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Genetic Characterization of Thyroglobulin and Leptin Genes in Pasundan Cattle at West Java

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ABSTRACT

The Thyroglobulin (TG) and Leptin (LEP) genes are two candidate genes that widely used for molecular selection to improve carcass traits in beef cattle. This research was carried out to identify the genetic characterization of TG and LEP genes from 47 heads of Pasundan cows at West Java using PCR-RFLP method. Research shows that TG gene of Pasundan cattle is monomorphic with C allele as the dominant allele (1.00). However, LEP gene of Pasundan cattle is polymorphic with C allele as the dominant allele (0.98) and T as the rare allele (0.02). The polymorphic informative content (PIC) and number of effective allele (n_e) values in the LEP gene in the animal studied were 0.04 and 1.04 respectively. It was concluded that TG/*Bst*YI and LEP/*Sau3*AI gene in the present study can not be used as molecular selection in Pasundan cattle. These results are important as the basic information for preparing the molecular selection program in the future.

Keywords: Leptin, Pasundan cattle, PCR-RFLP, Thyroglobulin

Introduction

Pasundan cattle is one of Indonesian native cattle from West Java province and was kept by smallholds as the beef cattle. This cattle was declared as Indonesian native cattle trough decision of Ministry of Agriculture of Republic Indonesia No: 1051/Kpts/SR.120/10/2014 (Anonymous, 2014). Recently, the population of Pasundan cattle is low because of reducing pasture area in West Java (Arifin *et al.,* 2015). In addition, low calving rate in the Pasundan herds was affected to the low number of Pasundan population (Said *et al.,* 2017). The genetic improvement program for Pasundan cattle is important to increase the productivity. Despite, Pasundan cattle with desirable traits can be increased the sell price in the market. This condition can affect for livestock demand and increasing to the number of Pasundan population. Genetic improvement in the livestock can be obtained through molecular selection in the some candidate genes that affecting productivity such as Thyroglobulin (TG) and Leptin (LEP) genes (Carvalho *et al.,* 2012).

The TG gene was mapped in the centromic region of bovine chromosome 14, covers at least 300 kb genomic DNA, contains 37 exons and encodes 8.7 kb mRNA (Baas *et al.,* 1986). The TG gene is important for metabolism regulation and affecting to adipocyte differentiation, growth and homeostatis of fat depots (Darimont *et al.,* 1993; Smas and Sul, 1995) and growth traits (Zhang *et al.,* 2015). A single nucleotide polymorphism (SNP) located at g.371C/T in the 5'untranslated region (5'UTR) of bovine TG gene was used for molecular selection in the marbling score (Barendse, 1999; Barendse *et al.,* 2004; Casas *et al.,* 2005), fat yield percentage (Sedykh *et al.,* 2016) and backfat thickness (Mears *et al.,* 2001; Moore *et al.,* 2003; Rincker *et al.,* 2006; Gan *et al.,* 2008) in some beef cattle breeds. Despite, Fernandez *et al.* (2014) reported that the TG gene polymorphism was influenced to puberty trait in Guzerat bulls.

The LEP gene was mapped in the bovine chromosome 4 along 16,735 bp and contains two introns and three exons (Pfister-Genskow *et al.*, 1996). The LEP gene is important for secretion of leptin hormone in the adipose tissue. This hormone is important for controlling body weight, feed intake and energy balance (Frunhbeck *et al.*, 1998). A SNP located at g.1926C/T in the intron 2 of LEP gene was used for molecular selection in the milk yield (Moravcikova *et al.,* 2012; Trakovicka *et al.,* 2013; Kiyici *et al.,* 2018), reproductive traits (Moussavi *et al.,* 2006; Oner *et al.,* 2017; Ferchichi *et al.,* 2018), body weight

(Almeida *et al.,* 2003; Nobari e*t al.,* 2010; Hussain *et al.,* 2017), fat yield percentage (Sedykh e*t al.,* 2016) and feed intake (Liefers *et al.,* 2002).

The study of molecular genetic in Pasundan cattle are limited, particulary no study has been made to assess the genetic diversity of TG and LEP genes in this breed. The objectives of the present study were to identify the typical allele and genotype of TG and LEP genes through PCR-RFLP method. The results of this study can be used as basic information for arranging the molecular selection program of Pasundan cattle in the future.

