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Identification of Single Nucleotide Polymorphism and Association Analysis of Alpha 2-Heremans Schmid Glycoprotein (AHSG) Gene Related to Fatty Acid Traits in Sheep

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ABSTRACT

Fatty acid (FA) composition of meat is regulated by many genes. The aim of this study was to identify Single Nucleotide Polymorphism (SNP) of Alpha 2-Heremans Schmid Glycoprotein (AHSG) gene and analyze its association with fatty acid (FA) traits in lambs. The study used a total of 67 rams of 12 months with average body size of 25-30 kg, consisted of 20 heads of Javanese Fat-Tailed (JFT) sheep, 17 heads of Javanese Thin-Tailed (JTT) sheep, 10 heads of Composite Garut (CG) sheep, 10 heads of Compass Agrinak (CA) sheep and 10 heads of Barbados Black Belly Cross (BC) sheep. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) were used to identify the single nucleotide polymorphisms (SNP) of AHSG gene. Association of AHSG genotypes with fatty acid traits was performed using General Linear Model by SAS 9.4 program. The SNP of AHSG gene was polymorphic with three genotypes (GG, GA and AA). In combined population, the genotype frequency of GG, GA and AA were 0.25, 0.13 and 0.62, respectively. The Chi-square test revealed that the locus of AHSG (g. 198655287 (G>A) was in Hardy-Weinberg equilibrium, except in Composite Garut (CG), Compass Agrinak (CA) and Barbados Black Belly Cross (BC) sheep breeds. The g.198655287 (G>A) SNP of AHSG gene was significantly associated ($P<0.05$) with saturated fatty acid, including capric acid (C10:0), palmitic acid (C16:0), heptadecanoic acid (17:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C22:0), tricosylic acid (C23:0), lignoceric acid (C24:0); with monounsaturated fatty acids, including palmitoleic acid (C16:1); oleic acid (C18:1n9c); eicosenoic acid (C20:1); nervonic acid (C24:1) and with polyunsaturated fatty acids, including linoleic acid (C18:2n6c); γ-Linolenic acid; α-Linolenic acid; eicosadienoic acid (C20:2); dihomo-gamma-linolenic acid; arachidonic acid; docosadienoic acid(C22:2); eicosapentanoic and docosahexaenoic acid. The SNP g. 198655287 (G>A) of AHSG gene may be a useful marker for selecting and producing sheep meat having desirable fatty acids.

Keywords : AHSG, Fatty Acid, Sheep, SNP

I. INTRODUCTION

Meat has a vital role in human diet as a source of protein, fats, minerals and essential vitamins (Wood et al. 2007; Cabrera and Saadoun 2014; Quiñones et al. 2017). Fatty acids are components of fats and are grouped into saturated fatty acids and unsaturated fatty acids, the latter are classified as monounsaturated fatty acids (MUFA) with one double bond and polyunsaturated fatty acids (PUFA) with the presence of more than one double bond (PUFA) (Hidayati et al. 2015; Gunawan et al. 2018). Fatty acids have many advantages including being source of energy, structure components of cell membranes, signaling molecules in immune system and regulators of gene expression (Kaić and Mioč

2016). Fatty acid composition of meat controls its quality and acceptance level by meat consumers (Sebsibe 2008). However, sheep meat is characterized by high level of saturated fatty acids and cholesterol which have been associated with cardiovascular diseases and cancer (Maharani et al. 2013). This affects sheep meat consumption.

Many researches were carried out to maintain saturated fatty acids at acceptable level and increase high levels of unsaturated fatty acids as higher consumption of unsaturated fatty acids including (PUFA) and (MUFA) has been reported to have a beneficial effects on human healthy (Maharani et al. 2013, Hidayati et al. 2015; Kaić and Mioč 2016). Fatty acid composition of muscle and adipose lipid tissue is under the control of breed, quality and quantity of feed, age/body weight, sex and level of fatness, where fatty acid composition affects the nutritive value and the sensational characteristics of meat (Sebsibe 2008).

