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

Asmarasari SA1,2, Sumantri C2, Gunawan A2, Taufik E2, Anggraeni A3

1Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
2Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
3Indonesian Research Institute for Animal Production, PO Box 221, Bogor 16002, Indonesia

E-mail: santi_ananda@yahoo.com

(received 01-05-2020; revised 19-05-2020; accepted 22-05-2020)

ABSTRACT

Asmarasari SA, Sumantri C, Gunawan A, Taufik E, Anggraeni A. 2020. Variasi genetik gen protein susu dan hubungannya dengan komponen susu sapi Friesian Holstein. JITV 25(3): 99-111. DOI: http://dx.doi.org/10.14334/jitv.v25i3.2502

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,
This research was conducted at the dairy cattle experimental station in the Indonesian Research Institute for Animal Production which located at Cawis-Bogor, West Java-Indonesia. A total number of 100 female HF cattle were used in this study with physiological status as pregnant, elution, and not pregnant. Blood samples were obtained from 100 pregnant cows before milking, from 50 pregnant cows after milking, and from 50 non-pregnant cows before milking. The location and sample size were determined based on the research objectives and the sample size needed for statistical analysis. The data were analyzed using SAS 9.1 software for statistical analysis. The results obtained were compared with the results from previous studies to understand the genetic variations of milk protein genes in cows' milk.

RESULTS AND DISCUSSION

Genetic variants of milk protein genes

| Genotype | Description | Frequency |
|----------|-------------|-----------|
| CSN1S1   | Known as CSN1S1, CSN2, and CSN3 genes | 28.0% |
| CSN2     | Known as CSN1S1, CSN2, and CSN3 genes | 28.0% |
| CSN3     | Known as CSN1S1, CSN2, and CSN3 genes | 28.0% |

DNA extraction was carried out from fresh HF blood samples using the Illustra Blood Mini Spin Kit. Detection of nucleotide diversity was carried out using the Real-Time Polymerase Chain Reaction (RT-PCR) technique. Genotyping was performed using the RT-PCR method, which was used to determine the genetic variations of milk protein genes in cows' milk. The results showed that the genetic variations of milk protein genes in cows' milk were the most common in the CSN1S1 gene, followed by the CSN2 and CSN3 genes.

Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR reactions were carried out for 20 seconds and 40 cycles consisting of denaturation at 95 °C for 20 seconds and annealing at 60 °C for 30 seconds. The primers used in this study were designed based on the nucleotide sequences of the milk protein genes, namely the CSN1S1, CSN2, and CSN3 genes. The primer sequences were chosen using the Primer3 software. The RT-PCR reactions were carried out in a reaction volume of 25 μl, containing 10 μl of genomic DNA, 12.5 μl of 2x PCR Master Mix, 0.5 μl of each primer, and 1.5 μl of dNTPs. The PCR reactions were carried out in a thermocycler with a denaturation step at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. The PCR products were visualized on a 3% agarose gel stained with ethidium bromide.

DISCUSSION

The results obtained in this study showed that the genetic variations of milk protein genes in cows' milk were the most common in the CSN1S1 gene, followed by the CSN2 and CSN3 genes. This finding is consistent with the results of previous studies conducted in other regions. The genetic variations of milk protein genes in cows' milk can provide information on the genetic potential of cows' milk and can be used as a basis for genetic selection in dairy cattle breeding programs.

CONCLUSION

The genetic variations of milk protein genes in cows' milk were analyzed using the Real-Time Polymerase Chain Reaction (RT-PCR) method. The results showed that the genetic variations of milk protein genes in cows' milk were the most common in the CSN1S1 gene, followed by the CSN2 and CSN3 genes. This finding can be used as a basis for genetic selection in dairy cattle breeding programs.
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. Ziyad & 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. Trakovićka 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 β-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 Reaction (PCR) technique

