Characterization of Indonesian Pesisir cattle

INTRODUCTION

In Indonesia, seven native cattle breeds, namely Aceh, Fillal Ongale, Galekan, Madula, and Pesisir cattle that belong to *Bos indicus* (zebu), as well as Bali cattle derived from *Bos javanicus* (banteng), have been raised in the different islands of this country. Pesisir cattle are traditionally reared in West Sumatra, especially in Pesisir Selatan District (Putra et al. 2016). This breed is characterized by small body size (average withers height are less than 100cm) and approximately 280,000 Pesisir cattle are reared in West Sumatra. They have great potential as a meat production and play an important role for the income of the local community in West Sumatra area. This beef cattle breed has well adapted on traditional livestock raising systems of the local community. They are released in field throughout the day without special attention from farmers, and adapt to relatively hot tropical environments and low quality and low quantity feeding.

Since the economically important traits of livestock

ABSTRACT

Pesisir cattle are one of the Indonesian native cattle in West Sumatra that are adapted to the local tropical environment. The aim of the present study is to investigate genetic characteristics of the Pesisir cattle by genotyping the SNPs within *SREBP1, SCD1, EDG1, NCAPG, DGAT1*, and *MC1R* genes by PCR or PCR-RFLP. We also determined the mitochondrial DNA and Y-chromosomal haplotypes by direct sequencing of PCR products. The results showed that *SREBP1, SCD1*, and *DGAT1* genes are monomorphic while *EDG1, NCAPG*, and *MC1R* genes are polymorphic in the population of Pesisir cattle.

The sequence analysis of the D-loop region of mitochondrial DNA indicated that the population of Pesisir cattle possesses five haplotypes that are classified into the I1 and I2 haplogroups of zebu cattle type haplotypes. The sequence analysis of the *SRY* gene on male specific region of Y chromosome also indicates that the cattle population possesses the zebu type Y-chromosomal haplotype. The present findings first report the presence of the desirable allele of *NCAPG* gene in zebu cattle, which is associated with increased carcass weight. These findings will be informative for future selective breeding of the Indonesian native cattle to increase their meat productivity.

Key words: Pesisir cattle, mt-DNA, Y chromosome, SNPs
such as growth rate, carcass weight, and meet quality are regulated by multiple genes, genotypes of these genes can be used to evaluate the genetic potential of livestock. For example, Cardoso et al. (2014), Hoashi et al. (2007), and Yamada et al. (2009) have reported the effects of genetic polymorphism of particular genes on the productivity of beef cattle. Furthermore, Nishimaki et al. (2016), Kaneda et al. (2011), and Okuda et al. (2017) have reported the frequency and distribution of genotypes in cattle breeds to investigate their genetic potential. Since the polymorphisms of such genes can be applied to marker-assisted selection (MAS) for economically important traits of livestock, the study to investigate distribution of genetic polymorphism in Pesisir cattle will contribute not only for the genetic characterization and conservation management strategies of traditional local breed, but also for improvement of their genetic potential.

In the present study, we genotyped the polymorphisms of stearoyl-CoA desaturase (SCD), sterol regulatory element-binding protein-1 (SREBP1), endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1), diacylglycerol o-acyltransferase 1 (DGAT1), non-SMC condensin I complex, subunit G (NCAPG), and melanocortin 1 receptor (MC1R) genes by PCR or PCR-RFLP. SCD and SREBP1 genes are associated with fatty acid composition (Taniguchi et al. 2004; Hoashi et al. 2007), EDG1, DGAT1, and NCAPG, and MC1R genes are associated with beef marbling (Yamada et al. 2009), milk composition (Grisart et al. 2002), carcass weight (Eberlein et al. 2009), and coat color determination (Klungland et al. 1995), respectively. We also investigated mitochondrial DNA (mtDNA) and Y-chromosomal haplotypes of Pesisir cattle. Since mtDNA and Y-chromosomal sequences show mode of maternal and paternal inheritance, respectively, the polymorphisms of mtDNA and Y-chromosome have been used for phylogenetic analysis of populations of livestock in maternal and paternal lines. Particularly, since the bovine mtDNA and Y-chromosomal haplotypes are clearly classified into Bos taurus (taurine) type and Bos indicus (zebu, indicine) type haplogroups (Achilli et al. 2009; Verkaar et al. 2003), investigation of the distribution of the mtDNA and Y-chromosome haplotypes will be informative for genetic characterization of Pesisir cattle.

