

Polymorphism of BGN gene (g.77807325 G>C) and its association with fatty acid and carcass characteristics of Indonesian meat lamb

M. F. Amin^{1,2}, C. Sumantri³, I. I. Arief³, A. Jayanegara⁴, K. Listyarini³, R. S. Harahap⁵,
and A. Gunawan^{3*}

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

²Department of Animal Science, Faculty of Fisheries and Animal Science,
Universitas Islam Lamongan, Lamongan 62211, Indonesia

³Department of Animal Production and Technology, Faculty of Animal Science,
IPB University, Bogor 16680, Indonesia

⁴Department of Animal Nutrition and Feed Technology, Faculty of Animal Science,
IPB University, Bogor 16680, Indonesia

⁵Faculty of Animal Science, Jambi University, Jambi 36122, Indonesia
Corresponding E-mail: agunawan@apps.ipb.ac.id

Received December 21, 2023; Accepted May 03, 2024

ABSTRACT

Fatty acid content and carcass characteristics are two meat quality traits consumers choose to obtain healthy and high-quality lamb. Genetic improvement of fatty acid content and carcass characteristics in sheep is important to increase the public appeal of lamb meat. The Biglycan gene (BGN) is a gene that functions to regulate the growth and maintenance of connective tissues, such as bone and cartilage. This study examined the polymorphism of the BGN gene to get a deeper understanding of the correlation between fatty acid content and carcass traits in Indonesian sheep. The PCR-RFLP approach was used to detect polymorphism in the BGN gene in meat samples obtained from the longissimus dorsi region of 115 rams aged between 10 and 12 months. The General Linear Model (GLM) was used test to analyze genotyping through association studies between genotypes and phenotypic attributes. The results indicated that the carcass' fatty acid composition, including tridecanoic acid (C13:0), arachidonic acid (C20:4n-6), and polyunsaturated fatty acids (PUFA), as well as the BGN gene with SNP point g. 77807325 G>C (GG and CC genotypes), were significantly ($P < 0.05$) related to these traits. Genotype GG was more associated with carcass percentage traits than other genotypes, whereas genotype CC was best associated with carcass length traits. The BGN gene with SNP point g. 77807325 G>C can be a potential genetic marker for selecting fatty acid (tridecanoic acid C13:0), polyunsaturated fatty acid (PUFA), arachidonic acid, carcass percentage, and carcass length in sheep meat in Indonesia.

Keywords: Biglycan, Carcass characteristic, Fatty acids, Sheep.

INTRODUCTION

Lamb consumption in Indonesia continues to grow annually, although it remains the lowest compared to other meats (OECD, 2023). This is primarily due to the unique needs of the lamb customer, as well as health concerns about the fatty acids and other characteristics of the carcass. Increasing the quantity and quality of sheep meat is crucial. The goal of improving sheep numbers and quality involves some selection. The two approaches to achieving selection are molecular and conventional, according to Sinha *et al.*, (2023). Many countries currently use a molecular selection technique known as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A simple, rapid, and affordable method for genotyping known SNPs is PCR-RFLP (Hughes and Moody, 2007). The liver of sheep with low tenderness reveals a down-regulated BGN gene out of 328 identified genes. The X chromosome contains the eight exons that make up the BGN gene. RNA sequencing in sheep was used to identify the unique SNP of the BGN gene (g.77807325 G>C). The SNP categorization is the third primary UTR variation (Listyarini *et al.*, 2023). The BGN gene encodes a protein that belongs to the short leucine-rich proteoglycan protein (SLRP) family. BGN genes mostly regulate the growth and preservation of connective tissues, such as cartilage and bone. According to previous studies, Korean cattle with greater marbling scores exhibit increased expression of BGN genes (Jeong *et al.*, 2017). Another study conducted on BGN genes demonstrated their essential role in maintaining the structural integrity of muscle cells in mice (Mercado *et al.*, 2006). The BGN gene shows regulated expression in carcass traits of Angus, Brangus, and Simental cattle (Oswalt *et al.*, 2021). Research related to the BGN gene in Indonesia is still in the field of human health (Inayati, 2020) and animal research on mice (Putri and Azminah, 2023). Research on the BGN gene related to fatty acids and carcass characteristics in Indonesian sheep has never been carried out. Therefore, there is an urgent need to investigate the association of BGN genes with fatty acids and carcass characteristics in sheep. The goal of this study was to examine the genetic variations in the BGN gene and determine

their correlation with the fatty acid composition and carcass traits of Indonesian sheep.

