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Variasi Sekuen gen *CCAAT/Enhancer Binding Protein α* (*C/EBP α*) Pengkode Kualitas Daging pada Bangsa Sapi Potong Lokal dan Sapi Eksotik

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ABSTRAK

Gen *C/EBPα* merupakan gen yang berasosiasi terhadap komposisi dan pendistribusian lemak, sehingga berkontribusi pada peningkatan kualitas daging. Informasi variasi sekuen gen *C/EBPα* pada sapi potong lokal dan eksotik belum tersedia di Indonesia. Penelitian ini bertujuan untuk mendeteksi adanya variasi sekuen gen *C/EBPα* pada sapi potong lokal maupun sapi eksotik. Sebanyak 12 sampel sapi digunakan dalam penelitian ini yang terdiri dari Bali (2), Peranakan Ongole (PO) (2), Pasundan (2), Friesian Holstein (FH) (1), Angus (1), Limousin (1), Simmental (2), dan Banteng (1). Tahapan yang dilakukan pada penelitian ini adalah esktraksi DNA, kuantifikasi DNA, PCR, elektroforesis, *sequencing* dan analisis sekuen gen *C/EBPα*. Koleksi DNA pada penelitian ini bersumber dari darah dan rambut. Penentuan basa homolog gen *C/EBPα* berdasarkan hasil BLAST dan variasi sekuens basa dianalisis dengan MEGA 6. Hasil penelitian ini menunjukkan bahwa semua sapi yang diteliti telah terkonfirmasi memiliki gen *C/EBPα* yang teramplifikasi (1.339 pb) pada suhu *annealing* 54°C. Ditemukan tiga variasi basa posisi 870 (G→A), 931 (A→G) dan 1196 (G→A) pada sapi Bali dan Banteng, tetapi tidak diperoleh pada sapi lokal lain, eksotik dan referensi sekuen *Bos taurus* (Japanese Black/Acc No. DQ068270.1, Hanwoo/Acc No. D82984.1, Qinchuan/Acc No. NM_176784.2). Variasi sekuens teresebut menyebabkan perubahan pada asam amino (Alanine → Threonine/931 dan Asparagine → Serine/1196). Mutasi pada posisi 271 menyebabkan perubahan basa sitosin menjadi adenine (C271A) yang ditemukan di sapi PO dan Simmental. Diperlukan penelitian lebih lanjut tentang perubahan asam amino gen *C/EBPα* pada sapi Bali, sapi PO dan Simmental terhadap kualitas daging.

Kata kunci: Gen *C/EBPα*, Variasi sekuen, Kualitas daging, Sapi potong lokal, Sapi eksotik

Sequence Variations of CCAAT/Enhancer Binding Protein α (C/EBPα) gene encoding meat quality in local and exotic cattle breeds

ABSTRACT

Gene of C/EBPα is a gene associated with lipid composition and distribution, the gene therefore contributes in enhancing of meat quality. There is no information of sequence variations of C/EBPα gene investigation in local and exotic beef cattle in Indonesia. This research was aimed to detect the presence of sequence variations of C/EBPα gene in local beef and exotic cattle. Twelve samples were used in this study such as Bali cattle (2), Ongole grade (2), Pasundan (2), Friesian Holstein (1), Angus (1), Simmental (2), Limousin (1) and Banteng (1). Several steps of the research were DNA extraction and quantification, amplification (PCR), electrophoresis, sequencing and sequence analysis of C/EBPα gene. DNA was collected either from blood or hair bulbs. Determination of homolog bases of C/EBPα gene was based on BLAST result while base sequence variation was analyzed by using MEGA 6. The result showed that all cattle samples were confirmed bearing the C/EBPα gene that amplified along of 1,339 bp. and annealing temperature of 54°C. It was obtained three nucleotide variations at the position of 870 (G→A), 931 (A→G) and 1196 (G→A) only found in Bali cattle and Banteng, but there were no nucleotide variations in the local beef cattle, exotic cattle and the sequence reference of Bos taurus (Japanese Black/Acc No. DQ068270.1; Hanwoo/Acc No. D82984.1; Qinchuan/Acc No. NM_176784.2). Those nucleotide variations caused changing of amino acid of Alanine to Threonine at 931 and Asparagine to Serine at 1196. Mutation at position 271 changed nucleotide Cytosine to Adenine (C271A) that found in PO and Simmental cattle. Further research is needed to confirm the changing of amino acid of C/EBPα gene in Bali cattle might affect to meat quality.

