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Genetic Diversity Study of Milk Protein Gene Code (κ-Cn) Using Restriction Enzyme PST 1 (PCR-RFLP) in Dairy Cows at Malang Region

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ABSTRACT

The objective of the research was to evaluate the genetic varieties of κ-casein gene of Holstein Friesian (HF) in Cooperative Village Unit Karangploso, Dau, Ngantang and Pujon, Malang region by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis (PCR-RFLP) used Pst-1. The research used Holstein Friesian dairy cows raised by farmers of Cooperative Village Unit Karangploso, Dau, Ngantang and Pujon, Malang Region. The blood samples were collected from 29 cows. The research activities were carried out by collecting cow's blood, DNA, extraction, and DNA amplification with PCR. PCR products were digested with Pst-1 enzyme restrictions, then allele gene of κ-casein were identified. The frequency of allele and genotype of κ-casein gene were calculated by Hardy-Weinberg. The results showed that cows raised by farmers of Cooperative Village Unit Karangploso, Dau, Ngantang and Pujon, Malang region, had two alleles κ-casein polymorphism, i.e. allele A (0.74) and B (0.26); therefore, two genotypes existed including AA (0.55) and AB (0.38). it can be concluded that genetic diversity κ-casein gene existed in Holstein-Friesian dairy cows raised by farmers of Cooperative Village Unit Ngantang, Pujon, Dau, and Karangploso, Malang Region.

Keywords: Pst-1, κ-casein gene, PCR-RFLP

INTRODUCTION

κ-casein gene has been widely studied in dairy cows abroad for the purpose of knowing the content of the milk protein casein. Until recently, it was 6 κ-casein gene variants, namely A, B, C, E, F and G [1]. Many studies indicate that genetic variation κ-casein gene influence was felt by the milk processing factories, especially for the factories that produce cheese. According Rahali and Menard [2] and Tsiaras et al. [3], milk from cows that have variant B seems to have the content and amount of protein higher than cows with variant A.

Variants that exist in the κ-casein gene caused by a point mutation so that diagnostic tests can be developed between the existing variants, one of which is a technique Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) [4][5]. Restriction enzymes that can be used for evaluation using PCR-RFLP technique, among others, Hind III, Hinf-I, Hae III, Mae-II and Pst-1 [6].

Based on these findings, it is necessary to carry out the research on a variety of genetic polymorphisms in dairy cattle of the people. The results are expected to be useful as a basis for genetic selection or quality improvement, more efficient and effective in improving the performance of dairy cattle of the people through an appropriate selection of genotypes. Purpose the study is to assess the genetic diversity of protein-coding genes κ-Cn milk of dairy cows in Cooperative Village Unit Ngantang FH, Pujon, Dau, and Karang Ploso Malang region with technical Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

MATERIALS AND METHOD

This study used 29 blood samples from the dairy farm owned by the people of Ngantang, Pujon, Dau, Karang Ploso Cooperative Village Units in Malang region.

Primers for amplification of κ-casein gene is kappafo (5'CGCTGTGAGAAAGATGAAAGATTC3') and kappare (5 'AGATTCAAGGAGTATACCAATTGTTG 3') [7]. Restriction enzymes were for digestion using Pst-1.

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DNA Extraction

Preparation of blood samples following Duryadi methods [8]. There were as many as 500-750 µ l of blood plus a volume X lysis solution {0.32 M Sucrose, 1% (V / V) Triton X-100, 5mm $MgCl_2$ and 10 mM Tris-HCl, pH 7.4}. Cell organelles in the solution were precipitated by centrifugation 6500 rpm for 1 minute. Deposition coupled with value 1X wash solution (75 mM NaCl, 50 mM EDTA, pH 8.0). The next blood coupled with digestion buffer (STES solution + 0.5 mg / ml Proteinase K), then incubated in a water heater temperature of 55 ° C for ± 16 hours or overnight. Purification of total DNA followed Sambrook et al.[9] modified Duryadi [8], with the addition of phenol: chloroform / isoamil alcohol (24:1). After the DNA was precipitated with absolute ethanol and washed using 70% alcohol. DNA dissolved in TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), then stored at -20 ° C.

