Association of the OLR1 Gene of the 3'UTR g.8232(A/C) genotypes on milk fatty acid components in Holstein Friesian

Anggraeni A1, Y P Nadapdap2, S A Asmarasari1, L Praharani1, A Hafid1 and A B L Ishak1

1Indonesian Research Institute for Animal Production, PO Box 221 Ciawi, Bogor.
2Faculty of Animal Science, Bogor Agriculture University, Bogor, Indonesia

Email: ria.anneke@yahoo.co.id

Abstract. Dairy cattle produce milk with a high proportion of saturated fatty acids (SFAs), instead of a low proportion of poly unsaturated fatty acids (PUFA). Oxidized low-density lipoprotein receptor 1 (OLR1) gene plays a key role in reducing oxidized low-density lipoprotein (oxLDL) involved in injury, dysfunction, and inactivation of endothelial cells. Association of the OLR1 gene in the 3'UTR of the g.8232(A/C) genotypes on 21 types of the milk fatty acids of Holstein Friesian (HF) cows were analysed kept at a breeding station in Central Java. Genotyping was analyzed by PCR-RFLP method using Pst1 restriction enzyme. Genotypic data were calculated for allele frequency, genotype frequency, and heterozygosity values. Effects of genotypes on individual fatty acid components were analyzed by General Linier Model. Genotyping resulted in three genotypes of AA, AC and CC with the frequencies successively 0.275, 0.525, and 0.200; and two alleles for A (0.463) and C (0.538). Genotypes affected medium-chain SFAs for Myristic (C14:0) and long-chain for Palmitic (C16:0) and Stearic (C18:0). The AA genotype generated the lowest levels of myristic and stearic, instead of the highest content of palmitic. Further the AA genotype resulted in the highest level of Oleic (C18:1) of MUFA. Therefore the OLR1 gene of the 3'UTR g.8232 (A>C) SNP is possible considered as a molecular-based selection in reducing SFAs (myristic and stearic) and increasing MUFA (Oleic) in domestic HF cattle.

1. Introduction
Milk production and milk quality are two essential economic traits in dairy cattle. Genetic improvement by selection of both traits is generally conducted by applying the quantitative breeding concepts. To provide genetic responses more quickly and accurately from selection on the quantitative traits, molecular-based selection can be assisted. Molecular selection can be done by exploring genetic variants of the main candidate genes from the genomic data. A strategy of identifying candidate genes has been proposed through direct search of loci at the nucleotide levels that influence quantitative traits (QTL). Genetic variation in one gene (and related genes) can affect physiological pathways and phenotypes, which can serve as a breeding strategy for the improvement of important quantitative traits [1–3]. Milk fat content and composition in dairy cattle are controlled by a number of major genes involving, among others, SCD1 gene (Stearoyl-CoA Desaturase 1), ACACA gene (Acetyl-CoA Carboxylase Alpha), OLR1 gene (Oxidized Low-Density Lipoprotein Receptor 1), and DGAT1 gene (Diacylglycerol O-Acyltransferase 1) [1,4–7].
The main protein of oxidizes low density lipoprotein receptor 1 (OLR1) presents in vascular endothelial cells which bind, internalize, and reduce oxidized low density lipoprotein levels [8]. The oxidized low-density lipoprotein (oxLDL) forms and its lipid constituents have many deleterious effects on endothelial secretory activity, causing the induction of apoptosis [9]. The OLR1 gene encodes vascular endothelial cell surface receptors that bind and degrade the oxidized low density lipoprotein (oxLDL) [10]. The OLR1 gene was one of the genes in QTL affecting milk fat percentage and milk fat yield [3]. The gene controlling the OLR1 synthesis in cattle is found on chromosome 5 (BTA5) with a sequence length of 11.373 base pairs, consisting of 5 exons and 4 introns (GenBank access number NW_215807). The bovine OLR1 gene encodes 270 amino acids that have 72% identity to the human protein [8]. A number of studies reported significant effects of the non-translation region (3'UTR) of the OLR1 gene as a DNA marker of rs109019599 or g.8232CNA or C223A on milk fat content and milk fat production [4,6,11]. Cows with CC and AC genotypes consistently produced higher milk fat against AA genotype [4,12]. Studying in domestic HF cattle [3] also reported that the CC and AC genotypes, against the AA genotype, resulting higher non-milking fat contents by 4.87% and 1.38%, respectively.

