# Associations between Lactoferrin (LTF) gene polymorphism exon 4 and milk compositions in Senduro goat

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**Abstract.** This research aimed to analyze the polymorphism of Lactoferrin (LTF) gene exon 4 associated with milk compositions in Senduro goats. A total of 42 DNA samples and milk compositions from Senduro goats were used in this study. The DNA sequence was amplified using Polymerase Chain Reaction (PCR) with a pair of primers. Genotyping was carried out using DNA sequencing and analyzed using FinchTV 1.4.0 and MEGA 6.0. In this research, the results showed that there were three genotypes (CC, CT, and TT) and two alleles (C and T). The frequencies of CC, CT, and TT genotypes were 0.381; 0.452; and 0.167, respectively. Furthermore, the frequencies of C and T alleles were 0.607 and 0.393, respectively. The genotype polymorphism did not affect on milk compositions. In conclusion, there was no association between polymorphism of LTF gene exon 4 and milk compositions in Senduro goats.

## 1 Introduction

Senduro goat is one of local goats in Indonesia. This breed is commonly raised for two purposes; milk and meat production [1]. As a dairy goat, the milk production of Senduro goat is 0.80-1.80 liter/head/day based on The Indonesian National Standard [2]. The variation of milk production could be affected by genetic and environmental factors. The animal selection could be conducted to reach optimal genetic quality that increase milk production. Commonly, the selection can be performed based on the phenotype and genotype.

The animal selection based on genotype can be conducted using molecular genetic approach in DNA level. Some functional genes associated with economical traits can be investigated through this approach. One of gene controlled specific protein in milk composition is Lactoferrin gene (LTF). Lactoferrin is a protein found in milk and colostrum that have biological functions in iron transport, antibacterial, antiviral, antimicrobial, and antiparasitic effects [3]. Some studies reported that polymorphism of LTF gene was analyzed and associated with milk composition [4,5]. The previous study explained that the variation of SNP in exon 4 (c.407C>T) related to milk composition on goats in Baghdad [6].

The genetic selection related to milk production and composition was not performed on Senduro goat. Regarding this issue, the genetic molecular approach can be conducted to

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improve the genetic quality. Furthermore, this research aims to analyze the polymorphism of Lactoferrin (LTF) gene exon 4 associated with milk compositions in Senduro goats.

## 2 Materials and methods

### 2.1 Data Collection and Animal Management

A total of 42 Senduro goats were used in this study consisted of 9, 22, 11 ewes on 2.0; 3.0 and 4.0 years old, respectively Milk productions and compositions of Senduro goats were collected at Senduro, Lumajang Regency, East Java from the first lactation to the fourth lactation. Milk compositions were analyzed in Laboratory of Dairy Sciene, Faculty of Animal Science, Universitas Brawijaya. Genetic molecular and data analysis was conducted in Laboratory of Animal Biotechnology, Faculty of Animal Science, Universitas Brawijaya. Senduro goats were fed and maintained under similar management and feeding system. Goats had free access to consume water and were housed in a barn individually.

### 2.2 DNA Isolation and Amplification

DNA was isolated from blood samples collected from Vena jugularis using vacutainer needles and Ethilenediaminetetraacetic Acid (EDTA) tubes. Goat genome was isolated using Genomic DNA Mini Kit for blood and cultered cell (Geneaid Biotech Ltd., China) and according to [7] method. The DNA quality and quantity were analyzed using 1.5% agarose gel electrophoresis and NanoDrop ND-1000 Spectrophotometer (Thermo ScientificTM, Massachusetts, USA) respectively.

DNA sequence of LTF gene exon 4 was amplified using PCR method. A pair of primers were used in this study consisted of forward and reverse primer (F: 5'- GCT AAG AGC TCT CGA TTC TC-'3 and R: 5'- CAG AGA GAA ATG AAC CAG GG-'3). These primers were designed to amplify a 541 bp fragment according to the goat genomic sequence in GenBank (accession number FJ609300). A solution mixture used in this study consisted of 1 µL DNA template (50 ng/µL), 0.3 µL of each primer (10 pmol/ µL), 15 µL Go Taq Green Master Mix (Promega, USA), and 14.4 µL Nucleus Free Water (NFW). The PCR method was conducted in Bio-Rad T100™ Thermal Cycler (Bio-Rad, USA) and the machine condition followed [8]. The machine condition was set in 5 steps; a pre-denaturation temperature at 94°C for 5 min, 35 cycles of 94°C for 10 s (denaturation), 60°C for 20 s (annealing), 72°C for 30 s (extension), and a final extension at 72°C for 5 min. The PCR products were checked using electrophoresis in 1.5% agarose gel and visualized using Glite 965 GW imaging system (Pacific Image Electronics Co., Ltd.)