To select and produce desirable and healthy meat meeting preferences of sheep meat consumers, direct selection of genes that affect fatty acids and meat quality is applied for breeding purposes. The fatty acid composition of meat is regulated by many genes involved in lipogenesis (Quiñones et al. 2017). AHSG gene was identified to be a potential candidate gene for fatty acid composition by a study of Gunawan et al. (2016) using RNA deep sequencing technology. Alpha2-Heremans-Schmid glycoprotein (AHSG), also known as Fetuin-A is coded by AHSG gene and is synthesized and secreted by the liver (Fisher et al. 2009). AHSG plays a role of a connection between fatty liver and insulin resistance where high AHSG plasma levels are associated with insulin resistance in humans resulting into accumulation of fat in the liver (Stefan et al. 2006). Elevated circulating levels of fetuin-A in blood have been linked to the increased risk of cardiovascular disease (Fisher et al. 2009). A Singlenucleotide polymorphism (SNP), rs4917, in the AHSG gene has been shown to be associated with reduced plasma levels as well as lower body fat in humans (Lavebratt et al. 2005). AHSG gene is mapped on chromosome 1, has 9 exons and 8 introns. There is no previous study conducted to identify and investigate the association of AHSG gene with fatty acid composition in sheep. Therefore, this study aimed to identify Single Nucleotide Polymorphism (SNP) of Alpha 2-Heremans Schmid Glycoprotein (AHSG) gene and analyze its association with fatty acid (FA) traits in sheep.

II. METHODS AND MATERIAL

Animals and samples

The present study was conducted at IPB University, Indonesia. Blood samples for DNA extraction were collected from jugular vein of one hundred and fifty one (151) male rams consisted of Javanese Fat Tailed (JFT) sheep (n=20), Javanese Thin Tailed (JTT) sheep (n=17), Composite Garut (CG) sheep (n=45), Compass Agrinak (CA) sheep (n=35) and Barbados Black Belly Cross (BC) sheep (n=34) while loin samples for fatty acid analysis were collected from sixty seven (67) sheep consisted of JFT sheep (n=20), JTT sheep (n=17), CG sheep (n=10), CA sheep (n=10) and BC (n=10). All sheep had average body weight of 25-30 kg and 12 months old, they were reared in groups of 5 animals in 14 covered pens equipped with troughs for food and water at *ad libitum*, were slaughtered according to the welfare ethics in a commercial abattoir in Indonesia. A total of 100µl of blood samples and 100 mg of loin samples for DNA extraction and fatty acids analysis were used, respectively. All samples were kept at -20° C until further usage.

Analysis of Fatty Acid Composition

Loin samples (100g) were collected and grounded for fatty acid analysis using the protocol of Association of Official Analytical Chemists (AOAC, 2012), the measured traits for fatty acids included total fatty acids, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids.

DNA Extraction and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

The g.198655287 (G>A) SNP of AHSG 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 AHSG gene. A pair of primers was designed using the software tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and PCR suitability tests were checked using Primer Stat. These primers (forward and reverse), 5'-GGAGGAATCAGGGCATTTTC-3' and 5'-CCCATATCCTTACGCAATCC-3', respectively were used to amplify a 473base pairs (bp) fragment according to the sheep genomic sequence in the GenBank database (accession number NC_019458.2). The PCR was performed under the following conditions, initial denaturation at 95 °C for 5 minutes and for 1 cycle. The second phase consisted of 35 cycles, each cycle consisting 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. The DNA amplification product of 473 bp was visualized by 1.5% agarose gel electrophoresis.

PCR products from polymorphic region of AHSG gene (473bp) were digested with EagI restriction enzyme which was selected according to the software (http://tools.neb.com/ NEB cutter2 /index. php) of the polymorphic sites. PCR product and EagI restriction enzyme were incubated at 37 °C for 2 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%, 0.6 g agarose powder was added to 30 ml of 0.5 x TBE, the mixture then heated to boiling and added to 1 µL fluoro safe, 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).

