Single Nucleotide Polymorphism on Exon 2 Leptin Gene of Pasundan Cattle

N Hilmia¹, D Rahmat¹, Dudi¹ and D N Hadi²

¹Animal Production, Faculty of Animal Husbandry, Universitas Padjadjaran
²Balai Pengembangan dan Perbibitan Ternak Sapi Potong Cijeungjing Ciamis

E mail: nena.hilmia@unpad.ac.id

Abstract. Single Nucleotide Polymorphism on exon 2 of leptin gene which changes encoding from Arginine to Cysteine could change the function of Leptin as an obese gene which regulated feed intake and energy expenditure. The aims to analyze point mutation based on SNP exon 2 of Leptin gene of Pasundan cattle. This research used 49 DNA of Pasundan cattle from BPPT SP Cijeungjing. Multiplication of DNA was done by Polymerase Chain Reaction (PCR). Analyze of SNP on exon 2 Leptin gene based on nucleotide sequence from direct sequencing of PCR product which was alligned with leptin gene sequence from gene bank using Bioedit and MEGA 5.2 program. The results showed nucleotide sequence of exon 2 Leptin gene in Pasundan cattle is different from Leptin gene sequence of Bos indicus and Bos taurus. It was found one synonymous SNP that did not change amino acids Serine encoding on S17S, and two non-synonymous SNP which altered amino acids encoding, i.e R25C and R25H. In Pasundan cattle, the frequency of C allele (42.86%) was higher than A allele (31.64%) and T allele (25.50%). Six genotypes were identified i.e. CC (24.49%), CT (32.65%), CA (4.08%), TT (8.16%) and TA (2.05%), AA (28.57%).

1. Introduction
In order to overcome increasing of food demand especially in meat, the empowerment of genetic resources of local cattle as the main producer of red meat, becomes an appropriate choice, since local cattle has been integrated and highly adaptive to the environment and farmer culture life. Although their productivity is still lower than imported cattle and their crossing, local cattle could survive and be productive with minimum inputs, more resistant to disease and more resistant to climate change. One of the local cattle in West Java, that has been established as a new breed with the Minister of Agriculture Decree no. 1051/kpts/SR.120/10/2014, namely is Pasundan cattle.

The molecular genetic technology was evolved rapidly, it is, a new breakthrough to be able to explore the genetic potential of beef cattle, quickly and precisely as a marked assisted selection (MAS). The Single Nucleotide Polymorphisms (SNP) analysis is one of technique to evaluate point mutation at nucleotide sequence level. SNP can be used discover and gene mapping, candidate gene analysis, and genetic disorder diagnosis, estimates of environmental stimulation responses, xenobiotic and diet, physiologic diversity and genomic testing.

Productivity as a quantitative trait is influenced by many gene, one of the potential genes that influence this trait is the leptin gene. The leptin gene is called the obese gene that acts as an anti-obesity gene. Leptin is a hormone, the product of obese gene, that was involved in physiological processes such as food intake, energy metabolism [1], regulation of body weight, reproduction, and immune system function [2]. It was assumed that point mutations at nucleotide sequence in this gene will affect the action of the leptin hormone as a leptin gene product in regulating of energy...
metabolism. Disorder on energy metabolism may influence livestock productivity, especially on feed intake, body weight, and fat deposition.

One of point mutation on exon 2 leptin gene that have been reported associated with productivity in beef cattle is SNP R25C. Several studies showed that SNP in exon 2 of Leptin gene contributed to fat accretion which was responsible for carcass fat quality, fat deposition, backfat thickness and butter fat [3-9]. Three of SNP on Leptin gene (promotor and exon 2) in Nellore cattle reported that there were differences in longissimus dorsi area among different genotypes [10]. Point mutation on exon 2 leptin gene is an important SNP that may influence productivity of beef cattle. The objective of this research was to identify Single Nucleotide Polymorphism on Leptin gene of Pasundan Cattle.