MATERIALS AND METHODS

Ethical Approval and Animal Collected

This research examined 115 rams weighing between 25 and 30 kg at 10 to 12 months of age for BGN gene polymorphisms. The Animal Ethics Commission of IPB University, under permission number 117-2018 IPB, granted authorization for all animal procedures conducted in this study. The samples consisted of 12 Jonggol sheep (JS), 10 Barbados cross sheep (BCS), 70 Javanese thin-tailed sheep (JTTS), 5 Garut composite sheep (GCS), 12 Jonggol sheep (JS), and 6 Compass agrinac sheep (CAS). The objective of this research was to examine the genetic variations in the BGN gene and determine their correlation with the fatty acid composition and carcass characteristics of Indonesian sheep. While JTTS, JS, and JTTS are local sheep, BCS, GCS, and CAS are crossbred sheep. CAS is a hybrid of 25% St. Croix, 25% Barbados black belly sheep, and 50% native Sumatran sheep. BCS is the result of crossing 50% local Sumatran sheep genetics with 50% Barbados Black Belly sheep genetics, while GCS is the result of crossing 50% local Garut sheep genetics with 25% St Croix sheep genetics and 25% Moulton Charolais sheep genetics. Each group of rams was housed in a cage and supplied fattening feed on a daily basis under identical management practices. Studies examining associations between fatty acid features and carcass characteristics will be conducted using data from 115 rams. Sheep were slaughtered for this investigation at the commercial slaughterhouse operated by PT Pramana Pangan Utama (PPU). The abattoir's personnel carried out the slaughtering procedure according to halal norms and met the necessary conditions for animal care. The abattoir provided the sheep with similar husbandry treatment, raised them on different farms, and allowed them to freely choose their food.

Fatty Acid Analyses

One hundred grams of lamb loin meat was used for the fatty acid content study. The fatty acid content was examined using AOAC 969.333 (AOAC, 2007) and AOAC 991.36 (Association of Official Analytical Chemists) standard extrac-

tion techniques. These are standard methods established by the Association of Official Analytical Chemists (AOAC) for extracting and analyzing the fatty acid content in food samples. Each method likely has its protocol for sample preparation and analysis, ensuring accuracy and reproducibility. Gas chromatography, an analytical method, was used to separate and measure the distinct constituents of a mixture, namely fatty acids. Gas chromatography separates the distinct constituents according to their characteristics by volatilizing the sample and directing it through a column. The quantity of each fatty acid may be ascertained by comparing the sample peaks with established standards. The reference citation is from Listyarini *et al.*, (2021). Fat, saturated, monounsaturated, polyunsaturated, and total fatty acids were the fatty acids that were tested (Listyarini *et al.*, 2021).

Carcass Characteristic Analyses

In this study, researchers meticulously documented several essential characteristics of carcasses, likely from livestock or other animals. These characteristics included the weight of the carcass when freshly slaughtered (referred to as the hot carcass weight), its length from the shoulder to the distal end of the tarsus (an important measure of overall size and proportions), the weight of the carcass after refrigeration for 24 hours at 4°C (known as the cold carcass weight), and a calculated value called the carcass proportion, which indicated the efficiency of meat production relative to the initial body weight. A systematic approach was employed to obtain these measurements. After slaughter, the researchers carefully measured the weight of the carcass and its length, ensuring accuracy by removing any non-carcass materials. The carcasses were then refrigerated for 24 hours at a specific temperature (4°C), a crucial step to facilitate preservation and rigor mortis, which improves the handling and accuracy of subsequent measurements.

After the refrigeration period, the weight of the carcasses was measured again to determine the cold carcass weight, providing insights into any changes in weight due to chilling and post-mortem processes. Finally, the carcass proportion was calculated based on the weight of the carcass after it had been emptied, indicating the

proportion of meat obtained relative to the initial weight. This study's methodology, outlined by Dagong *et al.* (2012), allowed for a comprehensive analysis of carcass characteristics, providing valuable information on meat production efficiency and quality.

DNA Extraction, PCR-RFLP Amplification and Genotyping

The experiment utilized the Geneaid gSYNC DNA Isolation Kit (GS050/100/300) following its provided procedure to extract genomic DNA from muscle samples. This kit likely offers standardized protocols and reagents optimized for efficient DNA isolation from tissue samples. For DNA amplification, the GeneAmp ESCO PCR machine was employed to amplify segments of the BGN gene. This process, known as polymerase chain reaction (PCR), enables the selective amplification of specific DNA sequences.

The PCR procedure involved several steps:

1. Denaturation at 95°C for one minute, which separated the double-stranded DNA template.
2. 35 cycles of amplification, each comprising denaturation at 95°C for 15 seconds, annealing at 65°C for 15 seconds, and extension at 72°C for 10 seconds.
3. An extra elongation step at 72°C for one minute is performed to ensure the full synthesis of any partly synthesized DNA strands.
4. Cooling the reaction to 15°C for five minutes to stabilize the DNA and halt enzyme activity.

Following PCR, the resultant products were identified using the *HinfI* enzyme through Restriction Fragment Length Polymorphism (PCR-RFLP), as detailed in the citation. *HinfI* cuts DNA at specific recognition sites, generating fragments of varying lengths. This step allows for the analysis of genetic variations within the target gene by examining the pattern of fragment sizes produced.