Materials and Methods

A total of 47 heads of Pasundan cattle from the breeding station (BPPIBT-SP Ciamis, West Java) were used for blood sampling purpose. Blood samples (3-5 mL) were taken from cocygeal vein using *venoject* and collected in *vaccutainer* tubes containing anticoagulant (EDTA). The bloods sample were used for DNA extraction process using the Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the manufacturer's protocol. Amplification of TG and LEP genes were performed in a Mastercycler® gradient (Eppendorf, Germany) with two different of PCR reagent composition volume. Total of 10 µL of PCR product per sample was used for TG gene amplification containing 1 µL of DNA template, 4 µL of PCR mix KAPA2G Fast ReadyMix (Kapa Biosystems Inc., USA), 4 µL of DDH_2O and 0.5 µL of each primer. Thus, total of 10 µL of PCR product per sample was used for LEP gene amplification containing of 4 µL of DNA template, 5 µL of PCR mix MyTag™ Hot Start Red Mix (Bioline, USA), 0.6 µL DDH_2O and 0.2 µL of each primer. The PCR programs for TG and LEP genes amplification were showed in Table 1.

The RFLP reagent of TG gene was performed in 10 µL per sample containing 5 µL of PCR product, 3.7 µL of DDH_2O, 1 µL of 10 x buffer and 0.3 µL of *Bst*YI restriction enzyme. Thus, the RFLP reagent of LEP gene was performed in 7 µL per sample containing 5 µL of PCR product, 1 µL of DDH_2O, 0.7 µL of 10 x buffer and 0.3 µL of *Sau3*AI restriction enzyme. The RFLP reagents of TG and LEP genes were digested at 60°C and 37°C respectively in the water bath along 1 h. Therefore, the PCR and RFLP products were visualized using 1% and 2% of agarose gels respectively and captured in GBOX Documentation System (Syngene, UK) with GelRed™ staining (Biotium, USA). The genotip of TG/*Bst*YI gene purposed be consisted of CC (295 bp, 178 bp and 72 bp), TT (473 bp and 72 bp) and CT (473 bp, 295 bp, 178 bp and 72 bp). The genotype of LEP/*Sau3*AI gene purposed be consisted of CC (389 bp and 32 bp), TT (304 bp, 85 bp and 32 bp) dan CT (389 bp, 304 bp, 85 bp and 32 bp).

The statistical analysis for the TG and LEP genes were consisted of allele frequency, expected heterozygosity (H_e), observed heterozygote (H_o), number of effective allele (n_e), polymorphic informative content (PIC) and Chi-square (χ^2) based on Nei and Kumar (2000).

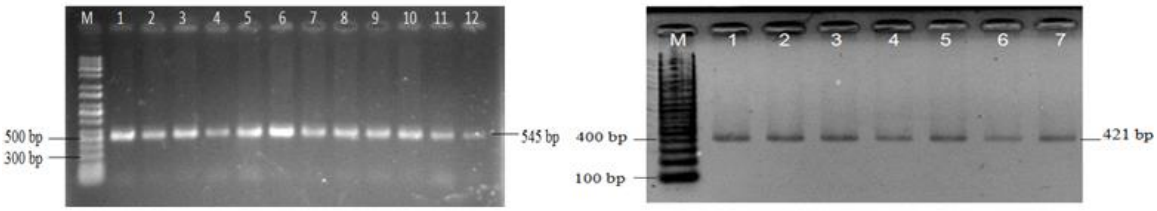
Result and Discussion

The TG and LEP genes of Pasundan cattle were successfully to amplify along 545 bp and 421 bp respectively (Figure 1). The results indicated that amplification fragment had good specificity, which could proceed directly to RFLP analysis. The RFLP analysis of TG/*Bst*YI gene in Pasundan cattle was monomorphic with CC genotype shows in all animal studied (Figure 2). Previous study reported that TG/*Bst*YI gene in Bali (*Bos javanicus*) and Nellore/Ongole (*Bos indicus*) cattle were monomorphic with C allele as the common allele (Table 3). It can be concluded that absence of T allele in Pasundan cattle reveals that this cattle had genetic material *Bos indicus a*nd *Bos javanicus* breeds.