Statistical Analysis

Gene frequency consists of genotype and allele frequencies were analyzed using genotyping data of four sheep breeds (JFT, JTT, CG, CA and BC). 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} is the 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 χ^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

Association between the SNP of AHSG gene and fatty acid traits was performed using the GLM procedure (SAS Inst, Inc., Cary, NC). The following model was used with the genotype as fixed effects (Genotype; 3 levels; GG, GA and AA) and breed (CG, CA, BC, JFT and JTT) :

$$Y_{ijk} = \mu + G_i + B_j + e_{ijk}$$

Where Y_{ik} is the observation on sheep meat fatty acid, μ is the populations mean for traits, genotype is the fixed effects, B_j is breed effects and e is random error. A p-value of <0.05 was considered to be statistically significant. In order to test the pair wise differences between the effects of genotype and breed, Duncan Multiple Range Test (DMRT) was performed.

III. RESULTS AND DISCUSSION

Results

AHSG Gene Polymorphism

The AHSG gene single nucleotide polymorphism (G>A) is located at the nucleotide position g.198655287, exon 2 of chromosome 1 with GenBank Accession Number: NC_019458.2. The 473 bp fragment of AHSG (g.198655287 (G>A) gene was successfully amplified as shown in (Figure 1). Figure 1. Amplification result of PCR for AHSG gene was performed on 1.5% gel agarose.

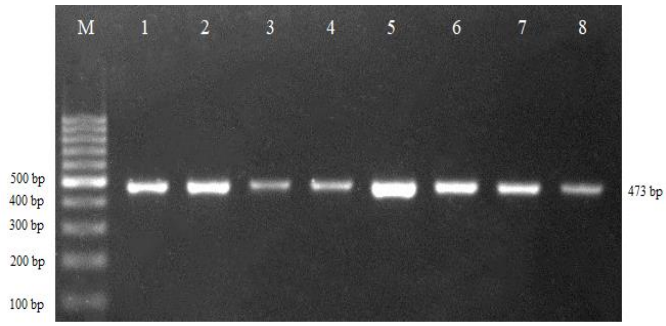

Figure 1. Amplification result of PCR for AHSG gene on 1.5% gel agarose; M=100 bp ladder size standard; Line 1-8=Individual sheep samples.

The 473 base pairs fragment was digested with EagI restriction enzyme and resulted into three genotypes designed as GG (two fragments of 200 and 273bp), GA (three fragments of 200, 273 and 473bp) and AA (a single, uncut fragment of 473bp) (figure 2). PCR-RFLP analysis of 473 bp fragment of AHSG gene by EagI restriction enzyme was performed on 2% agarose gel. Figure 2. PCR-RFLP analysis of 473 bp fragment of AHSG gene by EagI restriction enzyme on 2% agarose gel

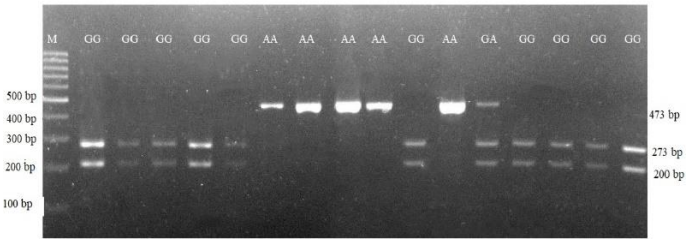

Figure 2. PCR-RFLP analysis of 473 bp fragment of AHSG gene by EagI restriction enzyme on 2% agarose gel. GG: GG genotype; GA: GA genotype; AA: AA genotype; M: DNA marker 100bp

They were two alleles (G and A). The allele G had a higher frequency in CA and BC sheep breeds, and allele A had a higher frequency in CG, JFT and JTT sheep breeds. The distributions of genotype and allele frequencies for AHSG/ EagI in all sheep are presented in Table 1.

Table 1. Genotype and allele frequencies of AHSG gene g. 198655287 (G>A SNP in JFT, JTT, CG, CA and BC sheep breeds.

