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Characterization and Association of *CFHR5* Gene Polymorphism with Fatty Acid Composition and Cholesterol in Sheep

Akbanugra Yudhananda[1], Kasita Listyarini[2], Cece Sumantri[2], and Asep Gunawan[2*]

[1]Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia
[2]Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia

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* Corresponding author:
E-mail: agunawan@apps.ipb.ac.id

ABSTRACT

The Complement Factor H Related 5 (*CFHR5*) gene is speculated to have an important role in regulating fatty acid composition in sheep. The aim of this study was to investigate the polymorphism of the *CFHR5* gene and its association with fatty acid composition and cholesterol of sheep in Indonesia. A total of 172 rams from 83 priangan sheep (PS), 20 sapudi sheep (SS), 19 Garut sheep (GS), 20 jonggol sheep (JS), 10 Garut composite sheep (GCS), 10 compass agrinac sheep (CAS), and 10 Barbados cross sheep (BCS) were used for this study. Identification of *CFHR5* gene polymorphism were performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) using AciI restriction enzyme. The results showed that the *CFHR5* gene in all sheep populations were polymorphic producing three genotypes, e.g. CC, CT and TT, respectively. The polymorphism had a significant effect ($p<0.05$) on fatty acid composition (miristoleic [C14:1], ginkgolic [C17:1], tridecanoic [C13:0], and heptadecanoic [C17:0] acids) and cholesterol. The breed and polymorphism had a significant effect ($p<0.05$) on fatty acid composition (JS with tridecanoic acid [C13:0] and BCS with miristoleic acid [C14:1]). The CC genotype is the preferred genotype and as it exhibits reduced levels of saturated fatty acids and cholesterol. The BCS sheep is the preferred genotype and as it exhibits high PUFA/SFA ratio. The *CFHR5* gene (SNP c.1011C>T) has the potential to be used as a genetic marker for the selection of low saturated fatty acid composition and cholesterol in sheep.

Keywords: Sheep, Fatty acid, Cholesterol, CFHR5 gene, PCR-RFLP

Introduction

Lamb meat is an important component of the human diet and health for being a relevant factor in consumers' meat preferences (Munyaneza *et al*., 2019). Sheep is one of the small ruminants that contribute to meeting the demand for meat in Indonesia (Rosyidi, 2009). Lamb meat production in Indonesia in 2018 reached 48.7 tons. Lamb meat contains higher saturated fatty acids than beef, thereby limiting the level of consumption of lamb meat (Gunawan *et al.,* 2018). Consumption of fat derived from ruminants is directly related to negative effects for human health as it is rich of cholesterol (Soeparno, 2011). Fatty acids are mostly classified based on the presence or absence of double bonds as saturated fatty acids (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) (Orsavova *et al.,* 2015). Animal products containing the proportion of polyunsaturated fatty acids (PUFA) need to be considered by many people as a substantial factor for the dietary value of meat (Perez *et al.,* 2010). Production of lamb with high PUFA content and low SFA is substantial for human health and is more in line with health recommendations, especially related to cardiovascular disease (CVD). Improving the quality of meat including the quality of fat, smell, and taste is an important factor in increasing the market demand for lamb. The properties of lamb meat can usually be measured after the lamb is slaughtered and sometimes has low heritability (Gunawan *et al.,* 2018). The heritability of the fatty acid composition in sheep has a moderate value about 0.25–0.46 (Rovadoscki *et al.,* 2018). Medium to high heritability values mean that phenotypic traits can be improved through selection (Hasan and Gunawan, 2014). Selection based on the molecular genetics of candidate genes which is approached through analysis of variants to identify single nucleotide polymorphism (SNP) is applied to this program.

Identification of genetic factors that control a phenotypic trait can provide opportunities for breeders to make selections to obtain the desired phenotypic value (Listyarini *et al.,* 2018). A difference in genetic interactions of several genes can affect the regulation of phenotypic traits (Sahadevan *et al.,* 2014). Selection with marker assisted selection can be one of the selection methods because it is more effective, efficient, and

accurate (Khasanah *et al.,* 2016). Several efforts need to be made to reduce levels of saturated fatty acids and cholesterol, one of which is through genetic selection, namely by using genes that affect fatty acid and cholesterol. Gunawan *et al*. (2021) stated that one of the genes that play a role in the production of fatty acids and cholesterol is Complement Factor H Related 5 (*CFHR5*). The *CFHR5* gene is a part of the small complement factor-H (CFH) gene cluster on the chromosome (Gale and Pickering, 2011). In the domestic sheep (*Ovis aries*), this gene maps to chromosome 12. The *CFHR5* gene provides instructions for the synthesis of a protein called complement factor H-5, which is produced in the liver and circulates in the blood (Gale and Pickering, 2011). The *CFHR5* protein has been detected on HDL lipoproteins and lipoproteins, molecules involved in lipid transport. This shows that the *CFHR5* gene is involved in lipid metabolism (McRae *et al.,* 2005). There has not been a similar study before regarding *CFHR5* gene polymorphism and its associations with fatty acid composition and cholesterol in sheep. This research was conducted to identify the polymorphism of the *CFHR5* gene in Indonesian sheep using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Materials and Methods

Experimental animals

The *CFHR5* gene polymorphism characterization were used a total of 172 rams from 83 priangan sheep (PS), 20 sapudi sheep (SS), 19 Garut sheep (GS), 20 jonggol sheep (JS), 10 Garut composite sheep (GCS), 10 compass agrinac sheep (CAS), and 10 Barbados cross sheep (BCS) which can be seen in Figure 1. Given the limited availability of longissimus dorsi muscles samples from sheep, a total of 100 sheep were included in the fatty acid analysis are 35 priangan sheep (PS), 20 sapudi sheep (SS), 15 jonggol sheep (JS), 10 Garut composite sheep (GCS), 10 compass agrinac sheep (CAS), and 10 Barbados cross sheep (BCS). In the cholesterol analysis, a total of 100 sheep included were 55 priangan sheep (PS), 6 sapudi sheep (SS), 15 jonggol sheep (JS), 10 Garut composite sheep (GCS), 8 compass agrinac sheep (CAS), and 6 Barbados cross sheep (BCS). Phenotypic characteristics were obtained from rams with body weight between 25 to 30 kg, and aged between 10 to 12 months in accordance with the Indonesian Inspection Service procedures and was approved by the 'Institutional Animal Care and Use Committee (IACUC)" issued by IPB University (approval ID: 117–2018 IPB). The rams were reared under the same management systems (high house system) and were fed the same fattening feed (grass by 10% of body weight and 1.5 % of body weight concentrate with 16% crude protein and 68% TDN) and water ad libitum. The samples were longissimus dorsi muscle samples were taken and stored in 1.5 mL tubes. Prior to slaughter, the carcasses were stored at -20°C. The longissimus dorsi muscles were analyzed for fatty acid by AOAC (2005) (Association of Analytical Chemist) standard and cholesterol by gas chromatography (GC) method. Analyses samples for fatty acid and cholesterol were secured in plastic and stored at -20°C before the analysis.