Subsequently, the samples were incubated for four hours at 37°C, likely to facilitate the activity of the *HinfI* enzyme during the PCR-RFLP analysis, as described in the citation. This incubation period optimizes the cleavage of DNA at the enzyme's recognition sites, aiding in the identification of genetic polymorphisms. (Thermo Fisher Scientific, EU, Lithuania). The digested products were separated using FluoroSafe, and an aliquot

Table 1. Primer sequence and size of PCR of gene BGN

| Gene | Accession number | Primer sequence | Application | TA (°C) | Size of PCR (bp) | Restriction Enzyme | SNP | Digest fragments length |
|------|------------------|--|-------------|---------|------------------|--------------------|-----------------------|--|
| BGN | NC_019484.2 | F:5' -GCT GAG GAG GGA TGT GTG TC-3' R:5' -CTT CAA CAA CCC CGT TCC CT-3' | Genotyping | 62 | 491 | Hinfl | g.7780 7325 G>C | GG = 491 GC = 491, 336 and 155 CC = 155 and 336 |

Note= designed using primer3; F= forward; R= reverse

of the PCR result from each reaction was examined on a 1.5% agarose gel (Fisher Scientific Ltd.) before digestion with *Hinfl* for BGN. The fragments were examined using a UV transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA) for genotyping. The observed fragment sizes were as follows: GG (491 base pairs), CC (155 and 336 base pairs), and GC (491, 336, and 155 base pairs) (Table 1).

Statistical Analysis

Genotype and allele frequencies

Statistical analyses performed included examining genotype frequencies, alleles were estimated using (Nei and Kumar, 2000) :

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

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

Where X_i = I allele frequency, x_{ii} = *ii* genotype frequency, N = total number of samples, n_{ii} = number of individuals with genotype *ii*, n_{ij} = number of individuals with genotype *ij*. The Hardy-Weinberg equilibrium was identified through the methods of Hartl and Clark (1997). The effect of genotypes on fatty acid and carcass characteristics was assessed with a fixed effects model (ANOVA) using PROC GLM, according to Ekawati *et al.*, (2022). The method was performed using Minitab® 18 software. Significance was defined as $p < 0.05$. Tukey's model was used to test for pairwise differences between genotype effects, with the formula:

$$Y_{ij} = \mu + \text{genotype}_i + E_{ij}$$

Where Y_{ij} = Composition of fatty acid and carcass characteristic, μ = population average, genotype_i = the fixed effect of *i*-th genotype, E_{ij} = the residual error.

RESULTS AND DISCUSSION

Polymorphism of the BGN Gene

Polymorphism of the biglycan gene was successfully performed with the amplified target SNPs using primers designed in the 3' UTR. A SNP of the BGN gene has been identified at SNP position g. 77807325 G>C (Figure 1). Genotyping of the amplified BGN gene target fragment was performed using PCR-RFLP analysis in this study. BGN gene polymorphisms amplified with SNP g.77807325 G>C were two genotypes, namely GG = (491 bp) and CC = (155, 336 bp) (Figure 2). We found gene polymorphisms in Compass agrinac sheep (CAS), Barbados cross sheep (BCS), Garut composite sheep (GCS), Jonggol sheep (JS), Java fat tail sheep (JFTS), and Java thin tail sheep (JTTS) (Table 2). Elea *et al.* (2017) state that genetic polymorphism is needed to map quantitative trait loci linked to productivity and improve breeding techniques. In Hardy-Weinberg equilibrium (HWE), the BGN gene is not present. This is because there is an uneven distribution of each genotype's quantity. Various variables, including natural selection, non-random mating, gene flow, mutation, and genetic drift, may all influence the state of Hardy-Weinberg equilibrium (Nature, 2023). Various factors, including as natural selection, non-random mating, mutation, genetic drift, gene flow, and other variables, may all contribute to a deviation from Hardy-Weinberg equilibrium (Graffelman *et al.*, 2017). HWE aberrations may also be caused by heterozygote excesses or defi-

ciencies, however these are less prevalent in humans (Thiessen and Gregg, 1980). This finding aligns with the research conducted by Garnier-Géré and Chikhi (2013), which suggested that heterozygote loss in large populations of diverse ethnic groups may be a prevalent source of HWE departure due to non-random pairing by geographic location. If a population has several sub-populations and individuals mate at random within these sub-populations, but not between sub-populations (Wahlund effect), homozygous alleles would be found more often in the population as a whole than would be predicted by HWE (Waples *et al.*, 2014). The GG genotype population makes up a larger share of the population, but the CC genotype is less common. Table 2 displays the genotypes and allele frequencies for the BGN gene.

