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The Occurrence of CSN1S1 E Allele on Saanen Goat Population at BBPTU-HPT Baturraden

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Abstract.
The *CSN1S1* gene is expressed as the milk alpha S1 casein which is valuable for cheese making. Allele E is a mutation of this gene, which is correlated with low quantity of expressed protein. This study aimed to identify the occurrence of *CSN1S1* E allele on the Saanen population at BBPTU-HPT Baturraden. Blood samples of 45 does were drawn, followed by DNA isolation using the column spin method. The identification of E allele was carried out by Allele Specific Polymerase Chain Reactions (AS-PCR) with forward primer 5-TCAGGAGCAGTGGGTATGTG-3 and reverse 5-CCTCCCAATGGAATAATGACA-3. Allele E was found as heterozygote genotype with non-E allele in 28 out of 45 samples. This means that F allele frequency was 0.31 and 0.6 for non-E allele. There was no significant difference of milk protein in both groups. In summary, E allele was found in the Saanen goat population in BBPTU HPT Baturraden with no effect on milk protein production.

Keywords: Saanen, *CSN1S1*, milk, goats, protein.

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1. Introduction

Cheese is a dairy product that has high economic value. Cheese is a favorite food in several countries including Indonesia. In fact, the growth in the level of consumption of cheese in Indonesia in 2017 increased by 8.17% and is predicted to continue to increase in the following years due to the characteristics of consumers that support this growth.

Cheese is a dairy product that uses protein as its raw material. Specifically, this protein is in the form of casein despite the presence another protein such as whey protein in milk. Casein which coagulating due to enzymatic processes or acidification is then processed in various ways into different types of cheese. The casein in milk is divided into several fractions which have different physicochemical properties [1,2]. The casein fractions are alpha S1, alpha S2, beta and kappa. In general, S1 alpha casein has the highest quantity in goat milk. This casein is known to have an influence on the quality

and quantity of cheese [3]. Several studies have shown that the quantity of S1 alpha casein has a positive correlation with the quantity of cheese produced, coagulation time and cheese texture [4].

However, there are variations in the quantity of alpha S1 casein from milk produced by dairy goats. Genetic variations in several breeds of goats have an influence on expression as well as the immaturity of the proteins that are formed or not even at all [5,6]. This variation needs to be studied in a population that has the potential to be used as a producer of milk as raw material for cheese.

One of the variations that occurred was the insertion of a DNA sequence called the Long Interspersed Nuclear Element (LINE) in the *CSN1S1* gene between the 19 exon and the untranslated last exon [7]. The insertion causes expression in the form of mRNA which is unstable, so that translation into alpha S1 casein is inefficient [3]. The mutation is then called the E allele.

Saanen goat is known to have a high quantity of milk production, so its milk can be used as a raw material for cheese. The higher the quantity of milk, the more cheese yields are produced. However, it is also known that the Saanen goat has genetic diversity, especially casein alpha S1 which has low quantitative characteristics [3]. This is a concern because it could be that the quantity of milk produced is high, but yield of the cheese is not optimum.

The purpose of this study was to determine the occurrence of the E allele from the *CSN1S1* gene in the Saanen goat population in BBPTU-HPT Baturraden. By knowing the presence of these alleles, it can be used as a reference in determining livestock breeding mechanisms that are suitable for livestock potential. For example, it can produce livestock that are able to produce cheese well, so that breeders who aim to produce milk for cheese making can use these goats.

2. Materials and Method

This research was conducted at the Balai Besar Pembibitan Ternak Unggul - Hijauan Pakan Ternak (BBPTU-HPT) Baturraden - A livestock breeding center that focuses on dairy animal breeding and located in Banyumas Regency, Central Java. The bloods of 45 does on same age and lactation period were drawn and collected on EDTA tube. Milk samples collected in a tube and stored at a temperature of -20^{o} Celsius until quantification of milk protein.

