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# Exploring effects of betaine-homocysteine methyltransferase (BHMT) gene polymorphisms on fatty acid traits and cholesterol in sheep

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**ABSTRAK**

Tujuan dari penelitian ini adalah untuk mengidentifikasi dan mengasosiasikan keragaman gen Betaine-Homocysteine Methyltransferase (BHMT) dengan komposisi asam lemak dan kolesterol pada daging domba. Penelitian ini menggunakan domba jantan sebanyak 147 sampel domba yang terdiri dari 5 bangsa yaitu 19 Domba Ekor Gemuk (DEG), 16 Domba Ekor Tipis (DET), 41 Domba Komposit Garut,(DKG), 35 Domba Compass Agrinak (DCA) dan 36 domba Barbados Cross (DBC) digunakan untuk identifikasi keragam gen BHMT. Sebanyak 61 domba yang mewakili kelima bangsa domba digunakan untuk menentukan asosiasi keragaman gen BHMT dengan asam lemak pada daging domba. Identifikasi keragaman gen BHMT dianalisis menggunakan metode PCR-RFLP. Asosiasi genotip BHMT dengan asam lemak dan kolesterol dilakukan dengan T-Test. Hasil penelitian menunjukkan bahwa SNP gen BHMT adalah polimorfik dengan tiga genotip yaitu CC, CT dan TT. Hasil penelitian menunjukkan SNP gen BHMT pada daerah exon g. 9947372 (C>T) ada asosiasi ($P<0.05$) dengan komposisi asam lemak jenuh (C18:0) dan tak jenuh yaitu, C14:1dan C17:1. Hasil ini menunjukkan bahwa SNP gen BHMT g. 9947372 (C>T) dapat dijadikan marker untuk seleksi serta produksi daging domba dengan kandungan asam lemak terbaik.

*Kata kunci: asam lemak, BHMT, domba, SNP*

**ABSTRACT**

This study was aimed to explore the effects of Betaine-Homocysteine Methyltransferase (BHMT) gene polymorphisms on fatty acid traits and cholesterol in lambs. This study used a total of 147 blood samples for genotyping including 19 Javanese Fat-Tailed (JFT), 16 Javanese Thin-Tailed (JTT), 41 Composite Garut (CG), 35 Compass Agrinak (CA) and 36 Barbados Black Belly Cross (BC). A total of 61 rams as representative from five breed of sheep were selected for association study. Identification of BHMT single nucleotide polymorphisms was analyzed by PCR-RFLP method. Association of BHMT genotypes with fatty acid traits and cholesterol was performed by T-TEST. BHMT genotyping resulted into three genotypes (CC, CT and TT). Gene frequency of BHMT (g. 9947372 C>T) was in Hardy-Weinberg Equilibrium, excluding Javanese Fat-Tailed sheep. Association of BHMT genotypes with fatty acid traits resulted into a significant association ($P<0.05$) with C14:1, C17:1 and C18:0 fatty acids but

not with cholesterol in sheep. SNP g. 9947372 (C>T) of BHMT gene might be a useful marker for selecting and producing sheep meat with desirable fatty acids.

*Keywords: BHMT, Fatty acid, Sheep, SNP*

## INTRODUCTION

Meat consumers are currently concerned with safe and high quality meat. Fatty acid and cholesterol contents in meat affect meat quality and human healthy (Karamichou *et al*., 2006; Ropka-Molik *et al*., 2016). Meat consumers tend to evaluate meat quality based on eating quality (palatability) including, tenderness, flavor and juiciness of cooked meat (Sebsibe, 2008) and meat tenderness was affected by the quality of fat. Fatty acids and cholesterol are major components of fat (Hidayati *et al*., 2015). When consumed, fat was a source of vitamins A, D, E and K and but also essential fatty acids (Kaić and Mioč, 2016).