MATERIALS AND METHODS

Total 28 blood samples were randomly collected from the population of Pesisir cattle in West Sumatra area of Indonesia. The blood samples were collected from the jugular vein using vacuum tubes containing EDTA. The isolation of DNA from whole blood was performed

### Table 1. Primer sequences, fragment lengths, annealing temperatures, and restriction enzymes for genotyping

| Gene       | Primer sequences (5’ to 3’) | Length (bp)* | Temp** | Enzyme   |
|------------|-----------------------------|--------------|--------|----------|
| SCD        | F : GTGTCTCGTTGTTGTGCTTCGCC 197 (G) 60 |
|            | R : AATATTCTCTCGGGGGTTGATGGTCTTG 156, 41 (A) NcoI |
| SREBP1     | F : CCACAAGCGCCATCGAGAAACGCTAC 348 (S) 65 |
|            | R : GGCTTCTCCTGAGCCACCCAATTTAG 432 (L) |
| EDG1       | F : GTCTCAGCTGCACAGATCC 378 (G) 62 |
|            | R : GAAGACCTCCGGCGCCGAT 163, 215 (A) MscI |
| DGAT1      | F : GCACCATCCTCCTCCTCAAG 411 (K) 66 |
|            | R : GGAGCCGTTCGCGATG 203, 208 (A) EaeI |
| NCAPG      | F : ATTTAGGAACGACTCTGG 129 (G) 51 |
|            | R : ATTTGATTCTCTATTATCAC 66, 63 (T) Tsp509I |
| MC1R (E, e) | F : AACCTGCACCTCCCCCATGTGACTACT 154 (E) 65 |
|            | R : ACAATTGCAACTCGCATCGACGAGGC 98, 58 (E) MspAlI |
| mtDNA      | F : CTGCAGTCTCCACATCAAC 1,593 57 |
|            | R : GATGATAGAGAAGATGATGACG 138, 81 (E) MspI |
| SRY        | F : CGGGCTTAAATATCGACCTCT 1,062 58 |
|            | R : GATGAAACCTTGGTCTCACAG  |

*Italicized letters indicate lengths of digested fragments.

**Annealing Temperature.

Letters in parenthesis indicate alleles of each gene.
Characterization of Indonesian Pesisir cattle

according to the method described by Yurnalis et al. (2013) using DNA Extraction kit (Promega Corporation, WI, USA) according to the manufacturing procedure.

For genotyping, SREBP1 gene were amplified by PCR using the primer pair listed in Table 1 and genotyped by the length of the amplified fragments, as described by Hoashi et al. (2007), SCD, EDG1, DGAT1, NCAPG, and MC1R genes were amplified using the primer pairs listed in Table 1 and genotyped by PCR-RFLP methods using NcoI, MscI, EaeI, Tsp509I, MspAI, and MspI restriction enzymes, according to Taniguchi et al. (2004), Yamada et al. (2009), Grisart et al. (2002), Eberlein et al. (2009), and Klungland et al. (1995) respectively (Table 1). PCR reactions were performed in 10 µl reaction mixtures containing 20 ng of genomic DNA, 0.2 µM primers, 0.2 µmol/L dNTP, 5 × PCR buffer, and 0.5 U Go Taq DNA polymerase (Promega Corporation, WI, USA), for 35 to 40 cycles of denaturation at 94°C for 30-120 sec, annealing at the temperatures indicated in Table 1 for 30-60 sec, and extension at 72°C for 30-120 sec. After PCR amplification and restriction enzyme digestion, the PCR fragments or restriction fragments were electrophoresed in an agarose gel in TAE buffer, stained with ethidium bromide, and visualized using UV transilluminator.

