

Detection of Gen C / $EBP\alpha$ Encodes the Meat Quality

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ABSTRACT

This study aims to confirm the presence of the gen $C / EBP\alpha$ beef in local and introduced beef cattle in local beef cattle, introduced beef cattle and determine gene base variations. $C / EBP\alpha$ in these cows. DNA analysis was performed by quantification, PCR, electrophoresis, *sequencing* and bioinformatics analysis. The results showed that, Gen $C / EBP\alpha$ has been successfully confirmed for local beef cattle (Pasundan, PO, Bali) and introduced beef cattle (Angus, Simmental, FH cattle). There are variations of base found in local beef cattle, namely Bali cattle. In the other five sample cows, base variations were not found. The conventional method can be used as an alternative method of extraction.

Keywords: Gen $C / EBP\alpha$, Meat Quality

INTRODUCTION

National beef consumption figures are increasing. This increase was 9.89% per year, starting from 1993 to 2015 (Secretary General of the Center for Agricultural Data and Information Systems, Ministry of Agriculture, 2016). An increasing population and public awareness of the importance of consuming animal protein are the causes of increasing national beef consumption (Wiyatna, 2007; Rusono, 2015). The provision of national beef is fulfilled by local cattle production as much as 70% (Rusono, 2015).

Bali cattle and Ongole or PO crossbred cattle are local beef cattle that are widely used as sources of national beef supply (Wiyatna, 2007; Yosita *et al.*, 2012). Other local beef cattle that have the potential as a source of fulfillment of national beef are Pasundan (Sulasmi *et al.*, 2017). Apart from local beef cattle, beef cattle originating from outside Indonesia (introduced) are also used as a source of national meat fulfillment, such as Angus, Simmental, Friesians Holstein or FH. These cows are able to adapt well to the environment in Indonesia.

Currently, the quality of local beef needs to be improved. Quality beef can be measured by parameters such as flesh color, the color of the fat, the amount of fat (Glitsch, 2000), low in fat (Dagong *etal.*, 2012), wetness, tenderness (Pearce *et al.* , 2011).

Provision of quality beef can be done through molecular selection methods using candidate genes associated with beef quality traits.

Several candidate genes associated with beef quality traits such as *Micromolar Calcium Activated Neutral Protease (CAPN1)* (Page *et al.*, 2002), *Leptin (LEP)* (Schenkel *et al.*, 2005), *Calpastatin (CAST)* (Schenkel *et al.*, 2006), *Fatty Acid Binding Protein 4 (FABP4)* (Barendse *et al.*, 2009), *Diacylglycerol O-Acyltransferase (DGAT1)* (Yuan *et al.*, 2013) and *CCAAT/ α Protein Binding Enhancer (C / EBP α)* (Shin *et al.*, 2007; Wang *et al.*, 2011). The *C / EBP α* gene can be associated with the composition and distribution of beef body fat (Shin *et al.*, 2007; He *et al.*, 2011; Wang *et al.*, 2011), thereby contributing to improving meat quality (Adoligbe *et al.*, 2015).

Data related to gen *C / EBP α* in cows were recently confirmed in Japanese Black, Qinchuan, Hanwoo. Research by Taniguchi & Sasaki (1996) confirmed the gen *C / EBP α* in Japanese Black cows. Research by Wang *et al.*, (2012) confirmed the gen *C / EBP α* in Qinchuan cattle with a length of 1062 *base pairs* (bp). The *C / EBP α* gene in Qinchuan cattle has values for *similarity* amino acids with *Sus scrofa* (97%), *Homo sapiens* (95%), *Rattus norvegicus* (94%), *Oryctolagus cuniculus* (94%) and *Mus musculus* (93%). Research Jeoung *et al.*, (2004) confirmed the gen *C / EBP α* in Hanwoo cattle with a length of 1059 *base pairs* (bp), which encodes 353 amino acids. In Hanwoo cattle, the highest expression of gen *C / EBP α* occurred in adipose tissue.