Sample	N	Genotype frequency			Allele frequency		χ^2
		GG	GA	AA	G	A	
JFT	20	0.00(0)	0.10(2)	0.90(18)	0.05	0.95	0.06^{ns}
JTT	17	0.00(0)	0.12(2)	0.88(15)	0.06	0.94	0.07^{ns}
CG	45	0.13(6)	0.11(5)	0.76(34)	0.19	0.81	18.18^{**}
CA	35	0.51(18)	0.00(0)	0.49(17)	0.51	0.49	35^{**}
BC	34	0.412(14)	0.323(11)	0.265(9)	0.57	0.43	3.88^{**}
Combined	151	0.25(38)	0.13(20)	0.62(93)	0.32	0.68	72.4^{**}

Note: ^{ns}=not significant at P<0.05, **=significant (P<0.01). χ^2: Chi-square: Chi-square from table=3.841(P<0.05), degree of freedom (df) is equal to the number of expected genotypes-1, number of genotypes is 3 and df=1.)

The SNP of AHSG (g.198655287 (G>A) was in Hardy-Weinberg Equilibrium, except in Composite Garut (CG), Compass Agrinak (CA) and BC sheep breeds. To the author's knowledge, no information is available on the SNP of AHSG (G>A) either in humans or animals which made difficult to compare the present results.

Association of AHSG gene polymorphism and fatty acid traits in CG, CA, BC JFT and JTT sheep breeds

Due to the fact that GG and GA genotypes were not segregated in all populations used in this study and due to the low frequency of G allele in JFT, JTT and CG sheep breeds, association analysis conducted between SNP of AHSG (g.198655287 (G>A) gene and fatty acid traits was carried out in combined population (BC, CA, CG, JFT and JTT). The association of SNP of AHSG (g.198655287 (G>A) gene and fatty acid traits in combined population (BC, CA, CG, JFT and JTT) is summarized in Table 2.

Table 2 Phenotype of meat fatty acid traits of JFT, JTT, CG, CA and BC sheep breeds (%).

Variable	BC, CA, CG, JFT, JTT(n=67)	BC(n=10)	CA(n=10)	CG(n=10)	JFT(n=20)	JTT(n=17)
Fat Content of meat	4.23±3.86	$1.68±1.86^{b}$	$0.00±0.00^{b}$	$1.95±0.91^{b}$	$7.08±4.01^{a}$	$6.21±2.65^{a}$
Total Fatty Acid	71.39±8.99	$64.90±15.52^{b}$	$79.78±7.32^{a}$	$69.08±5.93^{b}$	$71.26±6.09^{b}$	$71.79±5.50^{b}$
Saturated Fatty Acid	38.78±8.03	$34.36±10.71^{c}$	$47.84±6.15^{a}$	$41.78±5.53^{b}$	$35.28±6.67^{c}$	$38.42±5.06^{bc}$
Capric acid (C10:0)	0.08±0.05	$0.01±0.01^{d}$	$0.04±0.01^{cd}$	$0.05±0.01^{c}$	$0.10±0.03^{b}$	$0.13±0.05^{a}$
Lauric acid (C12:0)	0.48±0.54	0.47±0.40	0.54±0.20	0.35±0.10	0.68±0.89	0.29±0.19
Tridecylic acid (C13:0)	0.01±0.01	$0.00±0.00^{c}$	$0.01±0.01^{ab}$	$0.01±0.01^{ab}$	$0.01±0.01^{a}$	$0.00±0.01^{cb}$
Myristic acid (C14:0)	3.12±1.93	$1.97±1.28^{b}$	$3.32±1.07^{ab}$	$2.14±0.38^{ab}$	$3.55±2.64^{b}$	$3.74±1.84^{a}$
Pentadecanoic acid (C15:0)	0.48±0.15	$0.38±0.17^{b}$	$0.51±0.09^{ab}$	$0.48±0.10^{ab}$	$0.45±0.17^{ab}$	$0.55±0.13^{a}$
Palmitic acid (C16:0)	17.79±3.92	$13.15±6.06^{c}$	$18.36±3.02^{ab}$	$16.26±2.37^{b}$	$18.73±2.65^{ab}$	$19.99±2.20^{a}$
Heptadecanoic acid (17:0)	0.96±0.36	$0.60±0.19^{b}$	$0.80±0.12^{b}$	$0.80±0.08^{b}$	$1.20±0.47^{a}$	$1.09±0.21^{a}$
Stearic acid (C18:0)	15.52±5.98	16.67±4.30b	$23.86±2.60^{a}$	$21.33±3.14^{a}$	$10.43±2.80^{c}$	$12.52±3.36^{c}$
Arachidic acid (C20:0)	0.11±0.11	$0.33±0.06^{a}$	$0.00±0.01^{d}$	$0.16±0.03^{b}$	$0.06±0.02^{c}$	$0.07±0.03^{c}$
Heneicosylic acid (C21:0)	0.02±0.02	$0.05±0.01^{a}$	$0.03±0.01^{b}$	$0.02±0.01^{b}$	$0.01±0.01^{c}$	$0.00±0.00^{c}$
Behenic acid (C22:0)	0.07±0.10	$0.26±0.09^{a}$	$0.13±0.07^{b}$	$0.06±0.03^{c}$	$0.01±0.01^{d}$	$0.01±0.01^{d}$