To determine the mtDNA haplotypes, a 1,593-bp fragment of the hypervariable D-loop region of mtDNA were amplified by using a pair of primer listed in Table 1 (Loftus et al. 1994) and the amplified fragments were directly sequenced by using these primers. PCR reaction were carried out in 10 µl reaction mixtures containing 10 ng of genomic DNA, 0.2 µM primers, 0.2 µmol/L dNTP, 5 × PCR buffer, and 0.5 U KOD FX Taq polymerase (Toyobo, Osaka, Japan), for 35 cycles of denaturation at 94°C for 15 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec (Verkaar et al. 2004).

RESULTS AND DISCUSSION
In the present study, the genes associated with carcass weight, beef marbling, fatty acid composition, and coat color variations including SCD, SREBP1, EDG1, DGAT1, NCAPG and MC1R genes were genotyped in the Indonesian Pesisir cattle. As shown in Table 2, the results of the genotyping indicated that only one genotype of SS, VV, and KK were observed in SREBP1, SCD, and DGAT1 genes, respectively, suggesting that these genes are fixed to S, V, and K alleles in the populations of Pesisir cattle. On the
other hand, two genotypes of GG and GA, and GT and TT were observed in EDGI and NCAPG gene, respectively, indicating that both G and A, and G and T alleles of these genes are present in the population of Pesisir cattle. The frequencies of the A allele of EDGI gene and G allele of NCAPG gene were 0.02 and 0.29, respectively (Table 2). The observed distributions of the genotypes are not significantly different from those expected from Hardy-Weinberg Equilibrium Model.

For MC1R gene, two genotypes $E^+E^+$ and $E^+e$ were observed while no $E^D E^D$, $E^D E^+$, $E^D e$ and $e e$ genotypes were observed, suggesting that the e allele, in addition to the $E^+$ allele, are present in the population of Pesisir cattle at low frequency, but the $E^D$ allele are not present the populations. The frequencies of $E^D$, $E^+$, and e alleles were 0, 0.93, and 0.07, respectively. The coat color of Pesisir cattle is highly variable, and we observed various coat colors including red, red brick, yellow to brown, white and black colors in the population of Pesisir cattle. The contribution of the MC1R gene for these coat color variation is unclear, since only $E^+E^+$ and $E^+e$ genotypes were identified in the Pesisir cattle examined in the present study.

The sequence analysis of the D-loop region of bovine mtDNA in 18 of the 28 samples of Pesisir cattle indicated that these animals possess 5 haplotypes that belong to the zebu type haplogroups of the bovine mtDNA (Table 3). The bovine mtDNA haplotypes have been clustered into taurine type (T*, T1, T2, T3, and T4) and zebu type (I1 and I2) haplogroups (Achilli et al. 2009). The 20 samples of Pesisir cattle possess I1, I1_22, I1_35, and I2 haplotypes, as well as a new haplotype (Table 3). The new haplotype belongs to I1 haplogroup (Fig. 1) and we tentatively designated this haplotype as I1_Pe1. Therefore, all 18 samples of Pesisir cattle possess zebu type mtDNA haplotypes and no taurine type haplotypes were observed. In addition, the sequence analysis of the 1,062-bp segment of SRY gene on Y-chromosome in nine males of the 28 samples indicated that all nine male Pesisir cattle possess zebu type Y-chromosome haplotype (Table 4). Therefore, our investigation of both mtDNA and Y-chromosomal haplotypes suggested that Pesisir cattle originated from zebu cattle in both maternal and paternal lines. Genetic analyses of local breeds of Indonesian naive cattle showed gene-flow of banteng (Bos javanicus), Southeast Asian wild cattle, into some of these breeds (Namikawa et al. 1983; Nijman et al. 2003; Tanaka et al. 2011). Besides Bali cattle known as domesticated cattle originated from banteng and Madura cattle know to be originated from hybrid between banteng and zebu cattle (Otsuka 1983), some of zebu type cattle including Galekan and Filial Ongole cattle, also possess banteng type mtDNA haplotypes, showing gene-flow from banteng in maternal line (Mohamad et al. 2009). The results