According to Table 4, the C allele was more frequent in *Bos taurus* and *Bos indicus* cattle. Shin and Chung (2007) reported that homozygote TT animals in the TG/*Bst*YI gene of Hanwoo population had lowest of marbling score value ($P<0.05$) than other genotypes animals. In

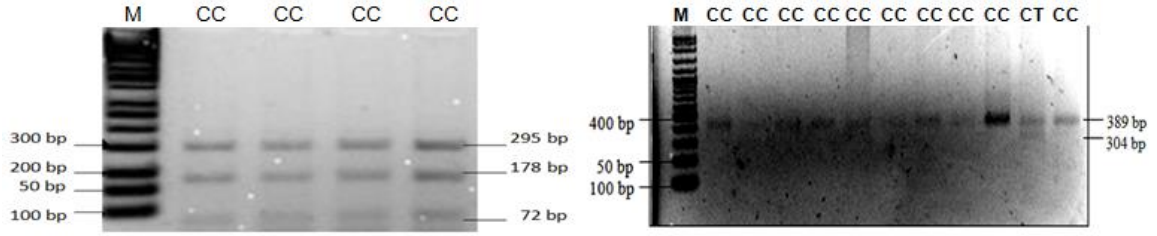
Table 1. Primer and PCR program in the TG and LEP genes

Gene	SNP	Location	Primer	Amplicon (bp)	PCR condition	GenBank	Reference
TG	g.371C/T	5'UTR	F: 5'-GGGGATGACTACGAGTATGACTG-3' R: 5'-GTGAAAATCTTGTGGAGGCTGTA-3'	545	95°C 1' (94°C 35", 57°C 30", 72°C 30") 35 cycles, 72°C 5'	AY615525.1	Barendse (1999)
LEP	g.1926C/T	intron 2 - exon 3	F: 5'-TGGAGTGGCTTGTTATTTTCTTCT-3' R: 5'-GTCCCCGCTTCTGGCTACCTAACT-3'	421	95°C 1' (95°C 15", 52.7°C 15', 72°C 10") 40 cycles, 72°C 5'	EU313203	Liefers *et al.* (2002)



TG gene LEP gene

Figure 1. The amplification of TG and LEP genes in Pasundan cattle were separated in 1% agarose gel along 545 bp (left) and 421 bp (right) respectively. M: DNA ladder 100 bp; line 1-12: number of sample.



TG/*Bst*YI gene LEP/*Sau3*AI gene

Figure 2. The RFLP analysis for TG and LEP genes of Pasundan cattle in 2% agarose gel. The TG/*Bst*YI gene is monomorphic with one genotype of CC (295 bp; 178 bp dan 72 bp) in all animal studied. The LEP/*Sau3*AI gene is polymorphic with two genotype of CC (389 bp) and CT (389 bp and 304 bp) in the animal studied. M: DNA ladder 100 bp.

Table 2. Statistical analysis in the TG and LEP genes of Pasundan cattle at West Java

Gene	Genotypic frequency (N)			Allelic frequency		H_o	H_e	n_e	PIC	χ^2
	CC	CT	TT	C	T					
TG/*Bst*YI	1.00 (47)	0.00 (0)	0.00 (0)	1.00	0.00	0.00	0.00	1.00	0.00	-
LEP/*Sau3*AI	0.96 (45)	0.04 (2)	0.00 (0)	0.98	0.02	0.04	0.04	1.04	0.04	0.02*

N: number of sample; H_o: observe heterozigosity; H_e: expected heterozigosity; n_e: number of effective allele; PIC: polymorphic informative content; X^2: Chi-square value; *genetic equilibrium (X^2<5.99).

addition, the C allele had trend to increase marbling score trait. Anton *et al.* (2012) reported that homozygote CC animals in the TG/*Bst*YI gene had the highest of milk yield than other genotype animals in Jersey and Hungarian Simmental populations (P<0.05). Bonilla *et al.* (2010) reported that homozygote CC animals in the TG/*Bst*YI gene of Mexican beef population had lowest of intramuscular fat content (IMF) than heterozygote animals (P<0.05).

The LEP/*Sau3*AI gene of Pasundan cattle in the present study was polymorphic with two genotype of CC and CT (Figure 4). According to Figure 2, two DNA fragments of 85 bp and 32 bp were not showed clearly in 2% agarose gel. The polymorphism of LEP/*Sau3*AI gene in the present study had low PIC value (0.04) and can not be used as molecular selection (Table 2).