Keywords: *C/EBPα gene, Sequence variation, Meat quality, Local beef cattle, Exotic cattle*

INTRODUCTION

Meat consumption in Indonesia increases up to 9.89% every year since 1993 to 2015 (Kementerian Pertanian, 2017). The national meat stock was dominantly covered 70% by local beef production (Rusono, 2015) while, the remain covered by importation. Both Bali cattle (*Bos sondaicus*) and Ongole Grade (PO) cattle (*B. indicus*) are more preferable fulfil the national beef demand (Wiyatna, 2007; Yosita *et al*., 2012). Another local beef cattle such as Pasundan (*B. indicus*) is one of potential cattle to be used as beef (Sulasmi *et al.*, 2017). Exotic cattle, which haveo tropical climate of Indonesia such as Angus (*B. taurus*), Simmental (*B. taurus*), and Friesian Holstein or termed as FH (*B. taurus*) also contribute to fulfil the national beef meat demand. Although the FH is dairy cattle and they still can be used as beef source when not productive anymore.

Improving of beef meat quality can used molecular selection through detecting genes associated with beef meat quality. There are several candidate

Author contributions: Endang Tri Margawati, Hajar Indra Wardhana are as main contributor; Nani Radiastuti and Slamet Diah Volkandari are as member contributor in this work.

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genes associated to the traits of beef meat quality. Those are *Micromolar Calcium Activated Neutral Protease* (*CAPN1*) (Page *et al*., 2002), *Leptin* (*LEP*) (Schenkel *et al*., 2005), *Calpastatin* (*CAST*) (Schenkel *et al*., 2006), *Fatty Lipid Acid Binding Protein 4* (*FABP4*) (Barendse *et al*., 2009), *Diacylglycerol O-Acyltransferase* (*DGAT1*) (Yuan *et al*., 2013) and *CCAAT/Enhancer Binding Protein α* (*C/EBPα*) (Shin *et al.*, 2007; Wang *et al*., 2011). One of those is *C/EBPα* gene associated to lipid composition and distribution in the body of cattle (Shin *et al*., 2007; He *et al*., 2011; Wang *et al*., 2011). The gene contributes in enhancing of the meat quality (Adoligbe *et al*., 2015).

The gene of *C/EBPα* plays an important role during differentiation of preadipocyte into adipocyte. The gene was identified as a Nuclear Factor (NF) that induces at differentiation time and binds specifically to promoter of several genes (Vasseur-Cognet and Lane, 1993). Besides the important role in adipocyte differentiation, the *C/EBPα* gene plays an important role in lipid deposition. The *C/EBPα* gene is one of important candidate genes that identified to be associated with carcass and meat quality traits in cattle (Shin *et al*., 2007).

The *C/EBPα* gene also regulates metabolism of energy and nutrition with activates several specific genes such as phosphoenolpyruvate carboxykinase and insulin receptor (Park *et al*., 1990). As like in the rat, the *C/EBPα* gene in bovine is free of intron. It has an *open reading frame* (nucleotide 169 – 1,230) encoding a protein of 353 amino acid residues which five amino acid are shorter than in the rat protein (Taniguchi and Sasaki, 1996). Those amino acid express in prior transcription of adipocyte specific gene which has site binding of the *C/EBPα* gene (Wang *et al*., 2011).

The data relating to *C/EBPα* gene in cattle has been confirmed in Japanese Black cattle (Taniguchi and Sasaki, 1996). Qinchuan and Hanwoo (*B. taurus*) cattle (Wang *et al*., 2012), *C/EBPα* gene in Qinchuan cattle has been confirmed in the fragment of 1,062 base pairs (bp). Moreover, the *C/EBPα* gene in Qinchuan cattle has similarity value of amino acid to *Sus scrofa* (97%), Homo sapiens (95%), *Rattus norvegicus* (94%), *Oryctolagus cuniculus* (94%) and *Mus musculus* (93%). Jeoung *et al*. (2004) confirmed that the *C/EBPα* gene in Hanwoo with a long of 1,059 bp encoded 353 amino acids. The highest expression of *C/EBPα* gene occurred in adipocyte tissue.

