

Contributions of *Bos Indicus* Breed to Genetic Diversity of Sumatra Native Cattle Based on Y-Chromosome Microsatellite Marker

A. Winaya¹, Muladno², R.E. Gurnadi² and A. Saefuddin³

¹Animal Husbandry Department, Faculty of Agriculture & Animal Husbandry, University of Muhammadiyah Malang

²Faculty of Animal Science, Bogor Agricultural University

³Faculty of Mathematics & Natural Science, Bogor Agricultural University

ABSTRACT

The genetic composition of cattle in the world generally tends to two dominated breeds, *Bos taurus* (European cattle) and *Bos indicus* (Asian cattle). From morphological phenotype could be differed by hump for Asian cattle and humpless for European cattle. Molecular study indicated that any alleles or haplotypes which tend to those dichotomy. Similar condition in Indonesian cattle, including Aceh and Pesisir cattle, previous study showed that any indication introduction of Taurine and Indicine breeds in those cattle, while in the other hand Indonesian native cattle is descendent from the one of common ancestor cattle in the world, Banteng. For advancing study in genetic introduction of other breeds in Aceh and Pesisir cattle, we assayed those Sumatra's breeds by using molecular marker of Y-chromosome microsatellite. The using of Y-chromosome marker by assumption could be a model for detection of male introduction in breed. From this research showed that all of locus have low allele number, both in Aceh and Pesisir cattle. Also from *Polymorphic Information Content (PIC)* value, this marker has lower value (less 0.5) than FAO recommended. But, in these result indicated that *B. indicus* is the one of the genetic composition of Aceh and Pesisir cattle. Because locus INRA 124 was could amplification in those breeds and these locus also the one of *B. indicus* specific allele.

Key words: *Bos taurus*, *Bos indicus*, *Bos javanicus*, Y-chromosome microsatellite, Aceh cattle, pesisir cattle

INTRODUCTION

Asia continent has more or less three hundred millions cows and two hundred millions of them reside in India sub-continent. Cattle breeds in Asia and Africa are generally categorized into hump and humpless. About 170 breeds are already known, including Bali cattle (domesticated from Banteng) in Southeast Asia, including Indonesia and Philippine. So, Asian farmers have been famous in their role in cattle domestication process and agriculture ecology in this continent (Schearf, 2003).

Bali cattle and other breeds have genetic relationship with Banteng (just like Madura cattle) and also have genetic admixture with *B. taurus* and *B. indicus*. Because of economic and political factors in the past (during colonialism era or after the independence), they are brought to Indonesia and finally could adapt to the environment and become part of local cattle. Aceh cattle are believed to be the local breed, but the previous studies by Muhamad *et al.* (2007) and Uгла (2008) shows that the genetic

compositions of those cattle came from *B. indicus*. So, the genetic study of native cattle in Indonesia is interesting because the genetic variations are great. This is important because it is related to the efforts characteristics improvement and keep genetic characters conserved. Finally, the quality of those native cattle does not decrease or even extinct.

The recent development on genome and genetic analysis on human population shows a tendency to haplotype of Y-chromosome which is an important tool in studying population naturally (Hurles & Jobling, 2001). Without neglecting pseudo-autosom, Y-chromosome action in general is a non-recombinant unit, which is male specific and effective haploid. This is to make sure that the combinations mutation along male offspring is concentrated as a single un-biased haplotype. Y-chromosome characters are needed in its context as male lineage just like mitochondrial DNA in female lineage. The level of polymorphism characters in non-recombinant area of Y-chromosome area is started from the lowest, that is biallele event in point mutation of

single nucleotide polymorphisms (SNPs) to the most common event in locus characters minisatellite or microsatellite (Short Tandem Repeat [STR]). Eventhough, the polymorphism of SNPs in Y-chromosome is often founded in specific population (Hammer *et al.* 1997). However, data of Y-chromosome in non-primate (like cattle, goat and lamb) genetic population is rare because of its rare marker in Y-chromosome in sequence information (Petit *et al.* 2002) and its low variation level (Hellborg & Ellegren 2004; Meadows *et al.* 2004; Queney *et al.* 2001).

Domestic cattle show indicated that individual variation into population based on microsatellite molecular marker is specific character to their sex chromosome (Y-chromosome), the existence of breed hybridization and their migration (McHugh *et al.* 1997; Bradley *et al.* 1998). Therefore, the existence of polymorphism in microsatellite specific to Y-chromosome can be used as a starting point in providing information about paternal genetic variation study on cattle or related species. The indication that growth hormone pseudogen (GH) is found in male domestic cattle shows that sex chromosome can influence individual differentiation process. It is important to know the role of sex chromosome in male cattle, for examples, in their reproduction ability.