The consumer's interests in the nutritional aspects of health have been increased recently. Fat in meat provides flavor, aroma and texture and are main factors that affect palatability of meat, thus affecting consumer acceptance (Maharani *et al*., 2012). Meat consumers dislike sheep meat due to off odour and flavour (Gunawan *et al*., 2018; Listyarini *et al*., 2018; Gunawan *et al*., 2019) but also complain for higher content in saturated fatty acids and cholesterol of sheep meat which are associated with cardiovascular diseases and some cancers (Kaić and Mioč 2016; Munyaneza *et al*., 2019). All saturated fatty acids (SFA) do not influence blood cholesterol at the same level (Sebsibe, 2008; Kaić and Mioč, 2016), myristic acid (C14:0) was the first saturated fatty acid to rise blood cholesterol level, palmitic acid (C16:0) comes next and lauric acid (C12:0), respectively (Sebsibe, 2008), the same author reported that myristic has four times the hypercholesterolemia action of other saturated fatty acids whereas all unsaturated fatty acids and stearic fatty acid are categorized as desirable fatty acids (DFA) (Frigolet and Gutiérrez-Aguilar, 2017). Cholesterol was a basic molecule for making steroid hormones, vitamin D and bile acids (Milićević *et al*., 2014).

Nutritionists suggest a reduction in total fat consumption; especially, saturated fatty acids as they have been linked with cardiovascular diseases and cancers (Milićević *et al*., 2014; Ye *et al*., 2016). Fat consumption ranged from 15%-30% of total energy needs, the consumption of saturated fatty acids was confined between 0-10% to maintain cholesterol levels in normal range to prevent from heart diseases, monounsaturated fatty acids are maintained to 16%, polyunsaturated fatty acids to 7% and cholesterol should be less than 300 mg/day (Hidayati *et al*., 2015). Consequenly, meat consumers need high quality meat with desirable fatty acids and cholesterol.

Through development of molecular techniques, it was possible in nowadays to select and produce animals having desired fatty acid traits and cholesterol. A study by Gunawan *et al*. (2016) using RNA deep sequencing technology identified Betaine-Homocysteine Methyl-transferase (BHMT) gene as potential candidate gene for fatty acid traits. Betaine-Homocysteine Methyltransferase was mapped on chromosome 7 of domestic sheep, it codes for an enzyme (BHMT) that converts homocysteine (Hcy) into methionine (Met), using betaine as methyl donor substrate (Ganu *et al*., 2015).

A study of Teng (2011) reported association of BHMT SNP rs3733890 (c.716G>A) with reduced risk for developing cardiovascular disease in humans. Deficiency of BHMT gene in mice resulted into accumulation of fat in liver due to elevated homocysteine concentrations leading to reduced phosphatidylcholine concentration, the molecule which was required for the synthesis of lipoproteins used to carry lipids from the liver to circulation (Teng, 2011). Xu *et al*. (2016) reported that the existence of BHMT−742G>A polymorphism which was associated with a protective role on the occurrence of uterine cervical cancer in humans. BHMT gene was highly expressed in the liver and kidney, and low levels in the brain, lenses, and other human tissues but in rodents, it was highly expressed only in liver (Teng 2011). There is no previous study carried out to identify polymorphisms of BHMT gene and investigate its association with fatty acid composition and cholesterol in sheep. Therefore, a study exploring genetic polymorphisms of BHMT gene and analyzing their effects on fatty acid traits and cholesterol in sheep is needed.

The objective of this study was to explore the genetic polymorphisms of BHMT gene and

their effects on fatty acid traits and cholesterol in sheep.

## MATERIALS AND METHODS

### Animal and Phenotypes

The present study was carried out at IPB University and Research Center for Development of Animal Production, Bogor, West Java, Indonesia. Blood samples for genomic DNA extraction were collected from jugular vein of one hundred and forty seven rams 147 consisted of Javanese Fat-Tailed (JFT) sheep (n=19), Javanese Thin- Tailed (JTT) sheep (n=16), Composite Garut (CG) sheep (n=41), Compass Agrinak (CA) sheep (n=35) and Barbados Black Belly Cross (BC) sheep (n=36). Due to limitations of loin samples from sheep, analysis of fatty acid was carried out on sixty one (61) from one hundred and forty seven (147) genotyped sheep. They were consisted of JFT sheep (n=19), JTT sheep (n=14), CG sheep (n=8), CA sheep (n=10) and BC (n=10). All Sheep had average body weight of 25-30 kg and 12 months old. All sheep were kept at Indonesian Center for Development of Animal Production, under the same management conditions and water was at *ad libitum*. They were slaughtered according to the welfare ethics in a commercial abattoir in Indonesia. A total of 1 ml of blood samples, 100 g and 100 g of loin samples for DNA extraction, fatty acids and cholesterol analysis were used, respectively. All samples were kept at -20°C for future utilization.

