The Potency of Leptin Gene as a Selection Marker of Economic Traits for Madura Cattle: Preliminary Study

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ABSTRACT
The Leptin gene, also known as 'obese', was an important regulator of growth traits which is the principal economic value of beef cattle. This paper aimed to investigate the potency of the Leptin gene as a selection marker of economic traits for Madura cattle. This study was performed by literature review from published studies. First, publications were collected to obtain a Leptin genes study on Madura cattle and Indonesian Cattle. Next, association study publications were collected to obtain Leptin gene polymorphism and its effect on the economic traits of various cattle. The selected economic traits were growth, carcass, milk, and reproduction. In total, 26 papers were used in this study. As a results, we found Leptin gene studies on nine Indonesian local cattle, including Madura, Kebumen Ongole Grade, Ongole Grade, Sumba Ongole, Bali, Pasundan, Bali Cross, Pesisir and Ciamis Local Cattle. However, most of this study is limited to polymorphism identification. Exon 2, Intron 2, and Exon 3 of the Leptin gene polymorphism were associated with four selected economic traits on several loci. However, Leptin gene SNP g.1180C>T (also referred to as R25C, Arg25Cys, 1047C>T, C305T, R4C, C73T, and *LepKpn2I*) in exon 2 was known to had association with growth, carcass, milk, and reproduction trait. It was concluded that SNP g.1180C>T had the potency to be used as a selection marker of economic traits for Madura cattle. Following marker selection, an association study on Madura Cattle was further to validate this result.

Keywords: *Leptin Gene, Selection Marker, Madura Cattle, Economic Trait*

1. INTRODUCTION

Madura cattle were meat-type cattle that originated from Madura Island, Indonesia. They were registered as local Indonesian cattle by the Ministry of Agriculture Republic of Indonesia (3735/Kpts/HK.040/11/2010) [1]. The origin of these cattle was divided into two versions. The first version explains that Madura cattle possibly derived from a crossbreed between Bali Cattle and/or the wild Banteng (*Bos (bibos) banteng*), *Bos Indicus*, and *Bos Taurus* [2]. It had supported by the finding of Taurine ZFY and SRY gene segments [3]. However, the famous version explains that Madura cattle are a crossbreed between Wild Banteng (*Bos javanicus*) and Zebu (*Bos indicus*) developed 1500 years ago [3,4].

Madurese culture had a heavy impact on the development of Madura cattle. Karapan Sapi (paired bull race) developed in all areas of Madura Isle starting around the 12-13th century. Madura cattle had been selected for light and small body size to support for run faster in the event. In the long term, it brought adverse effects on the genetic quality of Madura cattle [5]. Based on that condition, in the northern part of the island, farmers started another cultural event called *Sonok* (cow body conformation contest) in the '60s to stimulate farmers to improve their cattle performance. Nowadays, this traditional selection produces high cattle performance. This cultural event, however, generates three cattle classes called *Sonok* (selected for the contest), *Taccek*/*Pajengan* (*Sonok* candidate), and conventional (unselected for the contest), which had different characteristics [6].