This research was designed to embark the possibility of existing *C/EBPα* gene in local and exotic cattle in Indonesia, also focused on the sequence variation of the *C/EBPα* gene. The cattle samples involved were therefore focused on breeds consisting of local beef cattle and exotic cattle breeds. Up to present, there was no information relating to *C/EBPα* gene either in the local beef or exotic cattle in Indonesia. Finding of this research is expected to contribute in selection of beef cattle with a better meat quality since the *C/EBPα* gene associating with distribution of lipid in the meat or termed as marbling. This is a first step of molecular selecting for meat quality trait in cattle.

Table 1. Similarity of *C/EBPα* gene samples with data from the *GenBank*

Sample	References *(Bos taurus)*	*GenBank Accesion*	*Query Cover*	*Max Iden*
Bali (*B. sondaicus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	98%	99%
Banteng (*B. javanicus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	96%	99%
Pasundan (*B. indicus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	96%	99%
Ongole Grade (*B. indicus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	95%	99%
Friesians Holstein (*B. taurus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	98%	99%
Angus (*B. taurus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	98%	99%
Limousin (*B. taurus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	95%	99%
Simmental (*B. taurus*)	Japanese Black	DQ068270.1	100%	99%
	Korean/Hanwoo	D82984.1	100%	99%
	Chinese/Qinchuan	NM_176784.2	99%	99%

MATERIALS AND METHODS

Samples

Amount of twelve (12) samples were involved in this study. Those were three breeds of local beef cattle i.e Bali (2), Ongole Grade (PO) (2), Pasundan (2) and three exotic breeds i.e Angus (1), Simmental (2), Limousin (1), and Frisian Holstein /FH (1). In this study used Banteng (*Bos sondaicus*) as comparing breed. Bali cattle was from Nusa Penida, Bali, PO cattle was from Grobogan and Pasundan cattle was from BPPT (Balai Pengembangan dan Perbibitan Ternak) at Ciamis of West Java, while those exotic breed cattle were from BET (Balai Embio Ternak) at Cipelang of West Java which. Banteng from Prigen Malang.

DNA Extraction

DNA was extracted from blood samples (Bali, PO and Pasundan cattle) using high salt method (Montgomery *and* Sise, 1990) and follicle of tail hair (Angus, Simmental, Limousin, FH and Banteng) using DNA extraction kit (gSYSC™ DNA Extraction Kit). The blood samples were taken from *vena caudalis* and collected at vacutainer tube containing EDTA (anticoagulant). The DNA was quantified by using a spectrophotometer (GeneQuant).

Amplification of *C/EBPα* Gene

The gene of *C/EBPα* (1,339 bp) was amplified using PCR with a pair of primer according to Shin *et al*. (2007). The forward primer was 5′-ACAAACCGGTATAAATGCTG-3′ and reverse primer was 5′-AATCTCCTGGTCCTGCTTAC-3′. Total volume of PCR reaction was 12.5 µL consisting of 6.25 µL PCR master mix kit (KAPA2G Robust), primer of forward and reverse (100pmol/µl) was 0.5 µL of each primer, 4.75 µL DDW free nuclease) and 0.5 µL DNA template. The PCR was conducted in a thermocycler machine (Eppendorf) and set up as follows: pre denaturation 94°C for 5 minutes; denaturation 94°C for 30 second); annealing 54°C for 1 minute 20 second); extension 72°C for 1 minute15 seconds; final extension 72°C for 5 minutes. The PCR

Table 3. Changing of Amino Acid in the position of Base Variation in Bali Cattle

Sample	Triplet Codon			Amino Acid
Base Variation at Position 870	868	869	870*****	
Bali cattle	G	C	A*	*Alanine*
Angus, FH, Simmental, Pasundan, Ongole Grade	G	C	G	*Alanine*
Base Variation at Position 931	931*	932	933	
Bali cattle	G*	C	G	*Alanine*
Angus, FH, Simmental, Pasundan, Ongole Grade	A	C	G	*Threonine*
Base Variation at Position 1196	1195	1196*****	1197	
Bali cattle	A	A*	C	*Asparagine*
Angus, FH, Simmental, Pasundan, Ongole Grade	A	G	C	*Serine*

Remarks: *nucleotide variation position of Bali cattle

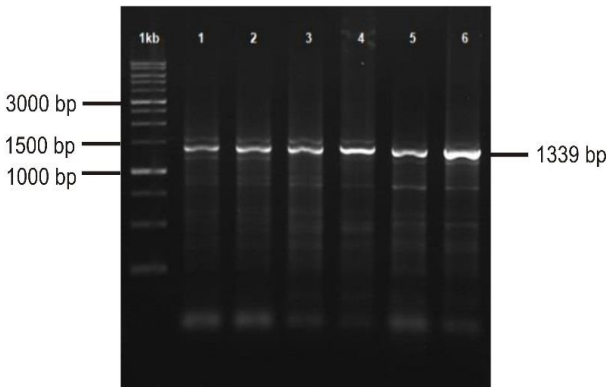

Figure 1. Visualization PCR product of *C/EBPα* gene.