Association of BGN Gene Polymorphisms with Fatty Acid Composition

The association analysis result shows a significant association ($P < 0.05$) between fatty acid levels and BGN polymorphisms (g. 77807325 G>C) (Table 3). CC genotype associated with fatty acids on arachidonic acid and polyunsaturated fatty acid (PUFA) traits had higher traits than GG genotype (Table 3). The GG genotype had a higher composition of tridecanoic acid (C13:0) than CC genotype (Table 3). Arachidonic acid (C20:4n6) is a polyunsaturated fatty

acid and is an omega-6 fatty acid. Omega 6 is one of the essential fatty acids that the human body cannot produce. Therefore, it must be externally obtained to meet the body's need for Omega 6. The fatty acid is a critical component of biological cell membranes. It is essential for the fluidity and flexibility of all cells, especially nerve, skeletal muscle and immune cells (Tallima and Ridi, 2018). The fatty acid is a fundamental constituent of the membrane phospholipids in sheep (Murariu *et al.*, 2023). The fatty acid is found in many cell membranes and is an essential compound in intercellular communication and a precursor compound for other important compounds in the body (Diana, 2013). The fatty acid is an essential fatty acid consumed in small amounts as part of our daily diet. Due to its contribution to the human body's proper functioning, it is considered an "essential" fatty acid. The body needs polyunsaturated fatty acids, also known as essential fatty acids (EFAs), but it cannot produce them; instead, it must obtain them from food. Through its conversion to PGE2 and subsequent activation of PKC (Protein Kinase C), the fatty acid also stimulates cell proliferation (Sellmayer *et al.*, 1996). The activities of the brain and muscles depend on fatty acids (Tallima and Ridi, 2018). The heating procedure converts fatty acids to 1-octen-3-ol and 1-octen-3-one. The three main components of lamb's taste are 2-Pentylfuran, 1-Octen-3-one, and 1-Octen-3-ol (Li

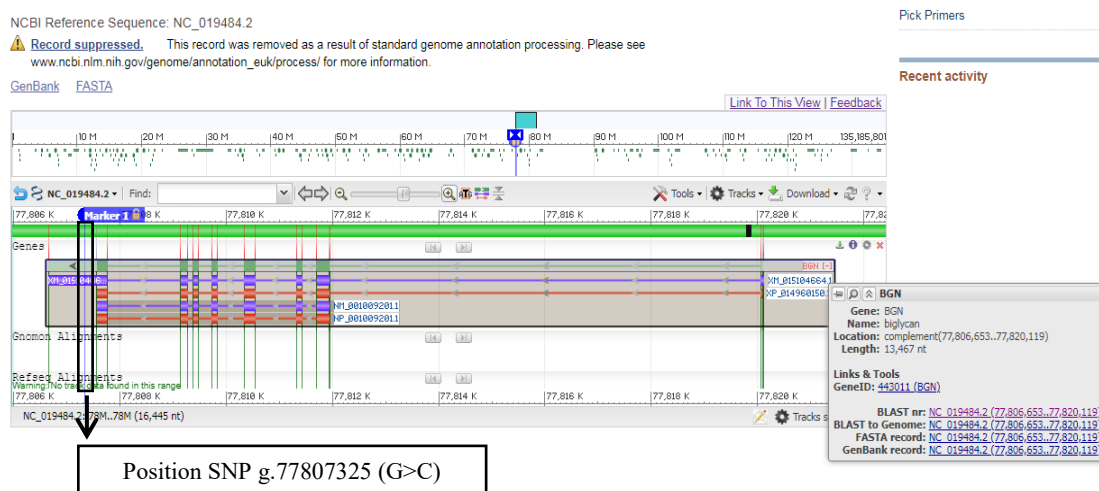


Figure 1. Primer position (underline) and the target sequence (498 bp) in ovine BGN (GenBank: NC_019484.2) belonging to 3'prime UTR position: 77807325 and *Hinf*I restriction site (AT*GG).

