# Genetic Variants of Milk Protein Genes and Their Association with Milk Components in Holstein Friesian Cattle

Asmarasari SA[1,3*], Sumantri C[2], Gunawan A[2], Taufik E[2], Anggraeni A[3]

[1]*Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*
[2]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*
[3]*Indonesian Research Institut for Animal Production, PO Box 221, Bogor 16002, Indonesia*
**E-mail:* santi_ananda@yahoo.com

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**ABSTRAK**

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Kandungan protein dalam susu sapi menjadi indikator kualitas susu sapi di masa yang akan datang. Atas dasar tersebut, perbaikan genetik untuk menghasilkan sapi perah Friesian Holstein (FH) menjadi penting dilakukan. Tujuan dari penelitian ini adalah untuk mengevaluasi varian genetik dari gen protein susu dan pengaruhnya terhadap sifat komponen susu sapi FH. Sapi yang diamati sebanyak 100 ekor. Sapi FH yang digunakan memiliki status fisiologis periode laktasi 1-3 dan bulan laktasi 1-12. Variasi genotipe gen protein susu diidentifikasi menggunakan metode RT-PCR *(Real Time-Polymerase Chain Reaction)*. Analisis komponen susu yang dilakukan meliputi kandungan protein, lemak, laktosa, dan *Solid Non Fat* (SNF) dengan menggunakan alat *Lactoscan*. Analisa genotyping dimulai dari ekstraksi DNA dan amplifikasi gen menggunakan metode RT-PCR. Hasil penelitian menunjukkan bahwa kadar protein susu dipengaruhi ($p<0.05$) oleh varian genetik gen CSN1S1-192 dan CSN2-67. Kadar lemak susu dipengaruhi ($p<0.05$) oleh varian genetik gen CSN1S1-192 dan CSN3. Sementara itu, kadar SNF susu dipengaruhi ($p <0.05$) oleh varian genetik gen CSN-BMC9215, CSN-BMC6334, CSN1S1-14618, CSN2_67, dan CSN3. Kadar laktosa susu dipengaruhi ($p<0.05$) oleh varian genetik gen CSN-BMC9215 dan CSN2-67. Disimpulkan bahwa varian genetik dari gen protein susu memiliki hubungan dengan komponen kimiawi susu sapi (protein, lemak, SNF, dan laktosa).

**Kata Kunci**: Varian Genetik, Gen Protein, Komponen Susu

**ABSTRACT**

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Protein content in milk is an important indicator of milk. Accordingly, genetic improvement to produce Holstein Friesian (HF) dairy cattle is important. The objective of this study was to evaluate the genetic variant of milk protein genes and its effect on milk component traits of Holstein Friesian (HF). A total of 100 HF were used in this study. The HF cattle used have physiological status in the lactation period 1 up to 3 and lactation change of 1 up to 12 months. Genotype variants of milk protein genes were identified using Real Time-Polymerase Chain Reaction method. Analysis of milk component was carried out covering the component of protein, fat, lactose, and solid non-fat (SNF) by using a milk quality measuring device (Lactoscan). Genotyping of cattle blood samples consisted of DNA extraction, genes amplification using the RT-PCR method. The result showed that protein milk was significantly affected ($p<0.05$) by the genetic variants of CSN1S1-192 and CSN2-67 genes. Fat milk was significantly affected ($p<0.05$) by the genetic variants of CSN1S1-192 and CSN3 genes. Meanwhile, solid non-fat milk was significantly affected ($p<0.05$) by the genetic variants of CSN-BMC9215, CSN-BMC6334, CSN1S1-14618, CSN2_67, and CSN3 genes. Lactose milk was significantly affected ($p<0.05$) by the genetic variants of CSN-BMC9215 and CSN2-67 genes. It was concluded that genetic variants of the milk protein genes have an association with the component of cow's milk (protein, fat, solid non-fat, and lactose).

**Key Words:** Genetic Variant, Protein Genes, Milk Component

## INTRODUCTION

Recently, genetic improvement in dairy cattle is not mainly to increase the amount of milk production but also to increase milk quality which is the high milk protein content. Milk has high-quality proteins, due to having sufficient amino acids to support the function of proteins in the human body (Davoodi et al. 2016). Franzoi et al. (2019) revealed that milk protein component is influenced by several factors, i.e., breed, lactation, season, and genetic polymorphism. Fresh milk with high protein is needed to meet the people nutrition requirement. The right component of milk protein is very useful to overcome health problems,

such as hypoallergenic. Besides, high protein milk is needed in the milk processing to produce high quality of dairy products. Therefore, genetic improvement is needed to produce Holstein Friesian cattle which have high milk protein carrier properties. One method for genetic improvement is a method of genotype selection. Genotype selection method in breeding programs is one of the newest methods to improve dairy cattle characters. Ozdemir et al. (2018) stated that the component of milk protein has a relationship with genetic polymorphisms. Genetic improvements to high protein milk will provide a fast and accurate selection response if the selection is applied at the DNA (nucleotide) level.

Ferretti et al. (1990) revealed that the protein component of cow's milk is controlled by two family genes of casein and whey. Casein, the largest component of milk protein (78-82%) is controlled by four casein genes, i.e., αs1-casein, β-casein, αs2-casein, and κ-casein. The location of these genes is very close to each other along 250 kilobases (kb) on chromosome 6 cattle (BTA 6q31). The close relationship, causing the casein gene inheritance often acts as a cluster. The protein component is predominantly produced by the αS1-casein (30.6%) and β-casein (28.0%) genes. Ferretti et al. (1990) stated that each gene was also known as CSN1S1, CSN2, CSN1S2, and CSN3 name genes. Farrell et al. (2004) explained that whey is a component of milk protein in a lower portion (12-18%). Laible et al. (2016) revealed that milk protein genes have the potential to be explored for genotypic selection to improve the cow's milk component. Bhat et al. (2019) added that the milk protein gene was reportedly relating to the nature of the cow's milk component. An association study between milk protein genes and milk components was carried out to see the impact of milk quality that occurs from the molecular breeding process in increasing protein component in milk based on milk protein genes. Holstein Friesian (HF) is a type of dairy cattle that is most widely imported by Indonesia. Many researchers stated that the milk component is an indicator of the quality of milk that determines the economic value of fresh milk (Davoodi et al. 2016; Thomas & Sasidharan 2015; Radika & Ajithkumar 2018; Ohlsson et al. 2017). Therefore, the aim of this study was to evaluate the genetic variants of milk protein genes and their effects on the milk component of HF cattle.