Hanotte *et al.* (2000) study using microsatellite characters in Y-chromosome of INRA23 locus showed that there is any introduction of Zebu male in local cattle, Mozambique and Zimbabwe. It is suspected that their alleles came from Mozambique bay. In the past, Mozambique bay is an international trading area including cattle trading, so that was able to make genetic introduction of cattle from other breeds.

Edward *et al.* (2000) study using four characters microsatellite DNA marker (INRA124, INRA126, INRA 189 and BM 861) showed an evidence that any male genetic flow from *B. Taurus* or Zebu on hybrid population of bovidae species, including cattle. A study on African cattle using microsatellite DNA on Y-chromosome showed that there is a high different in cattle in central Africa and Southwest Africa.

The specific aim of this study is to know the pattern of Y-chromosome microsatellite DNA allele polymorphism in Aceh and Pesisir cattle, and to get Y-chromosome microsatellite DNA specific alleles of Aceh and Pesisir cattle.

MATERIALS AND METHODS

Collection of DNA Samples

DNA was extracted from total blood-cells which were collected from cattle as many as 10 ml per sample and preserved using EDTA 10%. The two areas where the samples came from were Aceh cattle from Breeding Center of Promising Aceh Cattle (*Balai Pembibitan Ternak Unggul (BPTU) Sapi Aceh*), Indrapuri Sub District, Aceh Besar Regency, Nangroe Aceh Darussalam Province and Pesisir cattle from Painan Sub District, Pesisir Selatan Regency, West Sumatera Province.

DNA Amplification and Allele Microsatellite Detection

DNA extraction using phenol-chloroform standard method (Sambrook *et al.* 1989) was then preserved in TE buffer. Locus-microsatellite amplification used seven primers which is flanks Y-chromosome microsatellite locus (INRA008, INRA057, INRA062, INRA 124, INRA 126, DYS 199, and INRA 189). Microsatellite allele PCR product were separated using PAGE 10% and observation was done manually (Leung *et al.* 1993) after silver staining (Tegelstrom 1986).

Y-Chromosome Microsatellite Allele Analysis

Microsatellite alleles were obtained from observation result and were analyzed statistically to get frequency and distribution value of allele, heterozygosity (*h*) allele and *Polymorphic Information Content* (PIC) in sample population.

Total number and frequency of microsatellite alleles were calculated to get the value of heterozygosity and alleles frequency per locus using formula:

$$f(A) = \frac{A}{2n}$$

where:

f(A) = Frequency of microsatellite allele

A = Total number alleles of *i*th locus

N = Total number of individual observed

Locus is considered polymorphic if the value of f(A) is the same or less than 0,9. Genetics variation is determined from the average value of heterozygosity (*h*) for all locus.

Heterozygosity value per locus is calculate according to formula bellow (Nei 1987):

$$h = 2n \frac{(1 - \sum X_i^2)}{(2n - 1)}$$

where:

h = locus heterozygosity

X_i = alleles frequency of i^{th} locus

N = total number of individu observed

Allele frequency is also used to get Polymorphic Information Content (PIC) index, i.e. value to calculate marker informative level, calculated according to Botstein *et al.* (1980) formula:

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

Where k is total alleles number, P_i and P_j are allele frequency of i^{th} and j^{th} allele respectively at microsatellite locus observed.

RESULTS AND DISCUSSION

The developments of some genetic markers that are used in cattle breeding because of the molecular markers are more discriminative and accurate than their phenotypic characters. But, the utilizing of this marker has not been maximally yet. By using this marker would have helped in handling cattle breeding management system (Ge *et al.* 2002).

Breed specific allele information for Indonesian domesticated cattle until recently is limited. From previous studies (Muladno *et al.* 2000; Noor *et al.* 2000; Winaya 2000; Winaya *et al.* 2000; Mohamad *et al.* 2007; Uгла 2008) that use DNA microsatellite marker has been showed that these markers can also give description of breed specific allele and genetic relationship between Indonesian native cattle (i.e. Aceh, Pesisir, Madura, Bali, PO and PFH), but this result can not shows yet the description about the genetic variation of Indonesian native cattle based on Y-chromosome DNA microsatellite in more detail. So, this study may be consideration as a preliminary data base to determine the genetic composition of Indonesian native cattle in general. PCR product using seven primers which is flanks microsatellite sequence were loaded using polyacrilamide gel electrophoresis / PAGE 10% then continued with silver staining. Microsatellite allele was detected manually either its number and its size and next analysis.