Tricosylic acid (C23:0)	0.04±0.06	0.15±0.06^{a}	0.07±0.04^{b}	0.03±0.02^{c}	0.00±0.00^{d}	0.00±0.00^{d}
Lignoceric acid (C24:0)	0.06±0.11	0.28±0.12^{a}	0.11±0.08^{b}	0.03±0.02^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Unsaturated Fatty Acid	32.59±5.03	31.27±8.50	31.63±4.10	27.29±1.88	35.89±2.88	33.18±3.04
MUFA[1)]	28.10±6.01	21.93±7.61^{c}	25.60±4.06^{a}	22.78±2.87^{bc}	32.95±3.17^{a}	30.63±3.00^{a}
Myristoleic acid (C14:1)	0.15±0.11	0.26±0.21^{a}	0.12±0.05^{bc}	0.07±0.02^{c}	0.16±0.08^{b}	0.13±0.05^{bc}
Palmitoleic acid (C16:1)	1.64±1.31	2.03±3.40	1.25±0.28	1.17±0.19	1.90±0.25	1.63±0.30
C17:1[2)]	0.47±0.32	0.52±0.21^{b}	0.39±0.15^{bc}	0.28±0.03^{c}	0.76±0.34^{a}	0.27±0.30^{c}
Elaidic acid(C18:1n9t)	0.10±0.11	0.00±0.00^{d}	0.24±0.05^{a}	0.00±0.00^{d}	0.06±0.08^{c}	0.18±0.08^{b}
Oleic acid (C18:1n9c)	25.77±5.73	18.94±6.97^{c}	23.74±3.88^{b}	21.18±2.70^{bc}	30.11±2.98^{a}	28.57±2.88^{a}
C20:1[3)]	0.03±0.09	0.24±0.11^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Nervonic acid (C24:1)	0.05±0.10	0.23±0.17^{a}	0.08±0.05^{b}	0.02±0.02^{c}	0.00±0.00^{c}	0.00±0.00^{c}
PUFA[4)]	4.49±2.99	9.33±2.34^{a}	6.03±3.40^{b}	4.51±1.43^{b}	2.94±1.32^{c}	2.55±1.04^{c}
Linoleic acid(C18:2n6c)	2.72±1.31	4.50±20.83^{a}	3.22±21.50^{b}	3.05±20.71^{b}	2.26±20.90^{c}	1.72±20.82^{c}
C18:3n6[5)]	0.03±0.06	0.09±0.12^{a}	0.10±0.06^{a}	0.00±0.00^{b}	0.00±0.01^{b}	0.00±0.00^{b}
C18:3n3[6)]	0.21±0.19	0.21±0.14	0.14±0.06	0.13±0.01	0.23±0.12	0.29±0.32
Eicosadienoic acid (C20:2)	0.05±0.06	0.10±0.14^{a}	0.06±0.02^{b}	0.04±0.00^{b}	0.05±0.02^{b}	0.03±0.01^{b}
DGLA[7)] (C20:3n6)	0.08±0.13	0.31±0.20^{a}	0.12±0.09^{b}	0.08±0.04^{bc}	0.02±0.03^{c}	0.01±0.01^{c}
(AA)[8)](C20:4n6)	1.18±1.53	3.49±1.87^{a}	2.18±1.72^{b}	1.07±0.60^{c}	0.32±0.24^{c}	0.31±0.22^{c}
Docosadienoic acid(C22:2)	0.00±0.04	0.00±0.00^{b}	0.06±0.10^{a}	0.00±00^{b}	0.00±00^{b}	0.00±00^{b}
DPA[9)] (C20:5n3)	0.13±0.20	0.47±0.28^{a}	0.15±0.15^{b}	0.07±0.05^{bc}	0.01±0.04^{c}	0.09±0.10^{bc}
DHA[10)](C22:6n3)	0.05±0.08	0.14±0.16^{a}	0.03±0.03^{b}	0.05±0.03^{b}	0.01±0.02^{b}	0.06±0.05^{b}