Fatty acid composition measurement

The fatty acid composition was analyzed using AOAC (Association of Analytical Chemist) standard (2005). Longissimus dorsi muscles were used for the analysis of fatty acid composition including Saturated Fatty Acid, Octanoic acid [C8:0], Capric acid [C10:0], Lauric acid [C12:0], Tridecanoic acid [C13:0], Myristic acid [C14:0], Pentadecanoic acid [C15:0], Palmitic acid [C16:0], Heptadecanoic acid [C17:0], Stearic acid [C18:0], Arachidic acid [C20:0], Heneicosanoic acid [C21:0], Behenic acid [C22:0], Tricosanoic acid [C23:0], Tetracosanoic acid [C24:0], MUFA [Monounsaturated fatty acid], Myristoleic acid [C14:1], Palmitoleic acid [C16:1], Ginkgoleic acid [C17:1], Elaidic acid [C18:1n9t], Oleic acid [C18:1n9c], Gonodocic acid [C20:1], Nervonic acid [C24:1], PUFA [Polyunsaturated fatty acid]: Linoleic acid [C18:2n6c], γ-linolenic acid [C18:3n6], Linolenic acid [C18:3n3], cis 11,14-Eicosadienoic acid [C20:2], Homo-γ-linolenic acid [C20:3n6], Arachidonic acid [C20:4n6], Docosadienoic acid [C22:2], Eicosapentaenoic acid [C20:5n3], and Docosahexaenoic acid [C22:6n3].

In the first stage, the sample was extracted using the Soxhlet method. Then, the fatty acids were converted into fatty acid methyl esters (FAME) through a methylation process involving saponification with NaOH and treatment with BF3. After cooling, hexane was added to extract the FAME from the sample. The samples were analyzed using a gas chromatograph with Thermo Scientific ISQ 7610 single quadrupole GC-MS system detector. The column temperature had a gradient, starting at 130°C, increasing to 170°C, and then to 230°C. The detector temperature was set at 280°C. Before adding helium and nitrogen as the mobile phase, the sample flow was ensured. Various gas flow rates were used. The FAME standard mix and prepared sample were injected, and the retention time was compared to standards to determine the fatty acid composition. Calculation of the composition involved comparing sample area (Ax) to standard area (As), multiplied by standard concentration, adjusted for volume, and divided by 100.

Cholesterol measurement

Longissimus dorsi cuts were utilized for cholesterol analysis using the gas chromatography (GC) method. The GC method involves sample preparation, including saponification, extraction, and purification. Saponification is the hydrolysis of fatty acids using a strong base. For saponification, meat samples (2-3 g) were placed in a 500 mL Erlenmeyer tube with 40 mL of ethanol. The tube was stirred on a magnetic stirrer for 1.5 hours, then 8 mL of 50% KOH was added and stirred for 30 min. The solution was refluxed at 70-80°C for 60 min, followed by the addition of 60 mL of alcohol and refluxing for another 30 min. The tube was

closed and allowed to stand for 24 hours at room temperature. Next, the extraction process took place. The sample solution was mixed with 100 mL of toluene and stirred for 1.5 h. The solution was transferred to a separatory flask, allowed to separate, and the bottom layer was removed. Multiple extractions were performed using KOH solutions of varying concentrations, followed by a final wash with distilled water. The solution was then evaporated using an evaporator, leaving behind a residue with hexane.

Genotyping of *CFHR5* gene

Longissimus dorsi muscles samples for DNA extraction were extracted by utilizing the phenol-chloroform method (Sambrook *et al*., 1989). The *CFHR5* gene sequences (406 bp) (NCBI accession number NC_019469.2) were amplified by PCR technique using primers forward (5'-CTTTCCCAGTTTCTTGGG-3') and reverse (5'-GACCAGGCTGATAACAAATG-3') refers to Gunawan *et al.* (2021). Extracted DNA samples were amplified by placing 0.5-2 µL of DNA in a 1.5 µL tube and adding 14 µL of premix solution consisting of 0.2 µL forward primer, 0.2 reverse primer, 6.1 µL DW, 7.5 µL GoTaq® Green Master Mix (Promega, USA).

The mixture was incubated in an ESCO Maxi™ thermocycler machine for the amplification process. This DNA amplification process begins with a denaturation step at 95°C for 5 min. The second stage consisted of 35 cycles, each cycle consisting of denaturation at 95°C for 10 s, primary annealing at 60°C for 20 s, and DNA extension at 72°C for 30 s. The final stage was primer elongation at 72°C for 5 min. Primer attachment and the mutation site were presented in Figure 2.

Genotyping was carried out using the PCR-RFLP technique with the SNP from RNA sequencing results of Gunawan *et al*. (2018). In the PCR-RFLP method, 5 µL of DNA amplicon was added to a mix consisting of 0.9 µL DW, 0.7 µL buffer, and 0.4 µL AciI restriction enzyme, then incubated at 37°C for 4 h. 5 µL of DNA was cut and electrophoresed at 100 V for 35 min using 2% agarose gel.

The DNA fragments, were observed under UV light. DNA fragments that appear were compared with the ladder 100 bp marker. Genotyping was done based on the length of the visible DNA fragments. The same migration position was considered as one allele type and was used to determine the genotype of each sample presented in Table 1.

Data analysis

The statistical analyses conducted included the examination of genotype frequency, allele frequency, Hardy-Weinberg equilibrium, and the assessment of genotype and *CFHR5* gene polymorphism association using the PROC GLM procedure in Minitab 19 Software.

Genotype frequency was determined by calculating the ratio of the number of certain genotypes in each population using the formula Nei and Kumar (2000) as follows:

$$x_{ii} = \frac{n_{ii}}{N}$$

Where:
x_{ii} = *ii* genotype frequency
n_{ii} = individuals with *ii genotype*
N = total samples

Allele frequencies was calculated based on Nei and Kumar's formula (2000), as follows:

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

Where:
xi = *i allele frequency*
nii = Individuals with *ii* genotype
nij = Individuals with *ij* genotype
N = total samples

The Hardy-Weinberg equilibrium was tested using the Chi-Square calculation (Hartl and Clark, 1997):

$$x^2 = \sum \frac{(O - E)^2}{E}$$

Where:
x^2 = chi-square
O = observed value
E = expected value

Analysis of genotype differences associated with fatty acids and cholesterol according to Mattjik and Sumertajaya (2006), as follows:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij}$$

Where:
Y_{ij} = The fatty acid and cholesterol
μ = The population mean
G_i = The fixed effect of *i-th* genotype
ε_{ij} = The residual error

Results and Discussion

Fatty acid and cholesterol profile based on breed group

The fatty acid composition and cholesterol profile of the longissimus dorsi from priangan sheep (PS), sapudi sheep (SS), Garut sheep (GS), jonggol sheep (JS), Garut composite sheep (GCS), compass agrinac sheep (CAS), and Barbados cross sheep (BCS) are showed in Table 3 and 4, respectively. A total of 34 fatty acid profiles including saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA), were identified. There were 14 types of fatty acids in SFA (C8, C10, C12, C13, C14, C15, C16, C17, C18, C20, C21, C22, C23, and C24), 7 types of fatty acids in MUFA (C14:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1, C24:1), and 9 types of fatty acids in PUFA (C18:2n6c, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:4n6, C22:2, C20:5n3, and C22:6n3). The results showed that breed with high SFA was CAS, low SFA was BCS, high MUFA was SS, low MUFA was BCS, High PUFA was BCS, Low PUFA was SS, and high PUFA/SFA was BCS (Table 3). The cholesterol profile showed high cholesterol was BCS and low cholesterol was SS regardless of cholesterol type (HDL or LDL) (Table 4).

