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Polymorphism of B-Lactoglobulin (B-Lg) Gene and Its Association with Milk Yield and Milk Composition on Senduro Goats

Research Article

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ABSTRACT

The objective of this research was to examine the β-lactoglobulin gene polymorphism and its association with milk yield and composition in Senduro goats. A total of 60 lactation Senduro goats aged 2 to 4 years were used in this study. Milk yield and blood samples were collected from dairy goat farms in Burno and Kandangtepus Senduro villages. The 480 bp β-lactoglobulin gene DNA fragments were amplified using the polymerase chain reaction (PCR) method at 60 ˚C annealing for 35 cycles. The genotypes of the β-lactoglobulin gene were determined using the restriction fragment length polymorphism (RFLP) technique with the *Sac II* restriction enzyme. Association of β-lactoglobulin genotypes with milk yield and composition were analyzed using the ANOVA test. The result showed that the single nucleotide polymorphism (SNP) c.497G > A was detected. The β-lactoglobulin was polymorphic in Senduro goats. There were three genotypes (AA, AG, GG) and two alleles (A and G). The frequency of AA, AG, GG genotypes were 0.4, 0.52, 0.08, respectively, while the frequencies of A and G alleles were 0.66 and 0.34, respectively. There was no association between β-lactoglobulin gene polymorphism and milk yield and composition on Senduro goats. In conclusion, the genotype of the β-lactoglobulin gene did not effect on milk yield, protein, fat, density, lactose, salt, SnF, and total solid.

KEY WORDS allele, genotype, PCR, RFLP, SNP.

INTRODUCTION

Senduro goats have been registered by the Ministry of Agriculture decree number 1055/Ktps/SR. 120/2014 as an Indonesian local goat breed (Ministry of Agriculture, 2014). Senduro goat is a crossbreed goat from Etawah, Kacang, and Jawarandu goats (Gatot *et al.* 2019; Susilorini *et al.* 2020). The Senduro goats were originated in the Senduro Subdistrict, Lumajang Regency, East Java. Senduro goat are commonly raised by smallholders for sources of income and savings. The advantages of Senduro goats are prolific, high productivity, good adaptation, easy maintenance, dual-purpose type of meat and milk production, and produce milk about 1.3 ± 0.5 liters/day. Milk yield can be obtined by improving genetic quality through livestock selection. Currently, the selection is applied at the DNA level, namely marker assisted selection (MAS). This DNA level selection is more efficient, accurate, has short generation intervals, and allows genetic improvement. The β-lactoglobulin gene can be used as a marker gene for milk yield in animal breeding programs. The quality of milk is influenced by the quantity of milk, fat, and protein in milk (Volkandari *et al.* 2016). The physicochemical characteristics of milk are important for the efficient development of the milk industry and the marketing of its products. β-lactoglobulin locus has been studied as one of the genes that may affect the eco-

nomically important traits (El-Shazly *et al.* 2017). β-lactoglobulin is the major milk whey protein in the ruminants. β-lactoglobulin plays a protective role in the antioxidant nature of milk and has various biological functions to increase health and enhances immune responses. The biological activity of β-lactoglobulin is a source of bioactive peptides and antimicrobial activities against bacteria (El Hanafy *et al.* 2015; Tai *et al*. 2016). The impact of genetic polymorphism in the β-lactoglobulin gene on the protein content of milk has an important effect on products such as cheese (Hedayat-Evrigh *et al.* 2020). β-lactoglobulin gene in cattle and goats are located on chromosome 11 (11q28) consisting of exon 7 and intron 6, while β-lactoglobulin gene in sheep is located at chromosome 3 (Jawasreh *et al.* 2019; Joshi *et al*. 2019). β-lactoglobulin gene has a molecular weight of 18 kDa consists of 162 amino acids and forms stable dimers in milk (Gharedaghi *et al.* 2016).

Protein polymorphism of β-lactoglobulin gene in the first milk protein was found by Aschaffenburg and Drewy in 1995 (Rahmatalla *et al.* 2019) and is still being actively developed. Previous studies of β-lactoglobulin gene polymorphism and milk traits in goat were reported by El-Hanafy *et al.* (2015), Gharedaghi *et al*. (2016), Morammazi *et al.* (2016), Raja *et al.* (2019). A single nucleotide polymorphism (SNP) in the β-lactoglobulin gene has been reported in some goat breeds, such as Saanen goats (Ambarwati *et al.* 2019), Balady hybrid and damascus breeds (Kahilo *et al.* 2014), and African goat (Lekerpes *et al.* 2014), but no SNP information was reported in β-lactoglobulin gene in Senduro goat breeds.