2. Materials and Methods

2.1. Material and DNA Amplification

The identification of SNPs on Pasundan cattle Leptin gene was evaluated using 49 DNA samples which was taken from Balai Pengembangan Perbiban Ternak Sapi Potong (BPPTSP) Cijeungjing, Ciamis district West Java. The sequence target of Leptin gene was amplified through Polymerase Chain Reaction (PCR). Each PCR reaction contained buffer 10x, dNTP, primer, taq polymerase and dH2O. Leptin gene sequence with 620 bp of length, was amplified using forward primer 5’CTCACTGCTGCGTGGTCTAC3’; revers 3’ GCACTAGGATTCCGGTCTGG 5’ cover a part of intron 1, exon 2, and a part of intron 2. The initial denaturation at 95°C for 5 minutes, followed by 33 cycles of denaturized at 95°C for 45 seconds, 1 minute annealing at 58°C, extension at 72°C for 1 minute followed by polymerase at 72°C for 5 minutes.

2.2. Data Analysis

Genotyping of Leptin gene was analyzed by direct sequencing and was alignment using Bioedit and MEGA 4 program. The heterozygosity, alleles, and genotypes frequency of leptin gene based on SNPs R25C and R25H were calculated with Nei formula [11]. This analyzes was operated using POPGENE.32 program.

3. Results and Discussion

Amplification of the 620 bp leptin gene includes part of intron 1, exon 2 and intron 2 partially. Multiplication of DNA target on exon 2 Leptin using PCR machine. PCR product was evaluated by agarose electrophoresis, as shown in Figure 1 below.

![Figure 1. PCR product of Leptin gene along 620 bp](image)

The Single Nucleotide polymorphism (SNP) consists of two, i.e. synonymous and non synonimous SNP. Synonymous mutation is a change of one nitrogen base at codon that was not alter the coding of amino acids, thus have not an affect protein formed, also called silent mutation. Non synonimous
mutation is point mutation on nucleotide sequence that alters the coding of amino acids. Non synonymous mutation is divided into missense mutation, a mutation in the codon that affects previous amino acid coding and nonsense mutation changes in the codon into termination codon thus cutting off protein formation [12].

In these research were found three SNP on exon 2 leptin gene Pasundan cattle, one synonymous SNP and two non synonymous SNPs. Synonymous SNP was detected at g.1025T>C/g.1158T>C (access no NCBI EU313203.1 and U50365.1). There was a transition from tymin to cytosin, that was not altered encoded 17th amino acid (Serine). There were found two non synonymous mutation on R25C/g.1047C>T/g.1180C>T and R25H/g.1048G>A/g.1181G>A (NCBI acces no. EU313203.1 and U50365.1). This SNP altered amino acid 25th Arginine to Cysteine and Arginine to Histidin. There were base nitrogen transition from cytosine to thymine and guanin to adenine. There two kind of substitution mutation, transitions that changed base nitrogen A (adenine) to G (guanin) and C (cytosine) to T (thymine), or vice versa, (pyrimidine) and transversions are substitution mutation between purine and pyrimidine [12]. The aligned result of exon 2 leptin gene and allele determination using bioedit and MEGA 4, were showed at Figure 2. The SNP S17S was not included on allele determination, because, it was a silent mutation.

| Breed     | Allele Frequency   |
|-----------|--------------------|
| Bos_indicus_leptin | TAATTACGTGGAGGCTTGCCCATCCGCAGTTCCAGGATGACA |
| Bos_taurus_leptin  | TAATTACGTGGAGGCTTGCCCATCCGCAGTTCCAGGATGACA |

Figure 2. Alignment result of nucleotide sequence exon 2 leptin gene : SNP S17S/g.1025T>C/g.1158T>C, R25C/g.1047C>T/g.1180C>T and R25H/g.1048G>A/g.1181G>A(access no. NCBI EU313203.1 and U50365.1)

Those non synonymous mutation in line with [13] who revealed that in 78 head of the local cattle Ciamis that were taken from Tambaksari and Cijulang, Ciamis District, leptin gene was polymorphic. There were found non synonymous SNP R25C and R25H. The non-synonymous SNP R25H was specific mutation on Ciamis local cattle, based on there was not found in Bos indicus as well as in Bos taurus. Those point mutation were assumed had an effect to productivity, because alteration of amino acids on gene may change their protein function. The SNP R25C in leptin gene is causative mutation which could alter leptin function as an anti-obese hormone in the physiological processes [14].