### Genotyping of BHMT Gene using PCR RFLP

The g. 9947372 (C>T) SNP of BHMT gene used in this study was described by a study of Gunawan *et al*. (2016) using RNA sequencing. DNA extraction was performed using a reference (Sambrook *et al*., 1989). PCR was performed for amplification of polymorphic region of BHMT gene. A pair of Primers was designed for amplification of a fragment of 385 base pairs (forward and reverse, 5'-CACCATAACTTTAGGGCATC-3'and 5'-GACTTATCTAGAGCAAGGAG-3', respectively according to the sheep genomic sequence in the GenBank database (accession number NC_019464.2). The following are the conditions under which PCR was carried out; initial denaturation was at 95°C during 5 minutes and for 1 cycle. The second phase consisted of 35 cycles, each cycle consisted of denaturation process at 95°C for 20 seconds, primer annealing at 58°C for 30 seconds and DNA extension at 72°C for 30 seconds. The final phase was the primer elongation or final extension at 72°C for 5 minutes ESCO PCR machine was used. The DNA amplification product of 385 base pairs was visualized by 1.5% agarose gel electrophoresis.

PCR products from polymorphic region of BHMT gene (385bp) were digested with BsaAI restriction enzyme, selected according to the software (http://tools.neb.com/ NEB cutter2 /index. php**)** of the polymorphic sites. PCR product and BsaAI restriction enzyme were incubated at 30° C for 4 hours (Thermo Fisher Scientific, EU, Lithuania). The products of DNA fragments from PCR-RFLP were visualized using agarose gel electrophoresis with a concentration of 2%. Electrophoresis was run on average voltage of 100 volts for 45 minutes. Gels were visualized under ultra-violet transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA).

### Analysis of Fatty Acid Composition

Due to limitations of loin samples from sheep, analysis of fatty acid traits was carried out on sixty one (61) as a representing sample from one hundred and forty seven (147) genotyped sheep. Fats were extracted for each sample using a method described by Folch *et al*. (1957). Approximately 100 g of loin samples were collected and grounded for fatty acid analysis. Fatty acids analysis was done according to Association of Official Analytical Chemists (AOAC 2012).

### Cholesterol Analysis

Cholesterol was analyzed through saponification and measurement steps Due to the scarcity of laboratory products for cholesterol analysis; cholesterol analysis was conducted on twenty seven (27) loin samples, they include 10 loin samples from Compass Agrinak (CA), 9 loin samples from Javanese Fat-Tailed (JFT) and 8 loin samples from Composite Garut (CG) sheep breeds. Saponification: About 2 g of each sample were saponified by using a method described by Stewart *et al*. (1992) with little modification. Cholesterol Measurement: Samples were analyzed by high-performance liquid chromatography (HPLC) method. Cholesterol identification was performed by co-chromatography and by comparing sample retention times with standard retention times (Sigma and Polyscience, U.S.A. ® C8667). Data were expressed as mg/100g of loin

samples.

**Statistical Analysis**

Genotype and allele frequencies were analyzed using genotyping data of five sheep breeds; JFT, JTT, BC, CG and CA. Genotype frequency was calculated using the formula of Nei and Kumar (2000):

$$x_{ii} = \frac{\sum_{i=1}^{n} n_i}{N}$$

Allele frequencies (Nei and Kumar 2000):

$$x_i = \frac{(2n_{ii} + \sum_{i \neq j} n_{ij})}{2N}$$

Where $x_i$ is the i-th allele frequency, $x_{ii}$ is ii-th genotype frequency, i is the frequency of allele $i^{th}$; $n_{ii}$ is the total individuals with genotype ii; $n_{ij}$ total individuals with genotype ij and N is the population size. Hardy-Weinberg equilibrium (H-W) (Hartl and Clark, 1997):

$$\chi^2 = \sum_{i=1}^{N} \frac{(O-E)^2}{E}$$

Where $\chi^2$ is Chi Squared; O is total of observed genotypes and E is total of expected genotypes and i is number of observation.

**Association analysis**

Normality test of fatty acid traits and cholesterol data was performed. Association of BHMT genotypes with fatty acids traits and cholesterol was performed by T-TEST using MINITAB Program. The following statistical model was used:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where

$$S = \sqrt{\frac{\sum_{i=1}^{n}(\bar{X}_i - \bar{X}_1)^2 + \sum_{i=1}^{n}(\bar{X}_i - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

$\bar{X}_1 and \bar{X}_2$ = average traits for genotype 1 and genotype 2, n1 and n2 = individual number of genotype 1 and 2, S= combined variance.

