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Polymorphism of DNA microsatellite BMS 1248 and ILSTS 006 on taro white cattle in Bali, Indonesia

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Abstract

The population of Taro white cattle is only about 59 cattle. This small population which will result in the occurrence of inbreeding. This can lead to loss of genetic diversity. The purpose of this research was to characterize Taro white cattle breed using microsatellite BMS1248 and ILSTS006. A total of 20 Taro white cattle were blood drawn and extracted, and genome DNA were amplified by PCR. The result showed that the markers successfully amplified in 18 Taro white cattle. Microsatellites locus BMS1248 and ILSTS006 were shown to be highly polymorphic. In locus BMS1248 were found 5 alleles with allele's size ranged from 139 to 165 bp. The number of alleles in locus ILSTS006 were found 5 alleles with alleles size ranged from 162 to 168 bp. The average observed and expected heterozygosity were 0.25 and 0.75 (BMS1248) and 0.5 and 0.671(ILSTS006). The PIC value was 0.679 (locus BMS1248), and 0.58 (locus ILSTS006).

Keywords: Heterozygosity, microsatellite, PIC, taro white cattle

Introduction

The Bali cattle (*Bos sondaicus*) is an indigenous Indonesian cattle that is suspected originated from the banteng (*Bos javanicus*). They are thought to have originated in Bali. The Bali cattle have several advantages, including having a good adaptability to a bad environment, such as areas with high temperatures, low / rough feed quality, and others. In addition to bali cattle, recently there is also a group of cows with a very small population and limited life in the forest of Taro Village, District Tegallalang, Gianyar regency, Bali Province. People called it a Taro white cattle (Fig.1). Taro white cattle are considered as an endangered breed of cattle population in Bali (Sulabda *et al*., 2021) [14]. Indigenous to Taro Village in Gianyar, cattle which tropically well adapted to local environment and raised by farmers in limited area of Taro Village, are deemed holy or sacred (Oka *et al*., 2020) [9]. This cattle has a distinctive phenotype that distinguishes it from bali cattle in general, which is white hair, white skin, irises of eyes and pale or clear nails, and the color of the horns and tracer is more pale than ordinary bali cattle.

Population of Taro White Cattle is only about 59 cattle. This small population will result inbreeding. The most obvious effects of inbreeding are slow the population's ability to reproduce and poorer reproductive efficiency. This can lead to loss of genetic diversity. Consequently, there will be a decrease in adaptation to the environment. Referring to population risk status limitation, the Taro white cattle is categorized as critical (Critical breed) (Oka *et al*., 2020) [9]. To anticipate the loss of biodiversity in Indonesia, especially the extinction of Taro White cattle as germplasm, conservation efforts are needed. For that we need to learn more about the genetic characteristics of this Taro white cattle and explored the potential of genetic diversity to be used as a source of genetic improvement of cattle in Indonesia.

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Fig 1: Taro white cattle

Genomic technologies can contribute to genetic resource management by identifying biodiversity to prioritize for protection, and informing management actions that attempt to mitigate threats to endangered species (Eusebi *et al*., 2020) [4]. Molecular analysis can be used to determine the unique genetic characteristics of a particular population. The most widespread among analysis molecular, microsatellite markers are molecular markers that have recently been used in many animal species for various purposes including individual identification, paternity testing, conservation of endangered animals, and phylogeny based on origin and breeding history (Kim *et al*., 2021) [7].

Microsatellites are short sequences of simple (especially di-, tri- and tetranucleotide are repeated in sequence in the eukaryotic genome (Pokhriyal *et al*. 2012) [11]. Microsatellites are also excellent as tools for genetic studies and conservation (Kim *et al*., 2021) [7]. Microsatellite has several advantages including easy sample preparation, high information content, and co-dominant markers.

BMS 1248 is part of microsatellite DNA located on chromosome number 5 in cattle. In the studies that have been done to find the genetic characteristics of Locus BMS 1248 associated with birth weight of Bali Cattle show BMS1248 locus has 6 alleles with a length of 120-170 bp fragments, polymorphic information content (PIC) value ranged from 0.767 (BMS1248). The value of heterozygosity ranges from 0.00 (BMS1248) to 0.214 (ILST006). This indicates that the microsatellites loci has a high polymorphism in bali cattle (Puja *et al*., 2013) [12].

ILSTS006 is part of the microsatellite DNA located on chromosome number 7 in cattle Several studies using ILSTS006 have been done to study the population of genetic variation of bali cattle and the relationship between gene locus and body size. The results showed the long DNA fragment for ILSTS006 was 120-170 bp. (Puja, *et al*., 2013) [12]. Based on the above information, this study was conducted the genetic profile of Taro white cattle by using the locus and to see the pattern of marriage that occurs in the Taro white cattle population in the taro village.

Materials and Methods

Animal

A total of 23 Taro white cattle were used in this study. The age of the animals used for the study ranged from 2-5 years. The animal age is based on birth record. All cattle was healthy and were managed in an extensive system. The study was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine Udayana University.