Apart from genotypic factors, environmental conditions also affect beef quality (Schutt *et al.*, 2009). The phenotype in the form of quality meat is the result of the interaction between genotype and environmental factors (Peaston & Whitelaw, 2006). The difference in environmental conditions in the form of climate causes different characteristics of beef.

The intramuscular fat and internal fat content of *B. taurus* (subtropics) were higher than *B. indicus* (tropical) (Yosita *et al.*, 2012). Fat content in intramuscularly correlates with meat quality properties such as *tenderness* (Reverter *et al.*, 2003) and *lean meat* (low fat). (Dagong *et al.*, 2012). The difference in environmental conditions between local (tropical) beef cattle and introduced (subtropical) beef cattle allows for variations in the gen base *C / EBP α* .

THEORETIC

CCAAT/Protein Binding Enhancers (C / EBPs) is a family transcription containing the domain *leucine zipper* basic that is *conserved* or unchanged (Figure 7), at the C-terminus involved in dimerization and DNA binding (Ramji & Foka, 2002). The protein *C / EBP* was first identified in Steve McKnight's laboratory as a heat-stable factor in rat liver nuclei capable of interacting with the *CCAAT motif box* present in several cellular gene promoters (Johnson & McKnight, 1989).

The gen *CCAAT/Enhancer Binding Protein (C / EBP)* is a family of transcription factors expressed in the preadipocyte differentiation process (He *et al.*, 2011). The role of the family *C / EBP* includes cellular differentiation, controlling metabolism, cellular proliferation, regulation of inflammation (Ramji & Foka, 2002), cell cycle, hematopoiesis, skeletal development and *host* immuneresponse (Tsukada *et al.*, 2011).

The proximal promoter region of the *CCAAT/Enhancer Binding Protein α (C / EBP α gene)* in mice was first characterized and was shown to contain potential binding sites for *C / EBP*, *Sp1*, *Nuclear Factor (NF) -1*, *NF-Y*, *Upstream Stimulating Factor (USF)*), *Basic Transcription Element Binding Protein (BTEB)* and *NF-Kb* (Christy *et al.*, 1991). The gen promoter *C / EBP α* can activated automatically in cells that can be transferred through the expression of plasmids (Legraverend *et al.*, 1993).

The sequence of the gen *C / EBP α* bovine had 89.9% similarity to the sequence nucleotides and 92.5% amino acid sequences with mouse genes (Taniguchi & Sasaki, 1996). The *C / EBP α* cow's gen is like the mouse gene, which is intron free. *Open reading frame* (nucleotide 169 - 1,230) in gen *C / EBP α* bovine encodes 353 amino acid residue proteins which are five amino acids shorter than mouse protein (Taniguchi & Sasaki, 1996). These amino acids are expressed prior to transcription of the adipocyte-specific gene that has a binding site for the gen *C / EBP α* (Wang *et al.*, 2011). The TATA box-like sequence is located on the nucleotide, but the starting site for transcription is not determined (Taniguchi & Sasaki, 1996).

The transcription factor *C / EBP α* plays an important role during the differentiation of preadipocytes to adipocytes. The *C / EBP α gene* is identified as *Nuclear Factor (NF)* which is induced upon differentiation by binding specific to the promoter of multiple genes (Vasseur-Cognet & Lane, 1993). The *C / EBP α gene* plays an important role in fat deposition and adipocyte differentiation (Shin *et al.*, 2007). The *C / EBP α gene* is an

important gene candidate for genetic marker identification on carcass and quality traits of beef cattle (Shin *et al.*, 2007). The gen *C / EBPα* can also regulate energy and nutrient metabolism by activating specific genes such as insulin receptors.

Environmental conditions in the form of temperature will affect the physiological state of cows (Nardone *et al.*, 2006). Environmental conditions directly affect the metabolic processes of organs and muscles (Gregory, 2010). In the comfort zone, cows will spend less energy and are not affected by environmental temperature, on the other hand, if they are outside the comfort zone, cows will experience stress which aims to return to their normal body condition (Nardone *et al.*, 2006). These conditions will cause changes such as diet, physiology and metabolic function in the quantity and quality of production from the cow itself.