Table 1. Identified Leptin Gen in Indonesian Local Cattle

Breed	Research objective	SNP/Locus	Genbank	Primer	Bagian	Produk PCR	Result	Ref
Madura	Polymorphism determination	g.32A>G g.87T>C g.89G>A	FJ626856	F: 5'-CCATGGCAGACAGCAAATCTCGT-3' R: 5'- TGGTGTCATCCTGGACCTTCC-3'	Exon 2	234	Madura cattle close to wild cattle based on haplotype ATGCT and close to Bos turus based on haplotype GCATC.	[7]
Kebumen Ongole Grade	Polymorphism association with growth trait	g.1180C>T *HpyCH4V*	U50365	F: GATTCCGCCGCACCTCTC R: CCTGTGCAAGGCTGCACAGCC	Exon 2	466	Allele C 88.5%, T 11.5%; Genotype CC 78%, CT 21%, TT 1%. Genotype CT associated with higher weaning chest circumference than CC.	[8]
Ciamis Local Cattle	Polymorphism association with meat quality	g.1047C>T	EU313203.1	F: 5'CTCACTGCTGCGTGGTCTAC3' R: 3' GCACTAGGATTCCGGTCTGG 5'	Half Int 1, Exon 2, Half int 2	620	Genotype CC 42.9%, CT 42.9%, CH 14.2%. No association between genotype and meat quality.	[9]
Ongole Grade	Polymorphism identification	g.1047C>T and 1048G>A	EU313203.1	F: 5'CTCACTGCTGCGTGG TCTAC3 R: 3'GCACTAGGATTCCGGTC TGG5'	Exon 2	620	Allele C 33.3%, T 21.9%, A 44.8%; Genotype CC 20.8%, CT 20.8%, CA 42%, TT 10.4%, TA 2.1%, AA 41.7%	[10]
Bali Cattle	Polymorphism association with body weight	g.1047C>T and g.1048G>A	EU313203.1		Exon 2		Genotype CC 19.15%, CT 14.89%, CA 10.64%, AA 55.32%. No association between genotype and birth weight, weaning weight, daily weight gain.	[11]
Pasundan cattle	Polymorphism identification	g.1047C>T and g.1048G>A	EU313203.1 and U50365.1	F: 5'CTCACTGCTGCGTGGTCTAC3' R: 3' GCACTAGGATTCCGGTCTGG 5'	Exon 2	620	Allele C 42.9%, T 25.5%, A 31.6%; Genotype CC 24.5%, CT 32.7%, CA 4.1%, TT 8.2%, TA 2%, AA 28.6%.	[12]
Bali Cattle and Bali Cross	Polymorphism association with body condition score (BCS)	*LEP Sau3AI*		F: 5′- GTCACCAGGATCAATGACAT- 3′ R: 5′- CCTACGCAGGAGTAGGTGGT-3′		1820	Bali cattle allele A 100%, Bali Cross Allele A 85%, T 15%. Bali cattle genotype AA 100%, Bali Cross genotype AA 69.2%, AB 30.8%. No association between genotype and BCS.	[13]
Sumba Ongole	Polymorphism identification		U50365	F: 5'-AAGGCAGAAAACTGCGAGGC3' R: 5'- CAGAGCAATGCCATTGCAAACAC-3'	3'flanking region	514	Found high diversity with 17 mutations. Mutation type: transitions 58%, transversions 37% and insertion 5%.	[14]
Pasundan	Genetic characterization	g.1926C>T *Lep Sau3AI*	EU313203	F: 5'-TGGAGTGGCTTGTTATTTTCTTCT-3 R: 5'-GTCCCCGCTTCTGGCTACCTAACT -3	Intron 2 – Exon 3	421	Allele C 98%, T 2%; Genotype CC 96%, CT 4%.	[15]
Sumba Ongole	Polymorphism identification		U50365	LEP 3 (Intron 2): F: 5'-AGTCATGTCCAACTCTTTGAGAC-3 R: R: 5'-CCACGTGACCACCATGTTTCCAA-3 LEP 4 (Intron 2- Exon 3) F: 5'-TGACTGTGAGGGAGGAGTCTGC-3' R: 5'-GAGCTGGAACAGGGAGGAAGACT-3'	Intron 2 and Intron 2 – Exon 3	Int 2: 1002 Int 2 – Ex 3 : 1023	10 SNPs found in LEP 3 and 6 found in LEP 4. PIC value range were from 0.06 (low) to 0.37 (moderate)	[16]
Pesisir	Polymorphism identification	*LEP MspI*		F: 5'- TTG CTT GAT GGT CCA AAG GC -3' R: 5'- CGT GGG CAC AAG AAG TAA GG -3'	Exon 3	846	Allel (+) 65.5% and (–) 34.5%; Genotype (+/+) 48%, (+/-) 35%, (--) 17%.	[17]
Bali	Polymorphism identification	*Lep BsaA1*	HE605298.1	F:5'GTCTGGAGGCAAAGGGCAGAGT -3 R : 5' CCACCACCTCTGTGGAGTAG -3'	Intron2 – exon 3	552	Allele A 76%, G 24%; Genotype AA 72%, AG 20%, GG 8%	[18]

Based on the cultural event involving Madura cattle, it had proven that selection over the generation could change the character of the cattle. Selection aimed to produce livestock to reach the breeding objective or breeding goal, thus allowing for different economic values. Unfortunately, the traditional selection is no longer efficient, time-consuming, and inaccurate. Currently, a faster selection tool can be done early and accurately, called Marker-Assisted Selection (MAS). This method could be used for both quantitative and qualitative selection [19]. In the case of Madura cattle for the cultural event, which was selected based on body conformation, quantitative selection could make using associated genes. However, it should be noted that one gene can code for many traits. Many genes can code one trait; thus, careful consideration had needed. [20].