Table 2. Position of Base Sequence Variation of *C/EBPα* Gene with Reference of *GenBank* Data

Species _ Breed	Base Sequence Position																	
	88	97	103	271	567	733	832	855	870	921	926	931	957	1088	1149	1175	1196
B. taurus _Japanese Black(DQ068270.1)	G	T	C	C	A	A	A	C	G	G	T	A	C	A	C	T	G
*B. taurus*_Korean Hanwoo(D82984.1)	.	.	.	A	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. taurus*_Qinchuan(NM_176784.2)	A	.	T	.	.	C	G	T	.	.	C	.	.	T		C	.
*B. taurus*_Angus	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. taurus*_ Friesians Holstein	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. taurus*_ Simmental indv_1	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. taurus*_ Simmental indv_2	.	.	.	A	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. indicus*_ Pasundan indv_1	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. indicus*_ Pasundan indv_2	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. indicus*_ Peranakan Ongole indv_1	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. indicus*_ Peranakan Ongole indv_2	.	.	.	A	.	C	.	.	.	.	C	.	.	.	.	.	.
*B. sondaicus*_ Bali indv_1	.	.	.	.	.	C	.	.	A	.	C	G	.	.	.	.	A
*B. sondaicus*_ Bali indv_2	.	.	.	.	.	C	.	.	A	.	C	G	.	.	.	.	A
*B. javanicus*_ Banteng	.	.	.	.	.	C	.	.	A	.	C	G	.	.	.	.	A

Remarks: Numbering of base sequence based on Japanese Black (DQ068270.1)
Dot (.) Same base to Japanese Black (DQ068270.1)

was run for 40 cycles. The PCR products were visualized in 1.0% agarose gel by Electrophoresis (110 V; 30 minutes) with a 1 kb DNA ladder as molecular marker size. The gel was stained with Ethidium Bromide (Bio-Rad, USA) and documented with UV-Illuminator and Gel Doc.

Sequence Analysis of *C/EBPα* Gene

Sequencing of individual PCR product, in total of 12 animal samples was performed by 1st BASE Laboratory services, Malaysia. Total of 30 µl PCR product of each sample were used for sequence analysis. Electropherogram of *C/EBPα* gene was checked through (http://asparagin.cenargen.embrapa.br/phph/) to see their quality. The sequence results were assembly from two directions by using ChormasPro 1.5. The *C/EBPα* gene sequences were aligned and compared with *B. taurus* (Japanese Black cattle/Acc. No. DQ068270.1, Hanwoo cattle/Acc. No. D82984.1, Qinchuan cattle/Acc. No. NM_176784.2) using Multiple Sequence Alignment (MUSCLE) of MEGA ver. 6.0 program (Tamura *et al*., 2013). Sequence similarity was analysed using BLAST through NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

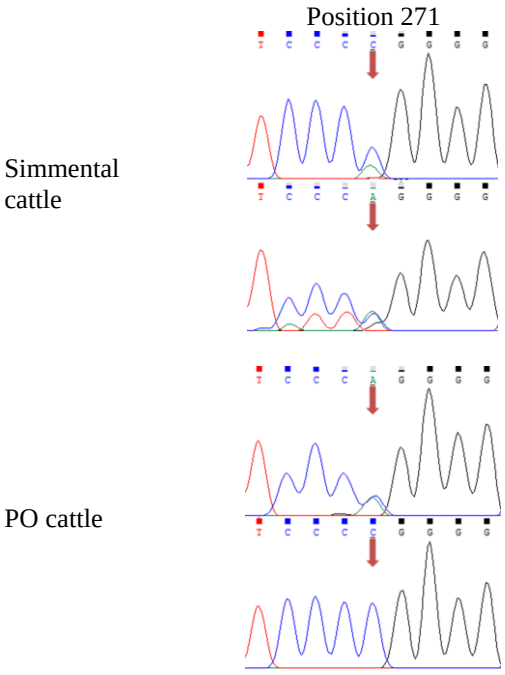

Figure 3. Nucleotide variations (heterozygous detect) of *C/EBPα* gene in Simmental and PO cattle.