The fatty acid composition in sheep is influenced by breed, as evident from the results

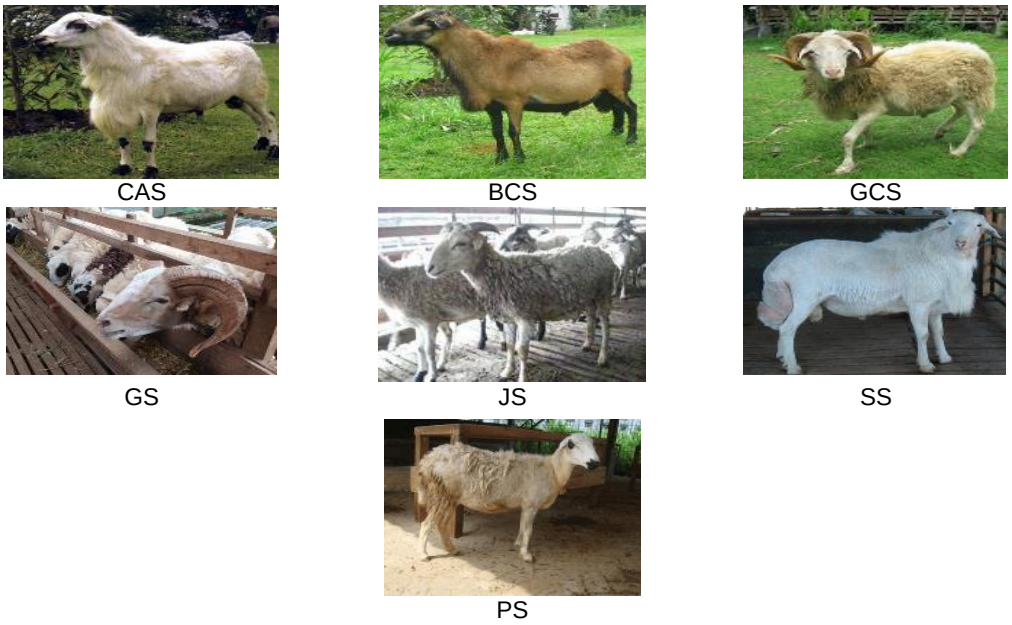

Figure 1. Sheep breeds (CAS = Compass agrinak seep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep).