## MATERIALS AND METHODS

### Location and sample

This research was conducted at the dairy cattle experimental station in the Indonesian Research Institut for Animal Production which located at Ciawi-Bogor-West Java-Indonesia. A total number of 100 female HF cattle were used in this study with physiological status between 1-3 lactation periods and 1-12 months lactation. Milk samples were obtained after milking of individual cow, each sample was 500 ml. Analysis of milk component was carried out covering the component of protein, fat, lactose, and solid non-fat (SNF) by using a milk quality measuring device (Lactoscan). Milk sample collection for milk component traits analysis was done once a month, each sampling was done morning and evening. Sampling was carried out for 12 months of lactation with 3 periods of lactation. Blood samples were collected from lactating cows collected from the vena coxygea. Genotyping series consisted of DNA extraction from blood samples, genomic DNA binding, elution, primer design for amplification of milk protein genes of CSN1S1, CSN1S2, CSN2, and CSN3 was used Primer Express. Some factors that must be considered in the primer design, i.e., the primer temperature must be between 58-60$^{o}$C, choose a minimum of 3 best primer designs that at least likely to form a secondary structure (primer-dimers and harpin loops), check with BLAST (Basic Local Alignment Search Tool) to see the specificity of each set of target primers, the range of acceptable amplicons was 50 between 250 base pair (bp) but the most recommended was around 70-130 bp. The primer designs of the milk casein genes for the Real-Time PCR technique were shown in Table 1.

### Real-Time Polymerase Chain Reaction (RT-PCR)

DNA extraction was carried out from fresh HF cattle blood samples using the illustra™ blood genomicPrep Mini Spin Kit. Detection of nucleotide diversity was carried out using RT-PCR with the composition of the reagents as in Table 2. The process of amplification and detection of diversity was carried out under pre-denaturation conditions at a temperature of 95 ºC for 20 seconds and 40 cycles consisting of denaturation of 95 ºC for 3 seconds and annealing at 60 ºC for 30 seconds.

### Data analysis

Relationship of genetic variants of milk protein genes to the component traits of milk was carried out with a general linear model using SAS 9.1 software.

## RESULTS AND DISCUSSION

### Genetic variants of milk protein genes

Genetic variants of milk protein genes that were identified using the RT-PCR method were presented in Table 3. Based on the genotyping of the CSN1S1_192 locus of the αS1-casein gene, three genotypes have been

identified, i.e., AA, AG, and GG genotypes, therefore have two types of allele (A and G). Genotype identification showed that the highest frequency was AG genotype (0.67), followed by AA genotype (0.33) and GG genotype (0.00). Genotype identification for CSN2_9215 locus of the β-casein gene was identified and resulting in three genotypes, i.e., TT, TG, and GG genotypes, so that there were two types of alleles, i.e., T and G alleles. The study showed that the highest genotype was TG (0.40), followed by GG (0.25) and TT (0.25).

Meanwhile, for the CSN2_BMC6334 locus, three genotypes were identified, i.e., AA, AG and GG genotypes, resulting in two types of alleles, i.e., allele A and G. Observations on 100 HF cows showed that 25 heads of HF cows had AA genotypes, 44 heads had AG genotypes, and 31 heads had GG genotypes. Genotyping at the CSN2_67 locus of the β-casein gene resulted in three types of genotypes, i.e., AA, AC and CC genotypes; so that there were two types of alleles, i.e., alleles A and C. Genotype identification showed that the highest frequency was AC genotype (0.43), followed by CC genotype (0.35), and AA genotype (0.22).

Results of CSN1S2 locus genotyping, there were three types of genotypes, i.e., TT, TA, and AA genotypes, with two types of alleles, i.e. T and A. alleles. Genotype identification showed that the highest frequency was TT genotype (0.79), followed by TA genotype (0.16), and AA genotype (0.06). The CSN3 locus of the κ-casein gene produced 3 genotypes, i.e., GG (0.06), GT (0.36), and TT (0.58). For the CSN1S1_14168 locus, there were identified three types of genotypes, i.e., CC, CT, and TT, and two types of alleles were obtained, i.e. C and T alleles. Genotype identification showed that the frequency of CT genotypes was highest (0.47), followed by TT genotypes (0.30), while the lowest was CC genotype (0.23).

In this study, it was known that the A allele dominant at the milk protein genes. Volkandari et al. (2017) stated that polymorphic which AA genotypes and A allele at locus κ-casein were frequently commonly found in Holstein Friesian cattle. Zyiad & Fawzi (2014) reported that A and B genotypes were favorable alleles in Palestinian Holstein-Friesian cattle. Similarly, some researchers found that A allele in Holstein-Friesian was as dominant allele in milk protein genes (Volkandari et al. 2017; Barbosa et al. 2019; Huang et al. 2012).

Variant genetic of milk protein genes was influenced by cattle breed. Trakovickà et al. (2012) found that in the crossbred of Simmental and Holstein's cattle, A allele was frequently higher than the B allele. Meanwhile, Deb et al. (2014) reported that A allele more frequent than B allele in Frieswal cattle (HF x Sahiwal). Zepeda-Batista et al. (2015) added that B allele more frequent than A allele and E allele in Mexican Jersey cattle. Furthermore, Ren et al. (2013) revealed that the B allele was higher than the A allele. Many researchers from different countries reported that A allele was more dominant in milk protein genes at dairy cows than other allele (Djedovic et al. 2015; Brka et al. 2010).

