Research Article

Identification of *CSN1S1* Gene Variations Between Dairy Goat Breeds and its Influence on Milk Protein Fractions in Indonesia

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Abstract | The *CSN1S1* gene variation may cause the discrepancy of αs1 casein. The information was lacking on Peranakan Ettawa (PE), Saanen (SA), and Sapera (SP). The objective of this study was to reveal the variation and its expression in those breeds. RFLP-PCR (*XmnI*) was applied to identify A, F, and N alleles with AS-PCR for the E allele on 44 PE, 121 SA, and 15 SP does then sequencing. SDS-PAGE fractionated then quantified the milk proteins from 34 PE, 57 SA, and 15 SP does. The study found the A allele was predominate followed by F and N on PE and SA. However, the SP goat only has A and F alleles in equal frequency, and the E allele was only found on SA. The AF genotype predominated on all breeds in almost half of its population, thus, the most diverse was SA. The Hardy-Weinberg disequilibrium of PE and SA indicated the influence of the breeding program. The genotypes affected the αs1 casein, total casein, and αs1/β ratio, in which AA was highest. The A, F, and N allele expressions were 3.21, 0.82, and 0.60g/L, respectively. It can be drawn that the *CSN1S1* gene variation has occurred in those breeds and an intermediate-expressing genotype was predominant, which affected the amount of some milk protein.

Keywords | Casein, *CSN1S1*, Goat, Milk, Gene variation

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INTRODUCTION

Goats milk demand is increasing and become the second largest consumed milk in Indonesia (Sumarmono, 2022). Dairy goat breeds have been developed for many years to respond to the opportunity. Peranakan Ettawa (PE), which a crossbreed between Jamunapari goat from India and a local Kacang goat, as well as Saanen (SA) goat which was brought in from Europe and Australia. Both goats have their own characteristic, which SA has better milk production, though PE is more adaptive than SA. Another breed is Sapera (SP), a crossbreed between SA and PE goats (Rusdiana *et al*., 2015). The SP breed were developed by farmers in respond to the demand of a goat breed that more adaptive but still has better milk production ability. Therefore, goats are more efficient than cows in milk production, so promoting dairy goats as more suitable animals for developing the dairy industry in Indonesia (Navarrete-Molina *et al*., 2020). Milk from SA breed are suitable and commonly used for cheese making, however the PE and SP is limited but prospective (Vacca *et al*., 2018; Yudatama *et al*., 2012).

Using goat's milk gives another advantage instead of cow's milk. Milk from both animals contains αs1 casein, which is known to be an allergen for some people (Ballabio *et al*., 2011). However, the disparity of αs1 casein in both milks can be the essential consideration. Goat's milk has less allergen because of the lesser αs1 casein amount. Cow's milk contains almost 40% αs1 casein; in contrast, goat's milk

contains less than 20% (Balthazar *et al*., 2017; Widodo *et al*., 2021). The casein content in the milk is a consequence of its gene expression (Chessa *et al*., 2003; Montalbano *et al*., 2016). The previous study shows a strong correlation between gene expression and milk protein quantity by altering the synthesis process (Janmeda *et al*., 2020; Rout and Verma, 2021).

The αs1 casein is encoded by the *CSN1S1* gene, which is known to be highly polymorphic. The polymorphisms modify nucleotide sequences of the gene aforesaid amino acid changes, incomplete protein, or even altering the casein production (Turhan *et al*., 2016). Moreover, this casein was related to others by assisting the transport process inside the micelle formation process (Rodrigues *et al*., 2015). Recent studies show some positive impacts of *the CSN1S1* gene on milk production efficiency, quality, and reproduction (Avondo *et al*., 2009; Zhang *et al*., 2019). On the other hand, αs1 casein plays a significant role in cheese making. This casein also positively correlates with milk coagulation properties, better cheese texture, and more cheese yield (Pazzola *et al*., 2014; Vacca *et al*., 2009). Cheese is needed for Indonesians as an alternative food to attain their protein requirements, however the consumption per people is about 151.2g/year on 2022. The consumption is increasing and dominantly supplied by importation (Kementan, 2022).

