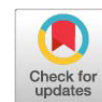


## Research Article



# Polymorphism of Kappa-Casein Gene (*CSN3*|*Alw44I*) and Its Effect on Milk Yield and Compositions in Indigenous Senduro Goat

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**Abstract** | Casein is one of the major milk proteins approximately 80-83% of total protein. The *CSN3* gene has been broadly studied due to its important influence on the milk properties. Senduro goat is local breeds in Indonesia that provide milk. This study was aimed to analyse *CSN3* gene polymorphism and its association with milk production and composition on Senduro goat. A total of 42 lactating Senduro goat were used in study from parity 1 to 4. Blood and milk samples were collected in Senduro district, Lumajang. The traits observed were milk yield, fat, lactose, solid non-fat, total-solid, and protein. The *CSN3*|*Alw44I* polymorphism was analysed using Polymerase Chain Reaction-Restricted Fragment Length Polymorphism method. The result showed that three genotypes (CC, CT, and TT) and two alleles (C and T) were found. The frequencies of CC, CT, and TT genotypes were 0.450, 0.500, and 0.050 respectively, while the frequencies of C and T allele were 0.702 and 0.298, respectively. The different genotypes didn't significantly affect milk production and compositions. Descriptively, Senduro goat with TT genotype had the highest milk production and compositions. In conclusion, the polymorphism of *CSN3*|*Alw44I* was polymorphic and could differentiate milk production and compositions among genotypes (TT>CT>CC).

**Keywords** | **Key words:** PCR-RFLP, Restriction enzyme, Polymorphic, Local goat, Kappa-casein

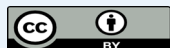
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## INTRODUCTION

Senduro goat is one of local breeds in Indonesia and commonly raised by the farmers as the dual-purpose goat. This goat has an important role to provide animal products such as meat and milk. The goat's milk could be processed into pasteurized milk, ultra-high temperature milk, powder milk, yoghurt, cheese, butter and ice cream as dairy products (Aryana and Olson, 2017; Fazilah *et al.*, 2018; Kalyankar and Patil, 2016). These products are important parts to fulfil the daily human diet. Recently, the goat's milk is well known as the functional food due to

nutritional properties and health promoting compounds, such as oligosaccharides, conjugated linoleic acids, and bioactive peptides or protein (Salva *et al.*, 2011; Abejón Mukdsi *et al.*, 2013).

Casein is one of the major protein of milk approximately 80-83% of total protein and consist of alpha ( $\alpha$ ), beta ( $\beta$ ), and kappa ( $\kappa$ ) casein (Fan *et al.*, 2020). Gipson (2019) reported that casein is the main protein in goat milk. Casein provides the body with all of the amino acids necessary to help build muscle and the main constituent of milk protein. The casein protein coding region have been

mapped on chromosome 6 with coding genes *CSN1S1* (*αs1-casein*), *CSN1S2* (*αs2-casein*), *CSN2* (*β-casein*), and *CSN3* (*κ-casein*) in goat (Rehman *et al.*, 2021). *CSN3* have an important role to influence the size of milk micelles, which has an impact on the coagulation features of the milk in cheese making process (Ahmed, 2011). The *CSN3* (*κ-casein*) gene has been broadly studied due to its influence on the properties of milk. Kappa casein is most important protein and it is being determined at chromosome number BTA6 that consists of five exons spread over about 13.06 kb and exon 4 is most of the protein-coding region (Akyuz *et al.*, 2012; Volkandari *et al.*, 2017; Barbosa *et al.*, 2019). *CSN3* gene in goat is highly polymorphic in several studies (Gupta *et al.*, 2009; Kiplagat *et al.*, 2010). Kumar *et al.* (2009) reported that there are 15 polymorphic sites in the exon 4 of *CSN3* gene. Some variants were reported to have a significant effect on the production and nutritional composition of the milk (Caravaca *et al.*, 2009, 2010).

The milk yield and composition in goats were reported have high variability (Gautam *et al.*, 2020), that might be influenced by genetic variability (Singh *et al.*, 2018). The selection for major genes will be profitable in efficient breeding scheme (Mucha *et al.*, 2018). *CSN3* was selected to support goat breeding program due to its effect on the milk and highly polymorphic gene. In addition, there needs to be an association between genotype and milk production. This study was aimed to analyse Kappa Casein gene (*CSN3*) polymorphism and its association with milk production and compositions on Senduro goat.