Several researchers had proven that milk protein genes were highly polymorphic, containing very large amounts of SNP polymorphism (Schopen et al. 2011; Huang et al. 2012). Those studies informed that a direct relationship to protein from both single SNP and haplotypes in CSN1S1-CSN2-CSN1S2 with block haplotypes. In contrast, there was no significant relationship for a single SNP or haplotype in CSN3 blocks. This proves that CSN2 and CSN1S2 contain the highest locus in causing causative DNA variants (SNP). The most significant results were found for the CSN2_67 SNP C allele which was consistently related to protein superiority. SNP CSN2_67, as a substitution of C to A, on codon 67 in the ß-CN gene, caused histidine to replace proline in the amino acid sequence (Schopen et al. 2011; Huang et al. 2012; Cecchinato et al. 2018).

**Association of genetic variants of milk protein genes on milk component traits**

Examination of the effect of milk protein genes of CSN-BMC9215, CSN-BMC6334, CSN1S1-192, CSN1S1-14618, CSN1S2, CSN2-67, and CSN3 on milk protein levels are presented in Table 4. During 12 months lactation, it was known that genotypes of milk protein genes of CSN1S1-192 and CSN2-67 had significant effect ($p<0.05$) on protein levels of cow's milk. Milk protein levels from the AA genotype of CSN1S1-192 were higher (3.63%) than AG genotype (3.37%). Meanwhile, in the CSN2-67 gene, the highest levels of milk protein were obtained from the AA genotype (3.75%), then followed by the CC (3.73%) and AC (3.68%). Previously, Hamza et al. (2011) reported that CN genotypes had significant effect on milk protein component. Sigl et al. (2012) revealed that milk protein gene expression has close relationship to component of milk protein. Furthermore, Sigl et al. (2012) explained that the process of milk protein synthesis, including transcription, post-transcription, translation, and amino acid supply was controlled at various levels in mammary epithelial cells. The gene that codes for this protein is regulated by a complex interaction of peptides and steroids hormones, especially the lactogenic hormone prolactin, insulin, and hydrocortisone; and cell and cell-substratum

**Table 1**. Primer sequences of milk casein gene for the Real-Time Polymerase Chain Reactin (PCR) technique

| Gene | Gene Bank | Position | Primary Sequence (5'->3') | Temperature ($^0$C) | Mutation |
|---|---|---|---|---|---|
| CSN1S1 | X59856 | 26181 | F: CCATCATTCTCTGACATCC | 61.2 | G>A |
| | | Exon 17 | R: AGGCAACAATATGCAGTC | 61.6 | |
| | | | VIC:CTCTGAGAACAGTGGAAAGACTACTAT GCC | 74 | |
| | | | FAM: CTCTGAGAACAGTGAAAAGACTACTAT GCC | 70.7 | |
| CSN1S2 | M94327 | 13231 | F: GCCGAATAAACATCCTGTCAACT | 58 | A>T |
| | | Intron 13 | R: CCCCTAAACAACCAGAGAGATTCA | 59 | |
| | | | VIC : CCTTCACCATAGTACT | 67 | |
| | | | FAM: TTCACCATAGTTCTAC | 67 | |
| CSN3 | AY380228.1 | 13975 | F: GAAGAGGTTAAACAGAAAGATCAATAAGATAG | 58 | G>T |
| | | Intron 4 | R: GACCAAAAATCATGTAGACAGTGTGA | 58 | |
| | | | VIC: AACATTTTGAGAGTCTAGGC | 66 | |
| | | | FAM: TTTGAGATTCTAGGCAAC | 67 | |
| CSN2 | NW_001495211 | 9215 | F:5'-CTTATGCACAATTATTTCACCACATG-3' | 58 | G>T |
| | | Exon 1 | R:5'-TCAGTATTTTTCCCTCATATGCTCAT-3' | 58 | |
| | | | VIC:5'- CTCATTTCACATCTTG-3' | 67 | |
| | | | FAM: 5'-TCACATCGTGTTTTTGA-3' | 67 | |
| CSN2 | M55158.1 | 6334 | F: 5'-CAGGATGATTGAGAGACATGTATGC -3' | 59 | A>G |
| | | Intron 4 | R: 5'-ACAGTCCATAGGGTCATACAGAGTTG-3' | 59 | |
| | | | VIC :5' -TGCAAAGTTGCTTCAG-3' | 67 | |
| | | | FAM :5'- CAAAGTTACTTCAGCCC-3' T | 66 | |

Source: Huang et al. 2012

**Table 2**. Components and compositions reaction of the Real-Time Polymerase Chain Reactin (PCR) process

| Compositions reaction | Compositions (µl) |
|---|---|
| DNA | 5 |
| Taqman GTXpress Master Mix (2x) | 12.5 |
| Custom Taqman SNP Genotyping Assays | 1 |
| PCR grade water | 6.5 |
| Total volume | 20 |

**Table 3.** Genotype and allele frequencies of milk protein genes in Holstein Friesian cattle

| Gene | N | Genotype Frequency | | | Allel Frequency | |
|---|---|---|---|---|---|---|
| CSN1S1_192 | 98 | AA(0.33) | AG(0.67) | GG(0.00) | A(0.66) | G(0.34) |
| CSN-BMC9215 | 100 | TT(0.25) | TG(0.40) | GG(0.35) | T(0.45) | G(0.55) |
| CSN-BMC6334 | 100 | AA(0.25) | AG(0.44) | GG(0.31) | A(0.47) | G(0.53) |
| CSN2_67 | 98 | AA(0.22) | AC(0.43) | CC(0.35) | A(0.44) | C(0.56) |
| CSN1S2 | 90 | TT(0.79) | TA(0.16) | AA(0.06) | T(0.87) | A(0.13) |
| CSN3 | 90 | GG(0.08) | GT(0.29) | TT(0.63) | G(0.22) | T(0.78) |
| CSN1S1_14168 | 100 | CC(0.23) | CT(0.47) | TT(0.30) | C(0.47) | T(0.54) |

