Genetic polymorphisms of the OLR1 and DGAT1 genes associated with milk components in Holstein Friesian dairy cattle under an intensive management in Central Java

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Abstract. Oxidized Low Density Lipoprotein Receptor 1 (OLR1) gene serves to encode the binding vascular surface receptors and to degrade the oxidized low-density lipoprotein. In dairy cattle a nucleotide mutation at 3'UTR location of the OLR1 gene results in C allele of which related to higher milk fat content than A allele. Diacylglycerol O-acyltransferase 1 (DGAT1) gene has a K232A mutation of which K allele also associated with higher milk fat content against A allele. This study was aimed to study the association of variant genotypes of the OLR1 and DGAT1 genes on milk fat and other milk components in Holstein Friesian (HF) dairy cattle. The target of this research was to obtain genotypes of the OLR1 and DGAT1 gene affecting highly to milk fat content and other milk components in domestic HF cattle. Animals observed were Holstein Friesian (HF) lactating cows for 53 heads kept intensively at one government dairy station at BBPTU Baturaden, Central Java. Base mutations at 3'UTR location of the OLR1 gene and at K232A mutation of the DGAT1 gene were identified by PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) techniques. Allele frequency of the base mutation of each gene was analyzed by PopGen 32 program. Milk components of fat, protein, solid non-fat (SNF) and lactose were generated from daily milk test for morning, afternoon and average of both. Study of the association of variant genotypes of the OLR1 and DGAT1 genes on individual milk component was analyzed by GLM considering the fixed effects of lactation period (1-3, 4-6), calving season (1-4 mo., and 5-8 mo.) and calving year (2011, 2012). Duncan Multiple Range Test was used to test significant differences of the averages among subclasses. The OLR1 gene resulted in A allele and C allele of whose the frequencies were respectively 0.457 and 0.543, while the DGAT1 gene resulted in A allele and K allele with the frequencies respectively 0.426 and 0.575. Variant genotypes of the OLR1 gene gave a significant different on milk fat production (P<0.05). Genetic polymorphism of the OLR1 gene had a significant effect on milk fat content in the noon, the highest was for CC genotype, followed by AC, and the lowest was AA, with the fat contents of CC and AC genotypes higher to AA one by 4.87% and 1.38% respectively. However variant genotypes of the OLR1 gene and K allele with the frequencies respectively 0.426 and 0.575. Variant genotypes of the OLR1 gene gave a significant different on milk fat production (P<0.05). Genetic polymorphism of the OLR1 gene had a significant effect on milk fat content in the noon, the highest was for CC genotype, followed by AC, and the lowest was AA, with the fat contents of CC and AC genotypes higher to AA one by 4.87% and 1.38% respectively. However variant genotypes of the OLR1 gene had no significant effect on other milk components (P>0.05). Similarly, variant genotypes of the DGAT1 gene consisting of genotype AK and genotype KK did not significantly affect on all of milk components. It was concluded that there was a fairly good control of the OLR1 gene to fat content in HF cows. The base mutation at the 3'UTR location of the OLR1 gene can be considered as an initial information in developing a genetic assisted selection (GAS) technique for milk fat content in domestic HF cows.

Keywords – genetic polymorphisms, OLR1, DGAT1, Holstein Friesian, milk components.
1. Introduction

Production of milk and other milk components are economically valuable traits for dairy cattle industry. To be able to improve the quality of milk, it is necessary to increase milk fat content and other milk components such as protein, dried matter, and lactose. High milk contents can increase the selling price of fresh milk from dairy cows. Based on the provisions of the Indonesian National Standard for fresh milk from dairy cattle, it is required, among others, to contain a minimum level of milk components for fat by 3%, protein by 2.8%, and non-fat dried-matter by 7.8% [1]. The quality of milk produced by dairy cows therefore needs to pay attention. Milk production and the quality of milk in dairy cattle are a quantitative trait that is controlled by a large number of quantitative trait loci (QTL) and influenced by the environment [2] [3] [4]. The biosynthesis of milk fat in a cow’s body is a complex series of processes, passing various pathways controlled by major genes with high genetic variation [5].