Note: [a, b,c,d] Mean value with different superscript letters in the same row differ significant at $P<0.05$. Numbers shown in parentheses are the number of individuals with the specified genotype. [1)] MUFA: Monounsaturated fatty acid; [2)] C17:1: Heptadecenoic acid; [3)]C20:1: Gondoic acid (Eicosenoic acid); [4)]PUFA: polyunsaturated fatty acid , [5)] C18:3n6: γ-Linolenic acid; [6)] C18:3n3: α-Linolenic acid; [7)]DGLA: Dihomo-gamma-linolenic acid; [8)] AA: Arachidonic acid; [9)] DPA : DPA docosapentaenoic acid (Eicosapentanoic); [10)]DHA: Docosahexaenoic acid).

The g.198655287 (G>A) SNP of AHSG gene was significant associated with fatty acids traits in combined population as shown in Table 2. There was a significant association with saturated fatty acids including, capric acid (C10:0), tridecylic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitic acid (C16:0), heptadecanoic acid (17:0), stearic acid (C18:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C22:0), tricosylic acid (C23:0) and lignoceric acid (C24:0), monounsaturated fatty acids including myristoleic acid (C14:1), heptadecenoic acid, elaidic acid(C18:1n9t, oleic acid (C18:1n9c), oleic acid (C18:1n9c), eicosenoic acid and nervonic acid (C24:1). It was also associated with polyunsaturated fatty acids including linoleic acid (C18:2n6c), γ-Linolenic acid, eicosadienoic acid (C20:2), dihomo-gamma-linolenic acid, docosadienoic acid, eicosapentanoic) and docosahexaenoic acid).

Individuals with homozygous GG genotype were associated with lower palmitic acid (C16:0) and lower unsaturated fatty acids in general but with higher polyunsaturated fatty acids (PUFA) as GA sheep had,

according to The table 3. However, this association should be validated with larger samples.

Table 3. Genotype and association analysis of AHSG gene (%)