The utilization of the β-lactoglobulin gene for selection based on genetic markers has been widely used in various types of dairy goats. However, unfortunately it was not studied on the Senduro goat. Therefore the current study was designed to examine the polymorphism of the β-lactoglobulin gene and its association with milk yield and composition in Senduro goats.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 95 blood samples from five different breeds were used in this study to identify polymorphism in the β-lactoglobulin gene (Table 1). For the association study, there were only 42 lactation Senduro goats aged 2 to 4 years (parity 1 to 4). Blood samples were collected from the jugular vein using a vacutainer needle. The sample was put into a labeled EDTA tube. Blood samples were kept in a cooler box immediately. Milk yield was collected every morning and analyzed for milk composition using Lactoscan (milk analyzer). Blood samples were extracted using Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the recommended protocol. The extracted DNA was labeled and stored at –20 ˚C. The DNA quality was checked using electrophoresis in 1.5% agarose gel and was measured using a spectrophotometer (NanoDrop ND-1000, Thermo Scientific, USA).

PCR amplification β-lactoglobulin gene

The primer sequences of the β-lactoglobulin gene in this study were designed by authors based on the GenBank database (accession number Z33881.1). The primers were: forward '5-CTGTGAGAATGGCTGGAAGC-3'; reverse '5-GGAGAGCACAGGTTTATGCC-3' amplifying the exon 7 of the β-lactoglobulin gene.

The 15 µL reaction volume included DNA template (50-100 ng/µL), primers (10 PM/µL), 1X Taq Polymerase (Promega), and Nucleus Free Water (Promega). The reaction conditions of PCR machine (Bio-Rad T100 Thermal Cycler, USA) were 95 ˚C for 5 min (initial denaturation), 35 cycles reaction of 95 ˚C for 30 sec (denaturation), 60 ˚C for 45 sec (annealing), 72 ˚C for 1 min (extension) and 72 ˚C for 5 min (final extension).

Genotype β-lactoglobulin gene using RFLP

An SNP of the β-lactoglobulin gene was detected by digesting 5 µL of the 480 bp PCR product with 4 U of the *Sac II* (Thermo Fisher Scientific Inc.) at 37 ˚C for 16 hours. The digested products were electrophoresed for 30 min at 100 V on a 2% agarose gel with Nucleic Acid Dye (Diamond, Promega). The gels were visualized under blue light (Glite 965 GW, Taiwan) to determine the genotypes.

Statistical analysis

Genotype and allele frequency

The allele and genotype frequency were calculated by using Nei and Kumar (2000) as follows:

$X_{ii} = n_{ii} / N$

$$X_i = \frac{(2n_{ii} + \Sigma_{i \neq j} n_{ij})}{2N}$$

Where:
X_{ii}: iith genotype frequency.
X_i: ith allele frequency.
N_{ii}: number of the sample of ii genotype.
N_{ij}: number of the sample of ij genotype.
N: total samples.

Heterozygosity

Genetic diversity can be determined by calculating the heterozygous value of the observations (H0) and expected heterozygosity (He) (Nei and Kumar, 2000):

$$H_0 = \Sigma_{i=1} \frac{n_{ij}}{N}$$

$$H_e = 1 - \sum_{i=1}^{q} x_i^2$$

Where:
H_0: observation heterozygosity.
n_{ij}: number of heterozygous animal.
N: number of observed animal.
He: expectation heterozygosity.
Xi: frequency of allele.
q: total alleles.

Hardy Weinberg equilibrium (HWE)
The Hardy Weinberg equilibrium was tested with chi–square (Harlt and Clark, 1997):

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

Where:
x^2: Hardy Weinberg equilibrium test.
O: observed number of iith genotype.
E: expected number of iith genotype.

Genotypic association with milk yield and composition
The association between the polymorphism with milk yield and composition (protein, fat, density, lactose, salt, SnF, and total solid) were analyzed using the General Linear Model (GLM) model in the ANOVA program of the Windows IBM SPSS Version 26 (IBM Corporation, New York, USA).