The PIC value is one of parameter for measuring the level polymorphism in genetic marker. According to Nei and Kumar (2000), PIC value was consisted of three category of low (PIC<0.25), moderate (0.25<PIC<0.50) and high (PIC>0.50). High PIC value indicated that the genetic marker had multiple allele and can be used for molecular selection (Chesnokov and Artemyeva, 2015). Therefore, the H_o and H_e values in the present study are similar and indicated that the crossing in Pasundan population was occured. Meanwhile, inbreeding in the population was detected when $H_o < H_e$ and random mating in the population was detected when $H_o > H_e$ (Chesnokov and Artemyeva, 2015). The LEP/*Sau3*AI gene in the population sample is under genetic equilibrium (χ^2=0.02) and can be caused by random mating and no selection program (Falconer and Mackay, 1996). The n_e value of LEP/*Sau3*AI in Pasundan cattle was 1.04 and explains that only one common allele that identify in LEP/*Sau*3AI gene.

Previous study reported that the frequency of C allele in *Bos indicus* breeds was 0.53 to 1.00 (Table 5). According to the Table 5, most of *Bos indicus* and *Bos taurus* cattle had T allele with lower frequency than C allele. Trakovicka *et al.* (2013) reported that CC genotype in the LEP/*Sau*3AI gene had highest of milk yield, protein yield and fat yield than other genotypes in mix population between Pinzgau and Slovak Spotted cows (P<0.05). In addition, heterozygote animals had lowest of age at first calving (P<0.05).

The TG and LEP genes can not be used as molecular selection for carcass traits of Pasundan cattle. The further research to identify SNP in other regions of TG and LEP genes are

Table 3. Allelic and genotypic frequency of TG/*Bst*YI gene in several breeds cattle

Breed	Species	N	Genotypic frequency			Allelic frequency		Reference
			CC	CT	TT	C	T	
Angus	*Bos taurus*	819	0.64	0.32	0.03	0.80	0.20	Barendse *et al.,* 2004
		39	0.21	0.62	0.17	0.51	0.49	Pannier *et al.,* 2010
		173	0.46	0.41	0.13	0.66	0.34	Anton *et al.,* 2012
Friesian Holstein	*Bos taurus*	1279	0.58	0.39	0.03	0.78	0.22	Khatib *et al.,* 2007
		415	0.75	0.24	0.01	0.87	0.13	Anton *et al.,* 2012
Simmental	*Bos taurus*	58	0.40	0.55	0.05	0.67	0.33	Pannier *et al.,* 2010
		438	0.53	0.39	0.08	0.73	0.27	Anton *et al.,* 2012
Hereford	*Bos taurus*	32	0.97	0.03	0.00	0.98	0.02	Pannier *et al.,* 2010
Belgian Blue	*Bos taurus*	19	0.74	0.16	0.10	0.82	0.18	Pannier *et al.,* 2010
Blonde d'Aquitaine	*Bos taurus*	13	0.62	0.31	0.07	0.77	0.23	Pannier *et al.,* 2010
Hanwoo	*Bos taurus*	309	0.41	0.46	0.13	0.64	0.36	Shin and Chung, 2007
Wagyu cross	*Bos taurus*	153	0.48	0.41	0.11	0.69	0.31	Casas *et al.,* 2007
Limousin	*Bos taurus*	123	0.67	0.32	0.01	0.83	0.17	Pannier *et al.,* 2010
Charolais	*Bos taurus*	80	0.56	0.35	0.09	0.74	0.26	Pannier *et al.,* 2010
Jersey	*Bos taurus*	283	0.60	0.35	0.05	0.78	0.22	Anton *et al.,* 2012
South Anatolian Red	*Bos taurus*	50	0.40	0.04	0.56	0.42	0.58	Yardibi *et al.,* 2013
Ukrainian Red & White	*Bos taurus*	93	0.74	0.25	0.01	0.88	0.12	Berezovsky, 2014
Ukrainian Black & White	*Bos taurus*	40	0.88	0.12	0.00	0.94	0.06	Berezovsky, 2014
Ukrainian Red	*Bos taurus*	38	0.82	0.18	0.00	0.91	0.09	Berezovsky, 2014
Ukrainian Grey	*Bos taurus*	84	0.31	0.57	0.12	0.60	0.40	Berezovsky, 2014
Anatolian Black	*Bos taurus*	36	0.56	0.36	0.08	0.73	0.27	Savasci and Fatih, 2016
East Anatolian Red	*Bos taurus*	51	0.45	0.41	0.14	0.65	0.35	Savasci and Fatih, 2016
Turkish Grey	*Bos taurus*	52	0.88	0.12	0.00	0.94	0.06	Savasci and Fatih, 2016
Brahman	*Bos indicus*	467	0.94	0.04	0.02	0.97	0.03	Casas *et al.,* 2005
		383	0.99	0.01	0.00	0.99	0.01	Smith *et al.,* 2009
Nellore	*Bos indicus*	46	1.00	0.00	0.00	1.00	0.00	Fortes *et al.,* 2009
Bali	*Bos javanicus*	200	1.00	0.00	0.00	1.00	0.00	Anwar *et al.,* 2016
Canchim	*B. tau* × *B. ind*	41	0.61	0.34	0.05	0.78	0.22	Fortes *et al.,* 2009

