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Allelic Frequency of Kappa-Casein Locus (Asp148/Ala) in F_1: Simmental (*Bos taurus*) x Ongole Grade (*Bos indicus*)

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ABSTRACT

This study was conducted to detect the genetic variants (single nucleotide polymorphism) of kappa-casein locus (Asp148/Ala) in F1: Simmental (*Bos taurus*) x Ongole grade (*Bos indicus*), SIMPO. Genomic DNA was isolated from blood sample of 40 SIMPO (21 males and 19 females). A 780 bp specific fragment of kappa-casein gene spanning from the forth exon region (517 bp) to forth intron (263 bp) was successfully amplified. The result of the PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphisms) analysis using *Hind*III enzyme showed that two genotypes (AA and AB) were found at this locus in SIMPO. The frequencies of A and B alleles in SIMPO were 0.79 and 0.21, respectively. The frequency lies between *B. taurus* (Simmental) and *B. indicus* group.

Key words: gene, locus, kappa-casein, PCR-RFLP, SIMPO

ABSTRAK

Penelitian ini bertujuan untuk mendeteksi varian (polimorfisme nukleotida tunggal) pada lokus kappa-casein (Asp148/Ala) pada F1: Simmental (*Bos taurus*) x Peranakan Ongole (*Bos indicus*), SIMPO. DNA genom yang digunakan diisolasi dari 40 sampel darah sapi SIMPO (21 jantan dan 19 betina). Fragmen DNA spesifik dari gen kappa-casein yang merentang dari daerah exon-IV (517 bp) hingga daerah intron-IV (263 bp) telah berhasil diamplifikasi. Hasil analisis PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphisms) menggunakan enzim *Hind*III ditemukan dua genotip (AA and AB) pada lokus tersebut pada sapi SIMPO. Frekuensi alel A dan B lokus ini pada SIMPO berturut-turut 0,79 dan 0,21. Frekuensi alel ini berada diantara *B. taurus* (Simmental) dan *B. indicus*.

Kata kunci: gen, kappa-casein, PCR-RFLP, SIMPO

INTRODUCTION

Recently in some areas of Indonesia, many cross breeding between superior bulls of Simmental (*Bos taurus*) and Ongole grade (*Bos indicus*) have been done. The Simmental cattle originated from Switzerland, they belong to dual-purpose type (beef and milk type) (Simmental Australia, 2012), and body weight of bulls and cows are about 1,000 kg (BBIB Singosari, 2012) and 605.7±51.8 kg (Pilarczyk & Wojcik, 2008), respectively. The Ongole grade (in Indonesia: *Peranakan Ongole*, PO) was the result of *grading up* between Java cattle and Ongole, and body weight of bulls and cows are about 315.6±39.46 kg (Carvalho *et al*., 2010) and 295±59.82 kg (Endrawati *et al*., 2010), respectively. The Ongole cattle breed, also known as *Nellore* cattle breed, came from Madras, India, they belong to dual-purpose type (beef-and work type) (Gaur *et al*., 2002). The result of crossing between superior bulls of Simmental (*B. taurus*) and Ongole grade (*B. indicus*) called as SIMPO (abbreviation: Simmental and PO). The cross breed of beef cattle are expected to be more rapid in growth as well more productive as a meat producer.

The pre-weaning growth of cattle highly correlated to post-weaning growth. The pre-weaning growth itself depends on milk intake from the dam. The dam which had good milk performance will have maternal ability as well. Calves which born from the dam like that will get a chance to consume a good milk so the pre-weaning growth would be better as well.