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

Where:
Y_{ijk}: milk yield and milk composition.
μ: overall mean.
G_i: fixed effect of ith genotype (i=AA, AG, and GG).
P_j: fixed effect of jth parity (j=1, 2, 3 and 4).
ε_{ijk}: random error.

RESULTS AND DISCUSSION

A 480 bp fragment of the β-lactoglobulin gene on the exon 7 was amplified by PCR (Figure 1). A pair of primers stretched along from nucleotide position 6374 to 6853 and the restriction enzyme was used to cut the PCR product of the β-Lactoglobulin gene, namely *Sac II*. The restriction site of *Sac II* (CCGCGG) was located at 6751 bp (Figure 2). Lekerpes *et al*. (2019) showed that two single nucleotide substitution were found in exon 7 at position 6705 (G/A) and 6751 (G/A) in African goat. This was in consistent with the present study, the SNP c. 497G > A of the β-Lactoglobulin gene was detected. The nucleotide change from G to A causes the codon CCG → CCA (Proline→Proline). This mutation did not change in the amino acids so it can be categorized as a silent mutation. It means that changes in DNA nucleotide bases have no effect on protein structure. The SNP at position 6751 in previous study, Ambarwati *et al*. (2019) resulting in two alleles (A, G) and three genotypes (AA, AG, GG) but the β-lactoglobulin genotyping association with milk protein and production in Saanen goats showed no significant differences. In another association study, Kahilo *et al*. (2014) revealed results that the β-lactoglobulin AB genotype a significantly higher milk production in balady hybrid breeds while in the Damascus breeds, AA genotype was significantly superior in milk production. In this study, the digested PCR product generated three patterns and two alleles (A and G alleles). The restriction patterns were singles fragment of 480 bp for AA genotype, three fragments of 480, 378, and 102 bp for AG genotype, and two fragments of 378 and 102 bp for GG genotype (Figure 3). In this study, the difference between the DNA bands of the AG and GG genotypes based on the top band of AG genotype (480 bp) is brighter than the lower band, while GG genotype (378 bp and 102 bp) showed that the lower band was brighter than the top band. In the GG genotype sample, the amount of DNA concentration was probably very high so that the restriction enzymes could not cut all the number of DNA copies.

Table 1 Blood sampel of goats based on five goat different breeds

Breed	Total	Location
Senduro	60	Burno and Kandang Tepus Village, Senduro Subdistrict, Lumajang Regency, East Java
PE	7	Agro Techno Park Universitas Brawijaya, Jatikerto Village, Kromengan Subdistrict, Malang Regency, East Java
Saanen	8	Animal Husbandry Training Center, Songgokerto Village, Batu Subdistrict, Batu Regency, East Java
Kacang	10	Sawohan Village, Buduran Subdistrict, Sidoarjo Regency, East Java
Boer	10	Sumbersekar Field Laboratory Faculty of Animal Science Universitas Brawijaya, Sumbersekar Village, Dau Subdistrict, Malang Regency, East Java

