# κ-CASEIN GENE POLYMORPHISMS IN RIVERINE AND SWAMP BUFFALO IN INDONESIA

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## ABSTRAK

κ-kasein dikenal sebagai gen yang berperan mengontrol protein susu, memiliki peran penting dalam koagulasi dan *curdling* susu, terutama dalam proses pembuatan keju. Penelitian ini bertujuan untuk mengidentifikasi keragaman gen κ-kasein kerbau lokal di Indonesia. Sampel yang digunakan adalah 40 ekor kerbau sungai dan 250 ekor kerbau rawa. Penelitian ini menggunakan metode PCR-RFLP, gen κ-kasein diamplifikasi menghasilkan fragmen sepanjang 157 pb di exon 4. Produk amplifikasi dipotong dengan enzim restriksi *Eco*RV yang mengenal situs potong gen κ-kasein pada ekson 4 di nukleotida GAT|ATC, menghasilkan keragaman pada posisi basa ke-23 pb akibat perubahan basa Ile (ATC) pada alel T menjadi Thr (ACC) pada alel C. Genotiping gen κ-kasein pada kerbau sungai menghasilkan dua tipe alel, yaitu alel C (157 pb) dan T (136 dan 21 pb). Kedua alel tersebut menghasilkan tiga genotipe, yaitu CC, TT, dan CT. Frekuensi alel C ditemukan lebih tinggi dibandingkan alel T. Gen κ-kasein pada kerbau rawa ditemukan monomorfik karena tidak adanya variasi alel (alel C). Nilai heterozigositas kerbau sungai dan rawa ditemukan rendah. Nilai PIC kerbau sungai dan kerbau rawa berkisar pada 0.000-0.288. Nilai indeks fiksasi gen κ-kasein pada kerbau sungai rendah (Siborong-borong SBBC = -0,0036; Deli Serdang = -0.025), namun pada kerbau rawa terjadi fiksasi. Penelitian ini menunjukkan bahwa terdapat keragaman gen κ-kasein pada kerbau sungai namun pada kerbau rawa ditemukan monomorfik.

*Kata kunci: Kerbau sungai, kerbau rawa, gen κ-kasein|EcoRV, PCR-RFLP, keragaman genetik*

## ABSTRACT

Kappa-casein (κ-casein) gene is known as a gene that plays a role in controlling milk protein and also play a crucial role in the coagulation and curdling of milk. This study was aimed to identify polymorphisms of the κ-Casein gene of local buffaloes in Indonesia. A total number of 40 heads of riverine buffalo and 250 heads of swamp buffalo. This study used PCR-RFLP method, which amplification of the κ-Casein gene resulted an amplicon with length of 157 bp, located in exon 4. The amplified fragment were digested with *Eco*RV restriction enzyme, which cut the κ-Casein gene in exon 4 at nucleotides of GAT|ATC, revealed the presence of one polymorphism at the base position of 23 bp that occurs with a substitution of Ile (ATC) of the T genetic variant into Thr (ACC) of the C genetic variant. Genotyping κ-Casein gene in riverine buffalo produced two types of allele, namely C allele (157 bp) and T allele (136 and 21 bp). These two alleles resulted in three types of genotypes, namely CC, CT, and TT. Frequency of the C allele was dominant to T allele. κ-Casein gene in swamp buffalo was monomorphic with one allele, namely C allele. Heterozygosity value of riverine and swamp buffaloes were low. PIC value in riverine and swamp buffalo ranged 0.000-0.288. Fixation index of κ-Casein gene in riverine buffalo was low (Siborong-borong SBBC = -0,0036; Deli Serdang = -0.025), but in swamp buffalo was in fixation. This study showed that κ-Casein|*Eco*RV were polymorphic in riverine buffalo and monomorphic in swamp buffalo.