The PCR DNA Fragment Amplification

Composition of 50 µ l PCR reagent mixture consisted of 2.5 mM $MgCl_2$, 10 mM dNTPs, 100-300 ng template DNA, 20-100 pmol of each primer and 2 U Taq polymerase (Gene ray) along the buffer. Amplification DNA fragments used in this study GeneAmp^{R}PCR engine system 2400 (Perkin Elmer). Amplification κ-casein gene PCR, performed with the following conditions: initial denaturation for 2 min at 94 ° C then followed by 94 ° C for 30 seconds for denaturation, 56 ° C for 45 seconds for annealing (annealing), 72 ° C for 1 min for elongation (elongation) for 35 cycles and then terminated by addition of elongation (extension) for 5 min at 72 ° C.

PCR Product Digestion

PCR products were digested with restriction enzyme Pst-1 [10][11]. Determination of genotypes of DNA samples by analyzing the results of digestion of DNA fragments is a method of agarose gel electrophoresis containing 1.5% ethidium bromide with the aid of UV light.

Data Analysis

Electrophoresis results were analyzed manually and derived alleles and allele frequencies were then calculated the genotypes, by the method of Hardy-Weinberg [12] as follows:

Alel Frequency

Alel A frequency = $p = f(AA) + ½ f(AB)$
Alel B frequency = $q = f(BB) + ½ f(AB)$

Genotype Frequency

AA frequency = $f(AA) = p^2$
AB frequency = $f(AB) = 2pq$
BB frequency = $f(BB) = q^2$
$p^2+2pq+q^2 = 1$

RESULTS AND DISCUSSION

κ-casein Gene Amplification

Amplification of the κ-casein gene in dairy cows using a Kappare and Kappafo primer generate DNA fragments measuring approximately 779 bp in all DNA samples. DNA profile of the amplification primers are presented in figure 1. DNA fragment size data obtained in accordance with Holstein Friesian cows sequin DNA (Gen Bank, AY380228).

κ-casein Gene Digestion with Pst-1

DNA amplification (Gen-κ Cn) is then digested using the restriction enzyme Pst-1. DNA profiles using restriction enzyme digestion results Pst-1 are presented in Figure 2.

Based on the Holstein Friesian cow DNA sequence (Gen Bank, AY380228) Pst-1 enzyme will cut the DNA into fragments of 171 bp and 608 bp for the cows that had allele B, and truncated to 171 bp, 302 bp and 306 bp for cows with allele A. Location of restriction enzyme cutting Skepa Pst-1 are presented in Figure 3. The results of PCR-RFLP using the restriction enzyme Pst-1 alleles A and B. found. This is evident from the results of agarose gel electrophoresis as shown in Figure 2. Allele A in the PCR-RFLP using the enzyme Pst-

1 (together with the genotype AA) cut DNA into fragments sized 306 bp, 302 bp and 171 bp, allele B DNA is cut into 608 bp and 171 bp. Genotop AB, DNA is cut into 608 bp, 306 bp, 302 bp and 171 bp.