### 2.3 Genotyping

Genotyping was performed based on the result of targeted sequencing. A total of 20 µL PCR product was used for targeted DNA sequencing using forward and reverse primers. The DNA sequencing was conducted using Applied Biosystem Genetic Analyzer platform through 1st BASE DNA sequencing services (Selangor, Malaysia). The sequencing chromatogram and alignment were analyzed using FinchTV and MEGA 6.0 respectively. The double peak found in the sequencing chromatograms indicated that the sample was heterozygous.

### 2.4 Data Analysis

The genotype and allele frequency were analyzed following the formula of [9]:

Genotype frequency:

$$xii = \frac{nii}{N}$$ (1)

Allele frequency:

$$xi = \frac{2nii + \sum nij}{2N}$$ (2)

Where:
χii  = frequency of ii genotype
χi   = frequency of i allele
nii  = number of individuals with ii genotype
nij  = number of individuals with ij genotype
N   = number of samples

The Hardy-Weinberg equilibrium was analyzed using Chi-square test [10]:

$$\chi2 = \sum \frac{(O-E)2}{E}$$ (3)

Where:
χ2   = HWE test
O   = the observed number of genotypes
E   = the expected number of genotypes
α   = 5% significance level
df   = number of genotype probabilities – number of alleles

LTF gene association with milk compositions. The association study between LTF gene polymorphism and milk compositions were analyzed using General Linear Model procedure in SPSS ver. 26.0. The mathematical model used in this research was:

$$Yijk = \mu + Gi + Pj + \varepsilon ijk$$ (4)

Where:
Yij = observation value (milk compositions) of jᵗʰ goat and iᵗʰ genotype
μ   = overall mean
Gi  = the effect of ith genotype
Pj  = the effect of jth parity
εij  : random error

## 3 Results and discussion

Lactoferrin (LTF) is an 80 kDa glycoprotein and had capability to bind and transfer irons. This protein is closely related to the serum transferrin gene family [4]. The previous studies were reported the biological functions of LTF to against bacteria [11], viruses [12], inflammation [13], and tumors [12]. LTF gene is a multifunctional gene for milk protein compositions, skeletal structure, and resistance against mastitis infection [4, 6, 14, 15].
In this research, a 541 bp of LTF gene sequence in exon 4 was successfully amplified using PCR technique at fixed annealing temperature of 60ºC. The visualization of PCR product was performed in 1.5% agarose gel electrophoresis. In this research, a Single Nucleotide Polymorphism (SNP) was successfully investigated at c.407C>T (rs636609419). This SNP

was a missense mutation and changed the amino acid from Threonine to Methionine (Thr136Met).

According to patterns of PCR product cut by MspI enzyme, there were three genotypes found in current study. The frequencies of CC, CT, and TT genotypes were 0.381; 0.452; and 0.167, respectively. Furthermore, the frequencies of C and T alleles were 0.607 and 0.393, respectively. Based on these allele frequencies, the SNP c.407C>T in this research was polymorphic. The calculated chi-square was lower than the chi-square table of 3.84, descriptively. This indicated that the population of Senduro goats in Senduro village was in Hardy-Weinberg Equilibrium. According to the breeding management of Senduro goats in Lumajang regency, the goats allowed to have random mating and there was no selection program. This situation probably affected the equilibrium that there was no change of allele frequencies in a gene pool from one generation to the next.

**Table 1**. Genotype frequencies, allele frequencies and chi-square value

| Breed | N | Genotype Frequency | | | Allele Frequency | | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| | | CC | CT | TT | C | T | |
| Senduro | 42 | 0.381 | 0.452 | 0.167 | 0.607 | 0.393 | $0.112^{ns}$ |

**Table 2**. Milk production and compositions of Senduro goat

| Parameter | Genotype | | |
|---|---|---|---|
| | CC | CT | TT |
| Milk production (L) | 974,85±381.50 | 1121.69±537.50 | 716±457.17 |
| Fat (%) | 26.42±1.77 | 25.15±3.60 | 26.83±2.06 |
| Lactose (%) | 4.79±0.38 | 4.69±0.31 | 4.85±0.33 |
| Solid non-fat (%) | 0.78±0.05 | 0.77±0.06 | 0.80±0.06 |
| Solid (%) | 8.27±0.55 | 8.07±0.70 | 8.45±0.61 |
| Protein (%) | 4.53±0.34 | 4.40±0.61 | 4.70±0.36 |

According to Table 2, the results showed that there was no association between LTF gene polymorphism and milk production and composition in this study (P>0.05). This case was probably affected by some factors such as the variation of parity, a few numbers of samples and different animal management. The goats with CT genotype had the highest milk production among genotypes, descriptively. The goats with TT genotype showed the highest milk compositions (fat, lactose, SNF, solid, and protein) although they had the lowest milk production. It indicated the negative correlation between milk production and milk composition.

## 4 Conclusion

In this research, the polymorphism of LTF gene exon 4 (SNP c.407C>T) was not associated with milk production and its composition. The SNP was polymorphic and needed further investigation on larger number of samples. This SNP was potential to be developed as a molecular marker to support the animal selection program that will improve the genetic quality.

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