The Fatty Acids (FAs) are a group of compounds with complex structures and different effects on organisms. Fatty acids are divided into short chain (4–10 carbon atoms) and long chain (more than 11 carbon atoms) fatty acids. Based on the presence of double chains, moreover fatty acids can be divided into saturated fatty acids and (SFAs) and unsaturated fatty acids (UFAs), providing monounsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) [7]. Several groups of fatty acids have a wide spectrum of activity and a great benefit to humans [13]. The PUFA group, which is very beneficial to humans, represents 4–5% of milk fat contents. Dairy cattle produce milk with a relatively high proportion of SFAs, instead of a low proportion of PUFAs. Some of FUPAs are a group of essential fats that protect against the incidence of heart diseases. This is because FUPAs act as anticoagulant, anti-inflammatory and anti-arteriosclerosis, besides of increasing endothelial cell functions and lowering blood pressures [9,10]. Oxidized Low-Density Lipoprotein Receptor 1 (OLR1) gene therefore plays a key role in reducing oxidized Low-Density Lipoprotein (ox-LDL) in reducing fat secretion to the mammary glands.

This research aims to study association between the OLR1 gene of the 3'UTR g.8232 (A/C) genotypes on milk fatty acid components of domestic Holstein Friesian (HF) cow kept in an intensive management. The expected result was the possibility of the g.8232(A>C) SNP of the OLR1 gene to be considered as a molecular markers of selection for improving MUFA and FUPA in domestic HF dairy cattle.

2. Materials and method

2.1. Material

2.1.1. Location and period. This research consisted of field and laboratory researches. The field research was carried out by collecting test day milk data from Holstein Friesian (HF) cows kept at BBPTU Baturraden Dairy Station, Banyumas Regency, Central Java. Laboratory research was conducted for genotyping of the OLR1 gene at the Laboratory of Animal Molecular Genetics, Breeding and Genetics Section, Department of Animal Production and Technology, Faculty of Animal Husbandry, Bogor Agricultural University, Bogor Regency, West Java. Research activities were carried out in 2011–2013.

2.1.2. Research material. Research samples were the bloods of HF cows (40 heads) in physiological status of lactation months of 1–8 mo. and lactation periods of 1–3. Data of one test day milk at morning and noon milking were used to study the association of genetic polymorphism of the g.8232(A/C) genotypes on each of milk fatty acids components (21 types) of the HF cows observed.
2.2. Method

2.2.1. Milk fatty acid analysis. Measurement of individual milk fatty acid components of domestic HF cows was analyzed using Gas Chromatography and Mass Spectrometry (GCMS) Method at the Technical Implementation Unit of Integrated Laboratory, Diponegoro University, Semarang [14].

2.2.2. Genotyping. DNA extraction of the blood samples from the HF cows followed the method of Sambrook et al (1989) modified [15]. Genotyping the OLR1 gene in the 3'UTR fragment at the g.8232(A>C) locus followed the method of Komisarek and Dorynek (2009) [11]. Genotyping phases through PCR-RFLP method briefly provided DNA extraction, amplification by PCR (polymerase chain reaction) technique of the base fragments of the 3'UTR of the OLR1 gene. The primer for amplifying the targeted segment of the OLR1 gene followed the method of Komisarek and Dorynek (2009) [11], with an amplified product of 143 bp. Primary sequences for forward and reverse for the OLR1 gene at the targeted amplification in the 3'UTR were forward F: 5'-TCCCTAACTTGTTCCAAGTCCT-3' and reverse R: 5'-GCTTTCTTCTAGGCATTGTAGAG-3'. The RFLP (restriction polymerase chain reaction) method was applied to cut DNA amplicons as PCR products. The PCR product (143 bp) was cut with PstI restriction enzyme which recognized the C*TGCAG as a restriction base.

2.3. Data analysis

2.3.1. Genotype and allele frequencies. Genotype frequency (Xii) is known by calculating the ratio of the number of a certain genotype in each population, using the formula of Nei and Kumar (2000) [16]. Allele frequency (X_i) was relative frequency of an allele in the population or number of a certain allele to the total number of alleles in a population.

2.3.2. Association of genotypes on milk fatty acid components. To study the association of the OLR1 gene of the 3'UTR g.8232 (A/C) genotypes on individual milk fatty acid components (21 types) was analyzed by General Linear Model (GLM) for unbalance data. The GLM considered the fixed effects of genotypes (CC, AC, and AA), month of lactation (1–4, 5–8), period of lactation (1, 2–3), season of calving (1–4 mo., and 5–8 mo.) and year of calving (2011, 2012/2013). Duncan Multiple Range Test was tested for mean differences among subclasses. Analysis was conducted by SAS Program Package ver. 9.1 [17].