Kappa-casein is one of the milk proteins that are essential for pre-weaning growth. Kappa-casein of cow consisted of 169 amino acids (Martin *et al*., 2002). Production of kappa-casein of cows was controlled

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by a gene located on chromosome 6, approximately 13 kb in size and was divided into 5 exons and 4 intron (Alexander *et al*., 1988; Rohallah *et al*., 2007). Exon 1 codes for 65 nucleotides of the 5'-untranslated region, exon 2 contains the remaining 5 nucleotides of the 5'-untranslated region and codes for 19 of the 21 amino acids of the leader peptide. Exon 3 contains only 33 nucleotides while exon 4 codes for the remainder of the mature protein as well as 37 nucleotides of the 3'-untranslated region, the remaining 173 nucleotides of which are contained in exon 5. Intron 1–4 are 2.5 kb, 5.8 kb, 2.0 kb and 1.8 kb in length respectively (Alexander *et al*., 1988). Along the gene it was found some genetic variants (Hamza *et al*., 2010).

By PCR-RFLP analysis using *Hin*dIII or *Hin*fI enzyme, some researchers have found a point mutation in exon 4 of kappa-casein gene in some breeds of cattle, and generally the locus is found two variant alleles: A and B. A and B alleles found in *B. taurus* and *Bos indicus*. The frequency of B allele of *B. taurus* is relatively higher than of *B. indicus*, but in *Bos sondaicus* was solely found B allele (Table 2). The sequencing of amino acid (nucleotides) showed that the B allele was found alanine (GCT) at position 148 of the amino acid sequence, while the A allele positions are occupied by aspartic acid (GAT) (Figure 3) (Alexander *et al*., 1988; Strzalkowska *et al*., 2002; Patel *et al*., 2007). Anggraeni *et al*. (2010) detected B allele of kappa-casein for Holstein-Friesian by PCR-RFLP analysis using *Pst*I enzyme, and they found B allele frequency of farm in west Java (Indonesia) which manage semi-intensive and intensively was 0.36 and 0.37, respectively. However, B allele frequency of two national AI station in Indonesia (BIB Lembang and BIB Singosari) was 0.26 and 0.45, respectively.

The presence of B allele of kappa-casein locus (Asp148/Ala) has given a positive effect on milk production and milk protein content in Holstein cows (Tsiaras *et al*., 2005). Alipanah *et al*. (2008) reported that in the two breed of Russian cattle -Russian Black Pied and Russian Red Pied, BB genotype had a milk protein content was higher than AA and AB genotype. Another study had reported that the BB genotype in Sahiwal (*B. indicus*) was able to produce milk monthly and 305 days, and protein monthly was higher than AA and AB genotypes (Rachagani & Gupta, 2008). Anggraeni *et al*. (2009) reported that high milk protein was affected by BB genotype of the kappa-casein gene (i.e. point mutation in exon 4 detected by PCR-RFLP/*Pst*I) in Holstein-Friesian female herd of West Java Province (Indonesia). The result suggested that it would be a good opportunity to be used as MAS (Marker Assisted Selection) in Holstein-Friesian domestic.

In the view of beef cattle, information concerning polymorphism effect of kappa-casein on growth performance was very rare. Lara *et al*. (2002) stated that variation of kappa-casein plays important role on growth and maternal effect of beef cattle breeds. Curi *et al*. (2005) found the same phenomenon such as dairy cattle, where frequency of A allele of kappa-casein was higher than B allele at beef cattle breeds (Nellore, Canchim, Simmental x Nellore, dan Angus x Nellore).

The study aimed to determine the allelic frequency of kappa-casein locus (Asp148/Ala) in SIMPO population by PCR-RFLP analysis using *Hin*dIII enzyme. The result of study will be the basis of efforts to improve the effectiveness and efficiency of crossing in order to generate new productive beef cattle breed as meat producers.

MATERIALS AND METHODS

The material used in the study were genomic DNA isolated from blood samples of 40 SIMPO, consisted of 21 males and 19 females (Mu'in *et al*., 2007). The SIMPO were owned by the farmers in Bantul Regency, Yogyakarta Province, Indonesia. Figure 1 shows some SIMPO and the dam. The study was conducted at the Laboratory of Biochemistry, Biotechnology Study Center, University of Gadjah Mada.

