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Polymorphism of CSN2 Gene Exon 7 in Indonesian Dairy Goat Breeds

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ABSTRACT

Milk production and composition are the main factors in the dairy industry. However, these traits are influenced by several factors, such as genetic factors. The CSN2 gene exon 7 is one of the genes that can affect the protein composition of goat milk. Several studies on the CSN2 gene exon seven have been conducted on Italian and Indian goat breeds, and their genetic variations have been found. Studies about CSN2 gene exon seven polymorphisms in Indonesia have been completed but found no variations. Therefore, this study aims to find genetic variations of CSN2 gene exon 7 using the Sanger Sequencing Method. Ninety-five blood samples of 66 Sapera, 15 Saanen, and 14 Etawa Grade goats were collected from the Livestock Research Center, Ciawi. Genetic diversity was calculated using PopGene32 programs. The results found two polymorphic SNPs in all three dairy goat breeds, namely g.8946C>T and g.8956G>A. Three genotypes found in SNP g.8946C>T are CC, CT, and TT, while a new SNP g.8956G>A found two genotypes (GG and GA). In conclusion, SNPs g.8946C>T and g.8956G>A are polymorphic. Additional research should be conducted to determine whether there is any association between these 2 SNPs and milk production and quality.

Keywords: CSN2 gene exon 7, Dairy goat, SNP

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Introduction

Dairy goat breeds that are commonly raised by Indonesian farmers are Etawa Grade and Saanen goats. The advantages of Etawa Grade goats are their adaptability to Indonesia's tropical climate. They can produce milk of around 1.5–3 L/day (Ratya *et al.*, 2017), while Saanen goats are well known for high milk production but can be limited due to the tropical climate. Strategies to overcome this problem are by a crossbreeding program designed to combine Saanen and Etawa Grade goats' characteristics to increase local goats' genetic potential. A Crossbreed of Saanen and Etawa Grade is known as Sapera, which can produce milk up to 4–5 L/day, good milk quality, and adapt to the Indonesian climate (Kaleka and Haryadi, 2013; Rusdiana *et al.*, 2015).

The quality of goat milk may vary depending on genetic traits, physiological factors, environmental factors, and maintenance management (Verruck *et al.*, 2019). Protein is an essential ingredient controlled by significant genes, divided into casein and whey. Casein content can reach 80% of the total protein in goat milk and other ruminants (Tortorici *et al.*, 2014). Besides that, casein has a moderate heritability value (0.2–0.5), so it can be

used as a marker to improve the quality of goat milk (Amills *et al.*, 2012). The most casein composition is β -CN which is encoded by the CSN2 gene. β -CN produces the bioactive peptide INK, which inhibits the ACE-i (Angiotensin Converting Enzyme Inhibitor) enzyme's role in increasing blood pressure or hypertension (Widodo *et al.*, 2019).

The CSN2 gene is located on chromosome 6, which has nine exons and is about 10.7 kb long (Sari, 2020). Exon 7 has a length of 492 bp and is the most extended exon in the CSN2 gene. Exon 7 also encodes 82% of mature proteins. Several mutation points were found that can affect the protein composition of goat milk (Li *et al.*, 2022). Previous studies revealed that there were 12 variations of the CSN2 gene allele in goats (Li *et al.*, 2022), one of which was a "null" allele found in exon 7, which was associated with the loss of β -CN content in milk (Martin *et al.*, 2013). According to Vacca *et al.* (2014) and Verma *et al.* (2022), in Italian and Indian dairy goats, the CSN2 gene had a significant effect ($P < 0.05$) on SNF and protein percentage.

Sari (2020) conducted molecular research on CSN2 gene polymorphism using the PCR-RFLP technique at locus g.8913 C>A, but the study did not find any polymorphism. Therefore, this study

uses the Sanger Sequencing method to identify the CSN2 gene exon 7 polymorphism in Saanen, Etawa Grade, and Sapera goats.

Materials and Methods

Animals and samples collection

Ninety-five heads of dairy goats consisting of 66 Sapera, 15 Saanen, and 14 Etawa Grade reared at the Livestock Research Center, Ciawi, Bogor Regency. Saanen and Etawa Grade goats compare CSN2 gene exon seven polymorphism with Sapera goats. All the goat breeds used in this research were not subjected to different management treatments to minimize environmental influences. The applied management practices included feeding (forage, concentrates, and mineral blocks), complete facilities and infrastructure, sanitation, waste processing into compost, and prevention and control of dairy goat diseases.