3. Isolation of DNA and Polymerase Chain Reactions

The blood samples were then isolated by DNA using the column spin method. The procedure for implementing this technique was in accordance with the manufacturer's recommendations until 100ul of DNA isolates were obtained for PCR. Previously, the isolates were quantified and calculated of its purity using a spectrophotometer. The primers design were according to [8] with forward primer 5-TCAGGAGCAGTGGGTATGTG-3 and reverse 5-CCTCCCAATGGAATAATGACA-3. Both primers will anneal with non E allele so it will amplify 583bp long amplicon and 1040bp long for E allele.

The PCR technique was carried out for genotype testing based on the manufacturer's procedure, however with minor modifications. The main reagent is 2X MyTaq HS Red Mix (Meridian Bioscience, USA) as a ready to use PCR Mix with 12.5ul per reaction usage. Then added 1ul of each primer (20mM), 3ul of DNA template, and Nuclease Free Water up to a total of 25ul reaction mixture. The reagent mixture then amplified with a PCR machine (PTC-100 Bio-Rad, USA). The cycle settings were 95^{o}C initial denaturation for 3 minutes followed by 35 cycles of 95^{o}C denaturation for 30 seconds - 48.8^{o}C annealing for 30 seconds - 72^{o}C extension for 30 seconds, ended with 72^{o}C final extension for 3 minutes. The obtained amplicon was then electrophoresed by agarose gel with concentration of 1.5% with the voltage of 50V for 1 hour.

3.1. Milk Protein Quantification

Quantification of milk protein was carried out using the Lactoscan instrument (Milkotronic, Ltd., Bulgaria). 50 ml of milk samples are tested by letting the instrument take a number of milk samples automatically and carry out the test. The results will be displayed on the screen and printed for further tabulation step. The results of testing the quantity of milk protein were then tested statistically between genotypes that had the E allele vs. non-E allele by statistical method 2 samples Kolmogorov-Smirnov test.

4. Results and Discussion

4.1. Identification of E and Non E Alleles

The results of the agarose electrophoresis assay are presented in **Figure 1** below. The results showed that there was genetic diversity in the *CSN1S1* gene of the Saanen goat population in BBPTU-HPT Baturraden. The results of electrophoresis resulted in

the identification of the E allele and the non E allele. The non E allele resulted in a band with a size of 583bp as predicted. These results appear because there is no LINE insertion in the DNA samples. In contrast to the previous results, the E allele was identified with a longer band due to the 457bp LINE insertion, so it is predicted to produce a band of 1040bp in length [7,8].