The gene variations were not well characterized in these breeds in Indonesia, mainly on government's breeding center. Anggraeni *et al*. (2021) were found the *CSN1S1* gene variations in Indonesia, however, the more comprehensive information on the variants is critical because the high demand for high-quality dairy goat breeds is intense. Moreover, the breeding program considering this gene is a propitious solution, which has been adopted in several countries (Carillier-Jacquin et al., 2016; Frattini *et al*., 2014; Pizarro Inostroza *et al*., 2019). This study is conducted to draw the findings of the lacking information above. Hence, it will heighten the efficacy of the dairy goat as a milk producer for specific products.

MATERIALS AND METHODS

This study used Peranakan Ettawa (PE) and Saanen (SA) goats that rised in a government's breeding center, "Balai Besar Pembibitan Ternak Unggul – Hijauan Pakan Ternak (BBPTU-HPT) Baturraden". The Sapera (SP) goat was from a medium size private dairy goat farm about 2 kms away from the breeding center. Both locations have relatively similar environments condition and feedstuffs.

The blood samples from 180 does consisting of 44 PE, 121 SA, and 15 SP. A total of 106 milk samples were also taken from previous does in lactation between the first to third periods. The milk samples were approximately 50mls as a composite of morning and evening milking consisting of 34 PE, 57 SA, and 15 SP.

The reagents were: blood DNA isolation kit (Geneaid, China), reverse and forward primers (IDT, Singapore), PowerPol 2X PCR Mix (AbClonal, USA), DNA Ladder (SmoBio, Taiwan), *XmnI* restriction enzyme (Thermo Fisher, Romania), RedSafe DNA Staining (iNtRON, South Korea), Tris-Borate-EDTA buffer, agarose (Himedia, India), bromophenol blue (Himedia, India), glycerol (Merck, Singapore), ExcelBand™ 50bp DNA ladder (Smobio, Taiwan), Sodium Dodecyl Sulphate (SDS) (Merck, Singapore), acrylamide/bisacrylamide ready mix solution 29:1 (30%) (Himedia, India), Ammonium persulphate (APS) (AR, China), β-mercaptoethanol (BME) (Merck, Singapore), tris base (Himedia, India), TEMED (Himedia, India), double distilled water, nuclease-free water (NFW) (Himedia, India), Coomassie Brilliant Blue R250 dye (Himedia, India), methanol (Merck, Singapore), and glacial acetic acid (Merck, Singapore).

The DNA extraction

The blood was extracted by spin column method to retain the DNA from white blood cells. The extracted DNA was then assessed in a spectrophotometer giving nucleotide yield of about 32-79ng/µl and 1.6-2.1 for a 230/260nm ratio.

Polymerase chain reaction for identifying *CSN1S1* gene variations

Restricted Fragments Length Polymorphisms (RFLP)-PCR were applied to identify A, F, and N alleles, then Allele-Specific (AS)-PCR for the E and non-E alleles. The RFLP-PCR was based on Ramunno *et al*. (2000) and AS-PCR from (Li *et al*., 2017) with some modifications. Table 1 is provided to show the PCR primer sequences and PCR steps setting.

The RFLP-PCR amplifies the 8th to 9th introns of the *CSN1S1* gene, producing 212 or 223bp amplicon containing a deletion of C and insertion of 11bp or even both. A 25µl reagent mixture was made between 12.5µl 2X PCR Mix, forward and reverse primer 0.5µl (10ng/µl) each, 7.5µl of NFW and 4µl (100-150ng) of DNA template. The PCR steps is described on Table 1. A restriction enzyme cutting process to the product then follows the PCR step by following manufacturer's manual. The *XmnI* restriction enzyme has restriction site 5'-GAANN|NNTTC-3'. The amplicon was then visualized by electrophoresis with 2.5% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer on 75V for 45 min, then observed and documented on the gel documentation system. The size of the bands that appeared was predicted using ImageLab software (Bio-Rad, USA) and compared with the DNA ladder to determine the size.

Table 1: The list of primer sequences for PCR and its steps.