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9901   TACAAGGCAT CTTTCCCAGT  TTCTCTTGGG  AAAATTTTAT  ATTATTCCTG  TGAATATAAT
9961   TTTATGTCTC CTTCAAAACA  CTTTTGGACT  CCCATAACAT  GCACAGAGGG  |C|GGATGGTCA
11021  CCGACTCCAA AGTGTCTCAG  TGAGTAAATG  CCTGATGGGG  ATGAACCGTT  CGGGGGGTGG
11081  AGAAGGGCGC AGCAGGTGGG  GTCCCAGGGT  CTTGACAGAG  AGTCACAGGG  GTCAGGACTA
11141  GAGGAGCCAG CAAAGAGGAG  AGATGCAGTC  CTTCATGTTT  TGAGAAGCCT  GTTTTGAAAA
11201  CCAAATGAAG AATGAATATA  CGAGCTTTCT  TTCATGTTGT  AAGCTTTAAA  ACTGATCATT
11261  AATATATTTC ACTCCTAATA  TTTATGTCCT  AATATTCATT  TGTTATCAGC  CTGGTCGTAA
```

Allele C : 5'----- AGAGG G|C GGAT ----3'
Allele T : 5'----- AGAGG G|T GGAT----3'

Figure 2. Primer sequence of CFHR5 gene (NCBI accession number: NC_019469.2) and mutation site **(**c.1011 **C>T).**

Table 1. PCR-RFLP cutting sites

Gene	Species	Accession number	Mutation point	PCR-RFLP enzyme		Cutting sites
CFHR5	*Ovis aries*	NC_019469.2	c.1011 C>T	AciI	CC CT TT	= 306 bp, 100 bp; = 406, 306,and 100bp; = 406bp

Table 2. Genotype and allele frequency of *CFHR5* gene

Sheep breed	N	Genotype frequency			Allele frequency		x^2
		CC (n)	CT (n)	TT (n)	C	T	
CAS	10	0.30 (3)	0.50 (5)	0.20 (2)	0.55	0.45	0.001
BCS	10	0.50 (5)	0.20 (2)	0.30 (3)	0.60	0.40	3.40
GKS	10	0.40 (4)	0.60 (6)	0.00 (0)	0.70	0.30	1.84
GS	19	0.37 (7)	0.70 (9)	0.16 (3)	0.61	0.39	0.001
JS	20	0.35 (7)	0.60 (12)	0.05 (1)	0.65	0.35	2.03
SS	20	0.20 (4)	0.60 (12)	0.20 (4)	0.50	0.50	0.8
PS	83	0.51 (42)	0.39 (32)	0.11 (9)	0.70	0.30	0.59
Total/average	172	0.42 (72)	0.45 (78)	0.13 (22)	0.65	0.35	

N = Number of samples; CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep x^2 table (0.05) = 3.84.

presented in Table 3. Specifically, BCS and SS breeds had lower levels of saturated fatty acids compared to CAS, CGS, PS, and JS breeds. Additionally, JS and PS breeds had high palmitic acid (C16:0), and were in agreement with Munyaneza *et al.* (2019). The cholesterol profile showed high cholesterol was BCS and low cholesterol was SS regardless of cholesterol type (HDL or LDL). Gunawan *et al*. (2018) investigated the breed effects on fatty acids in three sheep breeds: Javanese Fat-Tailed (JFT), Javanese Thin-Tailed (JTT), and Garut Composite (GCS). The results of both studies support the notion that different sheep breeds exhibit variations in fatty acid composition. It is worth noting that priangan sheep (PS) are characterized by their fat deposition in the tail region and are known to have lower levels of carcass and intramuscular fat (IMF) compared to other breeds, as indicated by Gunawan *et al.* (2018). Overall, the current findings were consistent with

Table 3. Phenotypic of fatty acid composition based on breed group

Parameters	Breed ($\bar{x}$ ± Std Dev)					
	BCS (n=10)	CAS (n=10)	SS (n=20)	PS (n=35)	GCS (n=10)	JS (n=15)
Fat content (%)	1.68±1.86	1.86±1.85	1.85±1.22	1.22±7.09	7.09±4.02	4.02±4.36
Saturated fatty acid (%)	33.3±9.45	9.45±47.85	47.85±6.16	6.16±35.28	35.28±6.68	6.68±41.07
Octanoic acid (C8:0)	0±0	0±0	0±0	0±0	0±0	0±0
Caprat acid(C10:0)	0.02±0.01	0.01±0.04	0.04±0.02	0.02±0.1	0.1±0.03	0.03±0.53
Lauric acid (C12:0)	0.47±0.4	0.4±0.55	0.55±0.2	0.2±0.68	0.68±0.9	0.9±0.35
Tridecanoic acid (C13:0)	0±0	0±0.02	0.02±0.01	0.01±0.02	0.02±0.02	0.02±0.01
Myristic acid (C14:0)	1.97±1.28	1.28±3.33	3.33±1.08	1.08±3.56	3.56±2.65	2.65±3.39
Pentadecanoic acid (C15:0)	0.39±0.18	0.18±0.51	0.51±0.1	0.1±0.45	0.45±0.18	0.18±0.58
Palmitic acid (C16:0)	13.15±6.06	6.06±18.37	18.37±3.02	3.02±18.73	18.73±2.66	2.66±19.78
Heptadecanoic acid (C17:0)	0.6±0.19	0.19±0.81	0.81±0.12	0.12±1.21	1.21±0.48	0.48±0.91
Stearic acid (C18:0)	15.6±5.61	5.61±23.86	23.86±2.61	2.61±10.43	10.43±2.81	2.81±15.34
Arachidic acid (C20:0)	0.34±0.07	0.07±0.01	0.01±0.02	0.02±0.07	0.07±0.02	0.02±0.11
Henecosanoic acid (C21:0)	0.05±0.02	0.02±0.03	0.03±0.02	0.02±0.01	0.01±0.02	0.02±0.04
Bahenic acid (C22:0)	0.27±0.1	0.1±0.14	0.14±0.07	0.07±0.02	0.02±0.02	0.02±0.02
Tricosanoic acid (C23:0)	0.15±0.06	0.06±0.08	0.08±0.04	0.04±0	0±0.01	0.01±0.01
Tetracosanoic acid (C24:0)	0.28±0.13	0.13±0.11	0.11±0.08	0.08±0	0±0.01	0.01±0.01
Unsaturated fatty acid (%)	31.6±10.14	10.14±31.94	31.94±4.14	4.14±35.96	35.96±2.89	2.89±35.45
MUFA (%)	21.19±6.8	6.8±28.82	28.82±4.12	4.12±33.02	33.02±3.21	3.21±32.06
Myristoleinic acid (C14:1)	0.27±0.22	0.22±0.13	0.13±0.06	0.06±0.16	0.16±0.09	0.09±0.13
Palmitoleic acid (C16:1)	0.98±0.21	0.21±1.25	1.25±0.28	0.28±1.91	1.91±0.25	0.25±1.58
Ginkgoleic acid (C17:1)	0.52±0.21	0.21±0.39	0.39±0.15	0.15±0.77	0.77±0.34	0.34±0.14
Elaidic acid (C18:1n9t)	0±0	0±23.74	23.74±3.89	3.89±0.07	0.07±0.09	0.09±1.71
Oleic acid (C18:1n9c)	18.94±6.98	6.98±3.22	3.22±1.5	1.5±30.11	30.11±2.99	2.99±28.41
Gonodoic acid (C20:1)	0.24±0.11	0.11±0	0±0	0±0	0±0	0±0.09
Nervonic acid (C24:1)	0.23±0.18	0.18±0.09	0.09±0.06	0.06±0	0±0	0±0.01
PUFA (%)	10.42±4.12	4.12±3.12	3.12±2.12	2.12±2.94	2.94±1.32	1.32±3.39
Linoleic acid (C18:2n6c)	5.58±3.83	3.83±0.25	0.25±0.05	0.05±2.27	2.27±0.9	0.9±2.03
Y-linolenic acid (C18:3n6)	0±0	0±0	0±0	0±0	0±0	0±0.12
Linolenic acid (C18:3n3)	0.21±0.14	0.14±0.15	0.15±0.06	0.06±0.23	0.23±0.13	0.13±0.5
Cis 11, 14-Eicosadienoic acid (C20:2)	0.1±0.14	0.14±0.06	0.06±0.03	0.03±0.05	0.05±0.03	0.03±0.04
Homo-y linolenic acid (C20:3n6)	0.31±0.2	0.2±0.13	0.13±0.09	0.09±0.03	0.03±0.03	0.03±0.02
Arachidonic acid (C20:4n6)	3.49±1.87	1.87±2.18	2.18±1.72	1.72±0.33	0.33±0.25	0.25±0.36
Docosadienoic acid (C22:2)	0±0	0±0.06	0.06±0.11	0.11±0	0±0	0±0
Eicosapentanoic acid (C20:5n3)	0.47±0.29	0.29±0.15	0.15±0.16	0.16±0.02	0.02±0.04	0.04±0.25
Dokosahelsanoic acid (C22:6n3)	0.15±0.17	0.17±0.03	0.03±0.04	0.04±0.01	0.01±0.03	0.03±0.05