*Keywords: Riverine buffalo, swamp buffalo, κ-Casein|EcoRV, PCR-RFLP, polymorphisms*

## INTRODUCTION

Beside as a meat producer and worker, buffalo also a producer of milk. In Indonesia, the production of buffalo milk is very slight. Contrast, in India buffalo is an important milch animal as more than 60% of the total milk produced is buffalo milk (Patel *et al.*, 2007). Buffalo is considered to be a better converter of fibrous feeds into milk, and to be more resistant to disease and local climatic condition. In Indonesia, there were very little work has been carried out on buffalo genetics that could give an effective implementation on breeding program. Nowadays selection program has come to molecular approach that can give fast and efficient result. Selection on molecular markers is more reliable than other methods (Othman, 2005). Progress in molecular technology allows selection to be done at molecular level and have already uncovered a large number of genetic polymorphisms at the DNA level which are being used as genetic markers.

Milk and milk products is important for feeding because supply nutrients, energy, high quality protein, vitamins and mineral requirements. The composition of the milk of different species varies in the percentages of these constituents. All milks contain the same constituents, but in varying amounts (Otaviano *et al*., 2005). Milk proteins are usually divided into two fractions. The first fraction is soluble fraction, named whey protein, constitutes the α-lactoalbumen and β-lactoglobulin. The second fraction is insoluble fraction, named whole casein, constitutes 4 different casein (alpha S1, alpha S2, Beta and kappa-caseins) (El-Rafey and Darwish, 2007; Rachagani and Gupta, 2008; Unsal *et al*., 2008). The casein fraction of milk proteins significantly influences the composition and physic chemical properties of the milk (Grosclaude, 1988).

κ-Casein is one of the most important milk proteins with 19,800 dalton molecular weight and 169 amino acids. The κ-Casein gene in buffalo is divided into 5 exon and 4 intron. In water buffalo the κ-Casein gene was mapped on chromosome 7 (Iannuzzi *et al.*, 1998). Variant B of κ-Casein gene result by means of point mutation (T/C) at position exon 4, due to increasing efficiency production cheese of milk (Henderson, 1971; Medrano and Aguilar, 1990). Mutations in this exon are responsible for quali-quantitative differences of gene expression in cattle and goat (Di Stasio *et al*., 2000; Chiatti *et al.*, 2005). Four single nucleotide polymorphisms are reported in literature: two at the exon 2: G versus T at codon 4 (*Arg*AGG versus *Ser*AGT) and T versus G at codon 8 (*Val*GTT versus *Gly*GGT) both in the signal peptide (EMBL a.n. DQ191173 and DQ191174); and two at the exon 4: T versus C at codon 135 (*Ile*ATC versus *Thr*ACC) and the silent mutation T versus C at codon 136 in Murrah Buffaloes (Mitra *et al*., 1998).

In sperm catalogs, BB or AB genotypes indicator ideal genotypes of milk production for use in cheese making (yielding) factories that due to decreasing coagulation time and increasing curdling stability of milk. In this reason, domesticator that their herd's milk are used for cheese making, use sperms with AA or BB genotypes until increase B allele frequency in herd and gradually increase ideal genotype of this gene in their domestics (Alipanah *et al*., 2008; Dayem *et al*., 2008).

Information on polymorphisms of κ-Casein gene in buffalo in Indonesia is very limited. This study was aimed to identify the polymorphism of κ-Casein gene by using PCR-RFLP technique as a fast efficient and low cost method in Indonesia local buffaloes.

## MATERIALS AND METHODS

### Sources of Sample

Blood samples used totally were 290 buffaloes, that were collected from 40 riverine buffaloes from Siborong-borong SBBC (20 heads) and Deli Serdang (20 heads); as well as a number of 250 swamp buffaloes from Banten (30 heads), Semarang (30 heads), Mataram (30 heads), South Sulawesi (30 heads), Aceh (67 heads), Riau (25 heads), North Sumatera (24 heads), dan West Sumatera (14 heads). Blood samples were collected from the jugular vein using vacuum tubes containing with heparin. Blood samples were stored in absolute alcohol.