interactions. Olenski et al. (2010) reported that there was a favorable genetic relationship of the A2 allele of the CSN2 gene with cow's milk protein. Ozdemir et al. (2018) reported that CSN1S1 gene affected milk protein component. Milk protein level was different between the CSN1S1 genotypes (Mangia et al. 2019). Zhou et al. (2019) stated that milk component traits were associated with the CN gene family, including CSN1S1 and CSN1S2. Bonfatti et al. (2010) explained that haplotypes that include CSN2 genes has been shown to influence milk protein component, suggesting that inheritance units can reach large genomic regions. Meanwhile, Huang et al. (2012) reported that the A and C alleles were associated with lower κ-CN concentrations. The other finding showed that there was relationship between the k-CN B allele and high protein component in Holstein breeds (Mohammadi et al. 2013). Furthermore, some researchers reported that B allele had a favorable and significant effect on milk protein components (Morkūnienė et al. 2016; Caroli et al. 2009). Relation of month lactation to protein component was reported by several researchers. In several previous studies, it was found that there was an inconsistency of the effect of lactation month on milk protein component. Some researchers (Jónás et al. 2016; Gurmessa & Melaku 2012) reported that milk protein component significantly influenced by lactation month. Meanwhile, Sudhakar et al. (2013) reported that the protein component in milk did not change in different lactation months. Çobanoglu et al. (2016) revealed that the highest protein component occurred in the first three months of lactation, where after that there was a decrease in protein component along with the increase in lactation month.

The effect of milk protein genes on milk fat levels are presented in Table 5. It was known that the genotype which affecting significantly ($p<0.05$) cow's milk fat levels were the genotype from CSN1S1-192 and CSN3 genes. The genotype of the CSN1S1-192 gene affected milk fat levels at four different lactation months, i.e., $2^{nd}$, $7^{th}$, $8^{th}$, and $11^{th}$. There was an inconsistency in the influence of the genotype of the CSN1S1-192 gene. At the lactation months $2^{nd}$ and $11^{th}$, the AA genotype showed higher milk fat levels (4.17% and 4.16%) compared to AG genotype (3.89% and 4.08%). Conversely, at the lactation months $7^{th}$ and $8^{th}$, the AA genotype (4.1% and 4.21%) of fat levels showed lower compared to AG (4.23% and 4.28%). Meanwhile, in the CSN3 gene, the highest levels of milk fat were obtained from the CG genotype (4.35%), and then followed by the TT (4.39%) and GT genotype (4.18%). Hamza et al. (2011) reported that CN genotypes had significant effect on milk fat component. Previously, Ardicli et al. (2018) reported that CSN1S1 genotypes were associated to milk fat. Besides, Dagnachew et al. (2011) stated that CSN1S1 had an association with milk fat. Le Parc et al. (2010) stated that CSN1S1 had the main function of the casein transportation efficiency from endoplasm to compartment Golgi. Bugeac et al. (2013) reported that

**Tabel 4**. The effect of milk protein genes (CSN-BMC9215, CSN-BMC6334, CSN1S1-192, CSN1S1-14618, CSN1S2, CSN2-67, and CSN3) on least squares means of milk protein component (%) in Holstein Friesian (HF) cattle for 12 months of lactation

| Month of Lactation | Genotype | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CSN-BMC9215 | | | | CSN-BMC6334 | | | | CSN1S1-192 | | | CSN1S1-14618 | | | | CSN1S2 | | | | CSN2-67 | | | | CSN3 | | | |
| | CG | GT | TT | Sig | AA | GA | GG | Sig | AA | AG | Sig | CC | CT | TT | Sig | AA | TA | TT | Sig | AA | AC | CC | Sig | CG | GT | TT | Sig |
| 1 | 3.58 | 3.62 | 3.66 | NS | 3.64 | 3.62 | 3.58 | NS | 3.59 | 3.57 | NS | 3.61 | 3.62 | 3.6 | NS | . | 3.62 | 3.62 | NS | 3.66 | 3.62 | 3.59 | NS | 3.65 | 3.47 | 3.66 | NS |
| 2 | 3.52 | 3.64 | 3.65 | NS | 3.54 | 3.54 | 3.37 | NS | 3.63 | 3.37 | * | 3.58 | 3.48 | 3.39 | NS | 3.89 | 3.62 | 3.47 | NS | 3.56 | 3.48 | 3.46 | NS | 3.71 | 3.46 | 3.67 | NS |
| 3 | 3.52 | 3.64 | 3.65 | NS | 3.54 | 3.54 | 3.37 | NS | 3.63 | 3.37 | NS | 3.58 | 3.48 | 3.39 | NS | 3.89 | 3.62 | 3.47 | NS | 3.56 | 3.48 | 3.46 | NS | 3.71 | 3.46 | 3.67 | NS |
| 4 | 3.53 | 3.55 | 3.59 | NS | 3.59 | 3.54 | 3.56 | NS | 3.61 | 3.53 | NS | 3.55 | 3.54 | 3.6 | NS | . | 3.52 | 3.55 | NS | 3.57 | 3.51 | 3.59 | NS | 3.57 | 3.47 | 3.45 | NS |
| 5 | 3.53 | 3.55 | 3.59 | NS | 3.52 | 3.55 | 3.58 | NS | 3.56 | 3.54 | NS | 3.5 | 3.56 | 3.62 | NS | 3.6 | 3.55 | 3.54 | NS | 3.49 | 3.55 | 3.58 | NS | 3.62 | 3.54 | 3.62 | NS |
| 6 | 3.55 | 3.57 | 3.56 | NS | 3.55 | 3.52 | 3.59 | NS | 3.6 | 3.55 | NS | 3.53 | 3.57 | 3.55 | NS | . | 3.49 | 3.56 | NS | 3.54 | 3.56 | 3.59 | NS | 3.54 | 3.63 | 3.62 | NS |
| 7 | 3.53 | 3.61 | 3.67 | NS | 3.67 | 3.55 | 3.56 | NS | 3.59 | 3.64 | NS | 3.62 | 3.57 | 3.55 | NS | 3.48 | 3.49 | 3.58 | NS | 3.66 | 3.57 | 3.55 | NS | 3.77 | 3.63 | 3.63 | NS |
| 8 | 3.76 | 3.79 | 3.83 | NS | 3.76 | 3.69 | 3.66 | NS | 3.67 | 3.71 | NS | 3.67 | 3.76 | 3.62 | NS | 3.61 | 3.7 | 3.71 | NS | 3.75 | 3.68 | 3.73 | * | 3.89 | 3.73 | 3.67 | NS |
| 9 | 3.82 | 3.88 | 3.89 | NS | 3.75 | 3.68 | 3.69 | NS | 3.68 | 3.7 | NS | 3.74 | 3.71 | 3.68 | NS | 3.55 | 3.7 | 3.71 | NS | 3.72 | 3.69 | 3.66 | NS | 3.56 | 3.84 | 3.86 | NS |
| 10 | 3.81 | 3.8 | 3.9 | NS | 3.87 | 3.82 | 3.8 | NS | 3.86 | 3.81 | NS | 3.86 | 3.8 | 3.71 | NS | . | 3.81 | 3.84 | NS | 3.87 | 3.81 | 3.82 | NS | 3.85 | 3.77 | 3.8 | NS |
| 11 | 3.58 | 3.62 | 3.66 | NS | 3.64 | 3.62 | 3.58 | NS | 3.6 | 3.56 | NS | 3.61 | 3.62 | 3.6 | NS | . | 3.61 | 3.62 | NS | 3.65 | 3.62 | 3.58 | NS | 3.7 | 3.51 | 3.73 | NS |
| 12 | 3.91 | 3.9 | 4.03 | NS | 4.03 | 3.87 | 3.93 | NS | 3.87 | 3.98 | NS | 3.93 | 3.96 | 3.83 | NS | 3.9 | 3.83 | 3.94 | NS | 4.08 | 3.99 | 3.93 | NS | 4.34 | 4 | 3.95 | NS |