Genetic improvement of milk quality is usually done through quantitative selection, but in accelerating the genetic response obtained, can be supported by molecular-based selection, namely through exploration of SNP variants from major genes or genome exploration. Identification of genetic polymorphisms in major genes that have a large influence on the milk components of dairy cattle can be used to accelerate the results of conventional selection programs. The Candidate gene strategy has been proposed by direct search for quantitative trait loci (QTL). Genetic variation in a gene, affecting the physiological pathways and phenotypes, further the proportion of genetic and phenotypic variation would likely be to affect the breeding strategy for improvement of important traits [4] [6]. Milk fat contents are controlled by a number of major genes, including SCD1 gene (stearoyl-CoA desaturase 1), ACACA gene (Acetyl-CoA Carboxylase Alpha), OLR1 gene (oxidized low density lipoprotein receptor 1), and DGAT1 gene [2] [3] [4] [5] [7].

Oxidized low density lipoprotein receptor 1 (OLR1) gene functions to encode oxidized low density lipoprotein receptors such as lectins, which are proteins that bind and degrade oxidized low density lipoproteins [8]. This gene in dairy cattle is located on chromosome 5 (BTA5) at intervals between 106 - 108 cM consisting of 5 exons with the function of encoding 279 amino acids of protein. It is known that there is a significant influence of the marker rs109019599 or g.8232CNA or C223A at the non-translational region or 3′UTR of this gene on milk fat content and milk fat production [2] [3] [9]. CC and AC genotypes consistently produce higher levels of milk fat against the AA cows [2] [10] [11].

Diacylglycerol acyltransferase 1 (DGAT1) gene is one of candidate genes that has a very high potency to become a gene marker in increasing milk fat content in dairy cattle. The DGAT1 gene encodes a microsomal enzyme by using diacylglycerol and fatty acyl coA as the terminal of substrates catalyzes and committed in triacylglycerol biosynthesis. The DGAT1 gene is also involved in triacylglycerol metabolism such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation and lactation [4]. This gene is located on bovine chromosome 14 with a total length of 14,117 bp consisting of two flanking regions, 17 exons and 16 introns. This gene encodes Acyl-CoA: diacylglycerol acyl transferase 1 as essential enzyme to role in the synthesis of triglycerides with the catalyst of the reaction of diacylglycerol and fatty acids [12]. A non-conservative substitution of lysine with alanine (K232A) at positions 10,433 and 10,434 in exon 8 showed an association to milk fat content and milk components.

This study was aimed to study the association of variant genotypes of the OLR1 and DGAT1 genes on milk fat and other milk components in domestic Holstein Friesian (HF) dairy cattle. The target of this research is to obtain genotypes of the OLR1 and DGAT1 gene affecting highly to milk fat content and other milk components in domestic HF cattle.

2. Materials and methods

The research materials were Holstein Friesian (HF) Lactating cows kept at BBPTU Baturraden, Purwokerto, Subdistrict. Banyumas District, Central Java. A total number of HF cows for 53 heads were observed at the satus of lactation months of 1-8 months, lactation periods of 1-6, and calving
years of 2011-2012. Data of milk production were collected from daily milk testing generated at morning, afternoon, and average of both of milking times per lactating cow.

All of those HF cows as samples were collected their fresh blood. Blood samples were taken for DNA analysis collected from jugular vein using a 21 G X 1½ sized vacutainer or 10 ml syringe containing heparin anticoagulant substance. A total of 1 to 2 ml of blood samples was stored in a 10 ml tube and added 8 ml of ethanol (EtOH) absolute containing 1 mM EDTA.

Milk component was obtained using a formol titration technique where p is the amount of NaOH for titration on milk samples and q is the amount of NaOH for blank titration. Components of milk (%) analyzed include fat content, protein, lactose, and non-fat dried-matter.

2.1. DNA extraction

DNA extraction using 0.2% NaCl, ethylene diamide tetra acetate (EDTA), 10% sodium diodecyl sulfate (SDS), sodium tris EDTA (STE 1.0x), proteinase k 5.0 mg / ml, phenol, CIAA (24 chloroform: 1 isoamyl alcohol), absolute ethanol, 70% ethanol and 80% EDTA (TE).