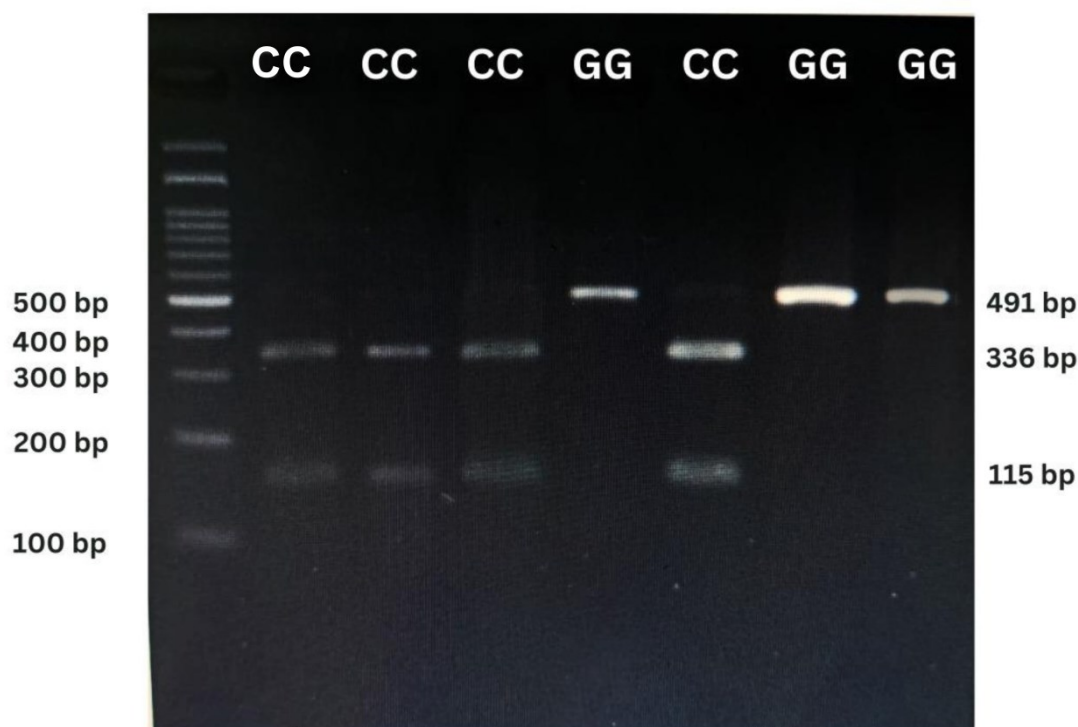


Figure 2. PCR-RFLP result of BGN gene; M= 100 bp ladder size standard; GG (491); CC (155, 336 bp); GC (155, 336, and 491 bp) genotype; bp= base pair

Table 2. The number of animals per genotype and allele frequency of each sheep breed

| Sheep Breed | N | Genotype frequency | | | Allele frequency | | Chi-square (χ^2) |
|-------------|-----|--------------------|----------|----------|------------------|------|-------------------------|
| | | GG (n=109) | CC (n=6) | GC (n=0) | G | C | |
| JFTS | 12 | 1.00 (12) | 0.00 (0) | 0.00 (0) | 1.00 | 0.00 | - |
| JTTS | 70 | 0.99 (69) | 0.01 (1) | 0.00 (0) | 0.99 | 0.01 | 0.51 |
| JS | 12 | 0.83 (12) | 0.17 (2) | 0.00 (0) | 0.83 | 0.17 | 12.00 |
| GCS | 5 | 0.80 (4) | 0.20 (1) | 0.00 (0) | 0.80 | 0.20 | 5.00 |
| CAS | 6 | 1.00 (6) | 0.00 (0) | 0.00 (0) | 1.00 | 0.00 | - |
| BCS | 10 | 0.80 (8) | 0.20 (2) | 0.00 (0) | 0.90 | 0.10 | 37.90 |
| | 115 | 0.95 (109) | 0.05 (6) | 0.00 (0) | 0.97 | 0.03 | 453.92 |

N, number of samples, (..) = number of samples which GG, GC, and CC genotype, χ^2 table = 3.84.

et al., 2023). Tachtsis *et al.*, (2018), Vaidya and Cheema (2014), Moreno-Aliaga *et al.* (2010), and others have all noted the importance of polyunsaturated fatty acids in controlling fat deposition, muscle growth, and glycolipid metabolism. Several studies on the impact of polyunsaturated fats (PUFAs) on meat quality have used feeding trials conducted on pigs in recent years, but the results have been mixed. Among PUFA supplements, conjugated linoleic acid (CLA) and linseed are the most popular. CLA is a linoleic acid

secondary product, and flaxseed is the mature flax plant seed. These therapy outcomes were inconsistent for several reasons. In subsequent studies, for instance, the varying PUFA sources (flaxseed or CLA), the added concentration (high or low), and the pigs' starting development stage (growing or finishing) produced conflicting findings (Wang *et al.*, 2021). One fatty acid molecule with the potential to act as an antibacterial is tridecanoic acid (C13:0). Nevertheless, tests on animals have shown that tridecanoic ac-

id (T13) is carcinogenic (Mboyazi *et al.*, 2020). Furthermore, breed, sex, body weight, environment, and interactions are among the elements that affect the content of fatty acids (Wood and Enser, 1997). Sheep fat stores differ in terms of

their fatty acid content as well (Vacca *et al.*, 2008). Every breed has unique genetic traits and mechanisms of production, particularly subcutaneous and intramuscular. The results of the investigation linking the BGN gene to fatty acids

Table 3. Association of BGN gene with fatty acid in the Indonesian sheep population