An environment with high temperatures will cause a decrease in the fat content of meat (Mitlohner *et al.*, 2001; Mader & Davis, 2004), which occurs in cattle *B. indicus* (Gregory, 2010). Temperature above 30 ° C will cause a decrease in total body weight (Nardone *et al.*, 2006). For a long period of time, environmental conditions will affect the phenotype and genotype changes in cattle (Nardone *et al.*, 2006; Gregory, 2010).

METHOD

The breeds cattle studied were divided into two groups, namely: 1) Beef cattle local (Pasundan cattle, PO, Bali); 2) Introduced beef cattle (Angus, Simmental, FH cattle). Sources of DNA come from: 1) Blood (Pasundan cattle, PO, Bali); Hair (Angus cow, Simmental, FH). Blood or hair that is taken, comes from two different individuals of each type, except for the hair sample of Angus cattle which is obtained from one individual only.

The types of cows studied came from different places, namely: 1) Pasundan cattle came from the Ciamis Beef Cattle Development Center (BPPT); 2) PO cattle are from BPPT Grobogan; 3) Bali cattle originating from the Nusa Peninda Bali Agricultural Technology Research Center (BPTP); 4) Angus, Simmental, FH cattle from the Livestock Embryo Agency (BET) Cipelang, Bogor. BET regulations that do not allow blood sampling are the main reason for the collection of hair samples from cows from Angus, Simmental, FH. All samples of cow hair obtained from BET are original cows (*origin*) that have not been cross-bred.

RESULT

Confirmation of *C / EBP α*

gene Confirmation of the presence of the gen *C / EBP α* in cows in this study was carried out by visualization of the electrophoresis of the PCR results, the visualization results are shown in Figure 8. The gen *C / EBP α* primers used in this study were designed by (Shin *et al.*, 2007), it is estimated that the base target to amplify is 1339 base pairs (bp) (Appendix 4). The estimated length of the *sequence* that will be amplified refers to *Gen Bank Accession* DQ068270.1.

Based on the results of visualization, the gen was *C / EBP α* confirmed to be owned by all the samples of cattle studied (Figure 8). The position of the gen band *C / EBP α* lies between 1000 bp and 1500 bp from the *marker* (Figure 8). This indicates that the length of the amplified base has matched the target size of 1339 bp. The base length of the gen *C / EBP α* refers to the study of Shin *et al.*, (2007).

Basic Local Alignment Search Tool

In this study, the BLAST results are intended to determine the percentage (%) similarity (homolog) of the tested sample bases with reference from *Gen Bank*, without specifying the type (species) or *breed* of the sample. The percentage of the suitability of the base sequence with the sample can be used later in the search for base variations.

Maximum identify is the percentage of identity or the similarity of a sample base sequence set to the base sequence available in *Gen Bank* (Madden, 2013). The value of 99% in this study does not show the similarity of the same species, but showed that the gen was confirmed *C / EBP α* in the sample. This is because the *C / EBP α* gene is not a gene identity used for the identification stage. The *C / EBP α* gene is a functional gene that plays a role in the fat content of meat (Tsukada *et al.*, 2011).

The cover query is the percentage length of the sample base sequence can be aligned with the order of bases available on *Gen Bank* (Madden, 2013). The value of 100% shows that the order of the sample bases can be completely aligned with the data on *Gen Bank*. The values *query cover* sample with Qinchuan cattle (NM_176784.2) ranged from 95% - 99%. This is because each sample is aligned at positions 62 (Simmental), 63 (Angus and FH) and 67 (Bali, Pasundan, PO) (Attachment 2). Qinchuan cattle (NM_176784.2) had a base sequence of 1231 bp, while each sample in this study had a base sequence of 1180 - 1216 bp (Appendix 1). This makes the end of

each sample not aligned. The PO sample has a value *cover query* of 95% where 52 bp of the sample is not aligned with Qinchuan cattle (NM_176784.2) (Appendix 3). The Simmental sample has a value *cover query* of 99% where 9 bp of the sample is not aligned with Qinchuan cattle (NM_176784.2).