PCR	Primer sequences and steps
RFLP-PCR for A, F, N alleles	F 5'-TTCTAAAAGTCTCAGAGGCAG-3' R 5'-GGGTTGATAGCCTTGTATGT-3' Predenaturation: 97°C for 3 min; denaturation: 97°C for 20 s; annealing 51°C for 20 s with 0.1°C step up per cycle; extension: 72°C for 20 s; repeated to denaturation for 29 times; final extension: 72°C for 5 min.
AS-PCR for E allele	F 5'-TCAGGAGCAGTGGGTATGTG-3 R 5'-CCTCCCAATGGAATAATGACA-3' Predenaturation: 97°C for 3 min; denaturation: 97°C for 20 s; annealing 54.5°C for 20 s; extension: 72°C for 30 s; repeated to denaturation for 29 times; final extension: 72°C for 5 min.

The AS-PCR amplifies the 19th exon of the *CSN1S1* gene and produces a 583bp or much longer band caused by the E allele's insertion. The mixture of the reaction was similar to the previous method. However, the amplicon were visualized in 1% agarose gel at 75V for 45 min.

Genotyping for each sample was done by comparing the band size to the reference's result. Samples for DNA sequencing were taken from homozygous genotypes per breed for the PCR step confirmation. The sequences were then interpreted to confirm the mutation point by BioEdit software, moreover the alignment between the sequences and a reference.

Milk protein fraction identification and quantification

The process uses a one-dimensional SDS-PAGE semi-quantitative electrophoresis technique adapted from de Jesus *et al.* (2019) and Rehbein and Schwalbe (2015). The quantity of milk protein is still relatively high for electrophoresis, so it needs to be diluted with 8M urea at a ratio of 10:1. The milk solution was calculated using the Bradford method to quantify the protein concentration. The BSA solution as standard was also prepared with 4 mg/ml and 40 mg/ml concentrations. The preparation was continued by dissolving each milk sample and BSA solution as much as 30µl with 30µl 2X SDS sample buffer. The solution was then heated at 95°C for 10 minutes, followed by immediate cooling.

Electrophoresis was carried out using a 1mm thick gel with a concentration of 15% acrylamides as separating gel, 8% for spacer gel to prevent smear and giving higher resolution, and 4% for stacking gel. Each well was filled with 10µl of the sample solution. Each gel electrophoresis was run simultaneously between milk samples, BSA standards, and protein ladder at 70V for 3.5 hours, followed with gel staining using CBB-R250. The image of the gel was taken in the gel documentation system. Each protein band was estimated for its molecular weight for protein identification. The protein quantity was calculated by rational comparison of each protein band density to BSA standards bands by the ImageLab software.

Statistical analyses

The calculation is comprised of the Hardy-Weinberg equilibrium test, observed and expected heterozygosity, that performed by PopGene v1.32 program. The Kruskall-Wallis is a non-parametrical test that was used to determine the effect of genotype on some parameters by IBM® SPSS v21 program. The Generalized Linear Model (GLM) was performed to estimate the expression of each allele on the αs1 casein quantity. The estimation assumed all alleles were in a dominant effect. However, overall mean was forced to zero as there were no any other factors that able to produce αs1 casein except the allele. The model was:

$$Yij = Ai + Ei + Fi + Ni + eij$$

Where Yij is quantity of the αs1 casein; Ai, Ei, Fi, Ni are fixed effect of allele; and eij is the random error.

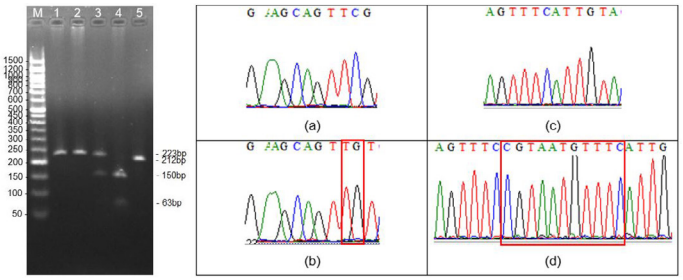

Figure 1: The visualization of band patterns from gel electrophoresis shows the appearance of the A (3, 4), F (1, 2, 3), and N (5) alleles of the CSN1S1 gene, then M as DNA ladder (left). Snapshots of chromatograms on the interested section (right) showing the C deletion (a vs b) and 11bp insertion (c vs d).