The Leptin gene was known to regulate adipose tissue and body weight by inhibiting feed intake and energy expenditure stimulation. The gene regulation was necessary for beef cattle, which aimed to produce high productivity. The Leptin gene in cattle had located on chromosome 4 (BTA4q32), has a length of 16,735 base pairs (bp) and consists of 2 introns and three exons as well [21]. The first intron is more than 8 kb and the second intron has a length of 1.6 kb [22]. Leptin consists of 167 amino acids and had a molecular weight of 16kDa [23]. The Leptin gene functions to produce the hormone Leptin, synthesized from adipose tissue (fat). The hormone Leptin then plays an essential role in controlling body weight, feed consumption, and energy balance [24]. Thus, this study aimed to investigate the potency of the Leptin gene as a selection marker of economic traits for Madura cattle.

2. METHODS

This study had performed a literature review from published studies. First, publications were collected to obtain a list of Leptin genes in Madura cattle. Because of the limited study found, we then collected Leptin gene study publications in various Indonesian cattle. We reviewed the references section for each manuscript to ensure that we included the highest number of relevant studies. Only electronic publications written in English and Indonesian had used in this study. Next, publications were collected to obtain the association of Leptin gene polymorphism to the economic traits in various cattle. The selected economic traits were body conformation, carcass, milk, and reproduction. Body conformation consisted of body weight and body measurement. Even though Madura cattle were meat-type cattle, the milk production trait had included because it relates to mothering abilities and the environmental dam effect. In total, 26 papers had used in this study.

3. RESULTS AND DISCUSSION

3.1. Leptin Gene Studies in Indonesia

In Indonesia, studies on the Leptin gene in local cattle were limited. Several studies of the Leptin gene in cattle breeds in Indonesia include Madura cattle, Kebumen Ongole Grade, Ciamis Local Cattle, Ongole Grade, Bali Cattle, Pasundan Cattle, Bali Cross, Sumba Ongole, and Pesisir Cattle (Table 1). Most studies use GenBank accession numbers U50365 and EU313203.1. The part that had often use was exon 2. The length of the PCR product fragment taken is between 234 to 1820 bp. Several mutations in the exon two portion of the Leptin gene cause changes in certain amino acids that can affect livestock productivity [25–27].

However, so far, the research has been limited to identifying polymorphisms. Therefore, it cannot be used for selection because it only knows the diversity. In order used as a selection tool, it was necessary to know whether or not there was an association of polymorphism with phenotypic traits. Some researchers have associated polymorphism with phenotypic traits, although results vary. Fathoni et al [28] had stated that the Leptin gene polymorphism g.1080C>T was associated with weaning chest circumference in Kebumen Ongole Grade cattle. Meanwhile, other researchers stated no association between genotype and meat quality, birth weight, weaning weight, daily weight gain, and body condition score. [9,12,13].

3.2. Leptin Gene Studies on Various Cattle

We found several SNPs associated with economic traits. SNPs had located in Exon 2, Intron 2, and Exon 3. Leptin gene association studies on various cattle showed in Table 2. Exon 1 of the Leptin gene had included in the promoter region with a small number of bases, so it assumed that it does not affect the association [29].

Exon 2 Leptin gene studies were found mostly in SNP 1180C>T and were associated with four selected economic traits. The CT genotype had a weaning chest circumference, 3.5% FCM, higher milk fat, and true milk protein than the CC genotype. The CC genotype had a higher backfat thickness in Korean and Hanwoo cattle but lower in crossbred cattle than the TT genotype. The CC genotype produced a higher Marbling score, yield grade, lean meat yield, longissimus muscle area than the TT genotype in Korean cattle, crossbreed steer, and Hanwoo cattle. In terms of reproduction, the CC genotype in Holstein cattle produced Estrous cyclic at 49 ± 3 DIM, which was higher than TT. The base mutation of Cytosine to Tymin at position 1180 converts the amino acid Arginine to Cysteine. SNP 1180C>T has several other names including R25C, Arg25Cys, 1047C>T, C305T, R4C, C73T and *LepKpn2I* [11,25,30–32]. This amino acid change had thought to be a causative mutation

that causes changes in the physiological work of the hormone Leptin in energy metabolism. The conversion of Arginine to Cysteine causes the binding capacity and function to return to the wild type, and this shows the importance of Cysteine Sulfihydryl [32]. Furthermore, this base change had proposed as a nonconserved substitution imparting a potential partial loss of biological function that is associated with fatter carcasses and higher Leptin mRNA levels [31,33]. Another hypothesis was that mutation changes the tertiary conformation of the Leptin protein by adding unpaired cysteine to its structure, thereby changing the affinity of the hormone for its receptor as evidenced by changes in Leptin concentrations. [34].