## RESULTS

**BHMT Gene Polymorphism**

A polymorphic region of BHMT was successfully amplified as presented by Figure 1. The single nucleotide polymorphism (C>T) of BHMT gene located at the nucleotide position g.9947372 segregated into three genotypes designed as CC (two fragments of 290 and 95bp), CT (three fragments of 295, 95 and 385bp) and TT (a single, uncut fragment of 385bp) (Figure 2). The resulting genotypes had two alleles (C and T). The allele T had higher frequency in all sheep breeds. The distributions of genotype and allele frequencies for BHMT in five sheep breeds are presented in Table 1. The SNP of BHMT (g.9947372 C>T) was found to be in Hardy-Weinberg Equilibrium in all sheep breeds, except JFT sheep breed.

**Effects of BHMT genotypes on fatty acid traits**

BHMT genotypes were significantly

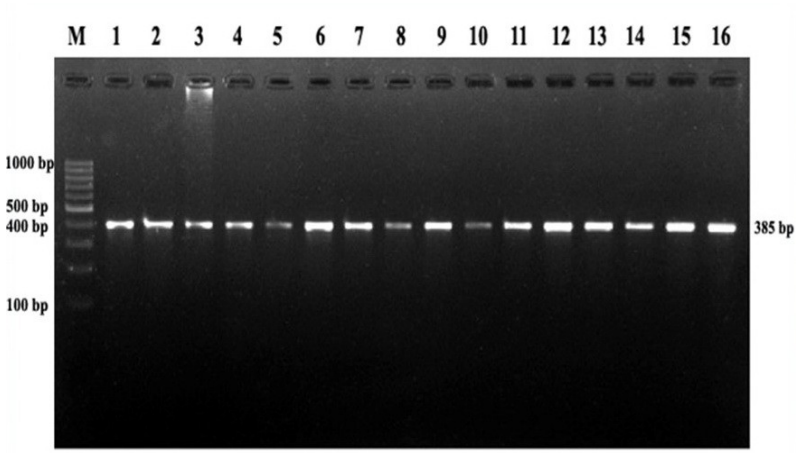


Figure 1 Visualization of BHMT Gene Amplification on a 1.5% Gel Agarose. M: DNA marker 100bp, sample 1-16: PCR product of BHMT gene.

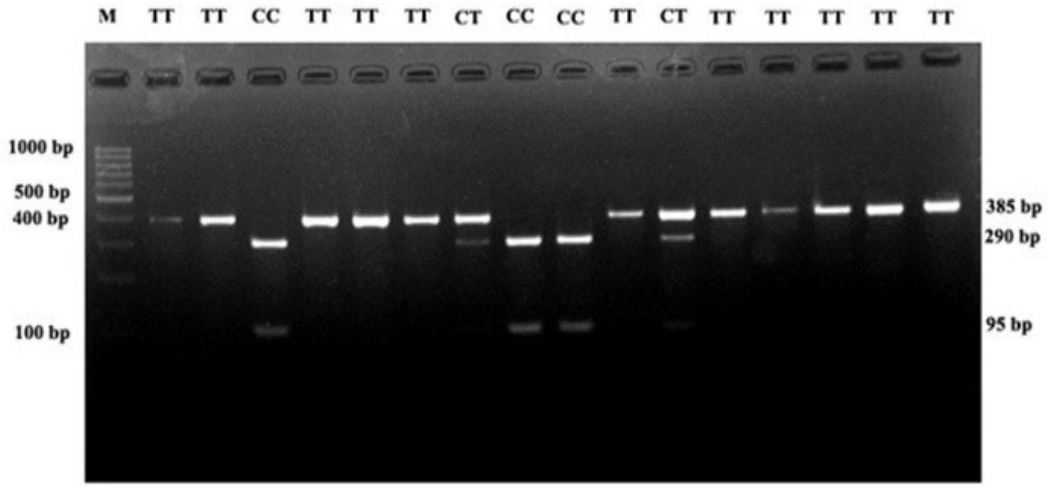


Figure 2 PCR-RFLP for a 385 bp Fragment of BHMT Gene by BsaAI Enzyme on 2% Agarose Gel.