Sig = Significance, NS = Non Significant difference (P>0.05), * = Significant difference (P<0.05)

**Tabel 5**. The effect of milk protein genes (CSN-BMC9215, CSN-BMC6334, CSN1S1-192, CSN1S1-14618, CSN1S2, CSN2-67, and CSN3) on least squares means of milk fat component (%) in Holstein Friesian (HF) cattle for 12 months of lactation

| Month of Lactation | Genotype | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CSN-BMC9215 | | | | CSN-BMC6334 | | | | CSN1S1-192 | | | CSN1S1-14618 | | | | CSN1S2 | | | | CSN2_67 | | | | CSN3 | | | |
| | CG | GT | TT | Sig | AA | GA | GG | Sig | AA | AG | Sig | CC | CT | TT | Sig | AA | TA | TT | Sig | AA | AC | CC | Sig | CG | GT | TT | Sig |
| 1 | 4.12 | 4.11 | 4.21 | NS | 4.17 | 4.11 | 4.14 | NS | 4.15 | 4.06 | NS | 4.08 | 4.14 | 4.2 | NS | Nd | 4.08 | 4.15 | NS | 4.18 | 4.15 | 4.1 | NS | 4.35 | 4.18 | 4.39 | NS |
| 2 | 4.04 | 4.14 | 4.17 | NS | 4.09 | 4.1 | 3.94 | NS | 4.17 | 3.89 | ** | 4.05 | 4.06 | 3.97 | NS | Nd | 4.32 | 4.1 | NS | 4.06 | 4.07 | 3.95 | NS | 3.87 | 3.97 | 3.95 | NS |
| 3 | 4.04 | 4.14 | 4.17 | NS | 4.09 | 4.1 | 3.94 | NS | 4.17 | 3.89 | NS | 4.05 | 4.06 | 3.97 | NS | 4.32 | 4.1 | 4.01 | NS | 4.06 | 4.07 | 3.95 | NS | 3.87 | 3.97 | 3.95 | NS |
| 4 | 4.09 | 4.21 | 4.16 | NS | 4.12 | 4.19 | 4.11 | NS | 4.15 | 4.15 | NS | 4.17 | 4.12 | 4.15 | NS | Nd | 4.1 | 4.15 | NS | 4.1 | 4.19 | 4.12 | NS | 3.99 | 3.96 | 4.08 | NS |
| 5 | 4.17 | 4.22 | 4.09 | NS | 4.1 | 4.18 | 4.17 | NS | 4.14 | 4.13 | NS | 4.07 | 4.16 | 4.26 | NS | 4.1 | 4.09 | 4.13 | NS | 4.01 | 4.15 | 4.15 | NS | 4.16 | 4.07 | 4.28 | NS |
| 6 | 4.18 | 4.25 | 4.23 | NS | 4.22 | 4.17 | 4.24 | NS | 4.2 | 4.24 | NS | 4.23 | 4.2 | 4.2 | NS | Nd | 4.13 | 4.23 | NS | 4.21 | 4.25 | 4.19 | NS | 4.06 | 4.26 | 4.29 | NS |
| 7 | 4.18 | 4.18 | 4.3 | NS | 4.3 | 4.18 | 4.17 | NS | 4.1 | 4.23 | * | 4.26 | 4.2 | 4.14 | NS | 4.08 | 4.24 | 4.23 | NS | 4.31 | 4.18 | 4.19 | NS | 4.34 | 4.19 | 4.2 | NS |
| 8 | 4.2 | 4.27 | 4.38 | NS | 4.35 | 4.23 | 4.16 | NS | 4.21 | 4.28 | * | 4.25 | 4.29 | 4.15 | NS | 4.09 | 4.3 | 4.25 | NS | 4.37 | 4.23 | 4.22 | NS | 4.42 | 4.28 | 4.29 | NS |
| 9 | 4.35 | 4.36 | 4.28 | NS | 4.23 | 4.34 | 4.28 | NS | 4.29 | 4.35 | NS | 4.24 | 4.36 | 4.2 | NS | 3.55 | 3.7 | 3.68 | NS | 4.22 | 4.33 | 4.28 | NS | 4.14 | 4.3 | 4.38 | NS |
| 10 | 3.93 | 3.88 | 3.86 | NS | 3.84 | 3.9 | 3.95 | NS | 3.92 | 3.95 | NS | 3.87 | 3.96 | 3.82 | NS | . | 3.91 | 3.94 | NS | 3.82 | 3.88 | 3.95 | NS | 4.11 | 4.01 | 3.82 | NS |
| 11 | 4.12 | 4.11 | 4.21 | NS | 4.17 | 4.11 | 4.14 | NS | 4.16 | 4.08 | * | 4.08 | 4.14 | 4.2 | NS | . | . | . | | 4.17 | 4.15 | 4.1 | NS | 4.18 | 4.09 | 4.3 | * |
| 12 | 4.46 | 4.63 | 4.6 | NS | 4.6 | 4.74 | 4.35 | NS | 4.56 | 4.39 | NS | 4.42 | 4.61 | 4.17 | NS | 4.12 | 4.86 | 4.54 | NS | 4.56 | 4.59 | 4.4 | NS | 4.85 | 4.72 | 4.26 | NS |