Amplification of specific DNA fragment (780 bp) that spans from the 4th exon (517 bp) to the 4th intron (263 bp) of kappa-casein gene, carried out using pair of primers [M1, (forward), 5'-CGCTGTGAGAAAGATGAAAGATTC-3'; M2 (reverse), 5'-AGATTCAAG GAGTATACCAATTGTTG-3', (Chikuni *et al*., 1991)]. Amplification procedure was as follows: a 2 μL solution of genomic DNA (± 100 ng), pair of primers (M1 and M2, each 2 μL, 16 pmol concentration), and 19 μL dH_2O inserted into a tube of 0.2 mL Ready- To-Go PCR Bead (Amersham Biosciences). PCR conditions was programmed as follows: initial denaturation 95 °C for 2 min, followed by amplification of 35 cycles with each cycle program: denaturation 94 °C for 1 min, annealing 55 °C for 1 min, extension 72 °C for 1 min. The final stage was an extra extension at 72 °C for 5 min.

PCR products obtained was digested by restriction enzyme *Hin*dIII. The procedure was as follows: 10 μL PCR product, 8 μL dH_2O and 2 μL restriction enzyme (*Hin*dIII) was put into 1.5 mL microtube, then incubated overnight at 37 °C. Digestion products were electrophorezed on 1.5% agarose gel containing Nucleic Acid Stain GoldView in TBE buffer. The TBE buffer was a mixture containing 108 g *Tris base* and 55 g *boric acid* in 700 mL aquabides, then added by 40 mL 0.5 M EDTA (pH 8,0), and added by aquabides in solution up to 1L of total volume. DNA marker (DirectLoadTM Wide Range DNA Marker, Sigma production) and one of the PCR products were included in the same gel to help the process of identification. Running of the gel was performed at a voltage 100 volts for 30 min. The results of electrophoresis were examined under ultraviolet light, then photographed. Identification of alleles and genotypes of kappa-casein locus was done by comparing band pattern of each sample to the DNA marker bands according to Chikuni *et al*. (1991).

Allele frequency of kappa-casein locus was calculated using the formula (Nei & Kumar, 2000):

$$X_i = (2n_{ii} + \Sigma n_{ij}) / (2n)$$

where: X_i = allele frequency of the i-th
n_{ii} = number of ii genotypies
n_{ij} = number of ij genotypies
n = number of samples

Figure 1. SIMPO and the dam (Ongole grade). Patterns and colors of calves skin of SIMPO are vary: the upper-left (grey and white striped body), upper right (brown and white stripes on the body), bottom left (brown without stripes, white in the foot), bottom right (dark brown without stripes).

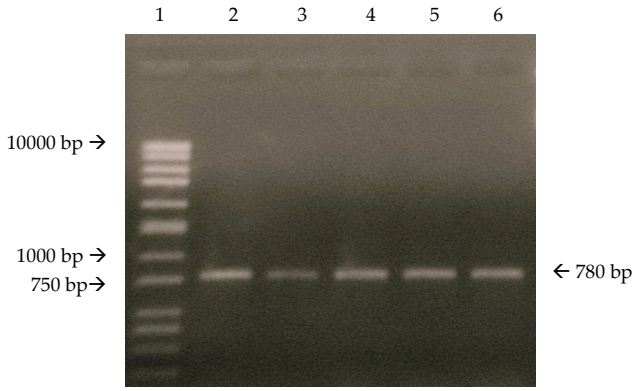

Figure 2. PCR products of SIMPO analysed in a 1,5% agarose gel containing Nucleic Acid Stain GoldView[Lane 1 = DNA markers, lanes 2-6 = PCR product (780 bp)].

RESULTS AND DISCUSSION

Specific DNA fragment, size 780 bp which extending from the 4th exon (517 bp) to the 4th intron (263 bp) of kappa-casein gene, containing the polymorphic region (Chikuni *et al*., 1991), were successfully amplified from 40 genomic DNA ofSIMPO [herd of first crossing (F_1) between Simmental and Ongole grade] using M1 and M2the primers (Figure 2). Figure 3 shows the nucleotide sequence of a specific DNA fragment (780 bp) of kappa-casein gene (Alexander *et al*., 1988; Genbank No.378700), and cutting sites of *Hin*dIIIenzyme (A↓AGCTT).