Separation result using polyacrylamide gel generally can determine number and allele frequency as shown in Table 1 below.

Table 1 showed that we found only two alleles for all of locus by means 1.7, both in Aceh or Pesisir cattle. So, this allele still could not be determined as polymorphic allele yet, because according to FAO minimum standard locus has minimally four different alleles to be used as judgment in determining genetic differentiation between groups of cattle (Pandey *et al.* 2006). From this study it is also revealed that genetic composition of Aceh and Pesisir cattle are also contain of *B.indicus* genetic. Because INRA124 also the one of *B. indicus* locus (Hanotte *et al.* 2000; Edwards *et al.* 2007). These result, therefore, are in line with the previous research results that *B indicus* is one of genetic source of the genetic composition of Aceh and Pesisir cattle (Mohamad *et al.* 2007; Uгла 2008).

From previous study (Winaya *et al.* 2008), the highest heterozygosity (h) value has been found in microsatellite locus of DYS 199 (68%) in Madura cattle population. It shows that the higher the number of allele, higher the heterozygosity tendency will be. Again, according to FAO guideline, here must be four different allele locus minimum to justify the differentiation or genetic variation between breed (Pandey *et al.* 2006).

From polymorphic information content (PIC) value, the Y-chromosome microsatellite marker has reveals PIC value from 0.18 to 0.23. This result could be explained that in generally all of the locus could not be determined as an informative allele for population genetic analysis, because the PIC value less than 0.50 (Botstein *et al.* 1980). As Meadows *et al.* (2006) study has been found the low value in nucleotide variation sequence of specific Y-chromosome area in some animal species, including cattle. So, from this study we still need more locus of Y-chromosome microsatellite marker for genetic analysis of Indonesian native cattle in generally. As we know that until recently for genetic evaluation is trend to use the Y-chromosome microsatellite marker. So, eventhough this study was not found the polymorphic locus, but for future study we still need more number of Y-chromosome DNA microsatellite marker, because this marker could be as a tool to determine the genetic specific of Indonesian native cattle in much more detail.

Table 1. Number of cattle assayed, allele number and frequency of seven Y-chromosome microsatellite locus in population of Aceh and Pesisir cattle

LOCUS	ACEH			PESISIR		
	1	2	3	1	2	3
INRA 008	18	2	A = 3 (0.17) B = 15 (0.83)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 057	18	1	A = 18 (1)	15	2	A = 2 (0.13) B = 13 (0.87)
INRA 062	18	2	A = 6 (0.33) B = 12 (0.67)	15	2	A = 7 (0.47) B = 8 (0.53)
INRA 124	18	2	A = 5 (0.28) B = 13 (0.72)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 126	18	1	A = 18 (1)	15	2	A = 1 (0.07) B = 14 (0.93)
DYS 199	18	2	A = 3 (0.17) B = 15 (0.83)	15	1	A = 15 (1)
INRA 189	18	2	A = 4 (0.22) B = 14 (0.78)	15	1	A = 15 (1)

Note: 1) number of cattle assayed; 2) allele number; 3) allele frequency.

Table 2. Heterozygosity (*h*) and Polymorphic Information Content (*PIC*) value of Y-chromosome microsatellite allele between Aceh and Pesisir cattle population

LOCUS	ACEH		PESISIR	
	<i>h</i>	<i>PIC</i>	<i>h</i>	<i>PIC</i>
INRA 008	0.30	0.24	0.34	0.27
INRA 057	0	0	0.24	0.20
INRA 062	0.47	0.34	0.53	0.37
INRA 124	0.43	0.32	0.34	0.27
INRA 126	0	0	0.14	0.12
DYS 199	0.30	0.24	0	0
INRA 189	0.36	0.28	0	0
Mean ± SD	0.27 ± 0.19	0.20 ± 0.14	0.23 ± 0.20	0.18 ± 0.14

CONCLUSIONS

The genetic flow of both *B taurus* and *B indicus* influencing genetic composition of Indonesian native cattle, including Aceh and Pesisir cattle, eventhough, we knew that Indonesian native cattle breed generally still have a lineage from Banteng (*Bos banteng*), the one of ancestor cattle in the world until nowadays. This trend has also been described in this study, by using Y-chromosome microsatellite markers, those cattle populations have indicated any genetic composition of *B indicus*, with locus INRA124 as reference.