| Position | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
|----------|---|---|---|---|---|---|---|---|---|
|          | 7 | 7 | 0 | 0 | 1 | 1 | 2 | 2 | 3 |
|          | 9 | 9 | 1 | 5 | 0 | 1 | 2 | 3 | 0 |
|          | 7 | 0 | 8 | 9 | 3 | 6 | 9 | 8 | 7 |
| AB039748 | G | T | A | C | T | A | C | C | C |
| AY079145 | T | C | T | G | C | G | T | T | T |
| AY079146 | T | G | C | T | G | T | T | T | T |
| Pesisir cattle(n=9) | T | C | T | G | T | T | T | T | T |

Reference sequences for 1* Bos taurus *, 2* Bos indicus *, 3* Bos javanicus *

| Table 3. Haplotypes of mtDNA D-loop region observed in Pesisir cattle |
|---------------------------------------------------------------|
| haplotype | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
|           | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| V00654*1  | T3 | C | G | C | T | A | G | T | G | T | T | G | T | T | T | T | T | T | T | T |
| L27733*2  | I1 | T | A | T | C | A | T | A | C | C | C | A | C | C | C | C | C | C | C | C |
| Pesisir cattle(n=13) | I1 | T | A | T | C | A | T | A | C | C | C | A | C | C | C | C | C | C | C | C |
| Pesisir cattle(n=1) | I1_22 | T | A | T | C | A | T | A | C | C | C | A | C | C | C | C | C | C | C | C |
| Pesisir cattle(n=1) | I1_35 | T | A | T | C | A | T | A | C | C | C | A | C | C | C | C | C | C | C | C |
| Pesisir cattle(n=2) | I1_Pe1*3 | T | A | T | C | G | A | T | A | C | C | C | A | C | C | C | C | C | C | C |
| Pesisir cattle(n=1) | I2 | T | A | T | C | A | T | A | C | C | C | A | C | C | C | C | C | C | C | C |

Reference sequences for 1* Bos taurus *, 2* Bos indicus *, 3* New haplotype *

"*" indicate 1-bp deletion.
of the present study indicated that the all 9 males of Pesisir cattle possess zebu type Y-chromosomal haplotypes but no banteng type haplotype (Table 4). These findings suggested less possibility of gene-flow from banteng in Pesisir cattle in paternal line that is in concordant with Mohamad et al. (2009).

The polymorphisms of the genes examined in the present study have been identified as the genes associated with particular traits of beef and dairy cattle in the taurine cattle. However distribution of these polymorphisms in the zebu cattle remains unclear. Since remarkable differences in allelic frequencies of various genes between taurine and zebu cattle populations have been reported (Lin et al. 2010; Kaneda et al. 2011; Yonesaka et al. 2016, Okuda et al. 2017), whether or not these polymorphisms observed in populations of taurine cattle are also observed in populations of zebu cattle is of particular interest. In the present study, we found that the polymorphisms of EDG1, NCAPG, and MC1R genes reported in taurine cattle were also observed in the population of Pesisir cattle. While the presence of A allele of SCD, L allele of SREBP1, and e allele of MC1R in zebu cattle have been reported (Kaneda et al. 2011; Okuda et al. 2017; Zhang et al. 2014), no polymorphisms of EDG1 and NCAPG genes in zebu cattle have so far been reported. Therefore, the present findings of the presence of the A allele EDG1 and G allele of NCAPG is the first report for the presence of the polymorphisms of these genes in zebu cattle populations. In particular, the presence of polymorphisms of the NCAPG gene in Pesisir cattle is important for animal production of Indonesia, since this polymorphism has been reported to be associated with carcass weight of beef cattle (Eberlein et al. 2009). The animals possessing the G allele of this gene showed significantly increased carcass weight. Therefore, our findings of the presence of the desired alleles of these genes in the populations will be informative for the genetic improvement of meat productivity of Pesisir cattle. In particular, since the frequencies of the G alleles are relatively low in the populations of Pesisir cattle, the selection of the animals possessing the desired alleles of these genes might be effective for improving the average carcass weight of the population.