Total fatty acid	64.9±15.53	15.53±79.79	79.79±7.32	7.32±71.26	71.26±6.09	6.09±76.67
MUFA/SFA ratio	**0.64**	**0.87**	**0.99**	**0.96**	**0.68**	**0.97**
PUFA/SFA ratio	**0.31**	**0.09**	**0.09**	**0.10**	**0.14**	**0.11**

CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep.

Table 4. Phenotypic of cholesterol based on breed group

Parameters	Breed ($\bar{x}$ ± Std Dev)					
	BCS (n=6)	CAS (n=8)	SS (n=6)	PS (n=55)	GCS (n=10)	JS (n=15)
Cholesterol	21.4±14.26	15.2±7.72	5.03±1.98	7.16±2.28	12.43±10.26	7.28±1.3

CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep.

previous research and demonstrate the impact of breed on fatty acid composition in sheep. However, it is important to note that further confirmation and validation of these associations are necessary. It is recommended to conduct larger-scale studies with diverse sheep breeds to ensure the robustness and generalizability of the findings.

Genotype variation of *CFHR5* gene

The genotyping was carried out using the PCR-RFLP technique using the restriction enzyme AciI (GGC|G), producing three genotypes namely CC, CT, and TT (Figure 3). The mutation identified in this study is a transitional mutation, namely a change from C to T in exon 2 at position c.1011. The CC genotype has 1 fragment with a length of 406 bp, the CT genotype has 3 fragments with a product length of 406 bp, 306 bp, and 100 bp, while the CT genotype has 2 fragments with a product length of 306 bp and 100 bp. The results of *CFHR5* fragments were visualized using a UV transilluminator on a 2% agarose gel, which can be seen in Figure 3. DNA sequences are cut specifically by restriction enzymes with different fragment sizes (Klug *et al.,* 2006). According to Gunawan *et al.* (2017), the restriction enzymes used must be able to recognize target DNA sequences and be able to cut DNA sequences at specific cut points. The *CFHR5* gene polymorphism was analyzed using the formula of allele frequency, genotype frequency, and Hardy-Weinberg equilibrium. The results of *CFHR5* gene genotype and allele frequency in sample sheep breed were presented in Table 2.

The results of this study indicate that the C allele is the dominant allele in the sample sheep population with a frequency value of more than 50% while the T allele frequency is below 50%. The polymorphism of the *CFHR5* gene in Indonesian sheep was divided into 3 genotypes, namely TT, CT, and CC. The most common genotype was the CT genotype with a relative frequency of 0.45, followed by the CC genotype with 0.42, and finally

the TT genotype with 0.13. The dominant allele identified was the C allele with a relative frequency of 0.65 and the T allele with 0.35. Genetic polymorphism between populations can be derived from gene frequencies and allele frequencies between populations (Li *et al.,* 2000). Gene frequency is the relative ratio of genes in a population (Nei and Kumar, 2000). Allele frequency is the relative ratio of alleles to alleles at a locus in the population (Nei and Kumar, 2000). Genotype frequency is the relative ratio of the number of genotypes in a population (Gunawan *et al.,* 2017). Gene frequency is affected by mutation, migration, selection, and genetic drift (Gunawan *et al.,* 2017). Chi-Square analysis (χ^2) on Indonesian sheep showed results that were not significantly different ($\chi^2<\chi^2$ table), so it could be concluded that the *CFHR5*|AciI gene was in Hardy-Weinberg equilibrium. Equilibrium in a population means that in that population there is no selection, mutation, migration, and genetic drift. The existence of gene polymorphism can be used as a reference for breeding programs in populations (Noor, 2010).

Association of *CFHR5* gene with fatty acid composition

The association analysis of *CFHR5* gene polymorphism (c.1011 C>T) showed a significant association (P<0.05) with fatty acid composition. The association analysis of *CFHR5* gene polymorphism and breed showed a significant association (P<0.05) fatty acid composition with tridecanoic acid (C13) in JS

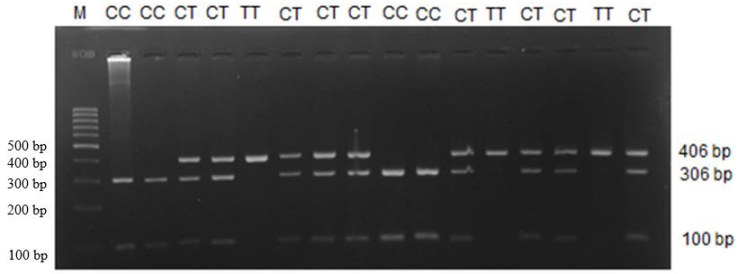

Figure 3. PCR-RFLP results of the *CFHR5*|*AciI* genotyping on 1.5% agarose gel. M : DNA marker 100 bp; CC,CT, and TT are genotypes.

Table 5. Association of the *CFHR5* gene with fatty acid composition

Parameters	Genotype *CFHR5* ($\bar{x}$ ± Std Dev)		
	CC (n=38)	CT (n=50)	TT (n=12)
Fat content (%)	**38.26 ± 7.58**	**38.62 ± 8.11**	**40.88 ± 9.18**
Saturated fatty acid (%)	**39.77±6.45**	**40.65±8.02**	**41.33±8.52**
Octanoic acid (C8:0)	0.04±0.11	0.05±0.13	0±0
Caprat acid(C10:0)	0.1±0.06	0.37±1.95	0.08±0.05
Lauric acid (C12:0)	0.48±0.39	0.46±0.53	0.51±0.46
Tridecanoic acid (C13:0)	0.01±0.01^b	0.02±0.01^a	0.02±0.02^a
Myristic acid (C14:0)	3.24±1.63	3.03±1.74	3.17±1.45
Pentadecanoic acid (C15:0)	0.49±0.15	0.52±0.15	0.56±0.15
Palmitic acid (C16:0)	18.75±4.19	18.55±4.25	18.81±3.5
Heptadecanoic acid (C17:0)	0.78±0.26^b	0.95±0.34^a	1.07±0.42^a
Stearic acid (C18:0)	15.57±4.27	16.43±5.38	16.76±6.41
Arachidic acid (C20:0)	0.13±0.09	0.11±0.09	0.12±0.12
Henecosanoic acid (C21:0)	0.03±0.03	0.03±0.06	0.02±0.02
Bahenic acid (C22:0)	0.07±0.09	0.05±0.08	0.08±0.09
Tricosanoic acid (C23:0)	0.03±0.06	0.03±0.04	0.05±0.07
Tetracosanoic acid (C24:0)	0.05±0.1	0.04±0.09	0.08±0.12
Unsaturated fatty acid (%)	**34.03±6.02**	**34.02±5.94**	**34.36±3.65**
MUFA (%)	**29.46±5.84**	**30.19±6.51**	**30.28±5.28**
Myristoleinic acid (C14:1)	0.14±0.07^b	0.13±0.07^b	0.23±0.2^a
Palmitoleic acid (C16:1)	1.43±0.49	1.59±0.4	1.61±0.4
Ginkgoic acid (C17:1)	0.23±0.26^b	0.33±0.37^b	0.56±0.34^a
Elaidic acid (C18:1n9t)	2.5±5.83	2.91±7.08	4.85±10.06
Oleic acid (C18:1n9c)	25.03±8.03	25.15±9.62	22.93±10.44