The use of molecular marker of Y-chromosome microsatellite has generally described potentials and genetic variations of Aceh and Pesisir cattle, eventhough there was not found the polymorphic and informative allele. But, for the next study we can use more locus of Y-chromosome DNA microsatellite marker, because we still need more data base for genetic

potential of Indonesian native cattle, especially the genetic composition of Aceh and Pesisir cattle. So, the genetic existence of those cattle as specific cattle can be improved.

ACKNOWLEDGEMENTS

The authors wish to thank the Head of Livestock Office of Nangroe Aceh Darussalam Province and the Head of Livestock Office of Pesisir Selatan Regency for providing blood cattle samples. Also grateful for Directorate of Higher Education, the Ministry of National Education for funding support through Research Competitive Grand Program, Year 2007-2008.

REFERENCES

- Bradley DG, Loftus RT, Cunningham P, Machugh DE. 1998. Genetics and Domestic Cattle Origin. Evolutionary Anthropology. Willey-Liss, Inc. hlm. 79-86.

- Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32 : 314-331.
- Edwards CJ, Gaillard C, Bradley DG, MacHugh DE. 2000. Y-specific microsatellite polymorphisms in a range of bovine species [short communication]. *Anim. Genet.* 31 : 127-130.
- Edwards CJ, Baird JF, MacHugh DE. 2007. Taurine and zebu admixture in Near Eastern cattle: a comparison of mitochondrial, autosomal and Y-chromosomal data [short communication]. *Anim. Genet.* 38 : 520-524.
- Hammer MF, Spurdle AB, Karafet T, Bonner MR, Wood ET, Novelletto A, Malaspina AP, Mitchell RJ, Horai S, Jenkins T, Zegura ZL. 1997. The geographic distribution of human Y chromosome variation. *Genetics* 145: 787-805.
- Hanotte O, Tawah CL, Bradley DG, Okomo M, Verjee Y, Ochieng J, Rege JEO. 2000. Geographic distribution and frequency of a taurine *Bos taurus* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan African cattle breeds. *Mol. Ecol.* 9: 387-396.
- Hellborg L, Ellegren H. 2004. Low levels of nucleotide diversity in mammalian Y chromosomes. *Mol. Biol. Evol.* 21 : 158-63.
- Hurles ME, Jobling MA. 2001. Haploid chromosomes in molecular ecology: lessons from the human Y. *Mol. Ecol.* 10 : 1599-1613.
- Leung H, Nelson RJ, Leach JE. 1993. Population structure of plant pathogenic fungi and bacteria. *Adv. Plant Pathol.* 10 : 157 - 205.
- MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics.* 146, 1071-1086.
- Meadows JRS, Hawken RJ, Kijas JW. 2004. Nucleotide diversity on the ovine Y chromosome. *Anim. Genet.* 35 : 379-385.
- Mohamad K, Olsson M, Anderson G, Purwantara B, van Tol HTA, Mikko S, Rodriguez-Martinez H, Colenbrander B, Lenstra JA. 2007. Genetic diversity and conservation of South-East Asian cattle : from Indian zebu to Indonesian Banteng, and then to the Cambodian Kouprey?. EU-Asia Link Project Symposium "Managing the Health and Reproduction of Elephant Population in Asia. 8 - 10 October 2007. Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand, pp. 120-124.
- Nei M. 1987. *Molecular Evolutionary Genetics.* New York : Columbia University Press.
- Noor RR, Muladno, Benyamin B, Hedah Z, Herliantini. 2000. Uji Kemurnian Sapi Bali Melalui Protein, DNA Mikrosatelit, Struktur Bulu dan Kromosom [laporan penelitian]. Bogor : Fakultas Peternakan IPB dan Pusat Inseminasi Buatan Singosari.
- Pandey, AK, Sharma R, Singh Y, Prakash BB, Ahlawat SPS. 2006. Genetic diversity studies of Kherigarh cattle based on microsatellite markers. *J. Genetics* 85 (2) : 117-122.
- Petit E, Balloux F, Excoffier L. 2002. Mammalian population genetics: why not Y? *Trends Ecol. Evol.* 17 : 28-33.
- Queney G, Ferrand N, Weiss S, Mougél F, Monnerot M. 2001. Stationary distributions of microsatellite loci between divergent population groups of the European rabbit (*Oryctolagus cuniculus*). *Mol. Biol. Evol.* 18 : 2169-78.
- Scheerf, B. (ed.). 2003. *World Watch List for Domestic Animal Diversity.* FAO 2003.
- Tegelström H. 1986. Mitochondrial DNA in natural population : an improved routine for the screening of genetic variation based on sensitive silver stain. *Electrophoresis.* 7 : 226 - 229.
- Ugla CM 2008. Investigating genetic variability within specific indigenous Indonesian cattle breeds.[dissertation]. Uppsala, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7080, 75007 Uppsala, Sweden.
- Winaya A, Herwintono, Amin M. 2008. Y Chromosomal Microsatellites Polymorphism in Madura Cattle (*Bos javanicus*). Proceeding of The 4th Indonesian Biotechnology Conference. Bogor, August, 5 - 7, 2008.