RESULTS AND DISCUSSION

The identification of *CSN1S1* gene variations

The RFLP-PCR method successfully demonstrated some results that were depicted on the electrophoresis gel by some band pattern. As expected from all breeds, the pattern can be identified as A, F, and N alleles of the *CSN1S1* gene. The appeared bands were 223bp, 212bp, 150bp, and 63bp long (Figure 1). The A allele is characterized by the appearance of 2 bands that had 150 and 63bp long. The A

allele bands appear as a result of *XmnI* restriction enzyme activity. The enzyme will cut a 5'-GAANN|NNTTC-3' sequence inside the PCR amplicon specifically on the 63rd nucleotide, reflecting if there were no C deletion. This result ties nicely with previous studies wherein similar band patterns were found by (Ramunno *et al.*, 2000) as the pioneer of the method, (Li *et al.*, 2017) on the Saanen goat in Taiwan as well as (Verma *et al.*, 2020) on the Jamunapari goat in India. The A allele is a mutation resulting from an ancestral B1 allele that still has the C nucleotide inside its ninth exon of the *CSN1S1* gene (Caroli *et al.*, 2009).

The appearance of some longer bands 212 and 223bp is a contrasting finding compared to the result above. The findings were indicated to the appearance of the F and N allele. Notably, F and N alleles are characterized by a single deletion of Cytosine positioned on the 67th nucleotide of PCR amplicon, causing the *XmnI* enzyme was failed to anneal to the restriction site (Figure 2). However, both bands have an 11bp discrepancy. The F allele is characterized by the deletion of C and 11bp insertion (Li *et al.*, 2017; Ramunno *et al.*, 2000). Indicates that the 223bp band likely belongs to the F allele, and the other 212bp band is supposed to be the N allele.

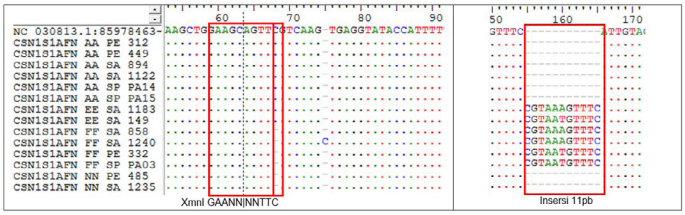

Figure 2: The alignment of RFLP-PCR amplicons showing two mutations: the C deletion (left) and 11bp insertion (right).

The upshots of the above analysis are then compared with the DNA sequencing results. Therefore, the A, F, and N alleles identification was in line with the DNA sequencing results. The chromatograms of all samples portray very well the expected mutations. The evidence of C nucleotide deletion was proven in Figure 1, which shows a disappearance of the C signal compared with 2(a). Figure 1 reveals the arise of many nucleotide signals, which indicates 11bp insertion compared with 2(c). Alignment was performed from the sequencing results to the reference of allele A CSN1S1 gene of *Capra hircus* (accession No. NC 030813.1). Furthermore, it brings more evidence of the *XmnI* deletion at the 63rd nucleotide and the 11bp insertion of 5' CGTAAAGTTTC 3' or 5' CGTAATGTTTC 3', as explained previously.

The AS-PCR was also able to perform the allele identification as expected. The method could discriminate between E and non-E alleles of the *CSN1S1* gene in all breeds. The non-E allele was indicated by the appearance of a 583bp band (Figure 3). Otherwise, the E allele has ~1500bp long. The 583bp band was formed by the functioning of both designed primers. On the other hand, the insertion of Interspersed Nuclear Elements (LINE) occurred on the 3' side of the 19th exon caused the reverse primer was failed to anneal (Dettori *et al.*, 2009; Turhan *et al.*, 2016). The only the forward primer made the DNA polymerase elongate much longer. However, Li *et al.* (2017) found a 1050bp band for the E allele. This may be caused by the difference in DNA polymerase's performance in their study. The limitation on this part was the error reading of the DNA sequencing, and then the data could not be shown.