3. Results and discussion

3.1. Genetic polymorphism

Genotyping of the OLR1 gene in the 3'non-translation region or the (3'UTR) using the PCR-RFLP method showed that the PstI restriction enzyme recognized the nucleotide substitution of A (Adenine) to C (Cytosine) at the 6th base cut site of C*TGCAG. The mutation was predicted as a silent mutation. In the absence of the cutting nucleotide site presented only one fragment of the PCR product (146 bp) stated as AA genotype, if the existence of three fragments (146 bp, 120 bp, and 26 bp) stated as AC genotype, and if the occurrence of two fragments (120 bp and 26 bp) stated as CC genotype [11]. Genotyping results from the HF cows observed produced three genotypes of AA, AC and CC with the frequicntest of 0.200, 0.525 and 0.275 respectively; therefore resulted in two alleles of A and C with the frequencies of 0.463 and 0.538 respectively. Genetic variant of the OLR1 gene in the 3'UTR at the g.8232 locus (C>A) locus reported in dairy cattle from the previous studies supported the results from this study. HF cattle in Iran had genotype frequencies of AA, AC and CC successively by 0.22, 0.50, and 0.28; with the allele frequencies of A and C respectively 0.47 and 0.53 [18].
Figure 1. Visualization of the OLR1 gene of the 3’UTR base fragments at g.8232 (A>C) locus on 2% agarose gel. Genotypes of AA (146 bp), AC (146 bp, 120 bp, and 26 bp) and CC (120 bp and 26 bp).

Similar results were found in HF cows from USA with the frequencies of the A and C alleles by 0.46 and 0.54 respectively [4]; and those in HF cattle from Poland by 0.43 and 0.57 respectively [11]. Whilst for red-and-white dairy cattle from Poland presented higher frequency of the CC to AC and AA, with the frequencies of CC (0.53), AC (0.34), and AA (0.13) [19], so the allelic frequencies of C (0.7) and A (0.3). Heterozygosity values can describe the genetic diversity of a population. For the g.8232 (A/C) SNP in HF cattle observed had the observed heterozygosity greater than the expected heterozygosity, namely Ho = 0.5250 vs He = 0.5035. This indicated that the alleles at this g.8232 (A/C) locus was at a high degree of heterozygosity.

3.2. Association of genotypes on milk fatty acids

Study of the association of the genotypes of the OLR1 gene in the 3’UTR of the g.8232 (A>C) locus on each of the fatty acid components of the HF cows observed are presented in Table 1. Individual fatty acid components were done by qualitative analysis using Gas Chromatography. There were 21 types of the milk fatty acids (FA) were identified, providing 13 types of saturated fatty acids (SFAs) and 9 types of unsaturated fatty acids (UFAs). For saturated fatty acids consisted of short-chain (C4-C8) (3), medium-chain (C10-C14) (3), and long-chain (C15-C24) (7) of the SFAs. While for unsaturated fatty acids (UFAs) provided monounsaturated fatty acids (MUFA) (C14:1) - (C24:1) (5) and polyunsaturated fatty acids (PUFA) (C18:2) - (C22:6) (4). The observed HF cows produced SFAs around 65.712–70.208%, consisted of short-chain by 1.736-2.654%, medium-chain by 12.166–21.271%, and long-chain by 46.882-51.810% respectively. Furthermore, the UFAs ranged from 29.545–32.084%, consisting of MUFA by 28.606-31.104% and FUPA by 0.939-1.081%. Genotypic polymorphism of the OLR1 gene of the g.8232 (A/C) genotypes affected on the milk fat composition of different dairy cattle populations [20].

The g.8232 (A/C) genotypes had significant effects on a number of milk fatty acid components, both saturated fatty acid levels (SFAs) and unsaturated fatty acids (UFAs). This study determined possible effects of the OLR1 gene of the 3’UTR g.8232 (A/C) genotypes on individual milk fatty acid components of the HF cows. The genotypes affected on the medium-chain SFA for Myristic (C14:0) (P<0.01) and long-chain ones for Palmitic (C16:0) (P<0.05) and Stearic (C18:0) (P<0.05). Moreover the genotypes affected MUFA for Oleic (C18:1) (P<0.05) but the effect was not significant for PUFA (P>0.05). Myristic (C14:0) of the AA genotype were at the lowest level against the CC and AC genotypes, by the lacks of 2.540% and 8.277% respectively. By contrast, Palmitic (C16:0) of the AA genotype were the highest level against the CC and AC genotypes, for the benefits of 2.912% and
3.966% respectively. Whereas for Oleic (C18:1) of MUFA that the AA genotype was at the highest content over the CC and AC genotypes, by the advantages of 1.918% and 1.557% respectively.