Sig = Significance, NS = Non Significant difference (P>0.05), * = Significant difference (P<0.05)

**Tabel 6**. The effect of milk protein genes (CSN-BMC9215, CSN-BMC6334, CSN1S1-192, CSN1S1-14618, CSN1S2, CSN2-67, and CSN3) on least squares means of milk solid non fat component (%) in Holstein Friesian (HF) cattle for 12 months of lactation

| Month of Lactation | Genotype | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CSN-BMC9215 | | | | CSN-BMC6334 | | | | CSN1S1-192 | | | CSN1S1-14618 | | | | CSN1S2 | | | | CSN2_67 | | | | CSN3 | | | |
| | CG | GT | TT | Sig | AA | GA | GG | Sig | AA | AG | Sig | CC | CT | TT | Sig | AA | TA | TT | Sig | AA | AC | CC | Sig | CG | GT | TT | Sig |
| 1 | 8.58 | 8.68 | 8.64 | NS | 8.63 | 8.61 | 8.65 | NS | 8.63 | 8.59 | NS | 8.6 | 8.58 | 8.72 | NS | . | 8.47 | 8.65 | NS | 8.63 | 8.7 | 8.54 | NS | 8.3 | 8.53 | 8.9 | * |
| 2 | 8.35 | 8.75 | 8.56 | NS | 8.62 | 8.59 | 8.35 | NS | 8.55 | 8.47 | NS | 8.59 | 8.47 | 8.43 | NS | 9.26 | 8.61 | 8.47 | NS | 8.05 | 8.52 | 8.33 | NS | 8.42 | 8.46 | 8.49 | NS |
| 3 | 8.39 | 8.23 | 8.46 | NS | 8.43 | 8.3 | 8.41 | NS | 8.43 | 8.36 | NS | 8.48 | 8.37 | 8.34 | NS | 8.53 | 8.5 | 8.36 | NS | 8.36 | 8.37 | 8.37 | NS | 7.88 | 8.4 | 8.32 | NS |
| 4 | 8.31 | 8.28 | 8.28 | NS | 8.3 | 8.25 | 8.3 | NS | 8.34 | 8.35 | NS | 8.27 | 8.24 | 8.38 | NS | . | 8.45 | 8.27 | NS | 8.32 | 8.22 | 8.49 | NS | 8.43 | 8.29 | 8.28 | NS |
| 5 | 8.53 | 8.41 | 8.43 | NS | 8.43 | 8.38 | 8.54 | NS | 8.38 | 8.45 | NS | 8.53 | 8.44 | 8.56 | NS | 8.57 | 8.7 | 8.43 | NS | 8.39 | 8.4 | 8.56 | NS | 8.59 | 8.48 | 8.48 | NS |
| 6 | 6.36 | 8.34 | 8.3 | NS | 8.29 | 8.3 | 8.4 | NS | 8.39 | 8.3 | NS | 8.37 | 8.31 | 8.36 | NS | . | 8.38 | 9.32 | NS | 8.3 | 8.34 | 8.37 | NS | 8.01 | 8.3 | 8.38 | NS |
| 7 | 8.33 | 8.44 | 8.61 | ** | 8.61 | 8.43 | 8.31 | ** | 8.4 | 8.47 | NS | 8.51 | 8.39 | 8.34 | NS | 8.22 | 8.46 | 8.41 | NS | 8.6 | 8.37 | 8.4 | * | 8.71 | 8.4 | 8.48 | NS |
| 8 | 8.47 | 8.36 | 8.66 | * | 8.64 | 8.44 | 8.42 | NS | 8.44 | 8.49 | NS | 8.48 | 8.58 | 8.3 | * | 8.19 | 8.47 | 8.43 | NS | 8.66 | 8.38 | 8.53 | * | 8.97 | 8.58 | 8.45 | NS |
| 9 | 8.28 | 8.33 | 8.41 | NS | 8.45 | 8.3 | 8.24 | NS | 8.31 | 8.33 | NS | 8.45 | 8.33 | 8.22 | NS | 7.92 | 8.42 | 8.29 | NS | 8.45 | 8.27 | 8.34 | NS | 8.4 | 8.47 | 8.15 | NS |
| 10 | 8.65 | 8.56 | 8.8 | NS | 8.75 | 8.65 | 8.62 | NS | 8.76 | 8.61 | NS | 8.72 | 8.61 | 8.48 | NS | .nd | 8.73 | 8.62 | NS | 8.75 | 8.6 | 8.67 | NS | 9.05 | 8.6 | 8.69 | NS |
| 11 | 8.56 | 8.68 | 8.67 | NS | 8.66 | 8.61 | 8.62 | NS | 8.63 | 8.57 | NS | 8.6 | 8.58 | 8.72 | NS | .nd | 8.48 | 8.65 | NS | 8.65 | 8.69 | 8.52 | NS | 8.69 | 8.53 | 8.91 | * |
| 12 | 8.79 | 8.84 | 8.78 | NS | 8.73 | 8.83 | 8.78 | NS | 8.55 | 8.8 | NS | 8.96 | 8.73 | 8.83 | NS | 9.07 | 8.75 | 8.95 | NS | 8.76 | 8.91 | 8.8 | NS | 7.87 | 8.67 | 8.81 | NS |