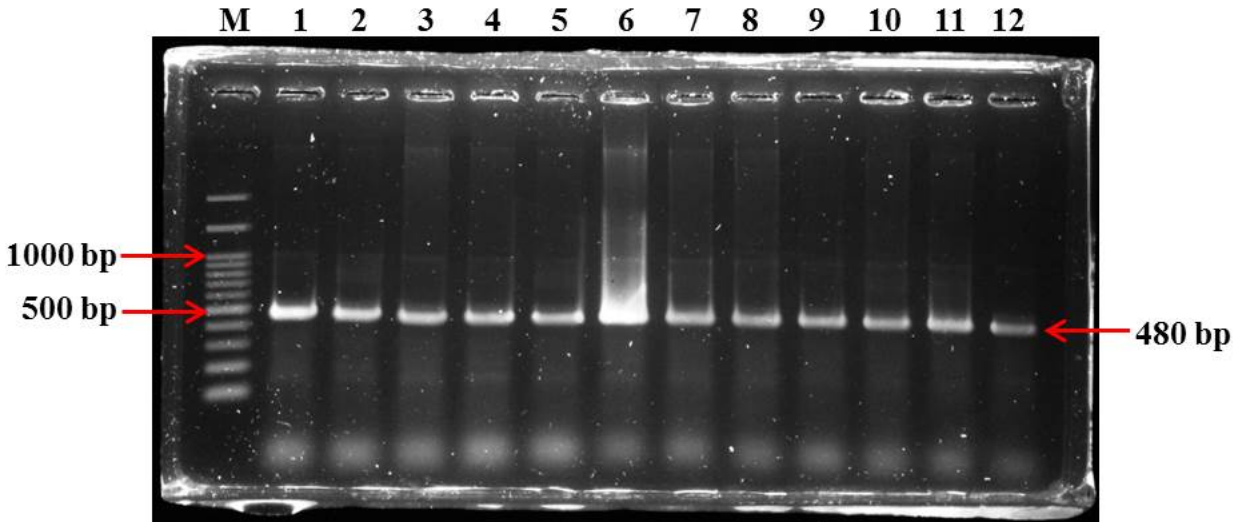

Figure 1 Amplification of β-lactoglobulin gene in Senduro goat (480 bp)
M: 100 kb DNA ladder, lane 1-12: samples

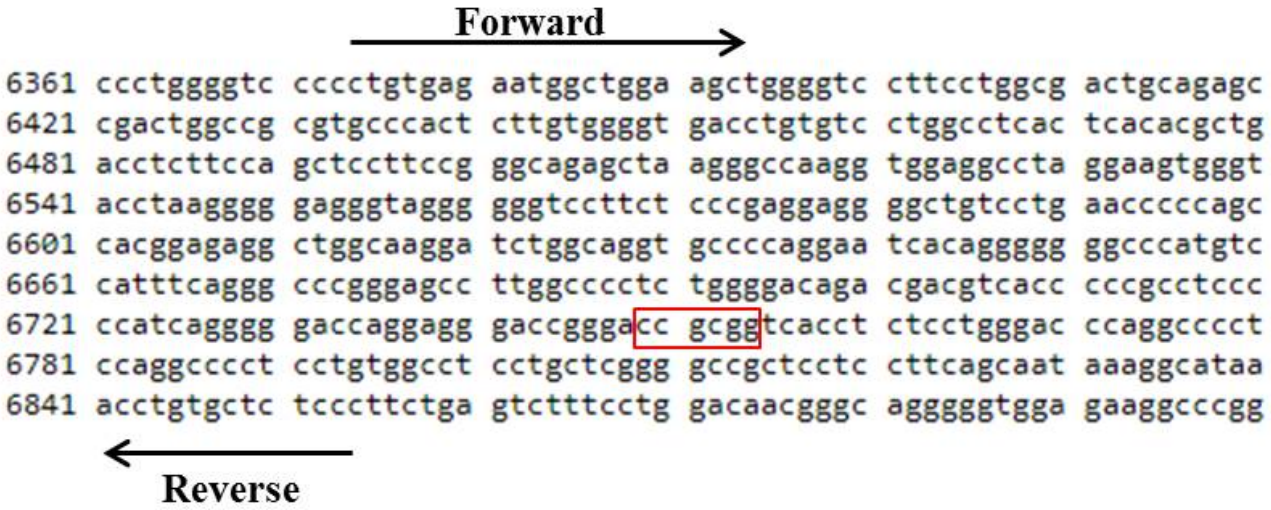

Figure 2 Primer and *Sac II* restriction site of β-lactoglobulin gene based on GenBank Accesion No. Z33881.1