2.2. Genotyping OLR1 gene

Genotyping the OLR1 gene included briefly DNA extraction activities, amplification by PCR (polymerase chain reaction) technique from base fragments in untranslational region or 3’UTR of the OLR1 gene. The RFLP (restriction polymerase chain reaction) method was applied to cut DNA amplicons as PCR products. The primary pairs namely forward: 5’-TCCCTAACTTGTTCACATCGCAGTG-3’ and reverse: 5’-GGAAAGCGCCATTTGAGG-3’ [9]. The PCR product (143 bp) was cut with the restriction enzyme PstI which recognized the C * TGCAG as a restriction base.

2.3. Genotyping DGAT1 gene

PCR analysis using DNA samples and premix PCR. Premix PCR consisted of 9.35 µl sterile distilled water, 0.3 µl triposfat deoxy nucleotide (dNTP), 3.0 µl buffer (5.0x), 1.0 µl MgCl2, 0.05 µl DNA polymerase taq enzyme and 0.3 µl primer. The primary used namely forward 5’s-GCACCATCCTCTCCTCAAGTGCCAG-3’ and reverse 5’-GGAAGCGCCTTTGAGG-3’ [12]. Analysis of RFLP using PCR and premix RFLP products. The premix consisted of EaeI restriction enzymes [13], sterile distilled water and buffer.

2.4. Data analysis

The frequency of a certain genotype was obtained by comparing the total number of that genotype cows to all genotypes obtained, while the Hardy-Weinberg equilibrium (HWE) was tested by Chi square analysis. To analyze gene frequency and H-W equilibrium were conducted by Gen Pop 3.2. Program.

To study of the association of variant genotypes of individual of the OLR1 and DGAT1 genes on each milk component observed was analyzed by the General Linear (GLM) Model by considering the fixed effects of lactation period (1-3, 4-6), calving season (1-4 mo., and 5-8 mo.) and calving year (2011, 2012). Duncan The Multiple Range Test is the difference between the averages among subclasses. Analysis was conducted by using SAS Program Package ver. 9.1 [14].

3. Results and discussion

3.1. Genotyping genes

Genetic polymorphisms of the OLR1 and DGAT1 gene can become potential molecular markers for selection on milk fat content and other milk components as important economic traits in dairy cattle [2] [10] [12] [13] [15] [18]. DNA amplification of both gene OLR1 and DGAT1 gene using PCR techniques were successfully performed on all of blood samples of the observed HF cows for the total 53 heads.
3.1.1. OLR1

Gene Genotyping of the 3' non-coding area of the OLR1 gene using PCR-RFLP with PstI restriction enzymes recognized the changes on base A to C at the 6-base cutting site from C*TGCAG. The mutation is thought to be a silent mutation. If there no cutting PCR product (146 pb) was declared as AA genotype, whilst three base fragments (146 bp, 120 bp, and 26 bp) found was identified as AC genotype, and if two fragments (120 bp and 26 bp) found was identified as CC genotypes [9]. The genotyping results in 53 HF cattle were found to result in three genotypes namely AA, AC and CC genotypes by the respective frequencies of 0.2075, 0.5283, and 0.2642; while the frequencies of A and C alleles were respectively 0.4574 and 0.5426. Chi-square test showed that the A and C alleles of the OLR1 gene had $\chi^2$ calculation = 0.1740 $< \chi^2 P_{0.05} = 0.6765$. This showed that the OLR1 locus was in the Hardy Weinberg (H-W) equilibrium. Similar results were obtained in HF cattle in Iran [11].

Variant genotypes of the OLR1 gene at the same base mutation of HF cattle in Iran that found the genotype frequencies were almost similar for AA, AC and CC genotypes, namely 0.22, 0.50, and 0.28 respectively; whilst the frequencies of A and C alleles were 0.47 and 0.53 respectively [11]. Almost similar frequencies for A and C alleles were also reported in HF cattle in USA, namely 0.46 and 0.54 [2], and HF cattle in Poland, namely 0.43 and 0.57 [9]. Another investigation at the same base markers g.8232CNA in the untranslated region of 3'UTR region of the OLR1 gene in Red-and-White strain Polish obtained genotype frequencies from the most consecutively for CC cattle (0.53), AC cattle (0.34), and AA cattle (0.13) [16]; so that the dominant for C allele (0.7) against A allele (0.3). CC genotype had the highest fat percentage but AA genotype for the lowest, while AC genotype for intermediate (P<0.05) [17]. The CC cows produced more milk fat yield against the AC genotypes AC (P<0.1) and AA ones (P<0.01). CC and AC cows had a higher milk protein content than AA cows P<0.01). These associations revealed, the SNP has the potential to be considered as a marker in marker-assisted selection.