| Parameters (%) | Osorio <i>et al.</i> | | | Osorio <i>et al.</i> |
|----------------------------------|-------------------------|------------------------|----------|----------------------|
| | GG (n=109) | CC (n=6) | GC (n=0) | |
| Fat content | 5.56±0.40 | 4.50±1.47 | - | 0.534 ^{ns} |
| Saturated fatty acid | 71.64±1.86 | 69.80±6.00 | - | 0.819 ^{ns} |
| Caprylic acid, C8:0 | 0.03±0.01 | 0.10±0.07 | - | 0.096 ^{ns} |
| Capric acid, C10:0 | 0.22±0.13 | 0.07±0.02 | - | 0.777 ^{ns} |
| Lauric acid, C12:0 | 0.34±0.04 | 0.23±0.05 | - | 0.531 ^{ns} |
| Tridecanoic acid, C13:0 | 0.01±0.001 ^a | 0.00±0.00 ^b | - | 0.026 |
| Myristic acid, C14:0 | 2.67±0.15 | 1.81±0.37 | - | 0.186 ^{ns} |
| Pentadecylic acid, C15:0 | 0.51±0.02 | 0.38±0.05 | - | 0.150 ^{ns} |
| Palmitic acid, C16:0 | 17.56±0.58 | 16.23±2.50 | - | 0.600 ^{ns} |
| Margaric acid, C17:0 | 0.80±0.04 | 0.71±0.05 | - | 0.628 ^{ns} |
| Stearic acid, C18:0 | 14.82±0.57 | 16.36±1.15 | - | 0.529 ^{ns} |
| Arachidic acid, C20:0 | 0.08±0.13 | 0.54±0.36 | - | 0.562 ^{ns} |
| Heneicosylic acid, C21:0 | 0.11±0.02 | 0.03±0.01 | - | 0.290 ^{ns} |
| Behenic acid, C22:0 | 0.05±0.01 | 0.10±0.05 | - | 0.134 ^{ns} |
| Tricosylic acid, C23 | 0.03±0.004 | 0.06±0.04 | - | 0.110 ^{ns} |
| Lignoceric acid, C24:0 | 0.03±0.01 | 0.10±0.06 | - | 0.092 ^{ns} |
| Unsaturated fatty acid | 33.19±1.04 | 33.06±2.31 | - | 0.976 ^{ns} |
| Monounsaturated fatty acid | 29.68±1.03 | 26.90±3.14 | - | 0.535 ^{ns} |
| Myristoleic acid, C14:1 | 0.13±0.01 | 0.12±0.04 | - | 0.803 ^{ns} |
| Palmitoleic acid, C16:1 | 1.32±0.07 | 1.33±0.19 | - | 0.975 ^{ns} |
| Ginkgoleic acid, C17:1 | 0.62±0.19 | 0.30±0.12 | - | 0.702 ^{ns} |
| Oleic acid, C18:1n9c | 24.30±1.06 | 24.51±2.96 | - | 0.963 ^{ns} |
| Elaidic acid, C18:1n9t | 3.13±0.51 | 0.48±0.48 | - | 0.223 ^{ns} |
| Paullinic acid, C20:1 | 0.12±0.03 | 0.08±0.05 | - | 0.739 ^{ns} |
| Erucic acid, C22:1n9 | 0.02±0.01 | 0.00±0.00 | - | 0.452 ^{ns} |
| Nervonic acid, C24:1 | 0.10±0.01 | 0.09±0.06 | - | 0.140 ^{ns} |
| Polyunsaturated fatty acid | 3.51±0.27 ^b | 6.16±1.94 ^a | - | 0.034 |
| Linoleic acid, C18:2n6c | 1.86±0.19 | 3.17±0.78 | - | 0.124 ^{ns} |
| Linolelaidat acid, C18:2n9t | 0.16±0.02 | 0.03±0.03 | - | 0.134 ^{ns} |
| γ-Linolenic acid, C18:3n6 | 0.05±0.01 | 0.07±0.03 | - | 0.697 ^{ns} |
| α-Linolenic acid, C18:3n3 | 0.35±0.03 | 0.32±0.11 | - | 0.818 ^{ns} |
| Eicosadienoic acid, C20:2 | 0.05±0.005 | 0.04±0.01 | - | 0.685 ^{ns} |
| Dihomo-γ-linolenic acid, C20:3n6 | 0.06±0.01 | 0.11±0.06 | - | 0.284 ^{ns} |
| Arachidonic acid, C20:4n6 | 0.68±0.10 ^b | 2.04±1.14 ^a | - | 0.007 |
| Docosadienoic acid, C22:2 | 0.003±0.001 | 0.00±0.00 | - | 0.608 ^{ns} |
| Eicosapentaenoic acid, C20:5n3 | 0.24±0.02 | 0.32±0.14 | - | 0.480 ^{ns} |
| Cervonic acid, C22:6n3 | 0.05±0.01 | 0.05±0.02 | - | 0.948 ^{ns} |
| Fat Acid Total | 71.64±1.86 | 69.80±6.00 | - | 0.819 ^{ns} |

\bar{x} = means of fatty acid; SE= standard error; ns = not significant; * Mean in the same row with different superscripts differ significantly (P<0.05). The numbers shown in parentheses are the number of individuals with the specified genotype.

in sheep may serve as a genetic marker for determining the composition of fatty acids in sheep (Osorio *et al.*, 2007). The body cannot create essential fatty acids (EFAs), which are polyunsaturated fatty acids that must come from the diet.