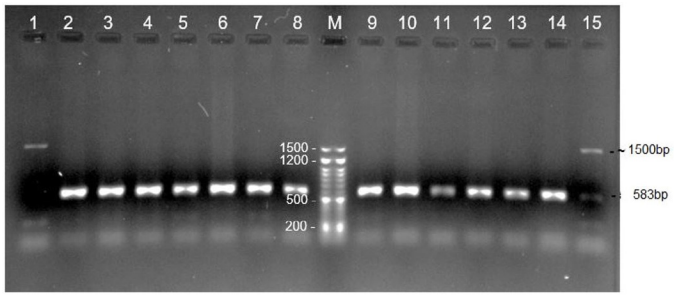

Figure 3: Three band patterns were appeared and indicated the E and non-E alleles forming EE (1), E/non-E (15), non-E/non-E (2-14) genotype and DNA ladder (M).

Table 2: Genotype and allele frequency of *CSN1S1* gene among all breeds.

Genotype and allele	Breed					
	PE		SA		SP	
	Frequency	Relative	Frequency	Relative	Frequency	Relative
Genotype frequency						
AA	12	0.27	38	0.31	3	0.20
AE	0	0.00	2	0.02	0	0.00
AF	22	0.50	46	0.38	9	0.60
AN	7	0.16	5	0.04	0	0.00
EE	0	0.00	3	0.02	0	0.00
FF	1	0.02	24	0.20	3	0.20
NN	2	0.05	3	0.02	0	0.00
Total	44	1.00	121	1.00	15	1.00
Allele frequency						
A	53	0.60	129	0.53	15	0.50
E	0	0.00	8	0.03	0	0.00
F	24	0.27	94	0.39	15	0.50
N	11	0.13	11	0.05	0	0.00
Total	88	1.00	242	1.00	30	1.00
HW Eq.	p=0.007		p=0.000		p=0.521	
Ho	0.659		0.438		0.600	
He	0.547		0.562		0.500	

HW Eq.=Hardy-Weinberg Equilibrium; Ho=Observed Heterozygosity; He=Expected Heterozygosity (Nei, 1973).

Table 3: Milk protein fractions between genotypes and alleles of *CSN1S1* gene among all breeds.

Proteins	The genotype of the *CSN1S1* gene					
	AA	AF	AN	EE	FF	NN
Caseins	(g/dL)					
αs2	0.35±0.09	0.33±0.09	0.29±0.07	0.34±0.10	0.33±0.06	0.43±0.16
αs1**	0.64±0.11^{a}	0.41±0.17^{b}	0.35±0.20^{b}	0.11±0.01^{c}	0.16±0.13^{c}	0.15±0.15^{c}
β	1.41±0.10	1.39±0.14	1.38±0.13	1.55±0.02	1.42±0.15	1.45±0.10
κ**	0.18±0.04^{b}	0.23±0.15^{b}	0.23±0.17^{b}	0.34±0.14^{a}	0.39±0.23^{a}	0.33±0.14^{ab}
Total**	2.57±0.15^{a}	2.36±0.21^{b}	2.25±0.16^{b}	2.35±0.18^{ab}	2.31±0.24^{b}	2.36±0.18^{b}
αs1/β**	0.46±0.80^{a}	0.29±0.13^{b}	0.25±0.14^{b}	0.07±0.01^{c}	0.11±0.89^{b}	0.10±0.10^{b}
Whey	(g/dL)					
β-Lg	0.34±0.03	0.33±0.03	0.33±0.05	0.26±0.01	0.32±0.04	0.36±0.02
α-La	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01
Total*	0.41±0.03^{ab}	0.40±0.03^{ab}	0.40±0.05^{ab}	0.33±0.01^{b}	0.40±0.04^{ab}	0.43±0.02^{a}
Caseins	(kDa)					
αs2	33.07±0.11	32.99±0.12	33.04±0.13	33.00±0.15	32.97±0.13	33.00±0.09
αs1	29.11±0.31	29.17±0.26	29.07±0.31	29.32±0.13	29.41±0.33	29.16±0.45
β	25.18±0.11	25.26±0.12	25.23±0.11	25.18±0.03	25.23±0.14	25.23±0.14
κ	22.27±0.12	22.29±0.11	22.29±0.10	22.27±0.13	22.27±0.12	22.33±0.13
Whey	(kDa)					
β-Lg	16.20±0.16	16.21±0.15	16.15±0.23	16.20±0.26	16.15±0.19	16.22±0.20
α-La	13.61±0.10	13.61±0.12	13.57±0.09	13.67±0.19	13.61±0.13	13.65±0.09