| Gene    | Gene Bank       | Position | Primary Sequence (5'→3') | Temperature (°C) | Mutation |
|---------|-----------------|----------|--------------------------|------------------|----------|
| CSN1S1  | X59856          | 26181    | F: CCATCATTTCTGACATCC     | 61.2             | G>A      |
|         |                 |          | R: AGGCAACAATATGCAGTC     | 61.6             |          |
|         |                 |          | VIC: CTCTGAGAACAGTGGAAAGACTCTATGCC | 74   |          |
|         |                 |          | FAM: CTCTGAGAACAGTGGAAAGACTCTATGCC | 70.7 |          |
| CSN1S2  | M94327          | 13231    | F: GCCGAATAAACATCTCTGCACT | 58              | A>T      |
|         |                 |          | R: CCCCCTAAACACCAGAGAGATTCA | 59   |          |
|         |                 |          | VIC: CCTCACCATAGTACT      | 67              |          |
|         |                 |          | FAM: TTCACCATAGTCTAC      | 67              |          |
| CSN3    | AY380228.1      | 13975    | F: GAAGAGGTTAAACAGAAAGACATCAATAAGATAG | 58   | G>T      |
|         |                 |          | R: GACAAAAATCATGTAGACAGTGTGA | 58   |          |
|         |                 |          | VIC: AACATTGGAGACTCTAGGC  | 66              |          |
|         |                 |          | FAM: TTTGAGATTCTAGGCAAC   | 67              |          |
| CSN2    | NW_001495211    | 9215     | F: 5'-CTTATGCAACTATTTTTCACACAT-3' | 58  | G>T      |
|         |                 |          | R: 5'-TCATATTTCCTCCTCATTGCTCAT-3' | 58  |          |
|         |                 |          | VIC: 5'-CTCATTTCACATCTT-3' | 67              |          |
|         |                 |          | FAM: 5'-TCACATCCTGTTTGTA-3' | 67              |          |
| CSN2    | M55158.1        | 6334     | F: 5'-CAGGATGATTGAGAGACATGTATG -3' | 59  | A>G      |
|         |                 |          | R: 5'-ACAGTCATAGGGTCATAACAGATGG-3' | 59  |          |
|         |                 |          | VIC: 5'-TGCAAAGTTGCTTCAG-3' | 67              |          |
|         |                 |          | FAM: 5'-CAAAGTTACTTCAGGCCC-3' | 66              | T        |

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)            | A(0.66) G(0.34) |
| CSN-BMC9215| 100 | TT(0.25)            | TA(0.16) T(0.87) |
| CSN-BMC6334| 100 | AA(0.25)            | AA(0.06) A(0.13) |
| CSN2_67    | 98  | AA(0.22)            | AA(0.06) A(0.13) |
| CSN1S2     | 90  | TT(0.79)            | T(0.87) A(0.13)  |
| CSN3       | 90  | GG(0.08)            | G(0.22) T(0.78)  |
| CSN1S1_14168| 100| CC(0.23)            | 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 k-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., 2nd, 7th, 8th, and 11th. There was an inconsistency in the influence of the genotype of the CSN1S1-192 gene. At the lactation months 2nd and 11th, 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 7th and 8th, 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. Bugaiea 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       | 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                 | CSN-BMC9215    | 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  |
|                   | CSN-BMC6334    | 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  |
|                   | CSN1S1-192     | 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  |
|                   | CSN1S1-14618   | 3.55| 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  |
|                   | CSN1S2         | 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  | 3.55| 3.54| NS  | 3.49| 3.55| 3.58| NS  | 3.62| 3.54| 3.62| NS  |
|                   | CSN2-67        | 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  |
|                   | CSN3           | 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                 | CSN-BMC9215    | 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  |
|                   | CSN-BMC6334    | 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  |
|                   | CSN1S1-192     | 3.81| 3.8 | 3.8 | 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  |
|                   | CSN1S1-14618   | 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  |
|                   | CSN1S2         | 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   | 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   | 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 | 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   | 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   | 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 | 4.38 | 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)
**Table 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 = Significance, 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   | 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     | 4.82 | 4.81     | 4.82 | NS    | nd   | 4.81 | 4.8     | 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     | 4.61 | 4.58     | 4.61 | NS    | 5.06 | 4.61 | 4.64     | 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     | 4.67 | 4.74     | 4.66 | NS    | 4.74 | 4.83 | 4.67     | 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     | 4.67 | 4.74     | 4.52 | 4.67 | nd   | 4.66 | 4.64     | 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     | 4.82 | 4.71     | 4.9   | NS    | 4.84 | 4.86 | 4.75     | 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     | 4.77 | 4.73     | 4.8   | NS    | nd   | 4.78 | 4.75     | 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     | 4.83 | 4.82     | 4.76 | NS    | 4.68 | 4.82 | 4.81     | 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     | 4.88 | 4.97     | 4.83 | NS    | 4.73 | 4.9 | 4.87     | 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     | 4.79 | 4.7     | 4.7   | NS    | 4.5  | 4.81 | 4.7       | 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     | 5.08 | 5.01     | 4.92 | NS    | .    | 5.01 | 5.06     | 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     | 4.78 | 4.8     | 4.81 | NS    | .    | 4.81 | 4.8     | 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     | 5.09 | 5.19     | 5.04 | NS    | 4.95 | 4.98 | 5.18     | 5.28 | 5.23     | 5.15 | NS   | 5.3  | 5.14 | 5.13 | NS   |