Digestion of amplicon (PCR product) on all samples using *Hin*dIII produced two genotypes: AA (indicated

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            Exon-4→
            Primer forward (M1)→
  1  ..5'  CGC TGT GAG AAA GAT GAA AGA TTC TTC AGT GAC AAA ATA
 40  GCC   AAA TAT ATC CCA ATT CAG TAT GTG CTG AGT AGG TAT CCT
 82  AGT   TAT GGA CTC AAT TAC TAC CAA CAG AAA CCA GTT GCA CTA
124  ATT   AAT AAT CAA TTT CTG CCA TAC CCA TAT TAT GCA AAG CCA
166  GCT   GCA GTT AGG TCA CCT GCC CAA ATT CTT CAA TGG CAA GTT
208  TTG   TCA AAT ACT GTG CCT GCC AAG TCC TGC CAA GCC CAG CCA
250  ACT   ACC ATG GCA CGT CAC CCA CAC CCA CAT TTA TCA TTT ATG
292  GCC   ATT CCA CCA AAG AAA AAT CAG GAT AAA ACA GAA ATC CCT
334  ACC   ATC AAT ACC ATT GCT AGT GGT GAG CCT ACA AGT ACA CCT
                                          HindIII
376  ACC   ACC GAA GCA GTA GAG AGC ACT GTA GCT ACT CTA GAA GCT
     Thr   Thr Glu Ala Val Glu Ser Thr Val Ala Thr Leu Glu Ala
     135   136 137 138 139 140 141 142 143 144 145 146 147 148
                                                      ⩛
                                                  B-allele
                                                     or
                                                    GAT
                                                    Asp
                                                    148
                                                     ⩛
                                                  A-allele
418  TCT   CCA GAA GTT ATT GAG AGC CCA CCT GAG ATC AAC ACA GTC
     Ser   Pro Glu Val Ile Glu Ser Pro Pro Glu Ile Asn Thr Val
     149   150 151 152 153 154 155 156 157 158 159 160 161 162
460  CAA   GTT ACT TCA ACT GCA GTC TAA AAACTCTAAGGAGACATCAAAGAAGA
     Gln   Val Thr Ser Thr Ala Val Stop
     163   164 165 166 167 168 169
           Intron 4→
510  CAACGCAG--GTAAATAAGGCAAAATGAATAACAGGCCAAGATTCATGGACTTATTAATAAAAT
572  CGTAACATCTAAACTAGCGTAGATGGATAAATTAAATCTGTTACAGAGAAGGCGAAATGGCTAA
636  TTATAACTTACATTTGCTGGTTCTTTATCATGTATATACTAGATTCTTTGCCAACAAGAAAGTT
700  TTAAAATATTTTACAAAATGAGTAAAAATTGCAGATTTTATTATTAAACCTTTTTCAACAATTG
764  GTATACTCCTTGAATCT-..3'
     ← Primer reverse (M2)
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Figure 3. The sequence of DNA fragment of kappa-casein gene bovine (PCR product, size: 780 bp) extending from the 4th exon (517 bp) to the 4thintron (263 bp), and their some of amino acid sequences (source: Alexander *et al*., 1988; Genbank No.378700), and *Hin*dIII enzyme cut sitesat nucleotide position 414 (A|AGCTT).