α-Lb=α-lactalbumin; β-Lg=β-lactoglobulin; *= significant effect ($p<0.05$); **= very significant effect ($p<0.01$); The difference of font superscripts in a row symbolizes a significant difference ($p<0.05$).

Table 4: The estimation of each allele expression in the *CSN1S1* gene.

Gene	Linear regression	Description
CSN1S1	Y=0.321(A)+0.057(E)+0.082(F)+0.060(N) R^2=0.871 dan R=0.933; p=0.001	Y: αs1 casein quantity (g/dL); A, E, F, N: allele (2=homozygote, 1= heterozygote, 0= null)

Allele and genotype frequency of the *CSN1S1* gene

In the Table 2, allele frequency of the *CSN1S1* gene in PE goat was predominated by the A allele, followed by F and N alleles. It influences the AF genotype being predominant. These results are in accordance with Singh *et al.* (2018) and Verma *et al.* (2020), who found the predominance of the A allele in the Jamunapari breed in India. This comparison is presented as an alternative because the genetic diversity of this gene in the PE goat population is still unavailable. The SA goat was also predominated by the A followed by F and N alleles, making AF the predominant genotype. However, the E allele was submissively found only in this goat, making the AE and EE genotypes have occurred only in the SA. Thus, this goat has a broader range of among all genotypes than others. A contrary result was obtained in Chinese and Mexican Saanen goats in which the AF genotype was submissive; thus, E and F alleles are predominant (Torres-Vazquez *et al.*, 2008; Yue *et al.*, 2011). The SP goat population has relatively the same results as the other two populations that predominated by the AF genotype; however, the goat only has A and F alleles. The results leading to similar finding where the AF genotype predominates two endangered Czech goats and Girgentana goat in Italy which have better milk production (Criscione *et al.*, 2019; Sztankóová *et al.*, 2008). From this standpoint, it brings more exciting results in the breeding center's SA population that maybe will give better casein yield compared to the similar breed worldwide.

The Hardy-Weinberg calculation shows that the PE and SA goats' genetic population was disequilibrium ($p<0.05$), except the SP goat population ($p>0.05$) (Table 2). The Hardy-Weinberg disequilibrium of PA and SA goats illustrates a shift in the genetic population that made the dominance of some alleles. The factors, i.e., non-random mating, mutation, natural selection, and genetic drift, can cause this shift (Paim *et al.*, 2019). We speculate this might be due to non-random mating as the most probable factor. It is essential to note that the evidence relies on reproduction management carried out by the breeding center that has applied artificial insemination (AI). The

AI tends to use repeated bucks, making it belong to non-random mating (David *et al*., 2008). However, it has been involved in maintaining the genetic quality of dairy goats, which occurred in France Saanen goat (Frattini *et al*., 2014). The A allele has been increased for 12% on the population, which may also happened in the breeding center.

The further finding was that heterozygosity of the *CSN1S1* gene in the PE goat population showed a higher value than expected. Kumar *et al*. (2007) reported the observed heterozygosity (Ho) in Indian Jamunapari goat is 0.395, which is lower than in this study. The combinations of several alleles made the heterozygosity in the population. The Ho exceeding the expected value reflects the excessive frequency of heterozygous genotypes; it was beyond Hardy-Weinberg's calculation and provoked the disequilibrium (Finocchiaro *et al*., 2008). Nevertheless, the SP goat population also has heterozygosity that exceeds the expected value but is still in the equilibrium. The Ho in the SA goat was lower than expected. The result demonstrates that the SA population's heterozygous genotypes are lower than the Hardy-Weinberg equilibrium calculation, even though the variety of the found alleles is numerous than the other two breeds. That could be due to a lesser entity of overall heterozygous than the homozygous in the population (Brito *et al*., 2015). This may offers an advantages in milk protein synthesis. The homozygosity of strong alelles are more preferred than heterozigosity of strong and weak allele which may causing lower rate.