Association of BGN Gene Polymorphism with Carcass Characteristic

Table 4 indicates, there is a substantial correlation ($P < 0.05$) between BGN genes and carcass features, specifically about carcass length and percentage. Table 4 demonstrates that the GG genotype is associated with more traits in carcass percentage than other genotypes, while the CC genotype is associated with traits that are more significant in terms of carcass length. Both carcass length and carcass percentage showed a good correlation with lamb body weight. There is a direct correlation between sheep's body weight, length, and carcass percentage. Compared to the CC and GC genotypes, the GG genotype's body weight has a larger carcass percentage. Yalcintan *et al.*, (2017), who examined the carcass and meat quality traits of sheep grown at different seasons of the year, agree with these findings. The percentage of feeding time in small ruminants varies greatly, depending on several parameters, including body weight, age at slaughter, breed, sex, and housing arrangement (Harahap *et al.*, 2023). It may range from 36% to almost 60% of feeding time. This suggests that a variety of genes, including BGN, affect the length of the carcass. Lopian *et al.*, (2013) stated, there exists a positive correlation between carcass length and carcass weight in sheep. Specifically, longer carcasses result in heavier meat.

The major component of the carcass is meat, which is very economically valuable. The components of carcasses include adipose tissue, connective tissue, bone, cartilage, and fat (Soeparno, 2011). The extracellular matrix of adipose tissue is influenced by the performance of the BGN gene (Jeong *et al.*, 2017). Factors affecting meat quality, including carcass length and conformation, are breed, age, sex, birth weight, feed, feed consumption, castration, stress, slaughter, and storage (Prache *et al.*, 2022). Factors that influence the length of the carcass are the permanent length of the bones (Priyanto *et al.*, 2015). Thus, the BGN gene association analysis results with sheep carcass traits can be considered a genetic marker for the selection of carcass percentage and carcass length in sheep.

CONCLUSION

Indonesian sheep breeds with JFT, JTT, GCS, BCS, CAS, GS, and JS all have polymorphisms in the BGN gene. The tridecanoic acid, polyunsaturated fatty acid, arachidonic acid, carcass percentage, and carcass length were all substantially correlated with the SNP g.77807325 of the G>C biglycan gene. Certain valuable quality have greater levels in sheep with the CC genotype. It is crucial to recognize the rarity of the CC genotype, which makes it desirable for several desirable qualities. Last but not least, the BGN gene SNP of G>C is a possible marker that may be used to choose sheep meat that has the right amount of fatty acids (triad decanoic acid, polyunsaturated fatty acid (PUFA), and arachidonic acid), as well as the right proportion of carcass length and percentage.

Table 4. Association of BGN gene with carcass characteristics

| Parameter | N | Genotype ($\bar{x} \pm$ SE Mean) | | | P Value |
|------------------------|-----|-----------------------------------|-------------------------|------------|---------------------|
| | | GG (n = 109) | CC (n = 6) | GC (n = 0) | |
| Live weight (kg) | 115 | 25.79±0.44 | 23.84±2.36 | - | 0.324 ^{ns} |
| Hot Carcass (kg) | 115 | 10.65±0.26 | 9.03±1.34 | - | 0.156 ^{ns} |
| Carcass Percentage (%) | 115 | 42.55±0.42 ^a | 38.78±2.36 ^b | - | 0.046 |
| Carcass Length (kg) | 115 | 64.96±1.19 ^b | 78.67±8.50 ^a | - | 0.013 |
| Cold Carcass (kg) | 115 | 9.95±0.33 | 8.71±1.41 | - | 0.391 ^{ns} |

\bar{x} = means of carcass characteristic; SE= standard error; ns = not significant; * Mean in the same row with different superscripts differ significantly ($P < 0.05$). The numbers shown in parentheses are the number of individuals with the specified genotype.