3.1.2. DGAT1 gene

Amplification product obtained on the DGAT1 gene had a base fragment length of 411 pb. Genotyping DGAT 1 gene by the EaeI restriction enzyme successfully cut the mutation site of the DGAT1 gene. The position of the base mutation occurred on the 8th exon, at the position of the 10th base of 433 which caused the change of adenine to guanine (A → G) or the transition mutation at the 10th base of 434. This caused the changes in adenine base to cytosine one (A → C) or in the form of transversion mutation at GC*GGCC bases (Kong et al., 2007). The mutation resulted in changes in amino acids from lysine (K = AAG) to alanine (A = GCG) for having a significant effect on milk fat content and other milk components of dairy cows [12].

Genotyping results of the DGAT1 in the HF cows observed resulted in A and K alleles. K allele was identified when the base fragment was not cut off by the EaeI restriction enzym, resulting in a base fragment of 411 bp. Conversely, the A allele was obtained when the base fragment was cut by the enzyme which resulted in the GC*GGCC mutation site, so that two base fragments were obtained by the fragment lengths of 203 bp and 208 bp. Genotyping DGAT1 gene in the observed HF lactating cows resulted in only two types of genotypes, namely KK genotype (411 bp), and KA genotype (203 bp, 208 bp and 411 bp), without AA genotype (203 bp and 208 bp). Genotype frequency of AK cow (0.8679) was dominant to that of KK genotype (0.1321), while frequency of K allele (0.5745) was higher than that of A allele (0.4255). The Chi Square test showed that for A and K alleles had $\chi^2$ calculation = 25.0734 $< \chi^2 P_{0.05} = 0.00001$. This shows that the DGAT1 gene at the observed locus was in H-W equilibrium. If Hardy-Weinberg equilibrium is constant from by generations indicates the accurances of selection, mutation, migration, and genetic drift [6].

Based on the results of studies on dairy cows of the Slovak Spotted breed and its crossbreds [18] frequency of genotype homozygotes for AA genotype (frequency 0.790), whilst only 2 were homozygotes for KK genotype in DGAT1 K232A locus (frequency 0.035). The rest of cows were AK heterozygotes (frequency 0.175). Further it was described allele frequentiest for A allele and K allele were successively 0.877 and 0.123. Hardy-Weinberg equilibrium was found that proved no significant
deviation of equilibrium in DGAT1 at K232A locus. It was assumed that the frequencies of A allele and AA (AK) genotypes were most probably caused by artificial selection of dairy products, which negatively influenced the occurrence and persistence of K allele.

3.2. Non genetic effects on milk components

Averages of milk fat content and other milk components as generated from milk daily testing in the morning, afternoon and both of the observed HF cows by the total number 53 heads were presented in Table 1. Examination of the influence of non-genetic factors included lactation period (lactation 1, 2), calving season (1-4 months, 5-8 months) and calving years (2011, 2012) on individual milk component showed that the morning milk fat content was significantly affected (P <0.05) by lactation period, calving season and calving year. So these three factors also had significant effect on the averages of individual milk component. Fat contents in the morning and average tests were higher for the 2nd lactation period to the 1st one by 5.67% and 3.64%; 2nd season (months 5-8) to the 1st one (January-April) by 4.15% and 3.60%; and calving year of 2012 to the 2011 one by 3.25% and 0.77%. Instead milk protein in the afternoon was significantly affected by the calving season and calving year. For HF cows in the 2nd calving season to that 1st one produced a higher protein content by 0.47%; while the calving year 2012 to that of 2011 produced a higher protein by 0.76%.