Gonodoic acid (C20:1)	0.08±0.21	0.04±0.19	0.05±0.09
Nervonic acid (C24:1)	0.04±0.08	0.04±0.09	0.07±0.11
PUFA (%)	**4.58±3.44**	**3.83±1.83**	**4.07±3.48**
Linoleic acid (C18:2n6c)	2.61±2.53	2.31±1.17	2.18±1.48
Y-linolenic acid (C18:3n6)	0.04±0.08	0.03±0.05	0.02±0.02
Linolenic acid (C18:3n3)	0.4±0.31	0.36±0.26	0.23±0.18
Cis 11, 14-Eicosadienoic acid (C20:2)	0.06±0.08	0.05±0.03	0.06±0.02
Homo-y linolenic acid (C20:3n6)	0.09±0.15	0.06±0.07	0.07±0.1
Arachidonic acid (C20:4n6)	1.01±1.4	0.76±1.01	1.28±1.87
Docosadienoic acid (C22:2)	0±0.02	0.01±0.04	0.02±0.05
Eicosapentanoic acid (C20:5n3)	0.24±0.21	0.2±0.2	0.16±0.25
Dokosahelsanoic acid (C22:6n3)	0.07±0.1	0.04±0.04	0.04±0.04
Total fatty acids	**73.81±10.11**	**74.78±9.35**	**75.69±8**
Ratio of MUFA/SFA	**00.74**	**00.76**	**00.76**
Ratio of PUFA/SFA	**00.16**	**00.13**	**00.14**

Numbers accompanied by different letters in the same row show significantly different (p<0.05) at the level of α=0.05.

and myristoleinic acid (C14:1) in BCS. The results of this study were consistent with a previous study by Harahap *et al.* (2021), Munyaneza *et al.* (2019), and Listyarini *et al.* (2022) with different genes named HSD17β13, BHMT, and OLFML3 respectively. Fatty acids that show significance were miristoleic acid (C14:1), ginkgoic acid (C17:1), tridecanoic acid (C13:0), and heptadecanoic acid (C17:0). The association of *CFHR5* gene with the fatty acid composition of Indonesian sheep was presented in Table 5. Association of CFHR5 gene with fatty acid composition based on genotype and breed group was presented in Table 6.

The composition of fatty acids in meat is influenced by the interaction of several genes that are additive (Hidayati, 2015). The *CFHR5* gene provides instructions for making a protein called CFH-5, which is produced in the liver and circulates in the blood (Gale and Pickering, 2011). The *CFHR5* protein has been detected in HDL and lipoproteins, molecules involved in lipid transport and involved in lipid metabolism (McRae *et al.,* 2005). *CFHR5* protein is detected in HDL lipoprotein which plays a role in the transportation of raw materials for lipid synthesis and the transportation of the resulting lipids. This shows that the *CFHR5* gene is involved in lipid metabolism (McRae *et al.,* 2005).

The sheep with the TT and CT genotypes had a higher composition of tridecanoic (C13:0) and heptadecanoic acid (C17) than the CC genotype. The TT genotype had a higher composition of myristoleinic acid (C14:1) and ginkgoic acid (C17:1) than CT and CC genotypes. The lowest saturated fatty acid composition and the highest PUFA/SFA ratio were found in the CC genotype. Meat with a higher ratio of PUFA:SFA is better for human health (Wood *et al.,* 2004). Better PUFA: SFA ratio in food is one of the means to reduce coronary heart disease (CHD) risk. Results showed that ratio of PUFA: SFA in the CC genotype is 0.16. Recommended PUFA:SFA ratio is above 0.12 with the prime value of above 0.40 (Wood *et al.,* 2008).

Tridecanoic acid (13) is one of the fatty acid compositions that has the ability to be an antimicrobial agent. On the other hand, tridecanoic acid (T13) poses a risk to cause a carcinogenic effect that was tested in mice (Mboyazi *et al.,* 2020). Heptadecanoic acid (C17) fatty acid is one of some odd chained fatty acids that have an association to lower disease risk of type II diabetes, coronary heart disease, increasing cell membrane fluidity, and insulin sensitivity disease suppressor (Jenkins *et al.,* 2015). The presence of myristoleinic acid (C14:1) can be used as a diagnostic indicator in patients who experience oxidation of long-chain fatty acids. These fatty acids are characterized in that it is cytotoxic to some tumor cells and can prompt apoptosis and necrosis in prostate cancer cells in humans (Iguchi *et al*., 2001). Myristolenia acid (C14:1) and palmitoleic acid (C16:1) are included in the monounsaturated fatty acids (MUFA). Beneficial properties of (C17:1) are anti tumor (Fukuda *et al.,* 2009), antibacterial effect (Hua *et al.,* 2017), as well as an inflammatory suppressan in company with PGE2 and level cyclooxygenase-2 (COX-2) expression reduction in human umbilical vein endothelial cells (Li *et al.,* 2018). Monounsaturated fatty acids (MUFA) are fatty acids that are correlated with a reduced risk of chronic heart disease (Mente *et al.,* 2009). According to Cao *et al.* (2009) consuming monounsaturated fatty acids in the short term can increase HDL and lower triglyceride levels in the blood.

Association of *CFHR5* gene with cholesterol

Association of *CFHR5* gene polymorphism (c.1011 C>T) showed a significant association ($p<0.05$) with cholesterol. The *CFHR5* gene polymorphism and breed showed no significant association ($p<0.05$) with cholesterol. The results of this study were different from a previous study conducted by Munyaneza *et al.* (2019) with other genes (BHMT) but in agreement with Aali *et al.* (2016) for different genes (CAST) and mutation (G>A). The CC genotype was significantly associated with lower cholesterol than CT and TT genotypes. Sheep with the TT and CT genotypes have higher meat cholesterol levels than the CC genotype. LDL or bad cholesterol have low protein/cholesterol levels while HDL or good cholesterol have high protein/cholesterol levels. Excess in LDL can cause artery clogging plaque meanwhile HDL is a cholesterol clearing agent in the blood (Ma and Shieh, 2006). Excess cholesterol accumulation can increase the risk of Alzheimer's disease, atherosclerosis islet β-cell dysfunction, testosterone deficiency, immune dysfunction, pituitary-thyroid axis dysfunction, liver dysfunction, renal dysfunction, osteoporosis, and osteoarthritis (Song *et al*., 2021). The association between the *CFHR5* gene and the cholesterol of sheep was presented in Table 7. Association of CFHR5 gene with cholesterol based on genotype and breed group was presented in Table 8.