Sig = Significancy, NS = Non Significant difference (P>0.05), * = Significant difference (P<0.05), ** = Highly significant difference (P<0.01)

**Tabel 7**. The effect of milk protein genes (CSN-BMC9215, CSN-BMC6334, CSN1S1-192, CSN1S1-14618, CSN1S2, CSN2-67, and CSN3) on least squares means of milk lactosa component (%) in Holstein Friesian (HF) cattle for 12 months of lactation

| Month Lactation | Genotype | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CSN-BMC9215 | | | | CSN-BMC6334 | | | | CSN1S1-192 | | | CSN1S1-14618 | | | | CSN1S2 | | | | CSN2_67 | | | | CSN3 | | | |
| | CG | GT | TT | Sig | AA | GA | GG | Sig | AA | AG | Sig | CC | CT | TT | Sig | AA | TA | TT | Sig | AA | AC | CC | Sig | CG | GT | TT | Sig |
| 1 | 4.79 | 4.77 | 4.86 | NS | 4.87 | 4.77 | 4.79 | NS | 4.83 | 4.74 | NS | 4.78 | 4.81 | 4.82 | NS | nd | 4.81 | 4.8 | NS | 4.87 | 4.77 | 4.78 | NS | 4.79 | 4.7 | 4.92 | NS |
| 2 | 4.54 | 4.66 | 4.74 | NS | 4.78 | 4.6 | 4.56 | NS | 4.7 | 4.61 | NS | 4.81 | 4.58 | 4.61 | NS | 5.06 | 4.61 | 4.64 | NS | 4.76 | 4.58 | 4.57 | NS | 4.66 | 4.55 | 4.7 | NS |
| 3 | 4.73 | 4.55 | 4.69 | * | 4.69 | 4.58 | 4.73 | NS | 4.68 | 4.68 | NS | 4.74 | 4.66 | 4.67 | NS | 4.74 | 4.83 | 4.67 | NS | 4.66 | 4.64 | 4.76 | NS | 4.36 | 4.65 | 4.68 | NS |
| 4 | 4.63 | 4.62 | 4.65 | NS | 4.67 | 4.59 | 4.62 | NS | 4.66 | 4.67 | NS | 4.74 | 4.52 | 4.67 | NS | .nd | 4.66 | 4.64 | NS | 4.69 | 4.56 | 4.79 | NS | 4.72 | 4.63 | 4.66 | NS |
| 5 | 4.83 | 4.65 | 4.72 | NS | 4.75 | 4.62 | 4.83 | NS | 4.71 | 4.76 | NS | 4.82 | 4.71 | 4.9 | NS | 4.84 | 4.86 | 4.75 | NS | 4.73 | 4.66 | 4.87 | NS | 4.83 | 4.75 | 4.8 | NS |
| 6 | 4.77 | 4.73 | 4.75 | NS | 4.75 | 4.74 | 4.77 | NS | 4.79 | 4.73 | NS | 4.77 | 4.73 | 4.8 | NS | .nd | 4.78 | 4.75 | NS | 4.75 | 4.72 | 4.8 | NS | 4.58 | 4.72 | 4.77 | NS |
| 7 | 4.76 | 4.85 | 4.9 | * | 4.89 | 4.83 | 4.75 | NS | 4.82 | 4.85 | NS | 4.83 | 4.82 | 4.76 | NS | 4.68 | 4.82 | 4.81 | NS | 4.88 | 4.81 | 4.8 | * | 4.98 | 4.81 | 4.8 | NS |
| 8 | 4.89 | 4.86 | 5 | NS | 4.99 | 4.76 | 4.78 | NS | 4.91 | 4.91 | NS | 4.88 | 4.97 | 4.83 | NS | 4.73 | 4.9 | 4.87 | NS | 4.99 | 4.86 | 4.94 | * | 5.19 | 4.92 | 4.89 | NS |
| 9 | 4.68 | 4.74 | 4.77 | NS | 4.83 | 4.72 | 4.63 | NS | 4.72 | 4.69 | NS | 4.79 | 4.7 | 4.7 | NS | 4.5 | 4.81 | 4.7 | NS | 4.79 | 4.67 | 4.7 | NS | 4.81 | 4.72 | 4.62 | NS |
| 10 | 5.02 | 5.01 | 5.12 | NS | 5.09 | 5.04 | 5.02 | NS | 5.08 | 5.03 | NS | 5.08 | 5.01 | 4.92 | NS | . | 5.01 | 5.06 | NS | 5.08 | 5.03 | 5.03 | NS | 5.07 | 4.97 | 5.01 | NS |
| 11 | 4.78 | 4.77 | 4.86 | NS | 4.87 | 4.76 | 4.78 | NS | 4.82 | 4.73 | NS | 4.78 | 4.8 | 4.81 | NS | . | 4.81 | 4.8 | NS | 4.87 | 4.76 | 4.78 | NS | 4.91 | 4.76 | 4.92 | NS |
| 12 | 5.13 | 5.17 | 5.24 | NS | 5.23 | 5.08 | 5.15 | NS | 5.1 | 5.17 | NS | 5.09 | 5.19 | 5.04 | NS | 4.95 | 4.98 | 5.18 | NS | 5.28 | 5.23 | 5.15 | NS | 5.3 | 5.14 | 5.13 | NS |

Sig = Significancy, NS = Non Significant difference (P>0.05), * = Significant difference (P<0.05)

the genetic variants of CSN3 affected milk fat levels. Hristov et al. (2011) added that milk fat component in the Bulgarian black pied cattle was associated with the genotype from the CSN3 gene. Meanwhile, Komori et al. (2013) explained that CSN3 had a function in regulating the formation and stabilization of micelles. The structure and component of the morphometry of the milk fat globules (MFGs) were reported to be influenced by a genetic polymorphism in αs1-casein (CSN1S1) (Cebo et al. 2012). Fleming et al. (2017) reported that there was a positive correlation between the component of milk fat and the diameter of MFGs. The previous study showed that HF cow which had BB genotypes resulted in higher milk fat components than other genotypes (AA and AB) (Vidović et al. 2013). Relation of lactation stage to milk fat component reported by Salamonczyk (2013) that the highest of fat milk components was recorded in milk which was produced at the last lactation stage (>300 days). Meanwhile, Januś & Borkowska (2011) found that lower calorific value of milk due to lower fat component obtained in the first 100 days of lactation. Stoop et al. (2009) explained that stage lactation contributed to variation in milk fat component which caused by the different activity of fatty acid pathways.