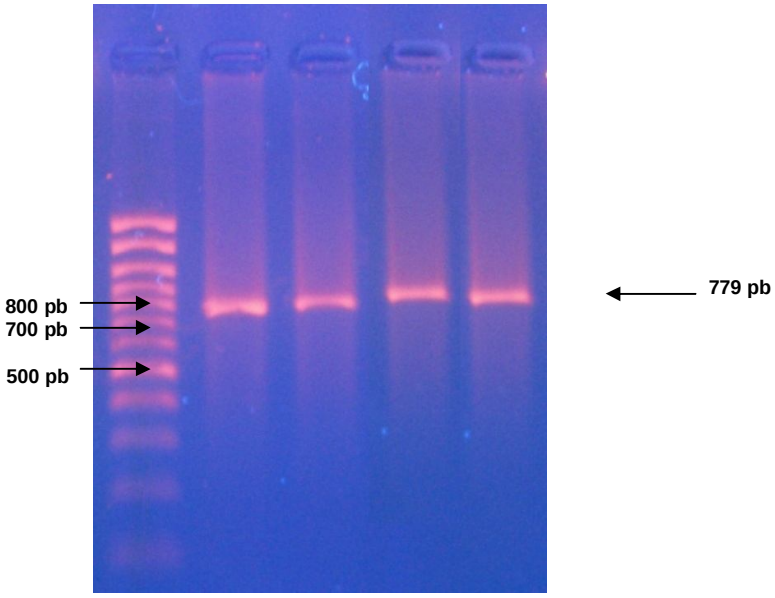

Figure 1 DNA profiles of dairy cows PFH amplification results using primer pair Kappare and Kappafo Description: Row 1: DNA marker 100 bp (Gene ray), row 2-5 DNA amplification using the primers and Kappare Kappafo.

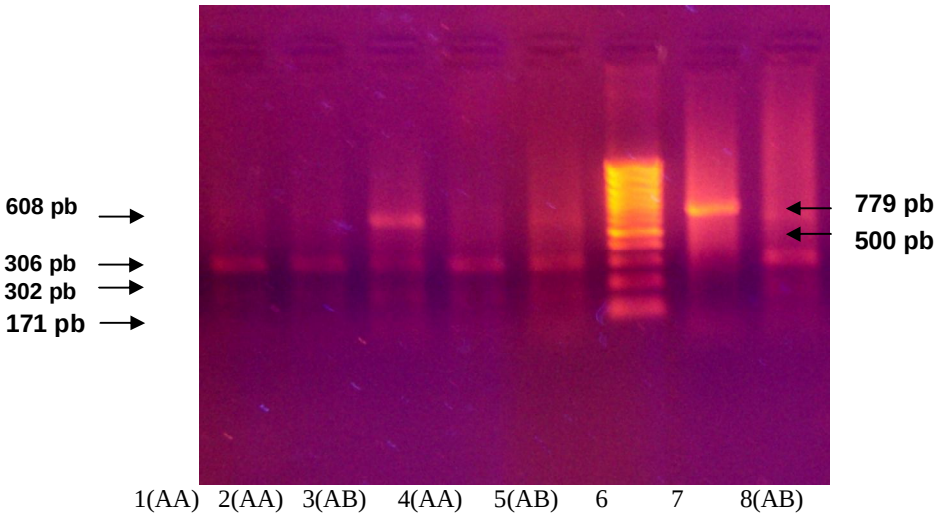

Figure 2 The results of PCR-RFLP DNA PFH dairy cows using the restriction enzyme Pst-1 on 1.5% agarose gel. Description: Row 1-2,3,4,8. PCR-RFLP results with Pst 1, Row 6.DNA marker 100 bp (Generay), Row 7. PCR results

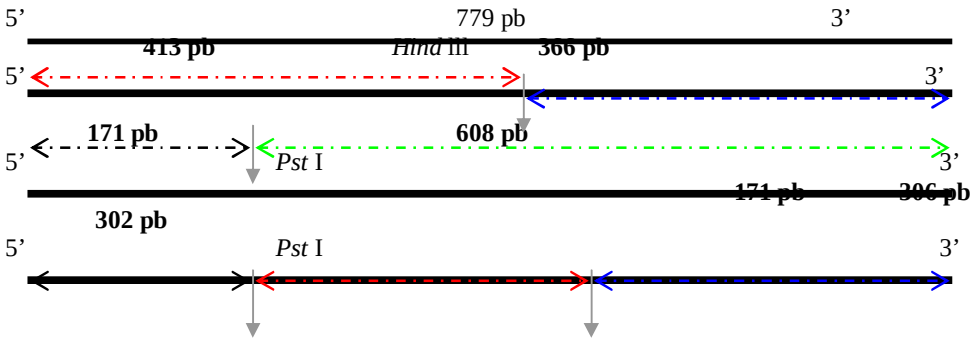

Figure 3. Scheme the cutting-Pst I restriction enzyme place to fragment DNA amplification result using the primer pair Kappafo and Kappare