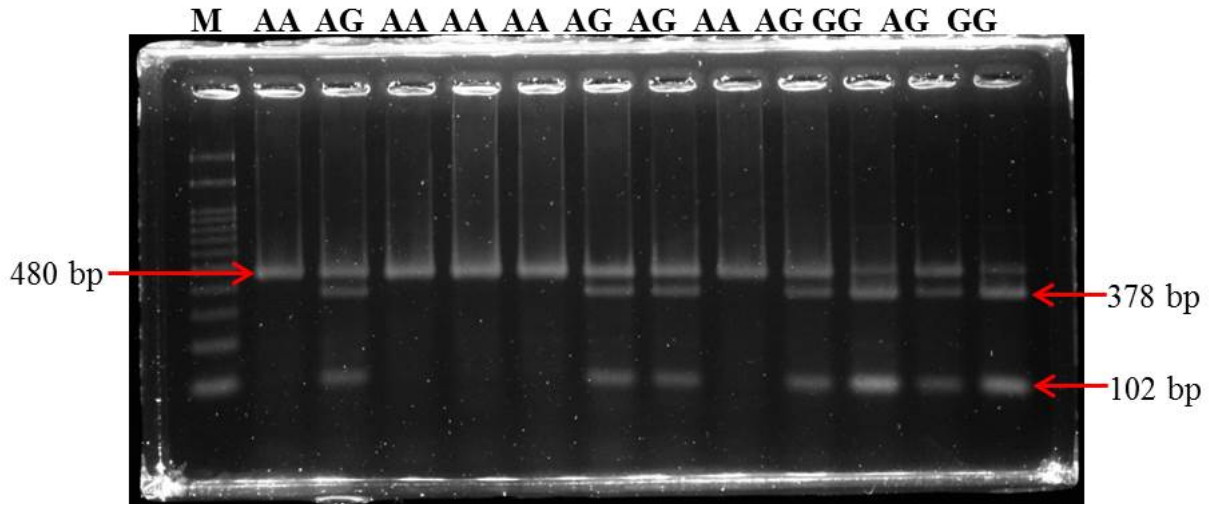

Figure 3 The RFLP pattern for β-lactoglobulin gene with *Sac II* restriction enzyme, M: Ladder 100 kb

Therefore, the amount restriction enzymes should be increased. RFLP becomes one of the ways to study genetic polymorphism. RFLP has been successfully used for the study of diversity and widely reported in Saanen goat, sheep, cattle, buffalo, pig, and chicken (Dincel *et al.* 2016; Feitosa *et al.* 2017; Furqon *et al.* 2017; Gunawan *et al.* 2018; Sihite *et al.* 2019; Nafiu *et al.* 2020; Rashaydeh *et al.* 2020).

The genotype and allele frequencies of the β-lactoglobulin gene in Senduro goats are shown in Table 2. The AG genotype was the highest frequency of Senduro, Saanen, Kacang and Boer goats. Meanwhile, PE goats had a higher AA genotype frequency than AG genotype frequency. The A allele was more frequent than the G allele in all the studied breeds. This was similar to the results of research by El Hanafy *et al.* (2010) that the genotype frequency of AB was the highest in Barki and Damascus and the A allele frequency was higher than the B allele frequency. There were A allele and G allele in this study shows the presence of heterozygous individuals, this was found in the presence of polymorphisms β-lactoglobulin gene at the study were located. Polymorphic will occur when an allele has an allele frequency equal to or less than 0.99, if the allele frequency is equal to or less than 0.01 then it is monomorphic. A population can be said to be diverse if it has more than one allele in one locus (Allendorf *et al.* 2010). Polymorphic results can be used as genetic markers, the use polymorphic genes as detecable molecular markers is an alternative methods of trait selection. The population of the five different breeds in this study was in genetic balance (Table 2).

Results of testing the Hardy Weinberg Equilibrium β-lactoglobulin gene with chi-square (X^2) showed that the population of the five different breeds in the Hardy Weinberg equilibrium (X^2 count < X^2 0,05). A population can be said to be balanced if there is no selection, migration, mutation, inbreeding, and random drift. The Chi-Square test was used to compare the number of genotypes observed with the expected genotypes (Lekerpes *et al.* 2014).

Milk yield and composition mean value for polymorphism β-lactoglobulin gene in Senduro goats were presented in Table 3. No significant association was found in milk yield, protein, fat, density, lactose, salt, SNF and total solid. The result of this research can be caused by differences in livestock breeding types, the limited number of animals, and interactions between genetics and the environment. Genetic environmental interactions can inhibit the expression of the superior traits that should be indicated by a particular genotype (Ambarwati *et al.* 2019).

Several studies have shown that the β-lactoglobulin gene polymorphism was not associated with milk yield in goats (Gheradaghi *et al.* 2016), sheep (El-Shazly *et al.* 2012), cattle (Barbosa *et al.* 2019).

Research about the association between polymorphism β-lactoglobulin gene with milk yield has been studied by El-Hanafy *et al.* (2010), El-Hanafy *et al.* (2014), Kahilo *et al.* (2014), El-Hanafy *et al.* (2015), Mahmood *et al.* (2016), and Işik *et al.* (2017). The average Senduro goat milk yield is 0.9 kg (Table 3), it is similar to the milk yield of Ettawa goats which is around 0.2-1.2 kg/day (Praharani *et al.* 2015).