The effect of the milk protein genes on solid non-fat milk levels are presented in Table 6. During 12 months lactation, it was known that genes genotype which had a significant effect ($p<0.05$) to the level of solid non-fat cow milk were genotypes for the CSN-BMC9215, CSN-BMC6334, CSN1S1-14618, CSN2_67, and CSN3 genes. The CSN-BMC9215 gene affected the levels of solid non-fat in the 7$^{th}$ and 8$^{th}$ lactation months. In the 7$^{th}$ lactation month, the highest solid non fat milk level was obtained from the TT genotype (8.61%), followed by the GT (8.44%) and CG genotypes (8.33%). In the 8$^{th}$ month, the highest level of solid non-fat milk was obtained from the TT genotype (8.66%), followed by the CG (8.47%) and GT genotypes (8.33%). Meanwhile, for the CSN-BMC6334 gene, the highest level of solid non-fat milk was obtained from the AA genotype (8.61%), followed by the GA (8.43%) and GG genotype (8.31%). In the CSN1S1-14618 gene, the highest level of solid non-fat milk was obtained from the CT genotype (8.58%), followed by the CC (8.48%) and TT genotype (8.3%). The CSN2-67 gene affected the levels of solid non-fat in the 7$^{th}$ and 8$^{th}$ lactation months. In those two months of lactation, it was found that the highest solid non-fat milk component was obtained from the AA genotype (8.6%), followed by the CC (8.4%) and AC genotypes (8.37%). The CSN3 gene influenced the level of solid non-fat milk at month lactation of 1$^{st}$ and 11$^{th}$. At 1$^{st}$ lactation month, the TT genotype produced the highest levels of solid non-fat (8.9%), followed by the GT (8.53%) and CG genotypes (8.3%). Meanwhile, on the 11$^{th}$ lactation, the TT genotype produced the highest levels of solid non-fat (8.91%), followed by the CG (8.69%) and GT genotypes (8.53%).This finding differed with Hamza et al. (2011) who reported that CN genotypes had no significant effect on milk solid non fat (SNF) component. Previously, Anggraeni et al. (2017) reported that there was no significant effect of κ-casein genotypes on the component of milk solid non-fat.

The effect of variant genetic of milk protein genes on the milk SNF component was reported in the previous studies. Deb et al. (2014) in Frieswal cattle showed that AB genotype resulted in higher milk SNF component compared to AA genotypes. Furthermore, Gurses & Yuce (2012) added that AB genotype affected higher milk SNF component than AA genotype in East Anatolian Red cattle (Turkey cattle). Radhika & Ajithkumar (2018) revealed that the component of milk SNF decreased along the increase of the age of cow. The component of milk SNF was relatively high in the first month, then dropped to a low in the second month, then raised as lactation progresses.

The effect of milk protein genes on milk lactose levels are presented in Table 7. During 12 months of lactation observation, it was found that the genetic variants of milk protein genes which significantly affected ($p<0.05$) level of cow's milk lactose were the CSN-BMC9215 and CSN2-67 genes. The CSN-BMC9215 gene affected milk lactose levels in the 3$^{rd}$ and 7$^{th}$ lactation months. In the 3$^{rd}$ lactation month, the CG genotype produced the highest lactose levels (4.73%), followed by the TT (4.69%) and GT genotypes (4.55%). Meanwhile, in the 7$^{th}$ lactation month, the TT genotype produced the highest levels of lactose (4.9%), followed by the GT (4.85%) and CG genotypes (4.76%). The CSN2-67 gene influenced milk lactose levels in the 7$^{th}$ and 8$^{th}$ lactation months. In the 7$^{th}$ lactation month, the AA genotype produced the highest lactose levels (4.88%), followed by the AC (4.81%) and CC genotypes (4.8%). Meanwhile, at the 8$^{th}$ lactation month, the AA genotype produced the highest levels of lactose (4.99%), followed by the CC (4.94 %) and AC genotypes (4.86%). This finding was similar to Hamza et al. (2011) who reported that CN genotypes significantly affected milk lactose component. Relation of lactation stage to milk lactose component reported by Salamonczyk (2013), who reported that the component of milk lactose decreased along with lactation stage increasement. The first two lactation stages (1-100 and 101-200 days) resulted in highest milk lactose component. Sigl et al. (2012) reported that in observations of the first 20 weeks of lactation in HF cattle, it was found that the highest milk lactose component occurred at 7$^{th}$ week lactation. In general, genetic variants of milk protein genes was associated with the chemical component of milk, i.e., protein, fat, solid non-fat, and lactose. The results of this study open the opportunity of genetic improvement of HF cattle based on milk protein genes to improve milk components, not only milk protein components but also the other component of milk components (fat, SNF, and lactose).

## CONCLUSION

Genetic variants of the milk protein genes have an association with the component of cow's milk (protein, fat, solid non-fat, and lactose). Protein milk was affected by the genetic variants of CSN1S1-192 and CSN2-67 genes. Fat milk was affected by CSN1S1-192 and CSN3 genes. Solid non-fat milk was affected by CSN-BMC9215, CSN-BMC6334, CSN1S1-14618, CSN2_67, and CSN3 genes. Lactose milk was affected by CSN-BMC9215 and CSN2-67 genes.

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