The interesting AF genotype is a combination of A and F alleles, whereas the A allele has a strong expression in producing the protein; otherwise, the F which a weak allele (Mastrangelo *et al*., 2013). Considering that the casein gene has a dominating effect, it may alter the αs1 casein production to be lower than the AA genotype. The AA genotype is a combination of a strong allele that may have higher αs1 casein production, The AA genotype is considered a strong expression, then the intermediate expression for AF. Deliberating the AA or AF genotype in the breeding program is essential because it could increase 2.2 or just 0.5g/L of αs1 casein in the progeny test of Saanen goat (Carillier-Jacquin *et al*., 2016). The breeding program may shift the genetic variation into one of the specific genotypes. However, this study suffers from a limitation in predicting which allele and genotype the genetic shift will proceed, making the justification for the breeding program undone. The result could be obtained by collecting the offsprings genotype (Mestawet *et al*., 2013).

Milk protein fractions

The further novel finding that the protein profile in goat's milk was successfully identified and quantified by the SDS-PAGE method. The casein protein fractions that could be identified were αs2, αs1, β, and κ caseins which similar with (de Jesus *et al*., 2019; Widodo *et al*., 2021). The results of the electrophoresis process are presented in Figure 4.

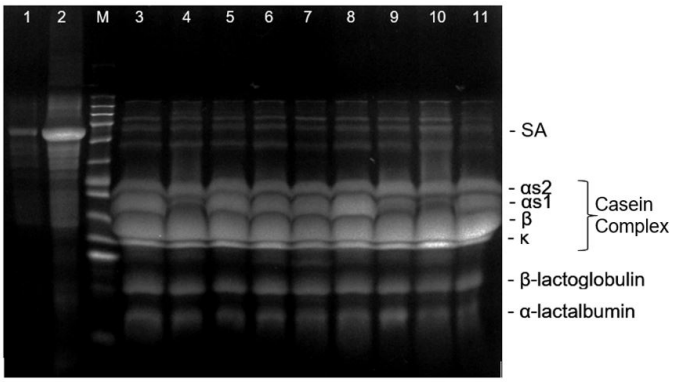

Figure 4: Milk protein fractions were separated adequately by SDS-PAGE in 15% acrylamide. BSA (1, 2), Protein marker (M), CSN1S1 genotype AA (3,8), AF (5, 6, 7), AN (11), EE (9), FF (10) and NN (4).

The variation of the *CSN1S1* gene has a significant effect on several measured parameters. It affected the quantity of αs1 casein, κ casein, total casein, and total whey in the milk (Tabel 3). Goats of these three breeds with the AA genotype produced the higher αs1 casein, which is up to four folds that FF and NN could be. This finding is consistent with what has been found by Verma *et al*. (2020) on the Jamunapari goat. In addition, the discrepancy between AA and FF genotype is almost 6g/L of αs1 casein, which might be essential for cheese production (Pizarro *et al*., 2020). The A allele in the *CSN1S1* gene is known to have a strong expression in producing αs1 casein. The E allele has moderate, and F has a weak expression in producing αs1 casein quantity. Heterozygous genotypes between A and E or F alleles made an intermediate expression of αs1 casein quantity due to the combination of alleles having strong and weaker expressions (Carillier-Jacquin *et al*., 2016; Turhan *et al*., 2016). The quantity of casein κ was highest in the FF genotype. The quantity of κ casein in the FF genotype was two folds higher than that of the AA genotype; otherwise, the genotype has lower αs1 casein than the counterpart, addressing a negative interaction of these proteins over the gene variations. These appear to be intergenic interactions or gene variations that may occur and cause this anomaly (Caravaca *et al*., 2011; Song *et al*., 2020). However, a result study from Vacca *et al*. (2014) revealed that *CSN3* plays no significant role in milk casein. That is inferred the amount of both caseins will be in opposite trend.

