this marker, the Expected and Observed heterozygosity in this study diversebetween 0.5981 (locus BMS2646) to 0.9048 (BMS1987 and INRA072) and 0.5981 (BMS2646 to 0.8943 (BM1248), respectively. The microsatellite loci INRA072 had a positive association with gestation period while BM2646 had a positive association with calving interval.These data provide evidence that bali cattle breed have good genetic variability, which prospects for future selecting programs, especially marker assistant selection.

Keywords | Bali cattle, Genotype, PIC, Polymorphisms, Reproduction trait

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INTRODUCTION

Bali cattle is one of the cattle played an important role in livestock development in Indonesia.Currently, breeding programs in conserving and improving the genetic potential of bali cattle are based on performance and pedigree information without any knowledge of the number of gen that effect the perpormance.

Recent advances in molecular technologies provide various approaches for DNA polymorphism studies (Teneva et al., 2014). Molecular genetic technologies offer to possibility to known of gen involved in the expression of a quantitative traitby combining information on DNA markers and phenotypicinformation on a trait. Microsatellites are the most popular genetic marker and useful markers, due to distributed randomly in genome, codominance, abundance, and high level of polymorphism. By analyzing the association of polymorphism of microsatellite between reproductive traits, these marker can applied to marker-assisted selection (MAS). Amomg spesific genes that may influence the economic traits in cattle such ILSTS026, BMS1987, BMS2646, INRA072, BMS1248 loci had been extensively studied. The information on the association analyses on calving interval and gestation period in bali cattle were not available. In this research, was carried out to analysis the genetic variation of these microsatellite markers and relation between calving interval and gestation period in bali cattle.

MATERIALS AND METHODS

Blood samples was collected from 42 cows from UD Sari Laba Bali cattle Farm, Bangli, Bali. The cows were unrelat-

Table 1: Primer sequences for microsatellite ILSTS026, BMS1987, BMS2646, INRA072, BMS1248.

Primer sequence forward and reverse (5'-3')	Annealing	Alelle (bp)	Microsatellite markers
Primer:CTGAATTGGCTCCAAAGGCC Primer: AAACAGAAGTCCAGGGCTGC	58°C	150-158 bp	ILSTS026
Primer:TGATGCAGAGAACGTTTTAATTT Primer: CTTGGGGTAGGCAGAGATTT	58°C	109 bp	BMS1987
Primer: CAAAGCCATAAGAAGCAATTATG Primer: CCTTCTATAGTGTGGTGACTACCC	58°C	286-298 bp	BMS2646
Primer: CTTAACTCATTCACCTCAACTG Primer: AGTGATTGAGCACATTGCGCAT	58°C	100-126 bp	INRA072
Primer: GTAATGTAGCCTTTTGTGCCG Primer: TCACCAACATGAGATAGTGTGC	58°C	122-164 bp	BMS1248

ed and selected randomly. All cows were keptunder identical and standard conditions. DNA was isolated using DNAzol method (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations from peripheral blood lymphocytes.Samples were amplified by Polymerase Chain Reaction (PCR). The description of the primer uses in this study were given in Table 1. The PCR reaction was continued the following component : 100 ng DNA template, 20 pM of each primer, 200 lM each dNTP, 1U Taq DNA polymerase. The final volume was 25 µl. The amplified PCR products were separated by electrophoresis onto non-denaturing polyacrylamidgel (8%) containing acrylamide and bis-acrylamide. The gel were stained with 0.1% silver nitrate and visualized thenphotographs under a white light gel documentation.

Statistical Analysis

All the alleles of microsatelitte and genotypic frequencies were identified directly from the gel. The Allele frequencies for microstaellite loci were calculated for the entire sample. polymorphism information content (PIC), the allele frequencies, Observed heterozigosity (HO) and expected heterozygosity (HE) were calculated using sofware MICROSATELLITE TOOLKIT V. 3.1 (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/). The polymorphism information content was estimatedusing following equation formula (Botstein et al., 1980). The data on calving interval and gestation period of different genotypes were analysis) using the SPSS V.23 software for Windows of Analysis of Variance using General Linear Model (Sharma, 1996). Significant test of difference in calving interval and gestation period between the different markers were analyzed by LSD.

RESULT AND DISCUSSION

All of five loci in bali cattle were successfully amplified. Genotypic and allelic frequencies estimated for the five microsatellite loci in are presented in Table 2. 28 alleles were detected in this study with an average of 5.80 alleles, and the observed number of alleles in each locus ranged from three to eight. In this present study, showed that all loci in bali cattle had polymorphism. The lowest and high-

Table 2: Number of allel perlocus,size and frequency in bali cattle

Locus	Number of Allele	Allele size	Allele Frequency
BM1248	8	133	14.29
		135	9.52
		139	11.90
		144	14.29
		147	11.90
		149	11.90
		153	14.29
		159	11.90
INRA072	8	118	47.62
		122	2.38
		127	4.76
		129	33.33
		133	2.38
		138	4.76
		141	2.38
		153	2.38
BMS2646	3	285	35.71
		292	52.38
		300	11.90
BMS1987	6	105	23.81
		110	38.10
		113	4.76
		116	19.05
		120	4.76
		121	9.52
ILSTS026	4	150	9.52
		155	45.24
		156	9.52
		160	35.71

est PIC values was0,503 (locus BMS2646) to 0,859 (locus BM1248). The result of this study, indicated that the INRA072 and BMS1987 showed the greatest heterozygosity (0.905) and ILSTS026 the lowest one (0.429) (Table 3).