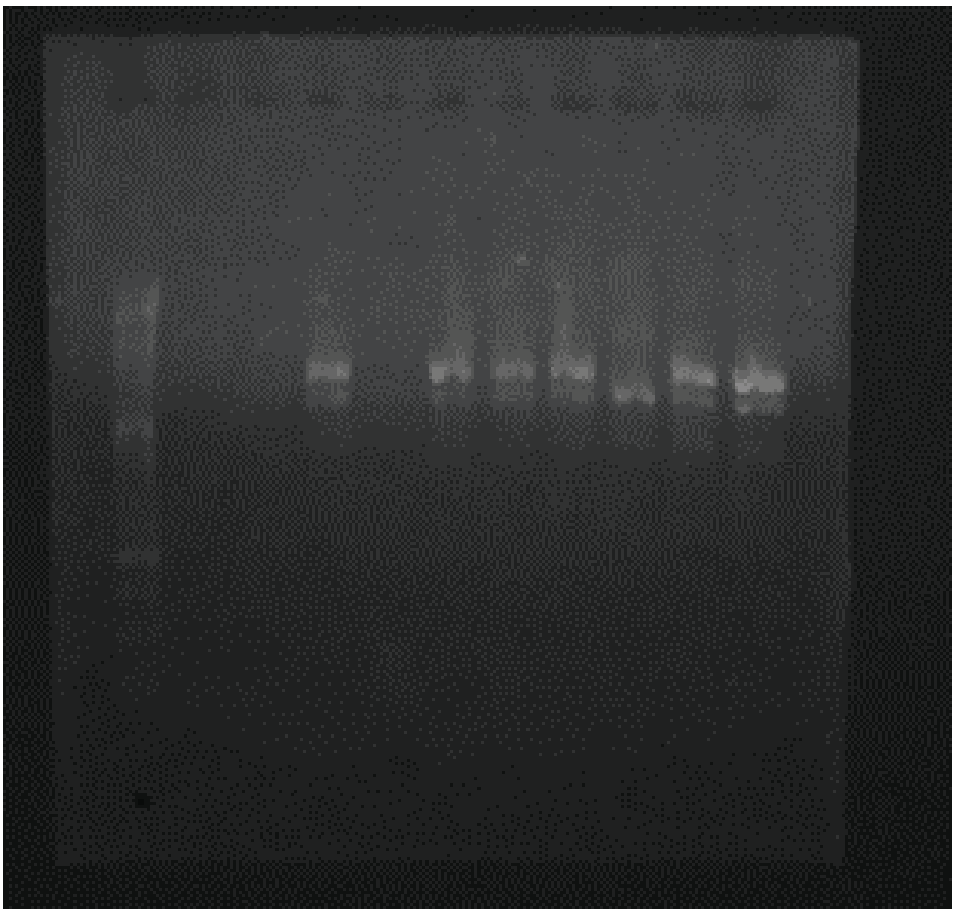

Figure 1: Agarose gel electrophoresis of amplicon (M=Marker; 1,2=Controls; 3,4,5,6,8,9=heterozygous E and non E allele genotype; 7=non E allele genotype).

The appearance of the E allele genotype in the population was identified heterozygously with the non E allele. The homozygous E allele genotype was not found in the sample population. This may occur because of the intensive mating between E and non E alleles from the parents of the population sample [9].

The existence of this heterozygosity will have an impact on the allele frequencies that appear in the calculation results. The calculation results show that the E allele frequency is 0.31 and the non E allele is 0.69. This shows that although the sample population is dominated by heterozygous genotypes of E and non E alleles, the e allele is still quite low in terms of frequency per allele. More in-depth testing of the non-E

allele frequencies is needed, so that more accurate results are obtained for each allele. The results of allele frequency calculations are presented in **Table 1** below:

Table 1: Tabulation of genotype and allele frequency of sample population.

Tally		Allele frequency	
EX	XX	E	X
28	17	0.31	0.69
45		1.00	

Note : the XX letter means non E alleles that may be homozygous or heterozygous between non E alleles.

4.2. Milk Protein Quantity

The sample milk protein quantity was carried out and then grouped between E allele and non E allele. The test results are presented in **Table 2** as follows:

Table 2: Means of milk protein of E allele and non E allele of sample population.

Alele		2 samples K-S test
E	Non E	
3,04%	3,26%	p>0.05

The test results showed that the milk of the population sample showed no significant difference between E and non E alleles (p> 0.05). It can be interpreted that the quantity of milk protein between the two allele groups is the same. This can occur because the E allele is an allele that has an expression in the form of an intermediate protein production ability. This is in accordance with the opinion [3,10,11] which states that the E allele is an intermediate allele compared to the A and B alleles which have high production capabilities and F and O alleles which have low production capabilities. The production capacity of A and B alleles can reach 3.5g/l while E allele is around 1.1g/l and N and 01 are around 0.45g/l [12].

The appearance of the difference in production capability can also affect the quality and quantity of the cheese product produced. The allele A of the *CSN1S1* gene with high ability correlates with the high production of cheese produced [3]. Thus, it will also affect the E allele which is intermediate. The allele in the *CSN1S1* gene also affects the size of casein micelles where the F allele is greater than E and the A allele is the smallest [13]. This may have an effect on the quality and quantity of cheese which is better in the A allele than the F allele because smaller micelles will reduce the distance between micelles during coagulation, so that more cheese is produced [14–16].

5. Conclusion

In summary, E allele was found on Saanen goat population in BBPTU HPT Baturraden as heterozygous with no effect to milk protein production.

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