Table 2 Genotype and allele frequencies, heterozigosity and Hardy-Weinberg equilibrium of β-lactoglobulin gene in five goat different breeds

Breeds	N[1]	Genotype frequency			Allele frequency		Ho	He	X^2 value[1]
		AA	AG	GG	A	G			
Senduro	60	0.4	0.52	0.08	0.66	0.34	0.52	0.4488	1.33^{ns}
Saanen	7	0.125	0.875	0	0.56	0.44	0.875	0.493	3.28^{ns}
PE	8	0.57	0.43	0	0.79	0.21	0.43	0.33	0.23^{ns}
Kacang	10	0.4	0.5	0.1	0.65	0.35	0.5	0.45	0.1^{ns}
Boer	10	0.4	0.4	0.2	0.6	0.4	0.4	0.48	0.28^{ns}

[1] N= total.
[2] X^2 (0.05:1)= 3.842.
NS: non significant.

Table 3 Means of β-lactoglobulin gene polymorphism on milk yield and composition in Senduro goats

Parameter	Genotype			Max	Min	Mean	SE
	AA (n=16)	AG (n=23)	GG (n=3)				
Milk yield (kg)	923.82±361.71	977.9±537.46	951.01±436.70	2141.67	95.71	955.38	461.34
Protein (%)	2.59±0.36	2.67±0.23	2.78±0.24	3.1	1.34	2.64	0.28
Fat (%)	6.89±2.66	6.56±1.45	6.61±0.70	15.04	4.02	6.69	1.94
Density (%)	25.47±3.73	25.86±2.30	27.08±2.30	30.16	12.47	25.8	2.89
Lactose (%)	4.72±0.32	4.76±0.35	4.92±0.43	5.73	3.94	4.75	0.34
Salt (%)	0.77±0.06	0.78±0.06	0.81±0.071	0.89	0.58	0.78	0.06
SnF (%)	8.15±0.71	8.20±0.56	8.58±0.78	9.34	5.88	8.21	0.63
Total Solid (%)	15.03±2.32	14.77±1.52	15.19±1.41	20.92	11.79	14.9	1.82

SE: standard error.

The average milk yield of various goat breeds such as Saanen, Toggenburg, Alpine, Anglo Nubian, Damascus and Boer is 2.5 kg, 3.46 kg, 2.66 kg, 0.9 kg, 1.88 kg, and 1.72 kg, respectively (Norris *et al*. 2010; Escareño *et al*. 2012; Ferro *et al*. 2017). Thus, the breeding program still needs to be developed. Milk yield was affected by goat breed, age, body condition score (BCS), parity, stage of lactation, feed nutrition, udder morphology, milking frequency, management systems, environmental conditions, and disease (Goetsch *et al*. 2011; Susilorini *et al*. 2014; Upadhyay *et al*. 2014; Idowu and Adewumi, 2017).

According to El-Shazly *et al*. (2012), the β-lactoglobulin gene polymorphism did not significantly effect milk composition in Saudi Arabian sheep. In contrast, several studies have stated that the β-lactoglobulin gene affects the composition of milk by Jawasreh *et al*. (2019) that the AA, AB, and BB genotypes showed were associated with fat, SNF, protein, and lactose. Hedayat-Evrigh *et al*. (2020) in Khalkhali goats, the genotypes of β-lactoglobulin gene significantly influenced the milk composition parameters, i.e. fat, protein and solid material percent but had no significant effect on lactose percentage of obtained milk samples. This different of result may be caused by breed differences, population size, frequency distributions of genetic variants, the structure of data analysed, the model used for statistical analysis and a failure to consider the relationship among animals (Karimi *et al*. 2009). The quality of milk depends on milk composition, milk composition depends on many factors, such as breed/genetic variant, age, parity, lactation stage, season of birth, period, udder size, environment, feed nutrition, management system, and health status (Toni *et al*. 2011; Gelasakis *et al*. 2016; Verma *et al*. 2017; El- Tarabany *et al*. 2018; Mohsin *et al*. 2019; Verma *et al*. 2019).

CONCLUSION

Based on the results of this study, it can be concluded that the polymorphism of β-lactoglobulin gene in Senduro goats is polymorphic. This study also showed the existence of genetic polymorphism at β-lactoglobulin gene in Senduro goat breed for the first time. The β-lactoglobulin genotype was not associated with milk yield and composition, but in the GG genotype resulted higher protein content descriptively. Therefore, the next study is needed to add up the sample items and uniformity in livestock populations to find out the effect of β-lactoglobulin gene polymorphism on Senduro goats.

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