## MATERIALS AND METHODS

### ANIMAL AND PHENOTYPIC PARAMETERS

Senduro goat is a dairy goat type that is mostly kept on the slopes of the Semeru Mountains, which includes 8 villages in Senduro District, Lumajang Regency, East Java. This area has a cold-humid climate with an annual temperature range of 25–27°C and humidity of 80–90%. The total population of Senduro goats almost reached 10,000 in 2018. This goat has an important role in supporting local economic society (Arsa, 2018).

All procedures related to animal use in this study were approved by the Animal Care and Use Committee of Universitas Brawijaya under regulation number 020-KEP-UB-2021 (Ethical Clearance). A total of 42 lactating Senduro goat aged 2–4 years were used in this study. The goats had parities ranged from 1 to 4. All goats were reared by farmers intensively. Milk yield was visually measured using the measuring cup of 1000 and 500 mL. Milk compositions (fat, lactose, solid non-fat, total solid, and protein) were analyzed using Lactoscan Milk Analyzer (MCC- Milk Analyzer) in Laboratory of Dairy Science, Faculty of Animal Science, Universitas Brawijaya.

Genomic analysis was performed in Laboratory of Animal Biotechnology, Faculty of Animal Science, Universitas Brawijaya.

### BLOOD SAMPLES AND DNA ISOLATION

Blood samples were collected from jugular vein using blood vacutainer containing EDTA. The DNA was isolated using Genomic DNA Mini Kit for blood and cultured cell (Geneaid Biotech Ltd., China). The procedures of DNA isolation followed the manufacture instructions. The quality of isolated DNA was checked using 1.5% agarose gel electrophoresis while the quantity was measured using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific™, Massachusetts, USA) respectively. The DNA concentration was adjusted to 50–100 ng/μL and stored at –20°C for the further analysis.

### GENE POLYMORPHISM

The *CSN3* gene polymorphism was analyzed using PCR-RFLP method in this study. A pair of primers were used in this study consisted of forward primer (F: 5'- GTA TGT GCT GAG TAG GTA TC -3') and reverse primer (R: 5'- CCT CTT TGA TGT CTC CTT AG -3'). These primers were designed to amplify a 455 bp fragment of *CSN3* gene according to the goat genomic sequence in GenBank (accession number NM\_001285587). The DNA amplification used the mixture of 1 μL DNA template (50–100 ng/μL) and 30 μL PCR premix. The PCR premix consisted of 0.3 μL primer (10 pmol/ μL), 15 μL Go Taq Green Master Mix (Promega, USA), and 14.4 μL Nucleus Free Water (NFW). The DNA amplification was performed in Bio-Rad T100™ Thermal Cycler (Bio-Rad, USA) and the machine conditions were shown in Table 1. The PCR products were checked using electrophoresis for 35 min at 100V on 1.5% agarose gel. The PCR products were visualized under blue light of Glite 965 GW imaging system (Pacific Image Electronics Co., Ltd.) after the gel was stained using Diamond™ Nucleic Acid Dye (Promega, USA).

**Table 1:** The condition of thermal cycler machine during PCR.

| PCR Step         | Temperature (°C) | Time      | Number of cycle |
|------------------|------------------|-----------|-----------------|
| Pre-denaturation | 95               | 5 minutes | Once            |
| Denaturation     | 95               | 30 s      | 35 times        |
| Annealing        | 60               | 45 s      |                 |
| Extension        | 72               | 1 minute  |                 |
| Final extension  | 72               | 5 minutes | Once            |

Furthermore, the PCR products were digested by a restriction enzyme of *Alw44I*. The *Alw44I* restriction enzyme has a specific restriction site on G|TGCAC. A total of 5 μL PCR product was mixed with 0.7 μL of 10 × reaction buffer, 4 units of *Alw44I* (Thermo Fisher Scientific,

Massachusetts, US) and 0.9  $\mu$ L nucleus free water (NFW). The mixed solution was immediately incubated in 37°C for 10-14 hr. Genotyping was performed based on the band pattern of digested products on 2% electrophoresed agarose gel.

## DATA ANALYSIS

### GENOTYPE AND ALLELE FREQUENCY

The genotype and allele frequency were calculated using the following formula of (Nei and Kumar, 2000):

Genotype frequency:

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Allele frequency:

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Where  $\chi_{ii}$  was frequency of ii genotype (CC, CT and TT);  $\chi_i$  was frequency of i allele (C and T);  $n_{ii}$  was number of individuals with ii genotype;  $n_{ij}$  was number of individuals with ij genotype; N was number of samples.

### HARDY-WEINBERG EQUILIBRIUM (HWE)

The Hardy-Weinberg equilibrium was calculated using Chi-square test (Hartl and Clark, 1997).

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Where  $\chi^2$  was HWE test; O was the observed number of genotypes; E was the expected number of genotypes;  $\alpha$  was 5% significance level; df was number of genotype probabilities – number of alleles.