The blood samples were collected under the guidelines of the Animal Ethics Committee of the Agricultural Research and Development Center with registration number Balitbangtan/Balitnak/Rm/11/2021. Blood samples were taken in the jugular vein and then stored in a 10 mL vacutainer tube containing anticoagulants (EDTA). Blood samples were then extracted for DNA using a modified Geneaid DNA Kit procedure.

PCR amplification and sequencing

CSN2 gene exon 7 PCR products are amplified using AB system machines. PCR volume of 25 μ l consisting of 2 μ l of DNA, 6.1 μ l of nuclease-free water, 0.3 μ l of primer F: 5'-GGC ACA GTC TCT AGT CTA TC-3' and 0.3 μ l primer R: 5'-CCT TTC TGC TGT ACC AGG AG-3', 16 μ l MyTaq HS Redmix. The primer of the CSN2 gene (AJ011018.3) was designed using the Molecular Evolutionary Genetic Analysis X (MEGAX) program and Primer Stats with a product length of 418 bp. The amplification process starts with predenaturation at 95° for 5 min, denaturation at 95° for

15 s, annealing at 60° for 15 s, extension at 72° for 10 s, and final extension at 72° for 1 min. PCR products are then sequenced using the services of 1st Base, Selangor, Malaysia.

Data analysis

PCR sequencing results were analyzed using the BioEdit, Finch TV, and Molecular Evolutionary Genetic Analysis (MEGA11) programs. In addition, exon 7's CSN2 gene diversity was analyzed using the PopGene32 program (Yeh and Boyle, 1997) by calculating allele frequencies, genotype frequencies, heterozygosity values, Hardy-Weinberg equilibrium, and PIC (Polymorphism Information Content).

Results and Discussion

Amplification and DNA variants of CSN2 gene exon 7

The CSN2 gene exon 7 in Sapera, Saanen, and Etawa Grade goats were successfully amplified at 418 bp (detailed in Figure 1). The amplification results are bright and unshaded bands; this indicates that PCR products have high specificity for subsequent processes.

The results of this study found 2 SNPs mutations, namely g.8946C>T and g.8956G>A (detailed in Figure 2). The mutation type of the two SNPs is a transitional mutation. Furthermore, three genotypes (CC, CT, and TT) at SNP g.8946C>T and two genotypes (GG and GA) at SNP g.8956G>A were found in all three dairy goat breeds.

Several studies on the polymorphisms of CSN2 genes have been widely carried out in various dairy goat breeds. For example, SNP g.8946C>T occurred in different goat breeds, including Banat's White, Carpathian, Girgentana, Sarda, Nubian, Desert, Nilotic, Taggar, Saanen, Bezoar ibex, and Nubian ibex goats (Tortorici *et al.*, 2014; Vacca *et al.*, 2014; Kusza *et al.*, 2016; Rahmatalla *et al.*, 2021). SNP g.8946C>T is also

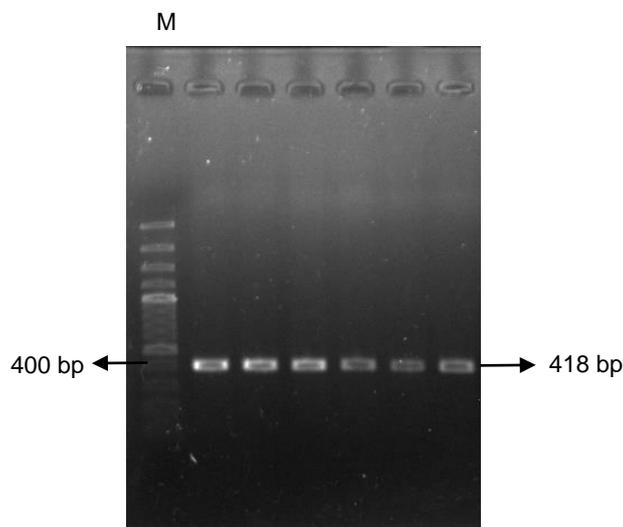


Figure 1. The amplification of CSN2 gene exon 7; M= 100 bp ladder size standard; bp= base pair.