ACKNOWLEDGEMENTS
This study was supported by a grant from Graduate School of Environmental and Life Science, Okayama University.

REFERENCES
Achilli A, Bonfiglio S, Olivieri A, Malusà A, Pala M, Hooshiar Kashani B, Perego UA, Ajmone-Marsan P, Liotta L, Semino O, Bandelt HJ, Ferretti L, Torroni A. 2009. The multifaceted origin of taurine cattle reflected by the mitochondrial genome. PLoS One, 4: e5753.
Beja-Pereira A, Caramelli D, Lalueza-Fox C, Vernesi C, Ferrand N, Casoli A, Goyauche F, Royo LJ, Conti S, Lari M, Martini A, Ouragh L, Magid A, Atash A, Zsolnai A,
Boscato P, Triantaphylidis C, Ploumi K, Sineo L, Mallegni F, Taberlet P, Erhardt G, Sampietro L, Bertranpetit J, Barbujani G, Luikart G, Bertorelle G. 2006. The origin of European cattle: evidence from modern and ancient DNA. Proceedings of the National Academy of Sciences of the United States of America, 103: 8113-8118.

Chen S, Lin BZ, Baig M, Mitra B, Lopes RJ, Santos AM, Magee DA, Azevedo M, Tarroso P, Sasazaki S, Ostrowski S, Mahgoub O, Chaudhuri TK, Zhang YP, Costa V, Royo LJ, Goyache F, Luikart G, Boivin N, Fuller DQ, Mannen H, Bradley DG, Beja-Pereira A. 2010. Zebu cattle are an exclusive legacy of the South Asia Neolithic. Molecular Biology and Evolution, 27: 1-6.

Cardoso DF, de Souza FR, de Camargo GM, Fonseca PD, Fonseca LF, Braz CU, Boligon AA, Mercadante ME, de Albuquerque LG, Tonhati H. 2014. Polymorphism analysis in genes of the somatotropic axis in Nellore cattle selected for growth. Gene, 545: 215-219.

Eberlein A, Takasuga A, Pfuhl R, Flisikowski K, Fries R, Weikard R, Kühn C. 2009. Dissection of genetic factors modulating fetal growth in cattle indicates a substantial role of the non-SMC condensin I complex, subunit G (NCAPG) gene. Genetics, 183: 951-964.

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M, Snell R. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Research, 12: 222-231.

Hoashi S, Ashida N, Ohsaki H, Utsugi T, Sasazaki S, Taniguchi M, Oyama K, Mukai F, Mannen H. 2007. Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Research, 12: 222-231.

Kaneda M, Lin BZ, Sasazaki S, Oyama K, Mannen H. 2011. Allele frequencies of gene polymorphisms related to economic traits in Bos taurus and Bos indicus cattle breeds. Animal Science Journal, 82: 717-721.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33: 1870-1874.

Klungland H, Vage DI, Gomez-Rayna L, Adalsteinsson S, Lien S. 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mammalian Genome, 6: 636-639.

Lin BZ, Sasazaki S, Mannen H. 2010. Genetic diversity and structure in Bos taurus and Bos indicus populations analyzed by SNP markers. Animal Science Journal, 81: 281-289.

Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham P. 1994. Evidence for two independent domestications of cattle. Proceedings of the National Academy of Sciences of the United States of America, 91: 2757-2761.