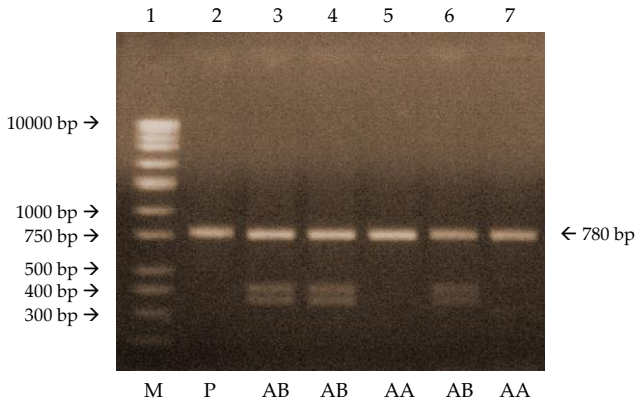

Figure 4. The PCR-RFLP electrophoresis products of kappa-casein locus (Asp148/Ala) in SIMPO using *Hind*III enzyme [Lane 1=M (DNA markers), lane 2=PCR product (780 bp), lanes 3, 4,and 6=AB genotype (780 bp, 413 bp, 367 bp), lanes 5 and 7=AA genotype (780 bp)]

by the presence of one band: 780 bp) and AB (three bands: 780 bp, 413 bp and 367 bp). BB genotype which is characterized by two bands: 413 bp and 367 bp (Chikuni *et al*., 1991) was not found in the study. Band pattern which characterizes a genotype that found in this study is the same as was found by Chikuni *et al*. (1991). Figure 4 shows the genotype of kappa-casein locus (Asp148/Ala) in SIMPO of the study.

Based on the genotype found of the study, it was known that the kappa-casein locus consisted A allele (a DNA fragment sized 780 bp) and B allele (two DNA fragments sized 413 bp and 367 bp). B allele of kappa-casein locus was demonstrated by the success of the *Hin*dIII (5 '.. A ↓ AGCTT .. 3') found DNA sequences that were recognized throughout the PCR products and success to cut them into two fragments sized 413 bp and 367 bp. Referring to the DNA sequences available on GenBank No. EF378700 and amino acid sequencing results reported by Alexander *et al*. (1988), it could be explained that the success of *Hin*dIII to find a sequence was due to the-416 nucleotide sequence (Figure 3) occupied by C (cytosine), so the sequence of triplet codons are GCT which encode alanine (Ala) at position 148 of the polypeptide chain of kappa-casein.In contrast, the A allele is indicated by the failure of *Hin*dIII find a recognizable DNA sequences along the PCR products. As a result the size of the PCR products before and after digested remain the same, which is 780 bp. The *Hin*dIII (5'.. A ↓ AGCTT .. 3') failed to find DNA sequences that were recognized due to the-416 nucleotide sequence (Figure 3) occupied by A (adenine), so the sequence of

Table 1. The frequency of A and B alleles of kappa-casein locus (Asp148/Ala) in SIMPO (*Bos taurus* x *Bos indicus*) and some breeds of *B. taurus, B. indicus,* and *B. taurus* x *B. indicus*

Group	Breed	n	Frequency of kappa-casein allele*)		Source
			A	B	
Bos sondaicus	Bali	60	0.00	1.00	Mu'in & Supriyantono, 2012
Bos taurus	Brown Swiss	93	0.49	0.51	Dogru & Ozdemir, 2009
	Holstein	224	0.59	0.41	Doosti *et al*., 2001
	Russian Red Pied	52	0.65	0.35	Alipanah *et al*., 2008
	Simmental	80	0.69	0.31	Vasconcellos *et al*., 2003
	Sarabi	66	0.76	0.24	Toorchi *et al*., 2006
	Iranian Native	210	0.81	0.19	Doosti *et al*., 2011
	Russian Black Pied	72	0.83	0.17	Alipanah *et al*., 2008
	Holstein-Friesian (HF)	304	0.83	0.17	Sitkowska *et al*., 2008
Bos indicus	Tharparkar	52	0.86	0.14	Rachagani & Gupta, 2008
	Sahiwal	252	0.87	0.13	Rachagani & Gupta, 2008
	Nellore	79	0.89	0.11	Curi *et al*., 2005
	Guzerat	25	0.92	0.08	Kemenes *et al*., 1999
	Gyr	746	0.93	0.07	Azevedo *et al*., 2008
Bos taurus x Bos indicus	Jersey x Zebu	112	0.52	0.48	Patel *et al*., 2007
	HF x Zebu	256	0.72	0.28	Patel *et al*., 2007
	Canchim (5/8 Charolais x 3/8 Zebu)	30	0.77	0.23	Curi *et al*., 2005
	Angus x Nellore	245	0.78	0.22	Curi *et al*., 2005
	SIMPO	40	0.79	0.21	In this study
	Simmental x Nellore	30	0.80	0.20	Curi *et al*., 2005