Table 1. Average of milk fat content and other milk components based on non-genetic factor and milking time in Holstein Friesian cattle.

| Milk component/ Factor | Variable | Cow (hd.) | Morning | Noon | Both |
|------------------------|----------|-----------|---------|------|------|
| Fat                    | Lact. Period | 1         | 39      | 4.321±0.380* | 4.420±0.255 | 4.372±0.223* |
|                        |           | 2         | 14      | 4.566±0.248  | 4.491±0.250  | 4.531±0.185   |
|                        | Calv. season | 1-4       | 16      | 4.262±0.393* | 4.346±0.212  | 4.306±0.188*  |
|                        |           | 5-8       | 37      | 4.439±0.343  | 4.479±0.261  | 4.461±0.223   |
|                        | Calv. year | 2011      | 45      | 4.364±0.374* | 4.450±0.264  | 4.409±0.236*  |
|                        |           | 2012      | 8       | 4.506±0.296  | 4.374±0.180  | 4.443±0.135   |
| Protein                | Lact. Period | 1         | 39      | 3.067±0.188  | 3.019±0.116  | 3.045±0.128   |
|                        |           | 2         | 14      | 3.015±0.100  | 3.006±0.121  | 3.011±0.056   |
|                        | Calv. season | 1-4       | 16      | 3.086±0.211  | 3.006±0.110* | 3.047±0.128   |
|                        |           | 5-8       | 37      | 3.039±0.149  | 3.020±0.121  | 3.032±0.108   |
|                        | Calv. year | 2011      | 45      | 3.034±0.171  | 3.012±0.120* | 3.026±0.116   |
|                        |           | 2012      | 8       | 3.160±0.124  | 3.035±0.102  | 3.099±0.077   |
| Lactose                | Lact. Period | 1         | 39      | 4.514±0.271  | 4.463±0.176  | 4.490±0.188   |
|                        |           | 2         | 14      | 4.444±0.160  | 4.440±0.193  | 4.444±0.089   |
|                        | Calv. season | 1-4       | 16      | 4.512±0.258  | 4.430±0.167  | 4.478±0.163   |
|                        |           | 5-8       | 37      | 4.488±0.245  | 4.465±0.180  | 4.478±0.172   |
|                        | Calv. year | 2011      | 45      | 4.461±0.238* | 4.452±0.184* | 4.456±0.169*  |
|                        |           | 2012      | 8       | 4.689±0.215  | 4.405±0.157  | 4.589±0.120   |
| Non-fat dried-matter   | Lact. Period | 1         | 39      | 8.322±0.458  | 8.225±0.308  | 8.275±0.321   |
|                        |           | 2         | 14      | 8.201±0.275  | 8.184±0.330  | 8.190±0.152   |
|                        | Calv. season | 1-4       | 16      | 8.338±0.449  | 8.183±0.298  | 8.263±0.291   |
|                        |           | 5-8       | 37      | 8.270±0.410  | 8.228±0.320  | 8.251±0.290   |
|                        | Calv. year | 2011      | 45      | 8.235±0.410  | 8.206±0.319  | 8.222±0.290*  |
|                        |           | 2012      | 8       | 8.603±0.338  | 8.261±0.279  | 8.434±0.205   |
| Average                |           | 53        |         | 8.290±0.419  | 8.215±0.311  | 8.254±0.287   |

Note: * at the same column showed significantly different (P <0.05).
However all of lactose and non-fat dried-matter were not affected by non-genetic factors, the exception was for morning lactose content that was significantly affected by the calving year. For lactose content in the morning test day was higher in 2012 (4.47%) against the previous year.

### 3.3. Effect of variant genotypes on milk fat content and milk components

#### 3.3.1. Effect of OLR1 gene

The results of the GLM analysis in Table 2 showed that the genetic polymorphism of the OLR1 gene had a significant effect on the level of milk fat content of HF cows milking in the afternoon, from the highest were for CC genotype cows, followed by AC cows, and the lowest AA cows. The fat contents of CC and AC cows were higher to the AA ones by 4.87% and 1.38% respectively. However, the fat contents in the morning and both (morning and noon) were not significantly (P> 0.05) influenced by the genotypes of the OLR1 gene. Other milk components, including fat, lactose, and non-fat dried-matter were also not significantly affected by the OLR1 gene.