Mohamad K, Olsson M, van Tol HT, Mikko S, Vlamings BH, Andersson G, Rodríguez-Martínez H, Purwantara B, Paling RW, Colenbrander B, Lenstra JA. 2009. On the origin of Indonesian cattle. PLoS One, 4: e5490.

Nakikawa T, Amano T, Takenaka O. 1983. Studies on the blood groups and biochemical polymorphisms in the different types of cattle and the Bantengs in Indonesia. Report of the Society for Researches on Native Livestock, 10: 68-81.

Nijman IJ, van Boxtela DJ, van Canna LM, Marnocha Y, Cuppenb E, Lenstra JA. 2008. Phylogeny of Y chromosomes from bovine species. Cladistics, 24: 723-726.

Nijman IJ, Otsen M, Verkaar EL, de Ruijter C, Hanekamp E, Ochieng JW, Shamshad S, Rege JE, Hanotte O, Barwegen MW, Sulawati T, Lenstra JA. 2003. Hybridization of banteng (Bos javanicus) and zebu (Bos indicus) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity, 90: 10-16.

Nishimaki T, Ibi T, Sqiuntuya, Kobayashi N, Matsuhashi T, Akiyama T, Yoshida E, Imai K, Matsu M, Uemura K, Eto H, Watanabe N, Fujita T, Saito Y, Komatsu T, Hoshiba H, Mannen H, Sasazaki S, Kunieda T. 2016. Allelic frequencies and association with carcass traits of six genes in local subpopulations of Japanese Black cattle. Animal Science Journal, 87: 469-476.

Okuda Y, Kanii T, Yamamoto Y, Kounnangvongsa B, Keonouchanh S, Bouaham B, Kunieda T. 2017. Genetic characterization of Laotian native cattle using mtDNA haplotype and loci associated with economical traits, coat color, and a hereditary disorder. The Journal of Animal Genetics, 45: 43-48.
Characterization of Indonesian Pesisir cattle

Otsuka J. 1983. General information about Indonesia and situation of livestock. Report of the Society for Researches on Native Livestock 10: 32-35.

Putra D, Sumadi, Kanazawa T, Hartatik T. 2016. Identification of growth hormone gene polymorphism for beef cattle in Pesisir Selatan District, West Sumatra, Indonesia. Biodiversitas, 17: 711-715.

Tanaka K, Takizawa T, Dorji T, Amano T, Mannen H, Maeda Y, Yamamoto Y, Namikawa T. 2011. Polymorphisms in the bovine hemoglobin-beta gene provide evidence for gene-flow between wild species of Bos (Bibos) and domestic cattle in Southeast Asia. Animal Science Journal, 82: 36-45.

Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, Ogino A, Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. Mammalian Genome, 14: 142-148.

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Bradley DG. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature, 410: 1088-1091.

Verkaar EL, Vervaecke H, Roden C, Romero Mendoza L, Barwegen MW, Susilawati T, Nijman IJ, Lenstra JA. 2003. Paternally inherited markers in bovine hybrid populations. Heredity, 91: 565-569.

Yamada T, Itoh M, Nishimura S, Taniguchi Y, Miyake T, Sasaki S, Yoshioka S, Fujita T, Shiga K, Morita M, Sasaki Y. 2009. Association of single nucleotide polymorphisms in the endothelial differentiation sphingolipid G-protein-coupled receptor 1 gene with marbling in Japanese Black beef cattle. Animal Genetics, 40: 209-216.

Yonesaka R, Sasazaki S, Yasue H, Niwata S, Inayoshi Y, Mukai F, Mannen H. 2016. Genetic structure and relationships of 16 Asian and European cattle populations using DigiTag2 assay. Animal Science Journal, 87: 190-196.

Zhang Y, Li Q, Ye S, Faruque MO, Yu Y, Sun D, Zhang S, Wang Y. 2014. New variants in the melanocortin 1 receptor gene (MC1R) in Asian cattle. Animal Genetics, 45: 609-610.