In this study, from each of the cooperatives are a sample of genetic diversity in cattle PFH used. The polymorphism genotypes and the number of alleles found in a row can be seen in table 1 and table 2. The results of PCR-RFLP using the restriction enzyme Pst-1 in all samples of this study the polymorphism seen cutting pattern; this suggests that there are polymorphism alleles in cattle. Alleles A and B are obtained in accordance with the previously performed Schellander et al. [13]. The frequency of each allele A and B and genotype frequency expectations AA, AB and BB in cattle Cooperative Village Unit of Karangploso, Dau, Pujon, and Ngantang were presented as in Table 3.

Table 1 Polymorphism genotypes found

Cooperative Village Unit	Actual Genotype (Pst-1)				Total
	AA	BB	AB		
Karang Ploso	0,80	0	0,20	1	10
Dau	0,33	0	0,67	1	6
Ngantang	0,50	0	0,50	1	6
Pujon	0,14	0	0,86	1	7
Total	14	0	15		29

Table 2 The number of alleles finding

Cooperative Village Unit	**Alel (Pst-1)**		
	A	**B**	**Total**
Karang Ploso	18	2	20
Dau	8	4	12
Ngantang	9	3	12
Pujon	8	6	14
Total	43	15	58

Table 3The frequency of alleles A and B; desired genotype AA, AB and BB Frequency

Cooperative Village Unit	Alel Frequency		desired genotype Frequency			Total	Total*
	A	B	AA	AB	BB		
Karangploso	0,90	0,10	0,81	0,18	0,01	1	0,99
Dau	0,67	0,33	0,32	0,49	0,18	1	0,81
Ngantang	0,57	0,43	0,56	0,38	0,06	1	0,94
Pujon	0,75	0,25	0,45	0,44	0,11	1	0,89

* number of genotypes AA + AB + BB frequency is actually on each Cooperative Village Unit because the study did not find any genotype BB.

The results of this study indicate that the frequency of allele A is greater than the frequency of allele B, meaning that most of the male parent and PFH has allele A. data are presented in table 3 indicate that all cows PFH of Cooperative Village Units are nothing with genotype AA and BB, just Ab. Whole Cooperative Village Units that allele A frequencies of 0.74 and allele B frequencies of 0.26, while the AA genotype frequencies of 0.55, genotype AB frequency of 0.38 and genotype BB of 0.00. According to the calculation of Hardy-Weinberg law of BB genotype frequency is equal to 0.07, but the study did not found a cow that has genotype BB. This is probably due to the amount of data that little or might be due to the origin of the cattle that are in the four Cooperatives Village Units are not a marriage in the population that are not found in the BB genotype in the population.

According to the Law of the Hardy-Weinberg genotype frequencies AA + AB + BB is 1, but in this study for each of the Cooperative Village Unit is <1 (table 3). This is presumably because there were no BB genotypes, although the results obtained are close to Hardy-Weinberg law.

According Rahali and Menard [2] and Tsiaras et al. [3], milk from cows that have content variant B protein and a higher number than cows with variant A. Therefore, the efforts to improve milk protein quality in dairy farms of the people in the Cooperative Village Unit in Malang to consider doing the selection of female and male parent who has allele B or had genotype BB.

CONCLUSION

There is genetic variability in cattle PFH in four Cooperatives Village Units with an allele a frequency of 0.74 and allele B frequency of 0.26; a genotype frequency of 0.55; AB genotype frequency of 0.38; and genotype frequency BB of 0.00. PCR-RFLP technique using the restriction enzyme Pst-1 can be used to detect the presence of alleles A and B on the κ-casein gene of dairy cows.

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