Previous studies showed that functional genes regulate cholesterol and marbling quality. Fat deposition in the muscle was based on a balance between catabolic and anabolic catabolic processes such as lipolytic and lipogenic, along with fatty acid transport and total fatty acid utilized. The balance for the processes was based on the amount of consumed fat, fatty acid transport, lipid degradation, triacylglycerol synthesis, and de novo fat synthesis (Zhao *et al.,* 2010). One type of regulatory gene is Complement Factor H related 5 (*CFHR5*) which encodes the *CFHR5* protein produced in the liver and is detected in the lipid transporter lipoprotein and plays a role in lipid transport (Gale and Pickering, 2011). The mechanism for transporting cholesterol from the blood to the tissues is by endogenous pathways, namely cholesterol in the chicken body through VLDL will be converted to LDL along the capillaries which then LDL will deliver cholesterol to the muscle tissue (Nelson and Cox, 2013). Some changes to certain genes could result in an accumulation of free cholesterol in tissues and blood (Qiao *et al.,* 2010). Consuming large amounts of cholesterol continuously will cause blockage of blood vessels and increase the risk of coronary heart disease (Linder, 2006).

Table 6. Association of *CFHR5* gene with fatty acid composition based on genotype and breed group

Parameters	Breed and Genotype *CFHR5* (x ± Std Dev)								
	BCS			CAS			SS		
	CC(5)	CT(2)	TT(3)	CC(3)	CT(5)	TT(2)	CC(4)	CT(12)	TT(4)
Fat content (%)	0.88±0.15	4.18±3.85	1.36±0.4	1.62±1.26	1.61±4.29	2.81±0.99	4.14±0.71	7.4±4.09	9.12±4.79
Saturated Fatty Acid (%)	30.68±6.11	27.2±8.99	41.73±11.23	41.99±7.76	49.36±10.53	52.86±1.08	36.83±9.91	34.96±6.26	34.7±6.04
Octanoic acid (C8:0)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Caprat acid(C10:0)	0.02±0.01	0.01±0.01	0.03±0.01	0.03±0	0.04±0.04	0.06±0.03	0.08±0.03	0.1±0.03	0.11±0.04
Lauric acid (C12:0)	0.47±0.46	0.21±0.2	0.65±0.41	0.39±0.07	0.55±0.51	0.77±0.23	1.05±0.71	0.63±1.01	0.46±0.75
Tridecanoic acid (C13:0)	0±0	0±0	0±0	0.01±0.01	0.01±0.01	0.03±0.01	0.01±0.02	0.02±0.02	0.03±0.02
Myristic acid (C14:0)	1.84±1.28	1.05±0.87	2.81±1.34	2.49±0.81	3.55±2.02	4.02±0.96	5.12±2.95	3.27±2.73	2.87±2.05
Pentadecanoic acid (C15:0)	0.33±0.12	0.28±0.11	0.57±0.2	0.48±0.16	0.52±0.1	0.55±0.05	0.36±0.05	0.43±0.17	0.59±0.24
Palmitic acid (C16:0)	13.55±7.91	9.37±2.84	15.02±4.02	15.7±4.18	18.95±2.49	20.89±1.98	19.1±4.07	18.68±2.58	18.54±1.89
Heptadecanoic acid (C17:0)	0.52±0.19	0.52±0.05	0.8±0.13	0.71±0.15	0.87±0.38	0.79±0.04	0.89±0.27	1.24±0.46	1.43±0.63
Stearic acid (C18:0)	12.91±4.49	14.48±4.19	20.85±5.66	21.66±2.63	24.5±8.21	25.58±2.13	10.11±2.77	10.49±2.97	10.57±3.11
Arachidic acid (C20:0)	0.33±0.03	0.38±0.18	0.32±0.04	0.01±0.02	0.01±0.03	0±0	0.07±0.02	0.07±0.03	0.07±0.02
Henecosanoic acid (C21:0)	0.05±0.02	0.05±0.03	0.05±0.01	0.04±0.02	0.03±0.02	0.02±0.02	0.02±0.02	0.01±0.02	0.01±0.02
Bahenic acid (C22:0)	0.26±0.08	0.34±0.18	0.23±0.08	0.17±0.09	0.14±0.07	0.08±0.02	0.02±0.01	0.02±0.02	0.02±0.02
Tricosanoic acid (C23:0)	0.14±0.06	0.18±0.08	0.15±0.07	0.11±0.06	0.07±0.04	0.04±0.01	0±0.01	0±0.01	0.01±0.01
Tetracosanoic acid (C24:0)	0.27±0.09	0.36±0.27	0.26±0.13	0.17±0.11	0.1±0.06	0.06±0.03	0±0	0±0.01	0.01±0.01
Unsaturated Fatty Acid (%)	33.38±14.14	28.46±6.41	30.73±4.84	31.09±2.97	32.03±4.05	33.01±0.57	36.16±1.51	35.87±3.7	36.04±0.58
MUFA (%)	21.6±9.54	18.93±5.48	22±2.12	26.84±5.27	28.96±3.61	31.44±0.38	33.16±1.27	32.95±4.03	33.1±1.93
Myristoleinic acid (C14:1)	0.15±0.09b	0.19±0.07b	0.52±0.24a	0.1±0.07	0.14±0.07	0.14±0.01	0.22±0.09	0.16±0.09	0.14±0.06
Palmitoleic acid (C16:1)	0.97±0.14	0.75±0.25	1.16±0.12	1.01±0.34	1.34±0.39	1.4±0.29	2.02±0.37	1.86±0.22	1.91±0.28
Ginkgoleic acid (C17:1)	0.48±0.23	0.58±0.25	0.55±0.22	0.37±0.08	0.43±0.36	0.33±0.02	0.64±0.28	0.78±0.36	0.86±0.4
Elaidic acid (C18:1n9t)	0±0	0±0	0±0	21.44±5.74	23.71±12.86	27.27±0.59	0.05±0.1	0.07±0.09	0.09±0.1
Oleic acid (C18:1n9c)	19.6±9.99	16.67±4.33	19.36±1.89	3.82±0.85	3.25±14.43	2.26±0.08	30.23±1.3	30.08±3.77	30.12±1.6
Gonodoic acid (C20:1)	0.21±0.09	0.38±0.19	0.2±0.04	0±0	0±0	0±0	0±0	0±0	0±0
Nervonic acid (C24:1)	0.18±0.11	0.37±0.38	0.22±0.14	0.1±0.08	0.09±0.05	0.05±0.02	0±0	0±0.01	0±0
PUFA (%)	11.79±4.72	9.54±0.93	8.72±4.65	4.25±3.05	3.07±1.2	1.57±0.19	3±0.45	2.93±1.45	2.94±1.76
Linoleic Acid (C18:2n6c)	7.04±5.2	4.39±0.22	3.96±1.12	0.28±0.08	0.25±0.94	0.21±0.02	2.26±0.33	2.26±0.98	2.3±1.24
Y-linolenic acid (C18:3n6)	0.16±0.15	0.04±0	0.03±0.02	0.15±0.08	0.11±0.06	0.04±0.02	0.01±0.01	0.01±0.01	0.01±0.02
Linolenic Acid (C18:3n3)	0.27±0.18	0.13±0.03	0.17±0.07	0.14±0.05	0.15±0.05	0.15±0.02	0.21±0.02	0.26±0.16	0.2±0.06
Cis 11, 14-Eicosadienoic acid (C20:2)	0.14±0.21	0.06±0.04	0.07±0.01	0.06±0.01	0.07±0.03	0.05±0.01	0.05±0.03	0.06±0.03	0.05±0.02
Homo-y linolenic acid (C20:3n6)	0.39±0.25	0.28±0.12	0.21±0.13	0.18±0.11	0.12±0.07	0.05±0.01	0.04±0.03	0.03±0.03	0.03±0.04
Arachidonic Acid (C20:4n6)	3.07±1.82	4.14±0.64	3.77±2.82	3.13±2.59	2.11±1.14	0.96±0.25	0.43±0.11	0.29±0.26	0.34±0.35
Docosadienoic acid (C22:2)	0±0	0±0	0±0	0.04±0.08	0.06±0.09	0.1±0.1	0±0	0±0	0±0
Eicosapentanoic Acid (C20:5n3)	0.52±0.26	0.43±0.28	0.42±0.44	0.2±0.09	0.18±0.14	0.02±0.02	0.01±0.01	0.02±0.05	0.02±0.03
Dokosahelsanoic acid (C22:6n3)	0.2±0.23	0.09±0.04	0.09±0.06	0.06±0.04	0.03±0.02	0±0	0.01±0.02	0.01±0.03	0.01±0.02