	Genotypes		
Variable	GG (n=7)	GA(n=9)	AA(n=51)
Fat Content of meat	1.24±2.53^{b}	2.68±2.29^{ab}	4.91±3.98^{a}
Total Fatty Acid	65.99±14.45	71.69±12.44	72.08±7.24
Saturated Fatty Acid	37.69±12.53	38.40±8.44	39.00±7.39
Capric acid (C10:0)	0.03±0.03^{b}	0.07±0.07^{ab}	0.08 ±0.04^{a}
Lauric acid (C12:0)	0.39±0.29	0.55±0.42	0.48±0.58
Tridecylic acid (C13:0)	0.01±0.01	0.00±0.01	0.01±0.01
Myristic acid (C14:0)	2.19±1.54	3.19±1.95	3.23±1.98
Pentadecanoic acid (C15:0)	0.41±0.13	0.44±0.18	0.49±0.14
Palmitic acid (C16:0)	14.14±5.63^{b}	17.23±6.25^{ab}	18.39±2.82^{a}
Heptadecanoic acid (17:0)	0.69±0.16^{b}	0.80±0.36^{ab}	1.03±0.36^{a}
Stearic acid (C18:0)	19.14±5.45	15.39±4.83	15.05±6.16
Arachidic acid (C20:0)	0.14±0.16^{ab}	0.23±0.15^{a}	0.08±0.07^{b}
Heneicosylic acid (C21:0)	0.04±0.02^{a}	0.03±0.02^{a}	0.01±0.01^{b}
Behenic acid (C22:0)	0.20±0.09^{a}	0.16±0.15^{a}	0.04±0.06^{b}
Tricosylic acid (C23:0)	0.11±0.05^{a}	0.09±0.10^{a}	0.02±0.03^{b}
Lignoceric acid (C24:0)	0.18±0.11^{a}	0.17±0.20^{a}	0.03±0.06^{b}
Unsaturated Fatty Acid	28.09±2.57^{b}	34.16±8.00^{a}	32.93±4.35^{a}
MUFA[1)]	20.40±4.86^{b}	27.13±7.21^{a}	29.33±5.14^{a}
Myristoleic acid (C14:1)	0.13±0.06	0.21±0.13	0.14±0.11
Palmitoleic acid (C16:1)	1.04±0.37^{b}	2.54±3.47^{a}	1.57±0.37^{ab}
C17:1[2)]	0.43±0.13	0.47±0.27	0.48±0.35
Elaidic acid(C18:1n9t)	0.14±0.14	0.04±0.09	0.11±0.11
Oleic acid (C18:1n9c)	18.58±4.76^{b}	23.82±7.31^{a}	27.10±4.73^{a}
C20:1[3)]	0.10±0.12^{a}	0.14±0.17^{a}	0.00±0.04^{b}
Nervonic acid (C24:1)	0.11±0.07^{a}	0.16±0.22^{a}	0.02±0.04^{b}
PUFA[4)]	7.69±3.10^{a}	7.02±4.10^{a}	3.60±2.14^{b}
Linoleic acid(C18:2n6c)	4.01±1.24^{a}	3.51±1.45^{a}	2.40±1.14^{b}
C18:3n6[5)]	0.13±0.10^{a}	0.05±0.10^{b}	0.01±0.03^{b}
C18:3n3[6)]	0.14±0.04^{b}	0.33±0.28^{a}	0.20±0.17^{ab}
Eicosadienoic acid (C20:2)	0.05±0.01^{b}	0.11±0.14^{a}	0.04±0.02^{b}
DGLA[7)] (C20:3n6)	0.18±0.12^{a}	0.24±0.25^{a}	0.04±0.06^{b}
(AA)[8)](C20:4n6)	2.89±1.88^{a}	2.24±2.48^{a}	0.76±0.94^{b}
Docosadienoic acid(C22:2)	0.04±0.07^{a}	0.00±0.00^{b}	0.00±0.04^{b}
DPA[9)] (C20:5n3)	0.22±0.17^{b}	0.37±0.36^{a}	0.07±0.11^{b}
DHA[10)](C22:6n3)	0.05±0.04^{b}	0.14±0.18^{a}	0.03±0.04^{b}

Note: $^{a, b, c,d}$ Mean value with different superscript letters in the same row differ significant at $P<0.05$. Numbers shown in parentheses are the number of individuals with the specified genotype. [1)] MUFA: monounsaturated fatty acid; [2)] C17:1: heptadecenoic acid; [3)]C20:1: gondoic acid (Eicosenoic acid); [4)]PUFA: polyunsaturated fatty acid , [5)] C18:3n6: γ-Linolenic acid; [6)] C18:3n3: α-Linolenic acid; [7)]DGLA: dihomo-gamma-linolenic acid; [8)] AA: arachidonic acid; [9)] DPA : docosapentaenoic acid (eicosapentanoic); [10)]DHA: docosahexaenoic acid).