### DNA extraction

Blood samples were collected from each buffalo in 10 mL non anticoagulan polypropylene tubes. Blood samples then were mixed with 96% ethanol. The process of DNA isolation used phenol-chloroform method (Sambrook *et al.*, 1989) modified by Andreas *et al.*, (2010) then were dissolved in TE buffer. Genomic DNA was stores at -20°C until amplification with polymerase chain reaction (PCR).

**Polymerase Chain Reaction (PCR)**

Amplification of Polymerase Chain Reaction (PCR) was carried out using specific primer by following from relevant references (Masina *et al.*, 2007) GenBank Acc No. AM900443 which previously being modified for parts of exon 4. The primer design using PIRA technique which replace (cc) base become (ga) base in *forward* primer (Table 1). The PCR was performed in a final volume of 15 µL for each reaction containing 0.5 µL of genomic DNA, 5.85 µL destilated water, 0.3 µL of each primer, 0.05 µL taq phire, and 7.5 µL 2x PCR buffer. The reaction mixture was subjected to an initial 5 min of denaturation 95°C, followed by 35 cycles of denaturation 95°C for 20 sec, annealing 60°C for 30 sec, extention 72°C for 40 sec, and a final extension 5 min at 72°C. Electrophoresis used to check the PCR product.

**Genotyping by PCR - RFLP**

Visualization of amplification was analyzed on agarose gel 1.5% containing 2.5 µL EtBr (ethidium bromide), 0.5X TBE buffer (1 M Tris, 0.9 M Boric acid, 0.01 M EDTA PH 8.0) with a 100 bp ladder as a molecular weight marker for confirmation of the length of PCR product. For digestion by using enzyme and determination of RFLP, 5 µL of PCR products was added to 0.4 µL *Eco*RV enzyme, 1 µL distilated water, and 0.6 µL R buffer. The mixture was then incubated at 37°C for 16 hour. The digestion products were separated by horizontal electrophoresis (100 volts, 40 min) in 2% agarose gel in 0.5X TBE, 2.5 µL EtBr and 20 bp ladder as a molecular weight marker for confirmation of the length of PCR-RFLP product visualized on UV transiluminator.

**Data Analysis**

**Genotype and Allele Frequencies**

PCR-RFLP data were analyzed by calculating the frequencies of allele and genotype (Nei and Kumar, 2000). The genotype frequencies can be determined by calculating the ratio of specific genotypes in each population, were calculated by the following formula

$$x_{ii} = \frac{n_{ii}}{N}$$

Allele frequency of an allele is the ratio of the overall alleles at a locus in a population. Allele frequency of κ-Casein|*Eco*RV were calculated by the following formula

$$x_i = \frac{2n_{ii} + \sum n_{ij}}{2N}$$

Description:

$x_{ii}$ = Frequency of genotypes AiAi
$x_i$ = Frequency of allele Ai
$n_{ii}$ = Number of genotype AiAi
$n_{ij}$ = Number of genotype AiAj
$N$ = Total sample

**Heterozygosity**

Heterozygosity was tested (Weir, 1996) by the following formulas

$$\mathbf{Ho} = \sum_{i \neq j} \frac{n_{ij}}{N}$$

Ho = Heterozygosity observation
nij = Number of heterozygous animal
N = Number of observed animal

Heterozygosity expectations (He) based on allele frequencies (Nei and Kumar 2000) were calculated by the following formulas

$$\mathbf{He} = 1 - \sum_{i=1}^{q} x_i^2$$

Description :

He = Heterozygosity expectation
*xi* = Frequency of allele
*q* = Total allele

**Polymorphic Informative Content (PIC)**

The level of informative alleles were calculated using PIC value (Bostein *et al.*, 1980)

Table 1. Forward and Reverse Primers for the Amplification of the κ-Casein Gene

| Primer Pair Name | Primer Sequence |
|---|---|
| κ-Casein F | 5'-GTTGAGCCTACAAGTACAgaTA-3' |
| κ-Casein R | 5'-TGTCTTCTTTGATGTCTCCTTAGAG-3' |

by the following formula

$$PIC = 1 - \sum_{i=1}^{n} pi^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2pi^2pj^2$$