Variation of Basa gen *C / EBPα* of Base Bali cattle

variation in the sample of cattle studied was only found in Bali cattle, while the other cattle samples had the same base as Japanese Black (DQ068270.1), namely 870 (*Guanine*), 931 (*Adenine*) and 1196 (*Guanine*). The variation in the base of the Balinese sample lies in three different positions. The variation is at positions 870 (G → A), 931 (A → G) and 1196 (G → A) (Table 4).

All the base variations of Bali cattle are located in exon 1, because the gen *C / EBPα* has only one exon. This is based on the characteristics of the exons gene *C / EBPα* in Japanese Black cattle (DQ068270.1). The overall variation is that *coding sequence* the gen(CDS) of *C / EBPα* Japanese Black cattle and Hanwoo cattle (D82984.1) is in the region 169 to 1230 (Taniguchi & Sasaki, 1996; Shin *et al.*, 2007). The location of the base variation in the exons is expected to affect the characteristics of Bali beef.

These base variations indicate genetic peculiarities in Bali cattle. Bali cattle is a species of *B. sondaicus* originating from the wild banteng (Talib, 2002; Purwantara *et al.*, 2012). Based on that, origin suggestion of Bali cattle is suspected to be the cause of base variations. In contrast to samples of Angus, FH, Simmental cattle which are species *B. taurus* and samples of PO, Pasundan cattle which are species of *B. indicus*. These cows are closely related to the offspring, most of the *breeds* of domestic cattle are *B. taurus* or *B. indicus* (Zebu) which are descended from *Auroch* wild (*B. primigeius*) (Mohamad *et al.*, 2012). The closeness of the origins of the samples of Angus, FH, Simmental, Pasundan and PO cattle is thought to be the cause of the absence of base variations found.

Bali cattle have smaller body characteristics when compared to *B. indicus* (tropical environment) and *B. taurus* (subtropical environment) (Soares & Dryden, 2011). This is thought to be influenced by Indonesia's different tropical environment. Environmental conditions in the form of temperature affect the physiological state of cows (Nardone *et al.*, 2006). Bali cattle genetic diversity (genotype) of the gen *C / EBPα*, which is

associated with the composition and distribution of body fat in cattle (Shin *et al.*, 2007; He *et al.*, 2011; Wang *et al.*, 2011) and Indonesian environmental conditions, allow affect the characteristics of Bali beef.

Balinese beef has tough meat characteristics (Navtalia *et al.*, 2014). This is presumably because of Indonesia's tropical environment temperature conditions that tend to be hot. An environment with high temperatures will cause a decrease in the fat content of meat (Mitlohner *et al.*, 2001; Mader & Davis, 2004). Predicted hot environmental conditions affect the fat composition in Bali beef. This is reinforced by the research of Yosita *et al.*, (2012) where the composition of fat in subcutaneous cows from subtropical cattle is more, compared to tropical cows (Yosita *et al.*, 2012). The composition of fat in the subcutaneous or just under the skin plays a role in maintaining the balance of body temperature with cold (subtropical) environmental temperatures.

CONCLUSION

Gen *C / EBPα* has been successfully confirmed for local beef cattle (Pasundan, PO, Bali) and introduced beef cattle (Angus, Simmental, FH cattle). There are variations of base found in local beef cattle, namely Bali cattle. In the other five sample cows, base variations were not found. The conventional method can be used as an alternative method of extraction.

The next stage of research can be carried out by increasing the number of sample populations of cows to find the presence of *Single Nucleotide Polymorphism* (SNP) of the gen *C / EBPα* in the cows studied. SNP is useful for selecting livestock with better meat quality. In addition, additional optimization needs to be done for conventional methods of extraction of cow hair DNA, in order to obtain maximum results.

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