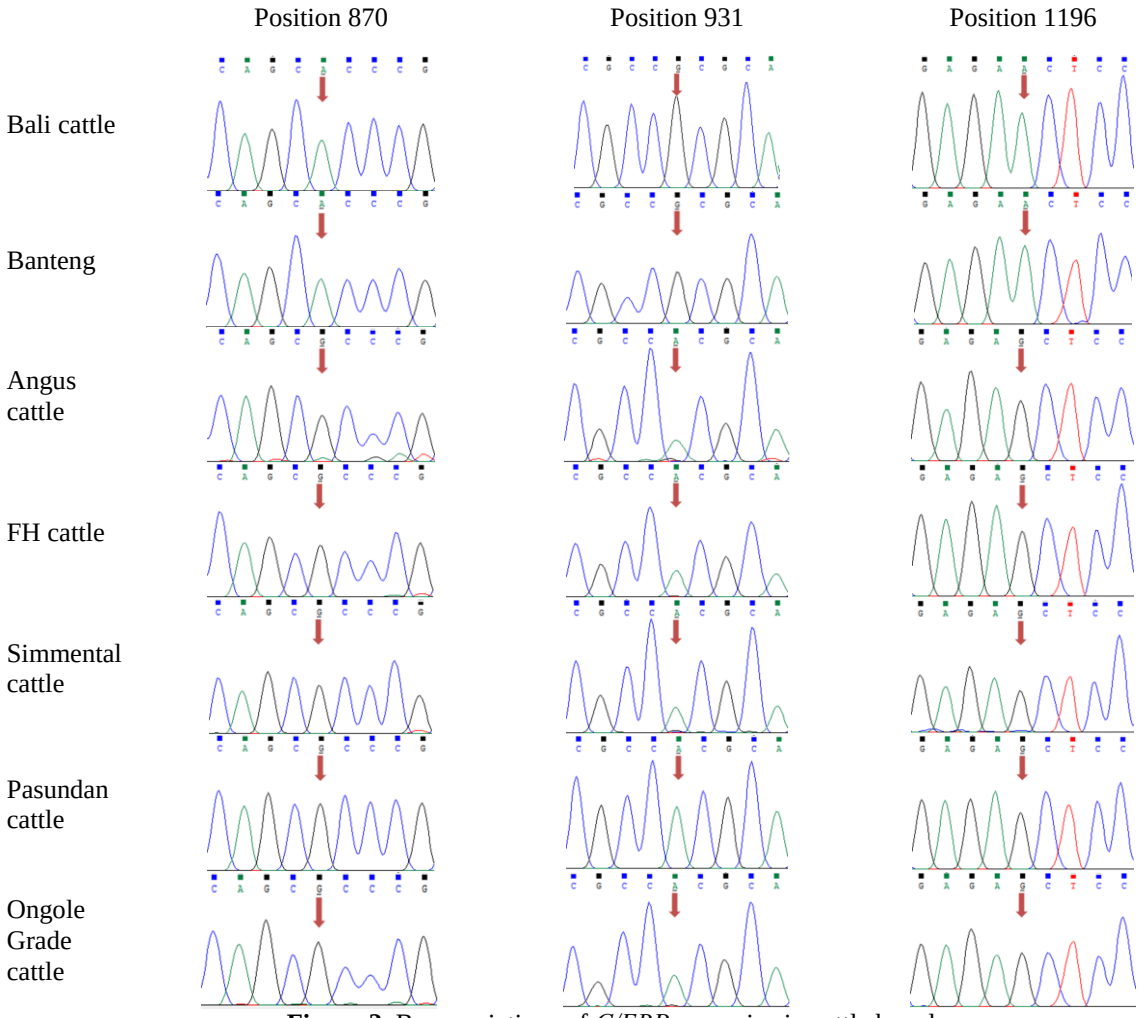

Figure 2. Base variations of *C/EBPα* gene in six cattle breed

RESULTS AND DISCUSSION

Size and Similarity of *C/EBPα* Gene

The *C/EBPα* gene was successfully amplified with the right size of about 1,339 bp (Figure 1) and it was successfully read 1,173 nt in all samples after alignment. Similarity sequences of all samples reached 99% comparing with all references (*B. taurus* cattle such as Japanese Black (DQ068270.1), Korean/Hanwoo (D82984.1), Chinese/Qinchuan (NM_176784.2)) (Table 1). *C/EBPα* gene has only one exon that spread 169 to 1,230 in Japanese Black cattle (DQ068270.1) (Taniguchi and Sasaki, 1996; Shin *et al.*, 2007).