Description :
$p_i$ = Frequency of allele-i
$n$ = Total allele

**Fixation Index**

Fixation index (Nei, 1987) in each source was obtained from equation

$$F_{ISki} = \frac{X_{kii} - X^2_{ki}}{X_{ki}(1 - X_{ki})}$$

Description:
$F_{ISki}$ = Fixation Index
Xkii = Frequency of homozygot genotype *i* in *k* population
Xki = Frequency of allele *i*

## RESULTS AND DISCUSSION

**κ-Casein Gene Amplification**

Amplification of the κ-Casein gene resulted an amplicon with the length of 157 bp, which is located in partial exon 4. The amplification fragment of the κ-Casein gene was performed in the thermocycler machine with an annealing temperature of 60 ºC. The amplification of κ-Casein gene fragment was carried on GeneAmp® PCR System 9700 (Applied Biosystem). Gene segment amplification products were visualized on 1.5% agarose gel as shown in Figure 1. Position of primers annealing on κ-Casein gene sequences shown in Figure 2.

**κ-Casein|*Eco*RV Gene Polymorphisms**

PCR-*RFLP* (restriction fragment length polymorphism) using *Eco*RV enzyme was used to genotype the Indonesian buffaloes. This enzyme recognized and cut at nucleotides of GAT|ATC sites. Forward primer that used in this research

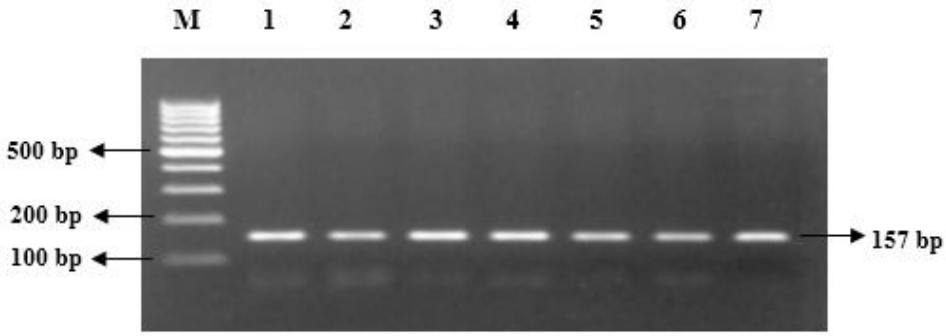


Figure 1. Visualization of κ-Casein Gene Amplification Results in 1.5% of Agarose Gel. M = Marker of 100 bp; No. 1-7 = Number of sample

Primer Forward

12001 taccatcaat accattgtta gt**gttgagcc tacaagtaca gata**tcactg aagcaataga
12061 gaacactgta gctactctag aagcttcctc agaagttatt gagagtgtac ctgagaccaa
12121 cacagcccaa gttacttcaa ccgtcgtcta aaaa**ctctaa ggagacatca aagaagaca**a

Primer Reverse

Figure 2. The Pattern Corresponding to the Sequence k-Casein Gene in Buffalo (GeneBank Accession AM900443). Restriction Site at Position 12043

was modified in order to get a restriction site by *Eco*RV enzyme. That technique known as PIRA (Primer Introduced Restriction Analysis), is widely used techniques in SNP detection. The method introduces an artificial restriction site into a PCR product by the use of a primer with a single-base mismatch close to its 3'end (Ke *et al.*, 2001). Based on amplification results of the κ-Casein gene sequences, it was found one point of *Eco*RV restriction enzyme, it was in 12043 or on base position 21 bp of the PCR products. RFLP resulted in two fragments with the base length of 21 and 136 bp, it showed C allele. If there was a base change at position 12045, from the base T (Thimin) changing to C (Citosin), it caused *Eco*RV did not recognized and cut the fragment (bands) resulting T allele (Figure 3). Patel *et al.* (2007) reported his study in riverine buffalo that the restriction enzyme digestion analysis of κ-Casein indicates the presence of the two types of restriction pattern, two fragments of 266 and 84 bp for BB-genotype were observed while in the second pattern three fragments 266, 134/132, and 84 bp for AB-genotype were observed, but none of buffaloes indicated AA-genotype.