DNA Extraction and Amplification

Blood sample for DNA for DNA extraction were obtained using 10 ml EDTA tubes. Genomic DNA extracted from blood by the DNA extraction kit (DNA Mini Kit Purelink). DNA were extracted according to the manufacture's protocol**.** Microsatellite BMS 1248 and ILSTS 066 were amplified by PCR technique. Amplification reaction in PCR were carried out in PCT 100 (MJ Research, Inc., Watertown, Mass, USA with the following program: initial denaturation at 940C (3 min), second denaturation at 940C (35 seconds), annealing at 52 °C during 35 seconds and extension at 720 C for 35 seconds. The amplification results are separated by bis-acrylamide 8% and visualization is done by silver staining. DNA typing is done by measuring the migration distance of each DNA band on the gel compared to the standard DNA bands of 100 bp ladder.

Data Analysis

The number of alleles, HO (observed heterozygosity, and He (expected heterozygosity) were analyzed using the microsatellite toolkit v.3.1 program (Park, 2001) [10]. The PIC was calculated on the basis of allele frequencies, (Botstein *et al*., 1980) [1].

$$PIC = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2P_i^2 P_j^2$$

The degree of heterozygosity was calculated using unbiased estimators (Nei, 1987) [8] as follows:

$$H = 2N (1-\sum X1^{2}) / 2N -1$$

Results

A total of 5 alleles were detected in microsatellite locus BMS1248 and 4 alleles in ILSTS066. The number of alleles, allele's size, PIC, and heterozygosity were presented in Table 1. Locus BMS 1248 has 5 different alleles, with alleles size ranged from 139 bp to 165 bp. Allele size 145 bp is the allele with the highest frequency of 0.38. The heterozygosity (Ho) and (He) values were 0.25 and 0.75, respectively. The value of PIC is 0.679. Locus ILSTS 006 has 4 different alleles, with alleles size ranged from 162 bp to 168 bp. The heterozygosity (Ho) and (He) values were 0.5 and 0.671, respectively. The PIC value is 0.585

Table 1: Number of allels, heterozygosity and polymorphism information content (PIC) in Taro white cattle using microsatellite locus BMS1248 and ILSTS006

Locus	Number of alleles	Allele Size	PIC	Heterozygosity	
				Observed	Expected
BMS 1248	5	139 – 65	0.679	0.25	0.75
ILSTS 006	4	162 - 168	0.585	0.5	0.671

Discussion

Genetic diversity is useful for determining genetic associations within and between populations (Hartl and Clark, 2000) [5]. One approach that can be used is DNA Microsatellite. This research uses two markers, BMS 1248 and ILSTS 006 which have been successfully amplified.

In Taro white cattle have been identified 5 alleles at the locus BMS 1248, with range from 139 bp to- 165 bp. The number of alleles identified in this study were lower compared to number obtained in bali cattle, wich number of alleles of 8 (Puja *et al*., 2018) [13]. In locus ILSTS006, have been identified 4 alleles. The number alleles of Taro white cattle were observed lower compared number obtained in Ongole cattle, which number of alleles was 15 (Thiagarajan, 2012) [16].

The observed heterozygosity (Ho) and expected heterozygosity values in BMS1248 for the Taro white cattle were 0.25 and 0.75. This value was lower to those reported for bali cattle (HO=0.809, HE=0.894) (Puja *et al*., 2013) [12].

The observed heterozygosity (Ho) and expected heterozygosity values in locus ILSTS006 for Taro white cattle 0.5 and 0.67 (Ho = 0.5 and He= 0.67). This HO value was higher to those obtained in bali cattle and was lower in He. In bali cattle, the average HO and He for locus ILST006 was 0.214 and 0.766.

The heterozygosity value is the most accurate way to measure gene variation, so heterozygosity is also called gene diversity (Nei, 1987) [8]. A heterozygosity value below 0.5 indicates low gene variation in a population (Javanherd *et al*., 2005) [6]. If the observed heterozygosity value (Ho) is lower than the expected heterozygosity value (He) indicates a genetic imbalance in the population and indicates the absence of random mating (Tambasco *et al*., 2003) [15]. If the observed heterozygosity value (Ho) is lower than the expected heterozygosity (He) value indicates a genetic imbalance in the population (Tambasco *et al*., 2003) [15].

Polymorphism Information Content (PIC) is used to determine variations of microsatellite markers. In this study the PIC value of BMS 1248 was 0.679, while ILSTS 006 was 0.585. The value of PIC in this study was similar to that of genetic diversity between Indian Gir, Deoni and Kankerj cattle based on microsatellite with the same locus and showed PIC value was above 0.5, (Brezinsky *et al*., 1993) [2]. According to Botstein *et al*. (1980) [1] that the PIC value is an index to measure the genetics assessment. If the value of PIC > 0, 5 then the locus has a high degree of polymorphism. But if the value of PIC lower than 0.5 locus has a low level of polymorphism. Based on the research results that the PIC value is quite high, it indicates that these loci are highly informative (Cervini *et al*., 2000) [3].

Conclusion

In this study demonstrated that microsatellite marker were use shown to be polymorphic. The Taro white cattle indicates low level genetic diversity. The results of this study indicate a genetic disequelibrium in the population and indicate the absence of random mating. The information elucidated through the present study would be useful for the formulation of conservation strategies.

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