Note: *) Single Nucleotide Polymorphism in exon 4 region in kappa-casein gene [Asp 148 (GAT, A allele)/Ala (GCT, B allele)].

triplet codons formed was GAT that encode amino acid aspartate (Asp) at position 148 of the polypeptide chain of kappa-casein.

Genotype frequencies of AA, AB and BB genotype were 57.5%, 42.5%, and 0%, respectively. So, the frequencies of A and B alleles at this locus were 0.79 and 0.21, respectively. The results of this study indicate that locus of the kappa-casein (Asp148/Ala) on the SIMPO was polymorphic, because allele frequencies which commonly found in the population (A allele), does not exceed 0.99 (Nei & Kumar, 2000). The distribution of genotypes of kappa-casein locus which found in SIMPO indicated that there were flow (migration) of B allele. Migration of B allele might come from the sire, Simmental (*B. taurus*), because in general the Simmental cattles which belonging to *B. taurus* are abundant in B allele compare to *B. indicus*.

Table 1 presents the frequency of A and B alleles of the kappa-casein locus of SIMPO (*B. taurus* x *B.indicus*) which found in the study. As comparison it was also shown A and B allele frequency of kappa-casein locus (Asp148/Ala) on some other breed (*B. taurus*, *B. indicus*, and *B. taurus* x *B. indicus*). Based on Table 1 it was known that the breeds of *B. taurus* generally had a higher frequency of B alleles than the breeds of *B.indicus*, while SIMPO which the result of crossing of *B. taurus* (Simmental) x *B. indicus* (Ongole grade) has the B allele frequency higher than the breeds of *B. indicus*, particularly when it was compared to Nellore breed (*B. indicus*) which was as ancestors to breed Ongole grade.

Based on the frequency of alleles of the kappa-casein it can be said that the frequency of B allele of SIMPO laid between *B. taurus* and *B. indicus*. Thus, cross breeds between Simmental and Ongole grade was able to increase the frequency of B allele in a population of progeny (SIMPO). Increasing frequency of B allele of SIMPO might have come from Simmental (*B. taurus*). Tabel 1 showed that some breeds of crossing between *B. taurus* and *B. indicus* had higher B allele frequency than those of parents of *B. indicus*. It was strongly evident that the crossing between *B. taurus* and *B. indicus* was able to increase of B allele frequency of the crossing breed.

Most reports suggest that the increased frequency of B allele of kappa-casein locus had a positive effect on performance and quality of milk production on some breeds of cattle (Alipanah *et al*., 2008; Rachagani & Gupta, 2008). This phenomenon might also apply to the SIMPO breed, where increasing of B allele frequency in SIMPO population would have positive effect on milk performance of SIMPO dams. The increasing of the milk performance would be positive effect on pre-weaning growth of calves in the next generation. The calves born will get a chance to consume good milk so the pre-weaning growth would be better. When the phenomenon is true, then the frequency of B allele should be increased in population by increasing the frequency of BB or AB genotype in SIMPO populations. The use of BB or AB genotypes of Simmental as bulls will be very effective in order to increase the frequency of B allele in the SIMPO population. Of this method crossbreeding in the framework of SIMPO breed will become more directional as expected to create a new productive breed of beef cattle as meat producers.

CONCLUSION

The SIMPO (*B. taurus* x *B. indicus*) had a frequency of B allele (0.21) was lower than A allele (0.79). However, the actual frequency of B allele was quite moderate, as occupying a higher position than that commonly found in *B. indicus*.

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