Table 1 Genotype and Allele Frequencies of BHMT Gene (g.9947372, C>T)

| Sample | N | Genotype frequency | | | Allele frequency | | $\chi^2$ |
|---|---|---|---|---|---|---|---|
| | | CC | CT | TT | C | T | |
| JFT | 19 | 0.05 (1) | 0.05 (1) | 0.90 (17) | 0.08 | 0.92 | $7.5^{s}$ |
| JTT | 16 | 0.125(2) | 0.25 (4) | 0.625(10) | 0.25 | 0.75 | $1.77^{ns}$ |
| CG | 41 | 0.03 (1) | 0.46 (19) | 0.51 (21) | 0.26 | 0.74 | $1.92^{ns}$ |
| CA | 35 | 0.06 (2) | 0.4 (14) | 0.54 (19) | 0.26 | 0.74 | $0.08^{ns}$ |
| BC | 36 | 0.14 (5) | 0.28 (10) | 0.58 (21) | 0.28 | 0.72 | $3.37^{ns}$ |

$^{ns}$=not significant at P<0.05, **=significant (P<0.05). $\chi^2$: Chi-square: Chi-square from table (P<0.05) = 3.841, degree of freedom (df=1). Numbers shown in parentheses are the number of individuals with the specified genotype. JFT: Javanese Fat-Tailed, JTT: Javanese Thin-Tailed, CG: Composite Garut, CA: Compass Agrinak, BC: Barbados Black Belly Cross.

associated (P<0.05) with stearic acid (C18:0), myristoleic acid (C14:1) and heptadecenoic acid (C17:1. Sheep with genotype CT had higher mean value of stearic acid (C18:0) and C14:1 compared to sheep with genotypes CC and TT (Table 2).

**Effect of BHMT Genotypes on Cholesterol in Sheep**

There was no effect (P>0.05) of BHMT genotypes on cholesterol in Javanese Fat-Tailed (JFT), Compass Agrinak (CA) and Composite Garut (CG) sheep as shown in Table 2.

## DISCUSSION

This study showed that Betaine-Homocysteine Methyltransferase (BHMT) gene is important in fatty acid composition of sheep as its genotypes were significantly associated (P<0.05) with monounsaturated fatty acids such as myristoleic acid (C14:1) and heptadecenoic acid (C17:1) and also with stearic acid (C18:0), a saturated acid which has beneficial effect on human health. BHMT gene plays a role in lipid movement from the liver to adipose tissue by increasing phosphatidylcholine concentration, this

Table 2. Table 2 Effect of BHMT Genotypes on Fatty acid Traits and Cholesterol in Sheep