Table 2. Association of Leptin gen with Economic trait on various cattle

SNP/Locus	Trait	Genotype	Best Genotype Frequency	Breed	Ref
Exon 2					
1180C>T	Weaning chest circumference	CT ↑, CC ↓	21%	Kebumen Ongole Grade	[8]
1180C>T	Body weight	CT ↑, TT ↓	47.7%	Crosbred Cattle	[25]
1180C>T	Backfat thickness	TT ↑, CC ↓	22.6%	Crosbred Cattle	[25]
1180C>T	Backfat thickness	CC ↑, TT ↓	29.2%	Korean Cattle	[35]
1180C>T	Marbling score	CC ↑, TT ↓	29.2%	Korean Cattle	[35]
1180C>T	Gain in backfat thickness	TT ↑, CC ↓	26.38%	Crossbreed steer	[33]
1180C>T	Ultrasound backfat thickness	TT ↑, CC ↓	26.38%	Crossbreed steer	[33]
1180C>T	Grade fat (backfat)	TT ↑, CC ↓	26.38%	Crossbreed steer	[33]
1180C>T	Yield grade	CC ↑, TT ↓	26.38%	Crossbreed steer	[33]
1180C>T	Lean meat yield	CC ↑, TT ↓	22.2%	Crossbreed steer	[33]
1180C>T	Back fat thickness	CC ↑, TT ↓	26.2%	Hanwoo Cattle	[36]
1180C>T	Longisimus muscle area	CC ↑, TT ↓	26.2%	Hanwoo Cattle	[36]
1180C>T	Estrous cyclic at 49 ± 3 DIM	CC ↑, TT ↓	34.6%	Holstein Cows	[34]
1180C>T	3.5% FCM	CT ↑, CC ↓	48.2%	Holstein Cows	[31]
1180C>T	Milk fat	CT ↑, CC ↓	48.2%	Holstein Cows	[31]
1180C>T	Milk true protein	CT ↑, CC ↓	48.2%	Holstein Cows	[31]
Intron 2					
BstMB1	Weight at 9 month	AB ↑, AA ↓	22%	Sistani Cows	[37]
BstMB1	Weight at 12 month	AB ↑, AA ↓	22%	Sistani Cows	[37]
BstMB1	Days open	AA ↑, AB ↓	64%	Brown Swiss	[37]
BstMB1	Age at conception	AB ↑, AA ↓	22%	Sistani Cows	[37]
BstMB1	1 month lactation	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	2 month lactation	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	3 month lactation	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	4 month lactation	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	60 days milk production	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	100 days milk production	AB ↑, AA ↓	35%	Brown Swiss	[37]
BstMB1	Milk days	AA ↑, AB ↓	64%	Brown Swiss	[37]
LEP/Sau3AI	Carcass weight	AA ↑, AC ↓	69.5%	Friesian Bulls	[38]
LEP/Sau3AI	Calving interval	+- ↑, -- ↓	12.7%	Angus-Nelore Cross	[39]
LEP/Sau3AI	Age at first calving	AB ↑, AA ↓	32.6%	Slovak Spotted and Pinzgau	[40]
LEP/Sau3AI	Milk yield	AA ↑, BB ↓	63.7%	Slovak Spotted and Pinzgau	[40]
LEP/Sau3AI	Protein yield	AA ↑, BB ↓	63.7%	Slovak Spotted and Pinzgau	[40]
LEP/Sau3AI	Fat yield	AA ↑, BB ↓	63.7%	Slovak Spotted and Pinzgau	[40]
Exon 3					
SSCP[1]	Birth weight	BB ↑, AA ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	6m withers height	BB ↑, AA ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	6m body length	BB ↑, AA ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	6m heart girth	BB ↑, AA ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	6m body weight	BB ↑, AA ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	6m average day gain	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	2y Withers height	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	2y Body length	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	2y Heart girth	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	2y Body weight	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
SSCP[1]	2y Average day gain	BB ↑, AB ↓	29.3%	Nanyang Cattle	[41]
A80V	Non-return rate in cows	TT ↑, CC ↓	7.1%	Polish Holstein-Friesian bulls	[30]
LEP/Msp I	Marbling score	AA ↑, BB ↓	29.4%	Hanwoo Cattle	[36]
rs29004509 (C>T)	Milk yield	CT ↑, CC ↓	20%	Karan Fries	[42]

Note: SSCP[1] = 66th bp position G→T transversion, 67th bp position A→C transversion and 299th bp position G→T transversion

Leptin gene mutations in intron two were associated with growth, carcass, reproduction, and milk traits. We found two positions based on published studies, namely *LEP/BstMB1* and *LEP/Sau3AI*. Sistani cattle with genotype AB in *LEP/BstMB1* had higher body weight and age at conception than genotype AA. Meanwhile, Swiss Brown Cows with genotype AB in *LEP/BstMB1* had higher milk production and shorter open days but had lower milk days than genotype AA. Genotype AA in *LEP/Sau3AI* Slovak Spotted and Pinzgau cattle had known to produce higher milk production and quality than genotype AB.