### CSN3 GENE ASSOCIATION WITH MILK YIELD AND COMPOSITIONS

The association study between *CSN3* gene polymorphism and milk compositions was analyzed using General Linear Model procedure in SPSS ver. 26.0. The fixed factor was the genotypes and parities while the dependent variables were milk yield, fat, lactose, solid non-fat, total solid, and protein. The mathematical model used in this research was:

$$Y_{ijk} = \mu + G_i + P_j + \epsilon_{ijk}$$

Where  $Y_{ijk}$  was observation value (milk yield and compositions);  $\mu$  was overall mean;  $G_i$  was the effect of  $i^{th}$  genotype (CC, CT and TT);  $P_j$  was the effect of  $j^{th}$  parity (1, 2, 3 and 4);  $\epsilon_{ijk}$  was random error.

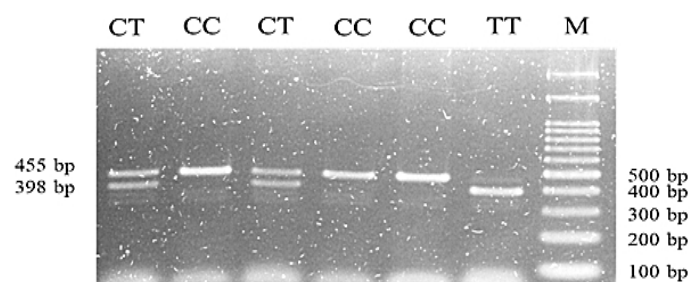
## RESULTS AND DISCUSSION

The production and composition of goat's milk are

influenced by many factors, one of them is parity. Effect of parity on milk yield and compositions on Senduro goats are presented in Table 2. The result showed that the parity had no effect on milk yield and compositions on indigenous Senduro goat ( $P > 0.05$ ), but it showed an almost steady increasing from first to third parity in all phases of lactation on milk yield. The first parity had the lowest milk yield and the third parity had the greatest milk yield (828.30 L and 1,114.53 L, respectively).

The increasing of goat age indicated the already developed alveoli that affecting the higher milk yield. On the other hand, there is a reduction in the use of energy for growth, favoring partitioning of nutrients toward milk production (Lérias *et al.*, 2014). Milk production will continue to increase from the first parity to the fourth parity and will decrease at the fifth parity, caused by the increasing of maturity and readiness of cells and hormonal systems associated with reproductive function in the same physiological status (Vacca *et al.*, 2018; Ishag *et al.*, 2012; Idowu and Adewumi, 2017; Awad *et al.*, 2022). Zamuner *et al.* (2020) showed that parity did not affect the percentages of milk fat or protein of commercial dairy goats in Australia. Another research reported that increasing parity increased the total fat yield (Šlyžius *et al.*, 2017), confirmed our present result that the highest total fat was found in the fourth parity (Table 2).

The visualization of PCR product of kappa casein gene using specific primer was a 455 bp fragment. The 455 bp fragment DNA of kappa casein gene after digested by *Alw44I* restriction endonuclease generated two fragments of 455 and 398 bp. Two fragments of 455 and 398 bp represented CT genotype and one fragment of 455 bp represented CC genotype while one fragment of 398 bp represented TT genotype (Figure 1). Kappa casein gene of local Tunisian goats using the *Alw44I* enzyme was found at 459 bp and their restriction fragments were 459 and 381 bp (Jemmali *et al.*, 2013). While on Zawaladi goats using *HaeIII* was at 645 bp and their restriction fragments were 416 and 229 bp (Patel *et al.*, 2011).



**Figure 1:** Visualization of restricted PCR products by *Alw44I* in 2% electrophoresis agarose gel. M DNA marker 100 bp.



**Table 2:** Effect of parity on milk yield and compositions on Senduro goat.

| Traits             | Parity 1 (n=4) | Parity 2 (n=23) | Parity 3 (n=11) | Parity 4 (n=4)  | Sig. |
|--------------------|----------------|-----------------|-----------------|-----------------|------|
| Fat (%)            | 6.39±1.50      | 6.88±2.24       | 6.18±1.10       | 7.33±2.56       | Ns   |
| Lactose (%)        | 4.88±0.33      | 4.80±0.37       | 4.71±0.27       | 4.49±0.24       | Ns   |
| SNF (%)            | 8.50±0.49      | 8.27±0.71       | 8.18±0.45       | 7.67±0.53       | Ns   |
| Solid (%)          | 14.88±1.90     | 15.14±1.98      | 14.36±1.38      | 15.00±2.32      | Ns   |
| Protein (%)        | 4.54±0.24      | 4.53±0.58       | 4.55±0.24       | 4.52±0.51       | Ns   |
| Milk yield (L/day) | 828.30±196.35  | 878.34±515.69   | 1,114.53±466.66 | 1,087.73±104.24 | Ns   |

Note: n: number of samples; Ns: not significant (P>0.05).