| Variable | BHMT Gene | | | P-Value | | |
|---|---|---|---|---|---|---|
| | Genotype, Mean ± SD | | | Genotypes | | |
| | CC (n=3) | CT (n=15) | TT (n=43) | CC vs CT | CC vs TT | CT vs TT |
| **Fat Content of Meat** | 1.64±0.33 | 1.05±0.89 | 1.37±0.79 | 0.28 | 0.55 | 0.21 |
| Total Fatty Acid | 74.95±1.63 | 73.7±11.3 | 70.60±8.71 | 0.85 | 0.39 | 0.27 |
| **Saturated Fatty Acid** | 41.81±2.11 | 41.81±7.75 | 37.90±8.47 | 0.99 | 0.43 | 0.12 |
| Capric acid (C10:0) | 0.61±0.10 | 0.75± 0.13 | 0.69±0.16 | 0.09 | 0.36 | 0.19 |
| Lauric acid (C12:0) | 0.53±0.38 | 0.57±0.16 | 0.57± 0.25 | 0.76 | 0.79 | 0.98 |
| Tridecylic acid (C13:0) | 0.87±0.22 | 0.73±0.23 | 0.79±0.25 | 0.35 | 0.61 | 0.40 |
| Myristic acid (C14:0) | 1.21±0.80 | 0.91±0.38 | 0.95±0.67 | 0.31 | 0.52 | 0.81 |
| Pentadecanoic acid (C15:0) | 0.40±0.37 | 0.48±0.12 | 0.48±0.15 | 0.47 | 0.42 | 0.97 |
| Palmitic acid (C16:0) | 20.44±1.49 | 18.44±3.44 | 18.12±3.29 | 0.45 | 0.23 | 0.48 |
| Heptadecanoic acid (17:0) | 0.50±0.89 | 0.54±0.10 | 0.51±0.09 | 0.61 | 0.88 | 0.42 |
| Stearic acid (C18:0) | 2.68±3.44 | 2.88 ±0.33 | 2.61±0.40 | 0.34 | 0.76 | **0.02*** |
| Arachidic acid (C20:0) | 0.62±0.85 | 0.65±0.25 | 0.63±0.24 | 0.85 | 0.93 | 0.81 |
| Heneicosylic acid (C21:0) | Nt | 0.18±0.03 | 0.19±0.03 | Nt | Nt | 0.20 |
| Behenic acid (C22:0) | 0.01±0.02 | 0.02±0.01 | 0.08±0.11 | 1.0 | 0.31 | 0.53 |
| Tricosylic acid (C23:0) | 0.00±0.01 | 0.04±0.05 | 0.04±0.06 | 0.27 | 0.33 | 0.79 |
| Lignoceric acid (C24:0) | 0.06±0.01 | 0.06±0.08 | 0.07±0.13 | 0.27 | 0.36 | 0.73 |
| **Unsaturated Fatty Acid** | 32.87 ±1.73 | 31.73±5.64 | 32.77±5.18 | 0.73 | 0.51 | 0.97 |
| **MUFA** | 29.72 ± 0.68 | 26.85±5.74 | 28.12±6.36 | 0.41 | 0.66 | 0.49 |
| Myristoleic acid (C14:1) | 0.55±0.25 | 0.62±0.14 | 0.50±0.18 | 0.47 | 0.70 | 0.02* |
| Palmitoleic acid (C16:1) | 1.59±0.31 | 1.30±0.32 | 1.88±1.69 | 0.16 | 0.77 | 0.19 |
| C17:1[1)] | 0.94±0.08 | 0.87±0.72 | 0.82±0.08 | 0.13 | 0.13 | 0.04* |
| Elaidic acid(C18:1n9t) | 1.98±0.08 | 2.23±0.29 | 2.19±0.24 | 0.29 | 0.26 | 0.71 |
| Oleic acid (C18:1n9c) | 27.85±1.18 | 25.06±5.76 | 25.5±6.0 | 0.42 | 0.51 | 0.78 |
| Gondoic acid (C20:1) | Nt | 0.02±0.07 | 0.04±0.11 | Nt | Nt | 0.42 |
| Nervonic acid (C24:1) | Nt | 0.73±0.20 | 0.71±0.20 | Nt | Nt | 0.90 |
| **PUFA** | 1.04±0.52 | 1.19 ±0.48 | 1.09±0.48 | 0.64 | 0.86 | 0.35 |
| Linoleic acid(C18:2n6c) | 2.33±1.40 | 2.91 ±1.38 | 2.78±1.33 | 0.51 | 0.57 | 0.75 |
| γ-Linolenic acid C18:3n6) | 0.00±0.01 | 0.04±0.09 | 0.03±0.06 | 0.44 | 0.50 | 0.42 |
| α-Linolenic acid (C18:3n3) | 0.08±0.15 | 0.24±0.25 | 0.22±0.18 | 0.30 | 0.21 | 0.70 |
| Eicosadienoic acid (C20:2) | 0.88±0.05 | 0.87±0.45 | 0.84±0.05 | 0.70 | 0.29 | 0.14 |
| DGLA[2)] (C20:3n6) | 0.03±0.04 | 0.13±0.20 | 0.08±0.10 | 0.41 | 0.47 | 0.18 |

Table 2 (continued)

| Variable | BHMT Gene | | | P-Value | | |
|---|---|---|---|---|---|---|
| | Genotype, Mean ± SD | | | Genotypes | | |
| | CC (n=3) | CT (n=15) | TT (n=43) | CC vs CT | CC vs TT | CT vs TT |
| Arachidonic acid (C20:4n6) | 0.59±0.16 | 0.61±0.22 | 0.66±0.21 | 0.83 | 0.57 | 0.51 |
| Docosadienoic acid (C22:2) | Nt | 0.02±0.07 | 0.00±0.03 | Nt | Nt | 0.39 |
| Eicosapentaenoic acid | 0.62±0.20 | 0.56±0.24 | 0.51±0.29 | 0.69 | 0.52 | 0.56 |
| Docosahexaenoic acid (C22:6n3) | 0.67±0.16 | 0.66±0.21 | 0.69±0.13 | 0.94 | 0.85 | 0.67 |
| | CC(n=0) | CT(n=9) | TT(n=18) | | | |
| **Cholesterol** | - | 2.34±0.81 | 1.88±1.05 | - | - | 0.252 |