**Sig** = Significance, **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 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 milk were 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 7th and 8th lactation months. In the 7th 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 8th 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 7th and 8th 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 1st and 11th. At 1st 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 11th 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 genotypes. The CSN-BMC9215 gene affected milk lactose levels in the 3rd and 7th lactation months. In the 3rd 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 7th 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 7th and 8th lactation months. In the 7th 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 8th 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 increase. 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 7th week lactation. In general, genetic variants of milk protein genes were 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.

ACKNOWLEDGEMENT

This study funded by the Indonesian Agency for Agricultural Research and Development.

REFERENCES

Angraeni A, Anneke A, Nury HS, Andreas E, Sumantri C. 2017. Genetic variants of k-casein and β-lactoglobulin genes and their association with protein and milk components of holstein friesian cows at small farmers in Lembang, West Java. KnE Life Sci. 2:86–94.

Ardicli S, Soyudal B, Samli H, Dincel D, Balci F. 2018. Effect of STAT1, OLR1, CSN1S1, CSN1S2, and DGAT1 genes on milk yield and composition traits of Holstein breed. Rev Bras Zootec. 47:e20170247.

Barbosa SBP, Araújo ÍM de, Martins MF, Silva EC da, Jacopini LA, Batista ÂMV, Silva MVB da. 2019. Genetic association of variations in the kappa-casein and β-lactoglobulin genes with milk traits in girolando cattle. Rev Bras Saúde e Produção Anim. 20:e0312019.

Bhat SA, Ahmad SM, Ibeagha-Awemu EM, Bhat BA, Dar MA, Mumtaz PT, Shah RA, Ganai NA. 2019. Comparative transcriptome analysis of mammary epithelial cells at different stages of lactation reveals wide differences in gene expression and pathways regulating milk synthesis between Jersey and Kashmiri cattle. Loor JJ, editor. PLoS One. 14:e0211773.

Bonfatti V, Di Martino G, Cecchinato A, Vicario D, Carner P. 2010. Effects of β-κ-casein (CSN2-CSN3) haplotypes and β-lactoglobulin (BLG) genotypes on milk production traits and detailed protein composition of individual milk of Simmental cows. J Dairy Sci. 93:3797–3808.

Brka M, Hodžić A, Reinsch N, Zečević E, Dokso A, Đjedović R, Rukavina D, Kapur L, Vegara M, Šabanović M, Ravić I. 2010. Polymorphism of the kappa-casein gene in two Bosnian autochthonous cattle breeds. Arch Anim Breed. 53:277–282.

Bugeac T, Bâlteanu V, Creanga S. 2013. Kappa-casein genetic variants and their relationships with milk production and quality in Montbéliarde dairy cows. Bull UASVM Anim Sci Biotechnol. 70:193–194.

Caroli AM, Chessa S, Erhardt GJ. 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. J Dairy Sci. 92:5335–5352.

Cebo C, Lopez C, Henry C, Beauvallet C, Ménard O, Bevilacqua C, Bouvier F, Caillat H, Martin P. 2012. Goat αs1-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. J Dairy Sci. 95:6215–6229.

Cecchinato A, Bobbo T, Ruegg PL, Gallo L, Bittante G, Pegolo S. 2018. Genetic variation in serum protein pattern and blood β-hydroxybutyrate and their relationships with udder health traits, protein profile, and cheese-making properties in Holstein cows. J Dairy Sci. 101:11108–11119.

Çobanoglu Ö, Gurcan EK, Çankaya S, Kul E, Abaci SH, Ulker M. 2016. Effects of lactation month and season on test-day milk yield and milk components in Holstein cows. Indian J Anim Res. 51:952–955.

Dagnachew B, Georg T, Lien S, Ådnøy T. 2011. Casein SNP in Norwegian goats: additive and dominance effects on milk composition and quality. Genet Sel Evol. 43:1–12.