Table 1. Average of milk fatty acid component (%) based on genotype of holstein friesian cows.

| Fatty acid component          | Genotype |
|------------------------------|----------|
|                              | AA       | AC       | CC       |
| Satated Fatty Acid (SFA)     |----------|----------|----------|
| a. Short chain (C4-C8)       |----------|----------|----------|
| Butyric (C4:0)               | 0.429±0.181 | 0.527±0.193 | 0.774±0.611 |
| Caproic (C6:0)               | 0.371±0.144 | 0.449±0.162 | 0.578±0.368 |
| Caprylic (C8:0)              | 0.937±0.341 | 1.080±0.405 | 1.302±0.709 |
| Sub total                    | 1.736    | 2.055    | 2.654    |
| b. Medium chain (C10-C14)    |----------|----------|----------|
| Capric (C10:0)               | 0.014±0.011 | 0.015±0.009 | 0.014±0.012 |
| Lauric (C12:0)               | 3.887±1.345 | 4.714±1.527 | 4.614±2.197 |
| Myristic (C14:0)             | 8.266±2.346 | 16.543±2.148 | 10.806±2.446 |
| Sub total                    | 12.166   | 21.271   | 15.435   |
| c. Long chain (C15-C24)      |----------|----------|----------|
| Pentadecanoic (C15:0)        | 1.112±0.242 | 1.114±0.235 | 1.048±0.161 |
| Palmitic (C16:0)             | 36.871±7.660 | 32.905±10.070 | 33.959±3.142 |
| Heptadecaenoic (C17:0)       | 0.502±0.079 | 0.526±0.103 | 0.520±0.087 |
| Stearic (C18:0)              | 13.225±2.731 | 12.227±3.864 | 15.691±4.120 |
| Arachidic (C20:0)            | 0.047±0.016 | 0.058±0.027 | 0.053±0.017 |
| Behenic (C22:0)              | 0.021±0.009 | 0.021±0.008 | 0.020±0.009 |
| Lignociric (C24:0)           | 0.034±0.011 | 0.031±0.018 | 0.033±0.011 |
| Sub total                    | 51.810   | 46.882   | 51.324   |
| Total SFA                    | 65.712   | 70.208   | 69.413   |
| Unsaturated Fatty Acid (UFA) |----------|----------|----------|
| a. MUFA (C14:1) - (C24:1)    |----------|----------|----------|
| Myristoleic (C14:1)          | 0.978±0.438 | 1.024±0.353 | 0.915±0.384 |
| Palmitoleic (C16:1)          | 2.352±0.556 | 1.888±0.522 | 1.755±0.708 |
| Oleic (C18:1)                | 27.636±9.508 | 26.079±10.410 | 25.718±5.670 |
| Eucir (C22:1)                | 0.086±0.106 | 0.012±0.023 | 0.165±0.446 |
| Nervonic (C24:1)             | 0.052±0.024 | 0.043±0.031 | 0.053±0.028 |
| Sub total                    | 31.104   | 29.046   | 28.606   |
| b. PUFA (C18:2) - (C22:6)    |----------|----------|----------|
| Linoleic (C18:2 n-6)         | 0.7149±0.186 | 0.7466±0.637 | 0.5956±0.279 |
| Linolenic (C18:3)            | 0.1505±0.084 | 0.2131±0.054 | 0.228±0.061 |
| Eicosatirnoic (C20:3)        | 0.0331±0.010 | 0.0347±0.009 | 0.0363±0.008 |
| Arachidonic (C20:4 n-3)      | 0.0823±0.018 | 0.0868±0.027 | 0.0795±0.032 |
| Sub total                    | 0.9808   | 1.0812   | 0.9394   |
| Total UFA                    | 32.0844  | 30.1274  | 29.5453  |

Note: Different letters in the same row indicated significant different (P<0.05) and very significant different (P<0.01).

From the previous results proved that the g.8232 (A/C) SNP caused of significant effects on the milk fatty acid compositions in dairy cows. The gene candidates underlying the variation on the fatty acid composition can be found in fat synthesis and metabolic pathways, which are under the control of a number of genes. The results from this study proved that the CC genotype was associated with low levels of Myristic and Palmitic, instead of AA and AC genotypes for a low Stearic. Further, the AA genotype presented a high level of Oleate from MUFA.
4. Conclusion
The GHR|Ssp exon 8 (T/A) and Pit1|Stu1 exon 3 (C/T) loci of the PO cattle from twin and multiple births had almost similar frequencies of the alleles and genotypes to single birth. Allelic frequencies of the GHR exon 8 g.914T>A locus of both birth types were high for T allele (M = 78.1–100%, S = 71.4–100%) over A allele. Genetic index values were also almost similar between the two birth types. Meanwhile Pit1 exon 3 c.577C>T locus was monomorphic with the only C allele (100%) without the T allele. The similar conditions of both genetic polymorphisms and genetic indexes between the two birth types indicated the two loci could not be considered as early indicators of genetic markers for exploring potency of twin and multiple births of the observed PO cattle.