| Breed     | Allele and Genotype Frequency based on SNP R25C (Arg25Cys/g.1047C>T) and R25H (Arg25His/g.1048G>A) on Pasundan Cattle |  |
|-----------|-------------------------------------------------------------------------------------------------------------------|---|
|           | Allele Frequency                                      | Genotype Frequency | |
|           | C     | T     | A     | CC    | CT    | CA    | TT    | TA    | AA    | |
| Pasundan  | 49    | 0,429 | 0,255 | 0,316 | 0,245 | 0,327 | 0,041 | 0,082 | 0,020 | 0,286 |

He = Expected Heterozygosity
Previous study on leptin gene showed that SNP R25C is a non-synonymous mutation that can change the biological function of leptin gene, its demonstrated by highly level of leptin mRNA in homozygous cattle (TT), that has an point mutation on R25C [4]. Based on those SNPs, on Pasundan cattle, leptin gene was polymorphic, as presented on Table 1 bellow.

The frequency of C allele/no mutation (42.9%) was higher than T allele (25.5%) and A allele (31.6%). Hereafter, CT genotypes (32.7%) were higher than CC genotype (24.5%), CA (4.1%), TT (8.2%), AA (28.6%), and TA (2.0%). The alleles frequency on this research in line with the previous on 78 DNA sample of Ciamis local cattle, indicated there were three alleles, C allele as the highest frequency (55.5%), than T (30%) and H/A allele (15%) [13]. The result of this study were different from leptin gene study on Bos taurus as well as on Bos indicus as is a new mutation SNP R25H. However the allele no mutation on this research was higher than allele mutation. These results were in accordance with other study which analyzed SNP in Leptin gene exon 2 (Arg25Cys) showing the frequency of C allele was higher than T allele. The study on 323 Fries Holland cattle, showed the frequency of C and T alleles were 67% and T 33% respectively [4]. The research on 169 individuals of Charolais X Fries Holland bull, indicated that C allele frequency was 65%, while T allele was 35% [15]. The SNP analysis on 55 Charolais cattle and 17 Simmental cattle found 34% and 32% for T allele respectively whereas C allele were 66% and 68% respectively[3]. In contrast, they reported different results on British cattle (Angus and Hereford) that the frequency of T allele (0.58% and 55 %), was higher than C allele (0.42% and 0.45%). Others study reported that there was low frequency of T allele and there was not found TT genotype in Nelore (Bos indicus) cattle, whereas it was found TT genotype with low frequency (19%) in Nelore X Bos taurus cattle [14]. There were mutation on leptin gen in Turkey cattle. The frequencies of T and C allele were 0.54±0.06 and 0.46±0.06 for East Anatolian Red cattle, and 0.48±0.05 and 0.52±0.05 for Anatolian Black [16].

Several studies revealed there were significant association between Leptin genotype based on SNP (Arg25Cys) with carcass grade fat, carcass yield grade, and lean meat yield. Thymine homozygotes (TT) had more carcass grade fat compared to heterozygotes and to animals homozygous for the cytosine allele, heterozygotes in turn had more carcass grade fat compared to cytosine homozygotes. Additionally, thymine homozygotes had lower lean meat yield and yield grades compared to heterozygotes and cytosine homozygotes [6]. Cattle with TT genotype had higher carcass grade compared to those with CT and CC genotypes [8]. This SNP on 154 bull is reported there were association between T allele with increasing fat deposition and higher levels of Leptin mRNA, the T allele was associated with fatter carcasses and conversely the C allele was associated with leaner carcasses[3]. The longissimus dorsi muscle tenderness from TT genotype was more tender than that from CC and CT genotypes [7]. There were significant association between Leptin genotype based on SNP Arg25Cys with carcass grade fat, carcass yield grade, and lean meat yield [3,6,8].