Meanwhile, genotype AA had a higher carcass weight than AC, and genotype (+-) had a higher calving interval than (--). Introns were known as non-coding regions because they remove before a protein was made. However, some non-coding DNA still had an essential role in regulating gene expression, that was to changes in introns, possibly affecting changes to the coding region (REF) portion. Allele B in *LEP/BstMB1* is the favorable allele in selected economic traits. However, this allele is recessive. In contrast to these mutations, the association of the *LEP/Sau3AI* gene did not form a uniform pattern. SNP *LEP/Sau3AI* are change of cytosine to thymine and results in amino acid change arginine by cysteine at position 2059 of the protein chain [40].

We did not find much research done on the Exon 3 Leptin gene. However, based on published research, it was found that 4 SNPs were associated with economic traits. The SNPs found were A80V, *LEP/Msp I*, and rs29004509 (C>T). In addition, mutations detected with SSCP were also found, with mutations at position 66th bp position G→T transversion, 67th bp position A→C transversion, and 299th bp position G→T transversion. Cows with the BB genotype in the SSCP study had a growth trait from birth to 2 years of age higher than the AA and AB genotypes. From birth to 6 months, genotype AB had also higher than AA, but because the average daily gain is lower, at the age of 2 years, genotype AB had a lower growth trait than AA. Although this study showed good results, further research is still needed to find a more specific effect on one of the mutation positions [41]. On the other hand, SNP A80V is a causative mutation that affects the non-return rate compared to other SNPs. Mutations at this position include conservative substitutions because alanine and valine are in the same non-polar amino acid group [30].

3.3. Potency of Leptin Gene as Selection Marker of Economic Trait for Madura Cattle

Madura cattle in the Madura island were kept based on the breeding goals of each farmer. Not only kept as beef cattle to produce meat, but Madura cattle had also kept for body conformation contests and racing. The main concern of breeders was body size and other qualitative traits. However, this selection had been shown to produce differences in body size characteristics. Based on this fact, it concluded that the selection of Madura cattle could run effectively. If Madura cattle had only improved for beef cattle whose economic value had to calculate from growth, carcass, milk, and reproduction traits, it would be better to become marker-assisted selection.

Sonok, *Taccek*, *Karapan*, and conventional cattle had different body sizes. It was necessary to measure their genetic profile through molecular analysis. Based on this profile, the differences between each cattle. If genetic profiles showed different profiles between types of cattle, this genetic profile could be used as the basis for selecting parent stock that produces the next generation. On the other hand, if the genetic profile between cattle is the same, basically every cow has the same genetic potential. Then, it can be a sign that the phenotypic differences are not genetic but were affected by non-genetic factors, such as livestock management.

In this study, it had found that leptin gene polymorphisms at various locations affected economic traits. However, the leptin gene polymorphism location that affects the four selected economic traits only SNP g.1180 C>T (R25C, Arg25Cys, 1047C>T C305T, R4C, C73T, and *LepKpn2I*), which had located in exon 2. Therefore, this SNP location is the most potential location to be used as the basis for selecting economic traits. The research results summarized in this paper indicate that the best genotype for each trait is different and depends on the breed of cattle. For example, the gain in backfat thickness trait in crossbreed cattle is TT genotype, but the best backfat thickness trait in Hanwoo cattle is CC. Based on this, it is necessary to conduct further research on Madura cattle to determine the best genotype for each trait.

Application of the use of the leptin gene in Madura cattle could be conducted by identifying the genotype of the leptin gene in a livestock population. The cattle population to be selected should meet the standards of good farming practices so that genetic potential can be expressed. The leptin gene genotype data obtained were then statistically associated with livestock productivity data [43].

4. CONCLUSION

The result concluded that Leptin SNP g.1180C>T (R25C, Arg25Cys, 1047C>T, C305T, R4C, C73T, and *LepKpn2I*) has the potency to be used as a selection marker of economic traits for Madura cattle. Following marker selection, an association study on Madura Cattle is further to validate this result.

AUTHORS' CONTRIBUTIONS

TN: Conception, conducted the literature review, data acquisition, data analysis and interpretation, manuscript drafting, and revision. TSMW: Conception and

manuscript correction. DM: Conception and manuscript correction.

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