The quantity of total casein in milk was significantly influenced ($p<0.01$) by the variations of the *CSN1S1* gene. The AA genotype had the highest amount of total casein compared to the other genotypes. The rising of the total casein in the AA genotype may occur due to the high quantity of αs1 casein. The speculation was drawn by comparing the

result to the data of αs2 and β casein quantities among genotypes. Thus, these caseins were relatively similar and increased along with the lower αs1 casein (Song *et al*., 2020; Zhang *et al*., 2019). The contradictory apparent of κ casein on the αs1 quantity provides a more compelling reason for the above assumption because the lower α casein of the AA genotype has no effect on the higher total casein in that genotype. This assumption may emphasize the reason of the total casein anomaly that was affected by the αs1 casein discrepancy due to gene variation. The total whey in milk was also significantly ($p<0.05$) influenced by the variations of the *CSN1S1* gene. The NN genotype registered as the highest total whey by altering the rational quantity of casein. Weak alleles of *CSN1S1* gene is tended to have higher quantity of whey proteins (Berget *et al*., 2010).

The ratio between αs1/β casein is a relative comparison of both caseins. If the ratio is higher, it represents that the quantity of αs1 casein is more than the β. This information is essential to characterize the milk by comparing both dominant caseins. The variations of the *CSN1S1* gene had a very significant effect ($p<0.01$) on the ratio αs1/β. The AA genotype had the highest ratio of the other genotypes. This result was also proven by the quantity of αs1 casein, which increased in mentioned genotype; in contrast, the NN genotype obtained the lowest ratio. Pizarro *et al*. (2020) reported β casein has no significant effects on other casein quantities, which implies the sole role of the *CSN1S1* gene.

The estimation of expression over alleles

The regression model to obtain the expression of each allele is presented in Table 4. The A allele was estimated to produce αs1 casein up to 0.32g/dL or 3.21g protein per liter of milk. Compared to previous studies, the quantity of allele A is slightly lower. Notably, the strong A allele is able to produce αs1 casein at almost 3.5g/L (Mastrangelo *et al*., 2013). The ability of the F allele to produce αs1 casein was 0.82g/L in this study. This allele is classified as a weak expression allele with production around 0.45g/L (Caroli *et al*., 2006). The estimation is almost two folds higher than the reference. This result may appear due to numerous AF genotype data, conducting a weighed expression mostly into these alleles. The N allele was classified as the null allele or almost no αs1 casein in the milk (Caroli *et al*., 2006). However, it was produce αs1 casein of about 0.60g/L in this study. This result is inconsistent with previous studies that claimed goat carrying the N allele was not producing αs1 casein (Mastrangelo *et al*., 2013; Ramunno *et al*., 2000). Although acrylamide electrophoresis was able to separate the protein by its molecular weight, the specificity of the protein needs further analysis which was limited in this study. This is may explain the inconsistency above, which also observed in 2D electrophoresis gel instead of genotyping (Santillo *et al*., 2022). The estimation of the E allele shows a result of 0.57g/L of αs1 casein. The result is below the reference, which has about ~1g/L αs1 casein production and classifies the allele as an intermediate expression (Mastrangelo *et al*., 2013). However, the effect of the E allele was statistically insignificant ($p>0.05$), so the result was rejected.

CONCLUSIONS AND RECOMMENDATIONS

In brief, variations of the *CSN1S1* gene were found on those goat populations and dominated by the strong-expressing allele, although, the genotype was mostly AF which influenced lower amount of αs1 and total casein than AA. The breeding program may shift the population into these genotypes on the breeding center.

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NOVELTY STATEMENT

This research reveals a significant evidence of the cheese making potency of local dairy goat's milk, which previously unavailable. This will encourage local dairy farmers and government in developing local dairy goats.

AUTHOR'S CONTRIBUTION

HSW: Data collection, analysis and manuscript drafting. TWM: Data analysis and manuscript drafting. AA: Manuscript drafting. AP: Manuscript drafting.

Conflict of interest

The authors have declared no conflict of interest.

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