Discussion

In combined population, the most abundant saturated fatty acid in muscle was palmitic acid (C16:0)

followed by stearic acid (C18:0) and myristic acid (C14:0), respectively, it is in agreement with the previous results Gunawan et al. (2018). Considering breeds, BC, CA and CG had higher levels of stearic acids compared to palmitic acids but JFT and JTT are dominated by palmitic acids (C16:0). The fatty acid composition was also affected by breed where results show that BC and JFT sheep breeds had lower levels of saturated fatty acids compared to CA, CG and JTT according to the Table 2. The current results are in agreement with previous results Harten *et al*. (2014) that reported breed effect on fatty acid composition in Dorper, Damara and Merino sheep breeds. They are also in agreement by Gunawan et al. (2018) that examined breed effects in fatty acids of three sheep breeds including, Javanese Fat-Tailed, Javanese Thin-Tailed and Composite Garuk sheep. Javanese Fat-Tailed (JFT) sheep store fat in their tail and they tend to have lower amounts of carcass and intramuscular fat (IMF) (Yousefi et al. 2012; Gunawan et al. 2018).

The SNP of AHSG gene (g. 198655287 G>A) was found to be in Hardy-Weinberg Equilibrium ($P<0.05$), except in Composite Garut (CG), Compass Agrinak (CA) and Barbados Black Belly Cross (BC) sheep breeds sheep breed. A population of large size is in Hardy-Weinberg equilibrium if genotype and allele frequencies remain constant 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 present study revealed the SNP of AHSG gene (g. 198655287 G>A) was significantly associated with saturated fatty acid, including capric acid (C10:0), palmitic acid (C16:0), heptadecanoic acid (17:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C22:0), tricosylic acid (C23:0), lignoceric acid (C24:0); with monounsaturated fatty acids, including palmitoleic acid (C16:1); oleic acid (C18:1n9c); eicosenoic acid (C20:1); nervonic acid (C24:1) and with polyunsaturated fatty acids, including linoleic acid C18:2n6c; γ-Linolenic acid; α-Linolenic acid; eicosadienoic acid (C20:2); dihomo-gamma-linolenic acid; arachidonic acid; docosadienoic acid(C22:2); eicosapentanoic and docosahexaenoic acid as presented in Table 3.

Analysis of SNP g. 198655287 G>A of Alpha 2-Heremans Schmid Glycoprotein (AHSG) mapped on chromosome 1 of BC, CA, CG, JTT and JFT sheep breeds resulted in a significant association ($P<0.05$) on composition of different sheep meat fatty acids from saturated to unsaturated fatty acids both monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). High consumption of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFA) in the human diet is healthy and desirable for meat consumers as they increase activity of hepatic low density lipoprotein (LDL) receptor thus reducing circulating concentration of LDL-cholesterol (Maharani *et al*. 2012) but high intake of saturated fatty acids has been linked to high saturated fatty acids with cardiovascular diseases and cancer (Hidayati *et al*. 2015; Kaić and Mioč 2016).

Consumption of saturated fatty acids is limited to 0-10%, MUF 16%, PUFA 7% and cholesterol is limited to 300 mg per day, excess consumption leads to cardiovascular diseases (Gunawan *et al*. 2018). Long chain n-3 PUFA such as Docosahexaenoic acid (DHA, also known as Cervonic acid) (C22:6n3) and eicosapentaenoic acid (20:5n3) are involved in the growth and development of the nervous and visual systems, actively participating in the processes of neurogenesis (Valenzuela *et al*. (2012; Gunawan *et al*. 2018).

Fetuin-A, also called Alpha 2-Heremans Schmid Glycoprotein (AHSG) is physiological inhibitor of insulin receptor tyrosine kinase and thus associated with insulin resistance, metabolic syndrome (MetS) and an increased risk for type 2 diabetes (Dabrowska *et al.* 2015). AHSG connects fatty liver and insulin resistance by which high AHSG plasma levels are associated with insulin resistance in humans leading to the accumulation of fat in the liver (Stefan *et al.* 2006). Elevated circulating levels of fetuin-A in blood have been linked to the increased risk of cardiovascular disease (Fisher *et al.* 2009).

IV. CONCLUSION

AHSG gene was polymorphic in BC, CG, CA, JFT and JTT sheep breeds with A allele more frequent in combined population. The SNP g.198655287 G>A of AHSG gene was significantly associated with different fatty acids including saturated and unsaturated fatty acids. This study showed that SNP (g.198655287 G>A) of AHSG gene might be a useful marker for selecting and producing sheep meat with desirable and healthy fatty acids.

V. CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest

VI. ACKNOWLEDGEMENT

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