**Table 3:** Frequencies of genotypes and alleles of *CSN3*|*A/w44I* in Senduro goat.

| Breed        | N  | Genotype frequencies |       |       | Allele frequencies |       | $\chi^2$ value      |
|--------------|----|----------------------|-------|-------|--------------------|-------|---------------------|
|              |    | CC                   | CT    | TT    | C                  | T     |                     |
| Senduro goat | 42 | 0.450                | 0.500 | 0.050 | 0.702              | 0.298 | 0.162 <sup>ns</sup> |

Note: n: number of samples;  $\chi^2$  (0.05;1) = 3.84; ns: not significant.

Genotype frequencies of kappa casein gene of Senduro goat in this study are 45.0%, 50.0%, and 5.0% for CC, CT and TT respectively while allele frequencies were 0.702 for C and 0.298 for T. The C allele was observed of high frequency compare to the T allele of *CSN3* in this study (Table 3). The results of this study are in accordance with the results of Orobica dairy goat in alpine valley of the Northern Region Italy for the locus of *CSN3* gene with the genotype frequencies of 51.2%, 37.7% and 11.1% for AA, AB and BB, respectively with allele frequencies of 0.72 for A and 0.28 for B (Chiatti *et al.*, 2007). The results of this study are contrast to the result from Jemmali *et al.* (2013) in local Tunisian goats with genotypes frequency were 12.5% AA, 27% AC, and 60.5% CC. The genotype frequencies for Sarda goats were 41.5%, 39.0%, and 7.5% for AB, BB, and AA, respectively (Pazzola *et al.*, 2014).

**Table 4:** Association of *CSN3*|*A/w44I* polymorphism and milk yield and composition.

| Traits             | CC (n = 19)   | CT (n = 21)     | TT (n = 2)      | Sig.* |
|--------------------|---------------|-----------------|-----------------|-------|
| Fat (%)            | 6.75±2.43     | 6.49±1.45       | 8.22±0.37       | Ns    |
| Lactose (%)        | 4.71±0.24     | 4.77±0.42       | 5.01±0.06       | Ns    |
| SNF (%)            | 8.13±0.62     | 8.25±0.67       | 8.54±0.08       | Ns    |
| Solid (%)          | 14.88±1.95    | 14.75±1.74      | 16.76±0.46      | Ns    |
| Protein (%)        | 4.46±0.59     | 4.52±0.4        | 4.58±0.01       | Ns    |
| Milk yield (L/day) | 858.16±431.47 | 1,092.48±516.39 | 1,337.86±136.37 | Ns    |

Note: n: number of samples; Ns: not significant (P>0.05), TT>CT>CC.

The genotypes obtained with two alleles were CC, CT and TT. This genotype was then associated with milk production and composition, as shown in Table 4. The TT genotype showed higher for milk yield and compositions compared to the CC and CT genotypes (P>0.05), TT>CT>CC. The result accordance with Wardani *et al.*

(2022) on Senduro goat using  $\beta$ -lactoglobulin gene that TT genotype had higher milk compositions than other genotypes. The results of Chiatti *et al.* (2007) showed significant association between genotypes and milk yield and composition in Orobica goats maintained in Alpine valleys (BB>AB>AA), with the same pattern of this present study. From their results they suggested that BB genotype could be used to exploit superior Orobica goat for improving milk yield, protein and casein percentages in the milk when cheese product is a selection objective.

## CONCLUSIONS AND RECOMMENDATIONS

The recent findings revealed that population of Senduro goat was polymorphic and in Hardy-Weinberg equilibrium based on *CSN3*|*A/w44I* locus. Milk yield and composition are higher for the goats with genotype TT than CT and CC. Goats having T allele tend to show higher milk yield and compositions. Extensive tracing of the TT genotype is needed for Senduro goats to obtain superior genetic goats in order to support breeding programs increasing milk yield and composition so that in the future it can increase farmers' income.

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1. Senduro goat is a local goat that until now distributed only in Senduro district, East Java, Indonesia.
2. Senduro goat is a local goat that is prioritized as a dairy goat breed in Indonesia, especially in East Java.

## AUTHOR'S CONTRIBUTIONS

TES and SS idea and design. AF, WAS and DW material sample collection and lab analysis. TES, SS, AF and DW write the manuscript. SS and TES revision.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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