The diversity of κ-Casein in this study was founded in exon 4 region. Masina *et al.* (2007) reported in their research in water buffalo that the comparison of the sequences obtained from exons 1,2,3 and 5 did not reveal any polymorphism. As for the exon 4, the comparison of the obtained sequences confirmed the two single nucleotide polymorphisms already reported in literature at the fourth exon (T versus C responsible for amino acid substitution at position 135 and the silent mutation T versus C at codon 136).

Results from the PCR-RFLP of the κ-Casein|*Eco*RV gene in riverine buffalo segments were polymorphic. There were three genotypes, namely genotypes CC, CT, and TT derived from two alleles, namely C and T allele. Contrast, the κ-Casein|*Eco*RV gene in swamp buffalo was monomorphic because it just showed one allele (T) and all of the genotypes is CC. Nei and Kumar (2000) stated that an allele was polymorphic if the frequency of allele was equal or less than 0.99.

Three variant genotypes found in riverine buffalo of this study were CC, CT, and TT (Figure 3). Genotyping the κ-Casein gene, showed for the resulted 157 bp, identified for CC genotype; 136 and 21 bp for TT genotype; and 157, 136, and 21 bp for CT genotype. The observation for κ-Casein gene polymorphisms in exon 4 are similar to the findings of Riaz *et al*. (2008) who noted one allele B in Nili-Ravi breed of Pakistan. Raj *et al.* (2008), El-Rafey and Darwish (2007) and Otaviano *et al.*, (2005) also found monomorphism (BB) for this gene in buffaloes. However, Patel *et al.* (2007) found two alleles A and B for κ-Casein locus in riverine buffalo, such as in Murrah, Surti, and Pandharpuri breeds of buffalo. The diversity of κ-Casein|*Eco*RV gene in buffalo (riverine buffalo and swamp buffalo) were indicated by the number of genotypes that appeared from each breeds.

**Genotype and Allele Frequency of the κ-Casein|*Eco*RV Gene**

Results of the κ-Casein|*Eco*RV gene analysis showed that the frequency of the CC genotype in all population of riverine and swamp buffaloes was higher than TT and CT genotype (Table 2). Frequency of CC genotype on riverine buffalo ranged 0.600 - 0.950. The highest frequency of

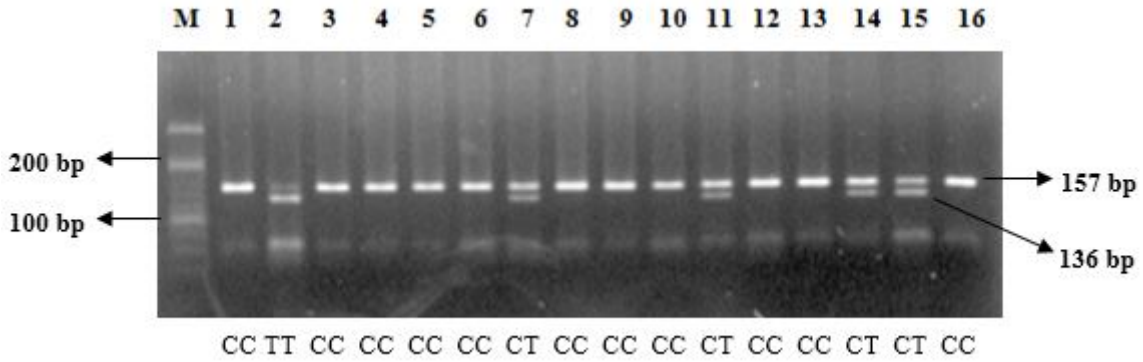


Figure 3. Result of κ-Casein Gene Fragment Using PCR-RFLP Method with *Eco*RV Restriction Enzyme on 2% of Agarose Gel. M = Marker 20 bp; 1-16 = Number of samples; CC, CT, TT = Genotype