| Milk component/Factor | Geno-type | Cow (hd.) | Morning | Noon | Both |
|-----------------------|-----------|-----------|---------|------|------|
| OLRI-genotipe         | AA        | 11        | 4.301±0.235 | 4.351±0.190 | 4.326±0.175 |
|                       | AC        | 28        | 4.430±0.342 | 4.411±0.225* | 4.423±0.219 |
|                       | CC        | 14        | 4.363±0.484 | 4.563±0.313* | 4.466±0.259 |
| DGAT-genotipe         | AK        | 46        | 4.401±0.368 | 4.448±0.256 | 4.427±0.218 |
|                       | KK        | 7         | 4.281±0.343 | 4.377±0.242 | 4.331±0.260 |
| OLRI-genotipe         | AA        | 11        | 3.052±0.100 | 3.044±0.119* | 3.050±0.073 |
|                       | AC        | 28        | 3.063±0.160 | 3.001±0.115 | 3.034±0.125 |
|                       | CC        | 14        | 3.033±0.231 | 3.024±0.121 | 3.031±0.121 |
| DGAT-genotipe         | AK        | 46        | 3.063±0.172 | 3.013±0.118 | 3.040±0.117 |
|                       | KK        | 7         | 2.987±0.149 | 3.037±0.115 | 3.014±0.092 |
| OLRI-genotipe         | AA        | 11        | 4.511±0.166 | 4.498±0.185 | 4.508±0.116 |
|                       | AC        | 28        | 4.530±0.265 | 4.437±0.174 | 4.484±0.198 |
|                       | CC        | 14        | 4.414±0.257 | 4.465±0.192 | 4.441±0.137 |
| DGAT-genotipe         | AK        | 46        | 4.508±0.248 | 4.454±0.180 | 4.482±0.172 |
|                       | KK        | 7         | 4.413±0.239 | 4.480±0.183 | 4.449±0.147 |
| OLRI-genotipe         | AA        | 11        | 8.313±0.279 | 8.285±0.325 | 8.300±0.195 |
|                       | AC        | 28        | 8.336±0.438 | 8.179±0.303 | 8.250±0.334 |
|                       | CC        | 14        | 8.181±0.476 | 8.231±0.328 | 8.209±0.265 |
| DGAT-genotipe         | AK        | 46        | 8.314±0.420 | 8.207±0.313 | 8.263±0.294 |
|                       | KK        | 7         | 8.131±0.406 | 8.261±0.320 | 8.199±0.249 |
| Average               |           | 53        | 8.290±0.419 | 8.215±0.311 | 8.254±0.287 |

The CC genotype cows produced a higher fat level in the noon compared to the AA ones were reported in some previous studies [2] [9] [10]. The highest milk fat content (3.31%) was produced by CC cows, the moderate one by the AC cows (3.17%), and the lowest by the AA cows (2.99%) [10]. AC and CC genotypes produced more fat than AA genotype [11]. In Holstein-Friesian Polish cattle (Red-and-White strains), where both AC and CC cows produced higher milk fat yield per lactation than AA cows, namely 280.19 ± 73.20 kg and 270.81 ± 59.43 kg vs. 249.32 ± 40.22 kg [16].
Variant genotype of the OLR1 gene also had a significant effect (P <0.05) on milk protein content in the afternoon of which AA genotype produced higher protein against AC and CC genotypes. Significant association between C allele and milk protein content from the 3’UTR region of the OLR1 gene seemed that this mutation base could be a potential gene assisted selection (GAS) for molecular selection for the increasing milk fat content program in domestic HF dairy cattle.

3.3.2. Effect of DGAT1 gene

Variant genotypes of the DGAT1 gene in related to the occasion of a base mutation at the base site of GC*GGCC base sequences the HF cows observed cows showed that both of AK and KK genotypes had no significant effect on all milk components. In the other side no AA genotype was found, so the effect of variant genotypes could not be compared among the three genotype. A non-conservative substitution of lysine by alanine (K232A) at the positions 10,433 and 10,434 in exon 8 in the DGAT1 gene can be considered for molecular selection for future breeding programs [19]. KK genotype had the greatest effect on all milk contents, while AA genotype had the greatest effect on milk yield.

4. Conclusion

Genotyping of the 3’UTR region of the OLR1 gene by the restriction enzyme Pst1 resulted two alleles (A and C), three genotypes (AA, AC, and CC), and in H-W equilibrium. While genotyping non-conservative substitution of lysine with alanine (K232A) of the DGAT1 gene produced two genotypes (AK and KK). The CC and AC genotypes of the OLR1 gene resulted higher milk fat content to the AA one. This SNP of the OLR1 gene can be initially considered as a candidate gene assisted selection (GAS) for increasing milk fat content in domestic HF cattle.

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