4. Conclusion

Leptin gene in Pasundan cattle was polymorphic. There were found one synonymous SNP that did not change amino acids Serine encoding on S17S, and two non-synonymous SNP which altered amino acids encoding, i.e. R25C and R25H. These non-synonymous mutations changed amino acids encoding from Arginine to Cysteine and Arginine to Histidine respectively. In Pasundan cattle, the frequency of C allele (42.86%) was higher than A allele (31.64%) and T allele (25.50%). Six genotypes were identified i.e. CC (24.49%), CT (32.65%), CA (4.08%), TT (8.16%) and TA (2.05 %), AA (28.57%).

References

[1] Hossner K L 1998 Cellular molecular and physiological aspect of leptin: Potential application in animal production Can. J. Anim. Sci. pp. 463-472.
[2] Van der L T, te Pas M F W, Veerkamp R F, Liefers S C 2005 Leptin gene polymorphisms and their phenotypic associations. *Vitamins and Hormones (71)*: 373 – 404 DOI: 10.1016/S0083-6729(05)71013-X

[3] Buchanan F C, Fitzsimmons C J, Van Kessel A G, Thue T D, Winkelman-Sim D C, Schmutz S M 2002 Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels *Genet Sel Evol.* 34: 105-116 doi: 10.1051/gse:2001006.

[4] Liefers S C, Veerkamp R F, Te Pas M F W, Delavaud C, Chilliard Y, Platje M, Van der Lende T 2002 Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in holstein heifers *J. Dairy Sci.* 85:1633–1638.

[5] Buchanan F C, Van Kessel A G, Boisclair Y R, Block H C, McKinnon J J 2007 The leptin arg25cys affects performance, carcass traits and serum leptin concentrations in beef cattle *Can. J. Anim. Sci.* 87:153–156.

[6] Nkrumah J D, Li C, Basarab J B, Guercio S, Meng Y, Murdoch B, Hansen C, Moore S S 2004 Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition *Can. J. Anim. Sci.* 84: 211–219.

[7] Schenkel F S, Miller S P, Ye X, Moore S S, Nkrumah J D, Li C, Yu J, Mandell I B, Wilton J W, Williams J L 2005 Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle *J. Anim. Sci.* 83: 2009-2020.

[8] Kononoff P J, Deobald H M, Stewart E L, Laycock A D, Marquess F L S 2005 The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle *J. Anim. Sci.* 83:927-932.

[9] DeVuyst E A 2010 The Economics of Gene Testing Cattle *Oklahoma Cooperative Extension Service* [http://osufacts.okstate.edu](http://osufacts.okstate.edu).

[10] Da Silva R C, Ferraz J B, Meirelles F V, Eler J P, Balieiro J C, Cucco D C, Mattos E C, Rezende F M, Silva S L 2012 Association of single nucleotide polymorphisms in the bovine leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle.

[11] Nei M 1987 Molecular Evolutionary Genetics New York (US) *Columbia University Press.*

[12] Graur Dan 2003 Single base mutation *Nature Ensilcopia of The Human Genomes Mcmillan Publisher Ltd. Nature Publishing Group* p. 287-290.

[13] Hilmia N 2013 Karakterisasi Fenotipe Dan Potensi Genetik Serta Hubungannya Dengan Produktivitas Dan Kualitas Daging Sapi Lokal Di Ciamis Jawa Barat *Disertation* Institut Pertanian Bogor Bogor.

[14] Fortes M R S, Curi R A, Chardulo L A L, Silveira A C, Assumpção M E O D, Visintin J A, de Oliveira H N 2009 Bovine gene polymorphisms related to fat deposition and meat tenderness *Genet. and Mol. Biol.* 32(1):75-82

[15] Lagonigro R, Wiener P, Pilla F, Woolliams J A, Williams J L 2003 A new mutation in the coding region of the bovine leptin gene associated with feed intake [Short Communication] *Anim Genet.* 34: 371–374

[16] Kaygisiz A, Bengi C, Cilek S 2011 Investigation of leptin gene polymorphisms in East Anatolian Red Anatolian and Black cattle and determination of genetic distance from Brown Swiss Cattle *J. Anim. Plant. Sci.* 21: 121-125.