Nt: Not tested using T-Test due to very small amount detected, means and standard deviations were calculated based on normalized data, SD: standard deviation, *: significantly at P<0.05. Numbers shown in parentheses are the number of individuals with the specified genotype, C17:1[1)]: Heptadecenoic acid, DGLA[2)]: Dihomo-gamma-linolenic acid. vs: Versus

molecule is important for the synthesis of lipoproteins used for transporting lipids from the liver to circulation, thus avoiding fat accumulation (Teng, 2011).

The SNP of BHMT gene (g. 9947372 C>T) was in Hardy-Weinberg Equilibrium (P<0.05), except in JFT sheep breed. A population of large size is in Hardy-Weinberg Equilibrium if gene frequency does not change from generation to generation in the absence of evolution forces including selection, mutation, gene migration, genetic drift (Noor, 2010; Allendorf *et al*., 2013; Gunawan *et al*., 2017).

The BHMT were significantly associated (P<0.05) with monounsaturated fatty acids such as myristoleic acid (C14:1) and heptadecenoic acid (C17:1) and also with stearic acid (C18:0). Stearic acid (C18:0) is a saturated fatty acid which is needed in human diet, it does not rise plasma Low density lipoprotein cholesterol (Baer *et al*., 2003) as other saturated fatty acids do. Stearic acid and unsaturated fatty acids are considered to be desirable fatty acids (DFA) (Sebsibe, 2008) as they contribute to the increased activity of hepatic low density lipoprotein (LDL) receptor resulting into reduced circulating concentration of Low density lipoprotein-cholesterol (LDL-C) also known as bad cholesterol (Maharani *et al*., 2012). They present benefits to human health, stearic acid and unsaturated fatty acids get beneficial effect because they protect against cardiovascular diseases (Kaić and Mioč, 2016). Myristoleic acid (C14:1) and heptadecenoic acid (C17:1) are monounsaturated fatty acids of cis-configuration with positive effects on human healthy as they do not reduce high density lipoprotein-cholesterol (HDL-C) which protects against coronary heart diseases by increasing circulation of low density lipoprotein (LDL-C) (Cifuni *et al*., 2003). Hameed *et al*., 2017 reported that myristoleic acid (C14:1) is thought to be a fatty acid that play a role against prostatic cancer.

Fatty acids are major components of fats, they are classified as saturated fatty acids and unsaturated fatty acids (Ratnayake and Galli, 2009). Unsaturated fatty acids are also grouped into monounsaturated fatty acids and polyunsaturated fatty acids (Hidayati *et al*., 2015). High consumption of unsaturated fatty acids presents healthy benefits because they accelerate activity of hepatic low density lipoprotein (LDL) receptor which reduces circulating concentration of LDL-cholesterol (Maharani *et al*. 2012). High intake of saturated fatty acids has been linked with cardiovascular diseases and cancer (Hidayati *et al*.; 2015; Kaić and Mioč, 2016).

BHMT gene (g.9947372 C>T) was not shown to affect significantly (P>0.05) cholesterol content in Javanese Fat-Tailed, Composite Garut and Compass Agrinak (Table 2). BHMT gene has

a role in regulating plasma total cholesterol and high density lipoprotein-cholesterol (HDL-C), which is known as good cholesterol (Teng, 2011). Selection of sheep having higher mean values of myristoleic acid (C14:1), heptadecenoic (C17:1) and stearic acid (C18:0) might provide healthy benefits for humans. We might speculate that BHMT genotypes present minor effects on cholesterol concentration in sheep.

## CONCLUSION

The BHMT gene was polymorphic in Barbados Black Belly Cross, Composite Garut, Compass Agrinak, Javanese Fat-Tailed and Javanese Thin-Tailed sheep with T allele more frequent. The SNP g. 9947372 C>T of BHMT gene was significantly associated with C14:1, heptadecenoic acid (C17:1) and C18:0. SNP (g. 9947372 C>T) of BHMT gene might be a useful marker for selecting and producing sheep meat with desirable fatty acids.

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