References
[1] Mohammed S A, Rahamtalla S A, Ahmed S S, Elhafiz A, Dousa B M, Elamin K M and Ahmed M K A 2015 DGAT1 gene in dairy cattle Glob. J. Anim. Sci. Res. 3 191–8
[2] Anggraeni A, Talib C, Asmarasari S A, Herawati T and Andreas E 2018 Genetic polymorphisms of IGF1, GH, and OPN genes in crosses Peranakan Ongole cattle based on birth type in Central Java J. Ilmu Ternak dan Vet. 22 165–72
[3] Anggraeni A 2019 Genetic polymorphisms of the OLR1 and DGAT1 genes associated with milk components in Holstein Friesian dairy cattle under an intensive management in Central Java IOP Conf. Ser.: Earth Environ. Sci. 287 12001
[4] Khatib H, Leonard S D, Schutzkus V, Luo W and Chang Y M 2006 Association of the OLR1 gene with milk composition in Holstein dairy cattle J. Dairy Sci. 89 1753–60
[5] Bouwman A C, Bovenhuis H, Visker M H P W and van Arendonk J A M 2011 Genome-wide association of milk fatty acids in Dutch dairy cattle BMC Genet. 12 1–12
[6] Wang X, Li T, Zhao H B and Khatib H 2013 A mutation in the 3′ untranslated region diminishes microRNA binding and alters expression of the OLR1 gene J. Dairy Sci. 96 6525–8
[7] Kesek M, Szulc T and Zielak-Steciwko A 2014 Genetic, physiological and nutritive factors affecting the fatty acid profile in cows’ milk--a review Anim. Sci. Pap. Rep. 32 95–105
[8] Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T et al 1997 An endothelial receptor for oxidized low-density lipoprotein Nature 386 73–7
[9] Imanishi T, Hano T, Sawamura T, Takarada S and Nishio I 2002 Oxidized low density lipoprotein potentiation of Fas-induced apoptosis through lectin-like oxidized-low density lipoprotein receptor-1 in human umbilical vascular endothelial cells Circ. J. 66 1060–4
[10] Mehta J L and Li D 2002 Identification, regulation and function of a novel lectin-like oxidized low-density lipoprotein receptor J. Am. Coll. Cardiol. 39 1429–35
[11] Komisarek J and Dorynek Z 2009 Effect of ABCG2, PPARGC1A, OLR1 and SCD1 gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein-Friesian bulls J. Appl. Genet. 50 125–32
[12] Soltani-Ghombavani M, Ansari-Mahyari S, Ghorbani G R and Edriss M A 2013 Association of a polymorphism in the 3′ untranslated region of the OLR1 gene with milk fat and protein in dairy cows Arch. Tierzucht. 56 328–34
[13] Nowakowski P, Patkowska-Sokóla B, Bodkowski R, Szulc T, Kinal S and Walisiewicz-Niedbalska, W Usy dus Z 2012 Modyfikacja składu chemicznego produktów mleczarskich Przem. Chem. 91 906–11
[14] Azis R, Anggraeni A and Gunawan A 2020 Acetyl-CoA carboxylase alpha gene polymorphism and its association with milk fatty acid of Holstein Friesian using real-time PCR method Trop. Anim. Sci. J. 43 306–13
[15] Sambrook J, Fritsch E F and Maniatis T 1989 Molecular Cloning: A Laboratory Manual (United State of America: CSH Laboratory Press)
[16] Nei M and Kumar S 2000 Molecular Evolutionary Genetics (New York: Columbia University Press)
[17] Software SAS®/STAT Software, Release 9.1. (Cary, NC, USA: SAS Institute, Inc)

[18] Hosseinpour Mashhadi M 2017 A research on association between SCD1 and OLR1 genes and milk production traits in Iranian Holstein dairy cattle *Iran. J. Appl. Anim. Sci.* 7 243–8

[19] Kowalewska-Łuczak I and Czerniawska-Piakatkowska E 2018 Polymorphism in the OLR1 gene and functional traits of dairy cattle *Vet. Arh.* 88 171–7

[20] Mele M, Conte G, Castiglioni B, Chessa S, Macciotta N P P, Serra A, Buccioni A, Pagnacco G and Secchiari P 2007 Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins *J. Dairy Sci.* 90 4458–65