Table 3: Parameter of genetic information conten, HE(Expected), and HO (Observed heterozygosities) of Bali cattle.

Locus	HE	HO	PIC Value
BM1248	0.8943	0.8095	0.859
INRA072	0.6713	0.9048	0.6003
BMS2646	0.5981	0.5714	0.5025
BMS1987	0.7666	0.9048	0.7106
ILSTS026	0.6655	0.4286	0.5852

In the present study observed the number of alleles varied from three to eight with a mean of 5.80. Microsatellite BMS1248 markers on bali cattle were different compared with other cows (Machado et al., 2003). In Bali Cattle, microsatellite BMS1248 observed had eight alleles with 133 – 159 bp range of fragment length and in Canchim cows had four alleles 130-140 bp range of fragment leght. In Bali Cattle, ILSTS026 had 4 alleles was different when compared with Bonsmara cattle (Greyling et al., 2008). Compared with other research in Indonesian cattle, the average number of alleles per locus in bali cattle population relatively low. In Katingan cattle was reported 13.6 allel, whereas in aceh cattle was reported 9.25 alleles (Utomo et al., 2011). However, the avarage number of alleles in bali cattle in this study much larger when compared with the other research. The avarage number of allelels per locus in bali cattle was reported 1.94 alleles (Winaya et al., 2007). The number of loci analyzed and sample size might be the reason for these different results. The avarage number of alleles depending on the number of loci analyzed (Rogic et al., 2011) and the number of alleles produced depending on the size of the sample (Fatima et al., 2008).

Based on the value of observed heterozygosity (Ho) for bali cattle, four microsatellite loci showed values above 0.5 and only one (ILSTS026) showed value below 0.5 and with avarage values 0.71. Locus INRA072 exhibits the greatest heterozygosity variability and was the most informative locus for bali cattle. According to observed heterozygosity, genetic variability level in bali cattle population were detected is higher compared to other Indonesian cattle. Polymorphism Information Content is an index which used to measure the informativeness of genetic marker and analyzed the degrre of gene variance. Microsatellite loci considered as high polymorphism if PIC ≥ 0.5 and medium polymorphism if 0.5 <PIC<0.25 (Puja et al.2013). In bali cattle, all of microsatellite loci has PIC values more than 0.5. Microsatellite loci showed PIC valued higher than 0.5 are considered as informative marker in genetic population analysis (Botstein et al., 1980). The high value of PIC indicated that the breeds are more heterogenous with no selection for certain traits. These findings are in agreement with research valeu in Hariana and Hissar cattle from Pakistan (Rehman and Khan, 2009). who used 30 microsatellite lociand obtained PIC values 0.749 in Hariana and 0.719 in Hissar cattle from Pakistan.

Association between the Genotypes of DNA Microsatellite with Calving Interval and Gestation Period

In bali cattle, the lenght of gestation period varied from 257 to 331 days. The average gestation length was 270.00±15.31 days.Calving interval is the avarage time interval (days or months) between succesive calving both from the same cow. Calving interval for bali cattle ranged from 112 to 497 days with avarage 238.80 ± 84.86 days. The results showed that for microsatellite loci INRA072 was detected a significant correlation of markers with gestation period (p≤0.05) while BM2646 was detected a significant correlation of markers with calving interval (p ≤0.05). Analysis on association between genotypes with calving interval and gestation period showed that the cattle possessing genotype of 118/129 bp on INRA072 loci had the short gestation periodand genotypes of 292/300 bp on BMS2646 loci had the short calving interval.

In cattle, gestation length is influenced by factors such as the breed. The breed of cattle has the greatest influence on gestation length. The gestation lenght of 279 days in this study was shorter than 284.4±5.7 days reported for the breed in in some districts in Bali province between 1997 and 2003 (Prasojo et al., 2010). This gestation lenght in this study shorter than the other study may be due to the farm management and nutrition were much better. Nutrient intake can play as the most important to the of reproductive performance in cattle (Alam and Sarder, 2010).

The average of238 days bali cowcalving interval in this study were shorter than 365.6 days reported for the breed who breed in high and low placesin West Nusa Tenggara Province, Indonesia (Pribadi et al., 2015). Moreover, average calving interval in this study wereshorter than 360.9 days reported by Breeding Centre of Bali Cattle in Bali province (Gunawan et al., 2011). Calving interval among the indigenous and crossbred cattle in the tropical country was reported within 365-536 days (Kamal, 2010).

The results of this study indicated associations of INRA072 and BM2646 markers on gestation length and calving interval. In relation to these results, calving interval and gestation lenght is areproductive traits that can be influenced by genotype. These data suggest these

microsatellites might play a role on the regulation of the gen that affect gestation length and calving interval. These facts suggest the potential role of microsatellites in the regulation of gene expression.

CONCLUSION

According to the research of bali cattle population from the 5 loci, bali cattle possessing typical genotypes of 118/129bp in INRA072 locus leaded to possess short gestation period, while bali cattle possessing typical genotypes of 292/300 bp in BMS1646 loci had the short calving interval. The associations observed in this study indicate the possible utilization of INRA072 and BMS1646 marker in this population to increase reproductive efficiency

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AUTHORS CONTRIBUTION

All of the authors have read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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