Davoodi SH, Shahbazi R, Esmaeili S, Sotrabbandi S, Mortazaviam AM, Jazayeri S, Talimi A. 2016. Health-related aspects of milk proteins. Iran J Pharm Res. 15:573–591.

Deb R, Singh U, Kumar S, Singh R, Sengar G, Sharma A. 2014. Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF x Sahiwal) cross breed of Indian origin. Iran J Ver Res. 15:406–408.

Djedovic R, Bogdanovic V, Perisic P, Stanojevic D, Popovic J, Brka M. 2015. Relationship between genetic polymorphism of κ-casein and quantitative milk yield traits in cattle breeds and crossbreds in Serbia. Genetika. 47:23–32.

Farrell HM, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, Hicks CL, Hollar CM, Ng-Kwai-Hang KF, Swaisgood HE. 2004. Nomenclature of the Proteins of Cows’ Milk—Sixth Revision. J Dairy Sci. 87:1641–1674.

Ferretti L, Leone P, Sgaramella V. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 18:6829–6833.

Fleming A, Schenkel FS, Chen J, Malchiodi F, Ali RA, Mallard B, Sargolzaei M, Corredig M, Miglior F. 2017. Variation in fat globule size in bovine milk and its prediction using mid-infrared spectroscopy. J Dairy Sci. 100:1640–1649.

Franzoi M, Niero G, Visentin G, Penasa M, Cassandro M, De Marchi M. 2019. Variation of Detailed Protein Composition of Cow Milk Predicted from a Large Database of Mid-Infrared Spectra. Animals. 9:176.

Gurmessa J, Melaku A. 2012. Effect of lactation stage, pregnancy, parity and age on yield and major
components of raw milk in bred cross Holstein Friesian Cows. World J Dairy Food Sci. 7:146–149.

Gurses M, Yuce H. 2012. Determination of kappa casein gene polymorphisms and their effects on milk composition in some native cattle breeds of Turkey. J Anim Vet Adv. 11:1023–1027.

Hamza A, Yang Z, Wang X, Chen R, Wu H, Ibrahim A. 2011. The impact of kappa casein gene polymorphism on milk components and other productive performance traits of Chinese Holstein cattle. Pak Vet J. 31:153–156.

Hristov O, Tsetsanova D, Radoslavov G. 2011. Effects of genetic variants of milk protein genes on milk composition and milk yield in cows of the bulgarian black pied cattle. Compt rend Acad bulg Sci. 64:75–80.

Huang W, Peñalver L, Ahmad K, Lucey J, Weigel K, Janus E, Borkowska D. 2011. A study of the relationship between bovine kappa casein and milk fat. Acta Fytotech Zootech. 3:61–64.

Janoš E, Borkowska D. 2012. Association between bovine milk protein gene variants and protein composition traits in dairy cattle. J Dairy Sci. 95:440–449.

Januš E, Borkowska D. 2011. Effect of selected factors on milk energy value of cow’s milk from PHF BW and Montbeliarde breeds. ZEYWNOŚĆ Nauk Technol Jakości. 5:141–149.

Jónás E, Atasever S, Gráff F, Erdem H. 2016. Non-genetic factors affecting milk yield, composition and somatic cell count in Hungarian Holstein cows. Kafkas Univ Vet Fak Derg. 22:361–366.

Khaizaran ZA, Al-Razem F. 2014. Analysis of selected milk traits in Palestinian Holstein-Friesian cattle in relation to genetic polymorphism. J Cell Anim Biol. 8:74–85.

Komori K, Kobayashi T, Matsuo H, Kino K, Miyazawa H. 2013. Csn3 gene is regulated by all-trans retinoic acid during neural differentiation in mouse P19 cells. Cooney AJ, editor. PLoS One. 8:e61938.

Laible G, Smolenisky G, Wheeler T, Brophy B. 2016. Increased gene dosage for β- and κ-casein in transgenic cattle improves milk composition through complex effects. Sci Rep. 6:37407.

Le Parc A, Leonil J, Chanet E. 2010. αS1-casein, which is essential for efficient ER-to-Golgi casein transport, is also present in a tightly membrane-associated form. BMC Cell Biol. 11:65.

Mangia NP, Saliba L, Zoumpopoulou G, Chessa S, Anastasiou R, Karayiannis I, Sgouras D, Tsakalidou E, Nudda A. 2019. Goat milk with different alpha-s1 casein genotypes (CSN1S1) fermented by selected Lactobacillus paracasei as potential functional food. Int J Food, Agric Vet Sci. 19:147–152.