Table 2. Genotype and Allele Frequencies of the κ-Casein Gene

| Buffalo Breed | Population | Genotype | | | Alelle | |
|---|---|---|---|---|---|---|
| | | CC | CT | TT | C | T |
| Riverine Buffalo | SBBC | 0.600 (12) | 0.350 (7) | 0.050 (1) | 0.775 | 0.225 |
| | Deli Serdang | 0.950 (19) | 0.050 (1) | 0.000 (0) | 0.975 | 0.025 |
| | Sub Total | 0.775 (31) | 0.200 (8) | 0.025 (1) | 0.875 | 0.125 |
| Swamp Buffalo | Banten | 1.000 (30) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | North Sumatera | 1.000 (24) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | Semarang | 1.000 (30) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | Mataram | 1.000 (30) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | Aceh | 1.000 (67) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | South Sulawesi | 1.000 (30) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | Riau | 1.000 (25) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | West Sumatera | 1.000 (14) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |
| | Sub Total | 1.000 (250) | 0.000 (0) | 0.000 (0) | 1.000 | 0.000 |

(...) = Number of samples; SBBC = Siborong-borong Buffalo Breeding Center

CC, CT, and TT genotype was found in Deli Serdang (0.950), SBBC (0.350), and SBBC (0.050), respectively. Genotype CC was not found in Deli Serdang, but in that location showed same result as SBBC that the genotype CC (0.950) was higher than CT (0.050). The κ-Casein gene of swamp buffalo in all populations were monomorphic with one genotype, namely CC genotype.

The C and T alleles of riverine buffaloes in SBBC were 0.775 and 0.225; then 0.975 and 0.025 for Deli Serdang. The T and C alleles in swamp buffalo in all populations were 1.000 and 0.000, respectively. Patel *et al.* (2007) who reported in their research with κ-Casein gene of water buffalo that the frequency of BB genotype (0.968) was very higher than AB genotype (0.032) and AA genotype (0.00). This result indicating that most buffalo population has allele (B) for higher casein production.

Monomorphic allele in swamp buffalo was confirmed in several research in other countries, such as: India (Shende *et al*., 2009; Gangaraj *et al*., 2008), Mishr (Othman, 2005; Mahmoud *et al*., 2010; Dayem *et al*., 2008), Pakistan (Riaz *et al*., 2008), Brazil (Otaviano *et al*., 2005), Iran (Abassi *et al*., 2009), dan China (Ren *et al*., 2011).

**Polymorphisms Degrees of the κ-Casein|*Eco*RV Gene**

Heterozygosity value is the most accurate way to measure the genetic diversity of population (Nei and Kumar, 2000) and to get an overview of genetic variability (Marson *et al*., 2005). Heterozygosity values are influenced by the number of samples, the number of alleles and allele frequencies. The result of heterozygosity analysis and PIC value in riverine and swamp buffalo shown in Table 3.

Javanmard *et al.* (2005) suggest that heterozygosity values below 0.5 (50%) indicate low variation of a gene in the population. Table 3 showed that the heterozygosity of riverine and swamp buffalo were low. In riverine buffaloes, the observation heterozygosity values were lower than expected heterozygosity (Ho = 0.200 < He = 0.219) indicated that κ-Casein gene had a low level of heterozygosity. Swamp buffalo was not showed heterozygosity value because it was not showed allele variation. This might be caused by a lack attention on breeding program and there were no intensive selection based on the milk quality of