N: number of sample.

Table 4. Allelic and genotypic frequency of LEP/*Sau3*AI gene in several breeds cattle

Breed	Species	N	Genotypic frequency			Allelic frequency		Reference
			CC	CT	TT	C	T	
Angus	*Bos taurus*	25	0.76	0.24	0.00	0.88	0.12	Rasor *et al*., 2002
Iranian Holstein	*Bos taurus*	238	0.89	0.11	0.00	0.95	0.05	Moussavi *et al*., 2006
Brown Swiss	*Bos taurus*	104	0.64	0.35	0.01	0.82	0.10	Nobari *et al*., 2010
Pinzgau	*Bos taurus*	85	0.45	0.49	0.06	0.69	0.31	Moravcikova *et al.,* 2012
Slovak Spotted	*Bos taurus*	110	0.69	0.28	0.03	0.83	0.17	Moravcikova *et al*., 2012
Hereford	*Bos taurus*	38	0.68	0.32	0.00	0.84	0.16	Sedykh *et al.,* 2016
Limousine	*Bos taurus*	26	0.39	0.61	0.00	0.69	0.31	Sedykh *et al.,* 2016
Holstein Friesian	*Bos taurus*	160	0.80	0.19	0.01	0.90	0.10	Oner *et al*., 2017
Tunisian Holstein	*Bos taurus*	412	0.52	0.32	0.16	0.68	0.32	Ferchichi *et al*., 2018
Brahman	*Bos indicus*	7	0.86	0.14	0.00	0.93	0.07	Rasor *et al*., 2002
Criollo	*Bos indicus*	11	1.00	0.00	0.00	1.00	0.00	Rasor *et al*., 2002
African Mashona	*Bos indicus*	6	1.00	0.00	0.00	1.00	0.00	Rasor *et al*., 2002
Sistani	*Bos indicus*	103	0.77	0.22	0.01	0.82	0.18	Nobari *et al*., 2010
Sarabi	*Bos indicus*	66	0.31	0.43	0.14	0.53	0.47	Javanmard *et al*., 2008
Bali	*Bos javanicus*	11	1.00	0.00	0.00	1.00	0.00	Mappanganro *et al*., 2014
Brangus	*B. ind* × *B. tau*	96	0.44	0.38	0.18	0.63	0.37	Almeida *et al*., 2003
Braford	*B. ind* × *B. tau*	18	0.94	0.06	0.00	0.97	0.03	Rasor *et al*., 2002
Santa Cruz	*B. ind* × *B. tau*	33	0.91	0.06	0.03	0.94	0.06	Rasor *et al*., 2002
Santa Gertrudis	*B. ind* × *B. tau*	25	0.92	0.08	0.00	0.96	0.08	Rasor *et al*., 2002
Native Iranian	-	132	0.59	0.36	0.05	0.77	0.23	Sharifzadeh and Doosti, 2012
Native Iraqi	-	60	0.67	0.33	0.00	0.83	0.17	Hussain *et al*., 2017

N: number of sample.

important in order to obtain marker assisted selection (MAS) for carcass traits of Pasundan cattle in the future.

Conclusions

The study showed TG/*Bst*YI and LEP/*Sau3*AI genes can not be used for molecular selection in Pasundan cattle. The 5'UTR of TG gene in Pasundan cattle was monomorphic with C allele as the common allele. The genetic diversity in the intron 2 of LEP/*Sau3*AI gene in Pasundan cattle was low (PIC<0.25) with C allele as the dominant allele.

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