Measuring the Responses of Different Genotypes of Slow Growing Broilers Toward Short-Term Heat Challenge Test

R. Afnan¹ and M. Gerken²

¹ Department of Animal Production and Technology, Bogor Agricultural University, Bogor, Indonesia

² Department of Ecology of Animal Farming and Behaviour, Georg August University, Goettingen, Germany

ABSTRACT

This study was performed to evaluate the responds of different genotype of slow growing broilers with regard to heat stress. A number of 102 females from the slow growing broiler hybrids (Hubbard ISA I657, S757N and I957) were raised from hatch until week 5 in 3 pens under the same room temperature of 30°C beginning from week 3 until 5. Twenty four experimental birds of each genotype were individually exposed for 15 minutes to a short-term heat test at 30°C (control) and 35°C between weeks 3, 4, and 5. The rectal temperatures before and after heat exposure were measured and the latency until panting was recorded. Strain differences were significant for body weight, daily weight gain and relative growth rate ($P < 0.01$). For I657, S757N and I957, respectively, body weight in week 5 averaged 815.8 ± 81.2 , 924.0 ± 87.9 and 1269.3 ± 136.3 g. Daily gain averaged 22.0 ± 9.8 , 25.5 ± 13.1 and 34.9 ± 17.6 g/d, whereas relative growth rate ranged between 11.5 ± 5.5 , 13.9 ± 6.9 and 13.0 ± 6.1 %. Rectal temperatures after short-term heat stress were $42.4 \pm 0.7^\circ\text{C}$, $42.4 \pm 0.7^\circ\text{C}$ and $42.7 \pm 0.7^\circ\text{C}$, with strains differing significantly ($P < 0.01$). The level of heat stress temperature significantly influenced latency until panting ($P < 0.01$). When exposed to 35°C, birds started panting within 10.95 ± 2.43 (I657), 12.26 ± 2.61 (S757N) and 10.16 ± 2.36 (I957) minutes. The chi-square analyses revealed significant influences of the heat level and the strain on the frequency of birds panting ($P < 0.01$). After 35°C test, 96% (I657), 100% (I957) and 67% (S757N) of birds demonstrated panting ($P < 0.01$), while strain differences were not significant for frequency of birds panting exposed to 30°C.

Key words: slow growing broilers, short-term heat stress, rectal temperatures, panting, growth

INTRODUCTION

Heat stress is one of the important stress factors especially in tropical and subtropical environments which affected the productive performance of broilers. High mortality decreased feed consumption and poor body weight gain as disadvantages have been reported by many authors. Beside high ambient temperature, the large contribution to heat production occurs in the bird itself since metabolic production increases as the body weight of bird progresses (Lott *et al.*, 1998).

Under hot environment, heat production decrease whereas heat dissipation increase. When air temperature climbs, the breathing frequency of birds increases and the evaporative heat loss enhances significantly (Wiernuz and Teeter, 1996) and dissipated through respiratory evaporation as the main avenue (Hillman *et al.*, 1985).

Increased heat tolerance is reflected in lower body temperature and the limit of temperature

tolerance is affected by body weight. Sykes and Fataftah (1986) reported the index of heat tolerance is the increasing rate of rectal temperature from the start and after one hour of exposure. Value of $2^\circ\text{C}/\text{h}$ or more reflects rapidly rising body temperature meanwhile, value of $\leq 0.5^\circ\text{C}/\text{h}$ indicates effective heat tolerance.

The intensive genetic selection for rapid growth rate has been associated with increased susceptibility of broilers to heat stress. Birds selected for rapid growth demonstrate higher body temperature (low heat tolerance) compared to slow growing birds which have a greater tolerance to high temperatures (Cahaner and Leenstra, 1992, Berong and Washburn, 1998).

The present experiment was conducted to develop a suitable method to measure reactions of slow growing broilers towards heat stress and to evaluate the differences between three genotypes of commercial slow growing broiler hybrids with regard to heat stress reactions.