Table 3. Observations Heterozygosity Values (Ho), Expected (He) and Polymorphic Informative Content (PIC) of κ-Casein Gene

| Buffalo Breed | Population | Ho | He | PIC |
|---|---|---|---|---|
| Riverine buffalo | SBBC (20) | 0.350 | 0.349 | 0.288 |
| | Deli Serdang (20) | 0.050 | 0.049 | 0.048 |
| | Sub Total | 0.200 | 0.219 | 0.195 |
| Swamp buffalo | Banten (30) | 0.000 | 0.000 | 0.000 |
| | North Sumatera (24) | 0.000 | 0.000 | 0.000 |
| | Semarang (30) | 0.000 | 0.000 | 0.000 |
| | Mataram (30) | 0.000 | 0.000 | 0.000 |
| | Aceh (67) | 0.000 | 0.000 | 0.000 |
| | South Sulawesi (30) | 0.000 | 0.000 | 0.000 |
| | Riau (25) | 0.000 | 0.000 | 0.000 |
| | West Sumatera(14) | 0.000 | 0.000 | 0.000 |
| | Sub Total | 0.000 | 0.000 | 0.000 |

(...) = Number of samples; SBBC = Siborong-borong Buffalo Breeding Center

κ-Casein gene in both location. Hartl and Clark (1997) reported that expected heterozygosity value can be used as a way to estimate the breeding value (inbreeding) in a group of livestock. Generally, the expectation value of heterozygosity is a good indicator as a genetic identifier that can explain the genetic diversity in a population of domestic livestock (Moioli *et al.*, 2004).

Besides heterozygosity value, polymorphism of a gene can be determined by calculating the PIC value. PIC value describe the values of corrected heterozygosity by the partially informative mating. PIC value have ranged from 0 – 1. PIC value equal null (PIC = 0) when only one allele is found in the genetic markers, whereas the PIC values equal one (PIC = 1) if there were an infinite number of alleles. If a gene has two alleles it will produce the maximum PIC value of 0.375 (Hildebrand *et al.*, 1992).

Based on Tabel 3, PIC value equal null (PIC = 0) were found in all populations of swamp buffaloes because in those location κ-Casein|*Eco*RV gene were monomorphic found only one allele, namely C allele. PIC value in riverine buffaloes was higher in SBBC (PIC = 0.288) than Deli Serdang (PIC = 0.048).

**Fixation Index of κ-Casein|EcoRV gene**

Fixation index can be used to determine breeding pattern and selection in population. The value of fixation index could be positive or negative, it was influenced by selection, inbreeding, and assortative mating. The highest fixation index values in this study were low (Table 4). The κ-Casein gene was in fixation in swamp buffaloes in all populations due to a monomorphic occurrence. The fixation process could be caused by inbreeding in all populations swamp buffaloes were observed (Nei and Kumar, 2000)

## CONCLUSION

κ-Casein|*Eco*RV gene in riverine buffalo in SBBC and Deli Serdang was polymorphic, with two alleles, namely C and T allele. κ-Casein|*Eco*RV gene in swamp buffalo was monomorphic with one allele, namely C allele. Heterozygosity value in riverine and swamp buffaloes were low (ranged from 0.000 – 0.350). Fixation Index values in riverine and swamp buffalo were low. The κ-Casein gene was in fixation in swamp

Table 4. Fixation Index of κ-Casein Gene

| Population | Allele | FISKI |
|---|---|---|
| Riverine Buffalo | | |
| SBBC | T | -0.0036 |
| | C | -0.0036 |
| Deli Serdang | T | -0.0256 |
| | C | -0.0256 |
| Swamp Buffalo | | |
| Banten | T | 0.000 |
| | C | 0.000 |
| North Sumatera | T | 0.000 |
| | C | 0.000 |
| Semarang | T | 0.000 |
| | C | 0.000 |
| Mataram | T | 0.000 |
| | C | 0.000 |
| Aceh | T | 0.000 |
| | C | 0.000 |
| South Sulawesi | T | 0.000 |
| | C | 0.000 |
| Riau | T | 0.000 |
| | C | 0.000 |
| West Sumatera | T | 0.000 |
| | C | 0.000 |

SBBC = Siborong-borong Buffalo Breeding Center; $F_{ISki}$: Fixation Index

buffaloes in all populations due to a monomorphic occurrence

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