Nucleotide variation of *C/EBPα* gene was successfully obtained in 17 different positions (Table 2). Interestly, three different nucleotides were found only in Bali (*Bos sondaicus*) and Banteng cattle at position 870 (G→A), 931 (A→G) and 1196 (G→A) based on Japanese Black (DQ068270.1) (*Bos taurus*). Those nucleotides were clearly different with the other samples (sharp and clear peak) (Figure 2). Furthermore, heterozygote was detected in PO and Simmental cattle at position 271 where cytosine changed to Adenine (C271A) but did not change of amino acid. This mutation could be detected using restriction enzyme *Sma*I (CCC^GGG) and produced three of genotype variant (AA, AC and CC). In Korean Hanwoo cattle (*Bos taurus*), three genotypes were successfully detected with allele frequencies A 0.374 and C 0.626. Genotype AA has the highest of carcass weight and meat quality compared with genotype AC and CC (Shin *et al.*, 2007). The two of three variants nucleotides at in Bali cattle caused changing of amino acid**.** Those changes were *Alanine* (GCG) to *Thereonine* (ACG), and Asparagine (AAC) to Serine (AGC) (Table 3).

Bali cattle (*B. sondaicus*) is domestication of wild Bante ng (Talib, 2002; Purwantara *et al*., 2012). Bali cattle has adapted with the tropical climate for hundred years it could contribute the genetic variation in Bali cattle. Bali cattle have been selected naturally and adapted to the tropical climate with lower forage quality (Margawati, 2012) and internal parasite, local disease so creates a specific genetic diversity (Sutarno and Setyawan, 2015). Bali cattle has characteristics of smaller body size compared to *B. indicus* (tropic) and *B. taurus* (sub tropic) (Soares and Dryden, 2011). Environment condition influences directly to the metabolism process of muscles and organs (Gregory, 2010). Bali cattle has higher percentage of carcass, 53.26% (Yosita *et al*., 2012), 54.0% (Wiyatna, 2007), than Madura, PO and Australian Commercial Cattle (ACC).

In quality meat trait, Marbling Score (MS) of Bali cattle in one years old reached 1.92 (Jakaria *et al*., 2017). Measuring of MS based on AUS-Meat Standard. Sumba Ongole (*Bos indicus*) has Marbling Score 2-3 (Priyanto *et al*., 2015) while Limousin and Shorthorn (*Bos taurus*) have 3 and 4 of MS, respectively (Cundiff *et al*., 1993). Marbling Score is intramuscular fat area devided by longissimus dorsi muscle area on 12^{th} -13^{th} ribs.

Tropical environment is predicted influencing the lipid composition of beef meat in Bali cattle. This opinion was supported by a research report of Yosita *et al*. (2012) that lipid composition in subcutan of sub tropic cattle are more excess compared to the cattle from tropical environment. In sub tropic, lipid in sub cutan plays an important role in protecting the body from low temperature environment. Composition of intramuscular lipid correlates to meat quality such as meat tenderness (Reverter *et al*., 2003). Intramuscular lipid content and internal lipid *B. taurus* in sub tropic is more excess compared to the cattle from *B. indicus* (Yosita *et al*., 2012).

The four nucleotide variantions of Bali cattle, PO cattle, Simmental cattle and Banteng in *C/EBPα* gene are need deep exploration to get more information about association with quality and quantity meat trait. Those information could be used to build of breeding strategy to improve genetic quality of Bali cattle and other Indonesia local cattle next.

CONCLUSIONS

The *C/EBPα* gene was successfully confirmed with size of 1,173 bp both in local beef cattle (PO, Bali and Pasundan cattle), in exotic cattle (FH, Angus, Limousin, and Simmental) and Banteng. Spesific sequence variantions was found in Bali cattle (*Bos sondaicus*) and Banteng, at the positions of 870 (G→A), 931 (A→ G) and 1196 (G→A). Those mutations caused amino acid changing *Alanine* to *Threonine* (at 931) and *Asparagine* to *Serine* (at 1196). Heterozygote genotypes were detected in PO and Simmental at position 271 of *C/EBPα* gene ytosine changed to Adenine (C271A).

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