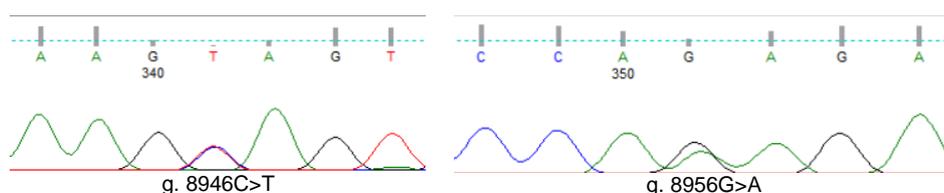


Figure 2. SNPs found in CSN2 gene exon 7; A= Adenine; T= Thymine; G= Guanine; C= Cytosine.

Table 1. Estimation of genetic diversity at g.8946C>T

Dairy goat breeds	N	Frequency					Ho	He	X ² test	PIC
		Genotype			Allele					
g.8946C>T		CC	CT	TT	C	T				
Sapera	66	0.197 (13)	0.439 (29)	0.364 (24)	0.417	0.583	0.439	0.486	0.711	0.368
Saanen	15	0.067 (1)	0.267 (4)	0.667 (10)	0.200	0.800	0.267	0.320	0.663	0.269
Etawa Grade	14	0.214 (3)	0.643 (9)	0.143 (2)	0.536	0.464	0.464	0.497	0.914	0.374

N= number of samples; (...)= number of samples within genotypes; Ho= observed heterozygosity; He= expected heterozygosity; X² table= 3.84; PIC= polymorphic informative content.

Table 2. Estimation of genetic diversity at g.8956G>A

Dairy goat breed	N	Frequency					Ho	He
		Genotype			Allele			
g.8956G>A		GG	GA	AA	G	A		
Sapera	66	0,636(42)	0,364 (24)	0 (0)	0,818	0,182	0,364	0,298
Saanen	15	0,733 (11)	0,267 (4)	0 (0)	0,867	0,133	0,267	0,231
Etawa Grade	14	0,929 (13)	0,077 (1)	0 (0)	0,964	0,036	0,071	0,069

N= number of samples; (...)= number of samples within genotypes; Ho= observed heterozygosity; He= expected heterozygosity.

known as a non-synonymous mutation because it changes the amino acid Alanine (GCA₁₇₇) to Valin (GTA₁₇₇) (Rahmatalla *et al.*, 2022). The novelty of this study is a new mutation in the position g.8956G>A found in the three goat breeds. Therefore, the g.8956G>A changes must be used in other dairy goats to generate more information and potentially become a significant point for selective breeding programs.

Genetic diversity of CSN2 gene exon 7

A population's genetic diversity can be determined by estimating genotype and allele frequencies, heterozygosity values (Ho and He), Hardy-Weinberg equilibrium, and PIC values. Based on the mutations at g.8946C>T, we obtained allele frequency, genotype frequency, heterozygosity values, Hardy-Weinberg equilibrium, and PIC (Table 1).

Genotype and allele frequency at g.8946C>T differ in three dairy goat breeds. The differences in allele frequency can be influenced by the type or breed used in the study (Pineda *et al.*, 2019). Furthermore, the observed heterozygosity (Ho) and expected heterozygosity (He) value at g.8946C>T revealed that Saanen goats have a low heterozygosity value, except for Sapera and Etawa Grade goats which have a heterozygosity value close to 0.5. According to Asmarasari *et al.* (2014), the population's high observed heterozygosity value may reflect the population's diversity of alleles and increased capacity for selection. The Hardy-Weinberg equilibrium test showed that all goat breeds were in Hardy-Weinberg equilibrium. The PIC calculation is informative in the three goats. The maximum value of PIC with two alleles is 0.5 (Chesnokov and Artemyeva, 2015). However, the value can be affected by the frequency distribution of alleles.

The allele frequency, genotype frequency, and heterozygosity values are also calculated for the new SNP position at g.8956G>A (Table 2). The calculation showed that SNP g.8956G>A is polymorphic because genotype and allele frequencies are less than 0.99. According to Allendorf *et al.* (2012), if the frequency of the most prevalent allele is less than 0.99, a locus is typically considered polymorphic. Furthermore, the heterozygosity value indicates a low degree of diversity since the range is less than 0.5. Therefore, SNP g.8956G>A changes must be used in other dairy goats and employ sufficient samples because genotype frequencies, alleles frequency, and heterozygosity value can differ depending on the breed and the number of samples used.

Conclusions

This study shows that the SNP g.8946C>T position is polymorphic with the discovery of three genotypes. In addition, this study also found the existence of a new SNP at the position of g.8956G>A, which was found in the three goat breeds. Additional research should be conducted to determine whether there is any association between these 2 SNPs and milk production and quality.

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