Mohammadi Y, Aslaminejad AA, Nassiri MR, Koshkioeh AE. 2013. Allelic polymorphism of K-casein, β-lactoglobulin and leptin genes and their association with milk production traits in Iranian Holstein cattle. J Cell Mol Res. 5:75–80.

Morkünlüenė K, Baltrėnaitė L, Puisištytė A, Bižienė R, Pečuliūtėnė N, Makštutienė N, Miškelienė R, Micleikienė I, Kerzienė Z. 2016. Association of kappa casein polymorphism with milk yield and milk protein genomic values in cows reared in Lithuania. Vet Med Zoot. 74:27–32.

Ohlsson JA, Johansson M, Hansson H, Abrahamsson A, Byberg L, Smedman A, Lindmark-Månsson H, Lundh Å. 2017. Lactose, glucose and galactose content in milk, fermented milk and lactose-free milk products. Int Dairy J. 73:151–154.

Olenski K, Kamiński S, Sydia J, Cieslinska A. 2010. Polymorphism of the beta-casein gene and its associations with breeding value for production traits of Holstein–Friesian bulls. Livest Sci. 131:137–140.

Ozdemir M, Kopuzlu S, Topal M, Bilgin OC. 2018. Relationships between milk protein polymorphisms and production traits in cattle: a systematic review and meta-analysis. Arch Anim Breed. 61:197–206.

Radhika G, Ajithkumar S. 2018. Low solids not fat percentage in milk of crossbred cows in waynad district of kerala, india – a retrospective study. Int J Sci Environ. 7:1962–1969.

Ren D, Miao S, Chen Y, Zhou C, Liu X, Liu J. 2013. Genotyping of the κ-casein and βlactoglobulin genes in Chinese Holstein, Jersey and water buffalo by PCR-RFLP. J Genet. 92:1–5.

Salamonzeky E. 2013. Cow’s milk quality and energy value during different lactation stages. Acta Pol Technol Aliment. 12:303–310.

Schopen GCB, Visker MHPW, Koks PD, Mullaat E, van Arendonk JAM, Bovenhuis H. 2011. Whole-genome association study for milk protein composition in dairy cattle. J Dairy Sci. 94:3148–3158.

Sigl T, Meyer HHD, Wiedemann S. 2012. "Gene expression of six major milk proteins in primary bovine mammary epithelial cells isolated from milk during the first twenty weeks of lactation. Czech J Anim Sci. 57:469–480.

Stoop WM, Bovenhuis H, Heck JML, van Arendonk JAM. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J Dairy Sci. 92:1469–1478.

Sudhakar K, Panneerselvam S, Thiruvenkadan A, Abraham J, Vinodkumar G. 2013. Factors affecting milk fat percentage and solids-not-fat percentage and milk price of dairy cattle in humid tropics. Int J Food, Agric Vet Sci. 3:229–233.

Thomas M, Sasidharan M. 2015. Factors affecting milk fat percentage and solids-not-fat percentage and milk price of dairy cattle in humid tropics. Adv Agric Sci. 3:11–17.

Trakovică A, Moravčíková N, Navrátilová A. 2012. Kappa-casein gene polymorphism (CSN3) and its effect on milk production traits. Acta Fytotech Zootech. 5:61–64.

Vidovč V, Nemes Z, Popović-Vranješ A, Lucač D, Cvitanovic D, Srbac L, Stupar M. 2013. Heritability
and correlations of milk traits in the view of kappa-casein genotypes in Vojvodina Holstein-Friesian dairy cattle. Mljekarstvo. 63:91–97.

Volkandari SD, Indriawati I, Margawati ET. 2017. Genetic polymorphism of kappa-casein gene in Friesian Holstein: a basic selection of dairy cattle superiority. J Indones Trop Anim Agric. 42:213–219.

Zepeda-Batista JL, Alarcón-Zúñiga B, Ruíz-Flores A, Núñez-Domínguez R, Ramírez-Valverde R. 2015. Polymorphism of three milk protein genes in Mexican Jersey cattle. Electron J Biotechnol. 18:1–4.

Zhou C, Li C, Cai W, Liu S, Yin H, Shi S, Zhang Q, Zhang S. 2019. Genome-wide association study for milk protein composition traits in a chinese holstein population using a single-step approach. Front Genet. 10:72.