Total Fatty Acids	64.07±19.26	55.66±15.42	72.45±7.21	73.07±10.55	81.39±8.95	85.86±0.52	72.98±9.36	70.86±5.42	70.75±5.92
MUFA/SFA Ratio	**0.70**	**0.62**	**0.72**	**0.87**	**0.94**	**1.02**	**1.08**	**1.07**	**1.08**
PUFA/SFA Ratio	**0.38**	**0.31**	**0.28**	**0.14**	**0.10**	**0.05**	**0.10**	**0.10**	**0.10**

CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep

Table 6. (continue)

Parameters	Genotype *CFHR5* ($\bar{x}$ ± Std Dev)								
	PS			GCS			JS		
	CC(18)	CT(14)	TT(3)	CC(4)	CT(6)	TT(0)	CC(4)	CT(11)	TT(0)
Fat content (%)	4.3±3.47	4.12±3.02	5.79±5.74	2.4±1.07	1.66±0.74	-	2.49±0.61	2.56±0.72	-
Saturated Fatty Acid (%)	41.77±4.38	39.97±8.56	42.1±5.57	41.72±6.27	41.83±5.62	-	41.47±1.88	45.55±3.68	-
Octanoic acid (C8:0)	0±0	0±0	0±0	0±0	0±0	-	0.34±0.09	0.23±0.18	-
Caprat acid(C10:0)	0.14±0.04	1.11±3.71	0.12±0.03	0.06±0.01	0.05±0.01	-	0.11±0.01	0.12±0.01	-
Lauric acid (C12:0)	0.43±0.34	0.27±0.18	0.27±0.13	0.29±0.11	0.39±0.09	-	0.43±0.2	0.56±0.32	-
Tridecanoic acid (C13:0)	0.01±0.02	0.02±0.01	0.01±0.01	0.01±0.01	0.02±0.01	-	0±0.002b	0.014±0.01a	-
Myristic acid (C14:0)	3.62±1.34	3.1±1.79	3.36±1.57	2.13±0.51	2.16±0.33	-	3.04±0.92	3.3±0.88	-
Pentadecanoic acid (C15:0)	0.57±0.15	0.6±0.16	0.53±0.08	0.44±0.1	0.52±0.11	-	0.5±0.07	0.58±0.1	-
Palmitic acid (C16:0)	20.73±1.84	18.17±5.59	21.57±3.27	17.11±2.54	15.69±2.31	-	19.9±1.1	21.92±1.99	-
Heptadecanoic acid (C17:0)	0.84±0.31	0.99±0.34	1.03±0.16	0.79±0.08	0.81±0.09	-	0.75±0.08	0.78±0.05	-
Stearic acid (C18:0)	15.25±3.18	15.51±4.7	15.04±3.42	20.58±3.11	21.84±3.34	-	16.22±1.29	17.82±1.01	-
Arachidic acid (C20:0)	0.11±0.05	0.1±0.07	0.09±0.06	0.16±0.03	0.18±0.04	-	0.12±0.01	0.14±0.05	-
Henecosanoic acid (C21:0)	0.03±0.03	0.05±0.11	0.02±0.03	0.03±0.02	0.03±0.02	-	0.02±0.02	0.03±0.02	-
Bahenic acid (C22:0)	0.02±0.02	0.02±0.02	0.02±0.02	0.06±0.03	0.08±0.03	-	0.03±0.02	0.03±0.01	-
Tricosanoic acid (C23:0)	0±0.01	0.01±0.01	0.02±0.02	0.04±0.03	0.04±0.02	-	0.01±0.02	0.02±0.02	-
Tetracosanoic acid (C24:0)	0±0	0.01±0.03	0±0.01	0.03±0.03	0.05±0.02	-	0.01±0.01	0.01±0	-
Unsaturated Fatty Acid (%)	35.52±4.38	35.1±7.81	36.64±4.53	28.45±1.66	26.44±1.68	-	33.8±1.67	36.69±2.66	-
MUFA (%)	32.1±3.71	31.58±8.09	34.04±3.96	24.48±2	21.58±2.9	-	30.6±1.8	32.73±2.17	-
Myristoleinic acid (C14:1)	0.13±0.06	0.12±0.06	0.12±0.06	0.07±0.03	0.07±0.03	-	0.13±0.04	0.15±0.07	-
Palmitoleic acid (C16:1)	1.48±0.54	1.65±0.47	1.79±0.41	1.23±0.15	1.14±0.22	-	1.68±0.16	1.73±0.12	-
Ginkgoleic acid (C17:1)	0.09±0.16	0.17±0.3	0.34±0.3	0.3±0.03	0.27±0.04	-	0±0	0±0	-
Elaidic acid (C18:1n9t)	1.69±1.38	1.86±1.82	1.09±1.71	0±0	0±0	-	0±0	0±0	-
Oleic acid (C18:1n9c)	28.58±2.64	27.7±8.45	30.69±2.54	22.85±1.91	20.07±2.7	-	28.77±1.85	30.81±2.09	-
Gonodoic acid (C20:1)	0.1±0.3	0.09±0.33	0±0	0±0	0±0	-	0±0	0±0	-
Nervonic acid (C24:1)	0.01±0.01	0±0.01	0.01±0.01	0.03±0.03	0.03±0.02	-	0.02±0.01	0.04±0.03	-
PUFA (%)	3.42±1.4	3.51±1.37	2.6±1.41	3.98±1.28	4.87±1.52	-	3.2±0.3	3.96±0.95	-
Linoleic Acid (C18:2n6c)	1.98±0.8	2.21±0.96	1.57±0.63	2.71±0.61	3.28±0.74	-	1.96±0.12	2.53±0.83	-
Y-linolenic acid (C18:3n6)	0.01±0.01	0.01±0.01	0.01±0.02	0±0	0±0	-	0.01±0.01	0.05±0.08	-
Linolenic Acid (C18:3n3)	0.55±0.36	0.45±0.35	0.4±0.35	0.12±0.01	0.14±0.02	-	0.54±0.11	0.61±0.08	-
Cis 11, 14-Eicosadienoic acid (C20:2)	0.04±0.02	0.04±0.03	0.05±0.01	0.05±0.01	0.05±0.01	-	0.02±0.01	0.03±0.01	-
Homo-y linolenic acid (C20:3n6)	0.02±0.03	0.03±0.03	0.02±0.03	0.07±0.05	0.09±0.04	-	0.04±0.01	0.04±0.01	-
Arachidonic Acid (C20:4n6)	0.38±0.31	0.35±0.2	0.27±0.17	0.91±0.57	1.19±0.66	-	0.33±0.04	0.34±0.03	-
Docosadienoic acid (C22:2)	0±0	0±0	0±0	0±0	0±0	-	0±0	0±0	-
Eicosapentanoic Acid (C20:5n3)	0.24±0.19	0.27±0.26	0.16±0.17	0.07±0.06	0.08±0.05	-	0.28±0.09	0.32±0.05	-
Dokosahelsanoic acid (C22:6n3)	0.06±0.06	0.05±0.04	0.06±0.03	0.05±0.04	0.05±0.03	-	0.03±0.01	0.04±0.02	-
Total Fatty Acids	77.29±7.72	75.42±9.8	78.73±9.88	70.21±6.91	68.33±5.75	-	75.26±1.45	82.25±5.95	-
MUFA/SFA Ratio	**1.05**	**1.03**	**1.11**	**0.80**	**0.70**	-	**1.00**	**1.07**	-
PUFA/SFA Ratio	**0.11**	**0.11**	**0.08**	**0.13**	**0.16**	-	**0.10**	**0.13**	-

CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep;
PS = Priangan sheep

Table 7. Association of the *CFHR5* gene with cholesterol

Parameter	Genotype *CFHR5* (x ± Std Dev)		
	CCC (n=39)	CTt (n=48)	TTt (n=13)
Cholesterol	7.15±2.88[b]	10.35±8.91[a]	11.77±7.44[a]

Numbers accompanied by different letters in the same row show significantly different (P<0.05) at the level of α=0.05.

Table 8. Association of *CFHR5* gene with cholesterol based on genotype and breed group

Parameters	Breed and Genotype *CFHR5* (x ± Std Dev)																	
	BCS			CAS			SS			PS			GCS			JS		
	CC(1)	CT(2)	TT(3)	CC(1)	CT(5)	TT(2)	CC(1)	CT(3)	TT(2)	CC(28)	CT(21)	TT(6)	CC(4)	CT(6)	TT(0)	CC(4)	CT(11)	TT(0)
Cholesterol	2.8	37.45	23.96	15.9	17.22	12.47	6.71	3.33	5.035	6.82	6.97	7.69	8.31	16.54	-	7.27	7.284	-

CAS = Compass agrinak sheep; BCS = Barbados cross sheep; GCS = Garut composite sheep; GS = Garut sheep; JS = Jonggol sheep; SS = Sapudi sheep; PS = Priangan sheep.

Conclusions

The SNP c.1011 C>T of the *CFHR5* gene in the studied sheep was polymorphic and affected the fatty acid composition (miristoleic [C14:1], ginkgolic [C17:1], tridecanoic [C13:0], and heptadecanoic [C17:0] acids.) and cholesterol. The CC genotype had highest PUFA/SFA ratio, low cholesterol, and low SFA categorized as good for health. The BCS breed had highest PUFA/SFA and categorized as good for health. It can be inferred that the *CFHR5* gene polymorphism has the potential in sheep breding for fatty acid composition and cholesterol.

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