REFERENCES

- AOAC, 2007. Official methods of analysis, association of analytical chemists. 18th ed., Washington D. C. Washington D. C. USA. 141–144.
- Dagong, M.I.A., R. Herman, C. Sumantri, R.R. Noor and M. Yamin. 2012. Carcass and physical meat characteristics of thin tail sheep (TTS) based on calpastatin gene (CAST) (Locus intron 5 – exon 6) genotypes variation. *Indones. J. Anim. Vet. Sci.* 17:13–24.
- Diana, F.M. 2013. OMEGA 6. *J. Kesehat. Masy.* 7:26–31.
- Edea, Z., T. Dessie, H. Dadi, K.T. Do and K.S. Kim. 2017. Genetic diversity and population structure of Ethiopian sheep populations revealed by high-density SNP markers. *Front. Genet.* 8: 1-14
- Ekawati, R.P., R.S. Harahap, K. Listyarini, C. Sumantri and A. Gunawan. 2022. Polymorphisms KIF12 gene/Bfal and its association on carcass characteristics and lamb meat quality. *Bulletin of Animal Science.* 46 (4):257–262.
- Garnier-Géré, P and L. Chikhi 2013. Population Subdivision, Hardy–Weinberg Equilibrium and the Wahlund Effect. In *eLS*, John Wiley & Sons, Ltd (Ed.), 1- 5.
- Graffelman, J., D. Jain and B. Weir. 2017. A genome - wide study of Hardy – Weinberg equilibrium with next generation sequence data. *Hum. Genet.* 136:727–741.
- Harahap, R.S., R.R. Noor, Y.C. Endrawati, H.S. Darusman and A. Gunawan. 2023. Novel polymorphisms of dopa decarboxylase gene and their association with lamb quality traits in Indonesian sheep. *Anim. Biosci.* 36:840–850.
- Hartl, D.L and A.G. Clark. 1997. Principles of population genetics. Sinauer associates Sunderland.
- Jeong, J.Y., M. Ibrahim, M.J. Kim, K. So, Y.D. Jeong, S. Park, M. Kim and H.J. Lee. 2017. Comparisons of extracellular matrix-related gene expression levels in different adipose tissues from Korean cattle. *Livest. Sci.* 198:138–146.
- Lapian, M., P. Siagian, W. Manalu and R. Priyanto. 2013. Carcass Qualities of Finisher Pig Born to Superovulated Sows Before Mating. *J. Vet.* 14:350–357.
- Li, J., C. Tang, Y. Yang, Y. Hu, Q. Zhao, Q. Ma, X. Yue, F. Li and J. Zhang. 2023. Characterization of meat quality traits, fatty acids and volatile compounds in Hu and Tan sheep. *Front. Nutr.* 10:1-12.
- Listyarini, K., C. Sumantri, S. Rahayu, A. Islam, S.H. Akter, M.J. Uddin and A. Gunawan. 2023. Hepatic Transcriptome Analysis Reveals Genes, Polymorphisms, and Molecules Related to Lamb Tenderness. *Animals.* 13(4):1-19.
- Listyarini, K., C. Sumantri, S. Rahayu, M.J. Uddin and A. Gunawan. 2021. Identification and association of polymorphism in THOC5 gene with fatty acid composition in Indonesian sheep. *Proceedings IOP Conference Series: Earth and Environmental Science. The 3rd International Conference of Animal Science and Technology, Makassar, Indonesia, 3-4 November 2020.* P. 1-8.
- Mboyazi, S.N., M.I. Nqotheni, T.S. Maliehe and J.S. Shandu. 2020. In vitro antibacterial and in silico toxicity properties of phytochemicals from *Ricinus communis* leaf extract. *Pharmacogn. J.* 12(5):977-983.
- Mercado, M.L., A.R. Amenta, H. Hagiwara, M.S. Rafii, B.E. Lechner, R.T. Owens, D.J. Mcquillan, S.C. Froehner, J.R. Fallon, M.L. Mercado, A.R. Amenta, H. Hagiwara, M.S. Rafii, B.E. Lechner, R.T. Owens, D.J. Mcquillan, S.C. Froehner and J.R. Fallon. 2006. Biglycan regulates the expression and sarcolemmal localization of dystrobrevin, syntrophin, and nNOS. *FASEB J.* 20:1724–1726.
- Moreno-Aliaga, M.J., S. Lorente-Cebrián and J.A. Martínez. 2010. Regulation of adipokine secretion by n-3 fatty acids. *Proceedings of the Nutrition Society. 3rd International Immunonutrition Workshop, Cambridge University, Inggirs. August, 2010.* P. 324–332.
- Murariu, O.C., F. Murariu, G. Frunză, M.M. Ciobanu and P.C. Boișteanu. 2023. Fatty Acid Indices and the Nutritional Properties of Karakul Sheep Meat. *Nutrients* 15:1–16.
- Nature, 2023. Hardy-Weinberg equilibrium [WWW Document]. URL [https://byjus.com/question-answer/why-is-population-not-in-](https://byjus.com/question-answer/why-is-population-not-in)

- hardy-weinberg-equilibrium/ (accessed 10.14.23).
- Nei, M and S. Kumar, 2000. Molecular evolution and phylogenetics. Oxford University Press, USA.
- OECD, 2023. Meat Consumption of sheep. Organization for Economic Co-operation and Development.
- Osorio, M.T.,J.M. Zumalacárregui, A. Figueira and J. Mateo. 2007. Fatty acid composition in subcutaneous, intermuscular and intramuscular fat deposits of suckling lamb meat: Effect of milk source. *Small Rumin. Res.* 73:127–134.
- Oswalt, H., S. Smith, B. Williams, F. Ferdous, M. Burns, W. Bridges, T. Scott and H.W. Dunn. 2021. Methodology to identify candidate genes from beef carcass traits at weaning: A pilot study. *Anim. Gene.* 20:1-8.
- Priyanto, R., A.M. Fuah, E.L. Aditia, M. Baihaqi and M. Ismail. 2015. Improving Productivity and Meat Quality of Local Beef Cattle Through Fattening on Cereals Based Feed with Different Energy Levels. *Indones. J. Agric. Sci.* 20:108–114.
- Putri, A.W and A. Azminah. 2023. Diabetic Foot Ulcer (DFU) Gene Expression in Homo Sapiens Species. *J. of World Scie.* 2:901–908.
- Rizqa Inayati, A. 2020. Osteogenic Differentiation of Mesenchymal Stem Cells Cultured on Polyvinyl Alcohol and Human Fibroblast-Derived Matrix Hybrid Scaffold. University of Indonesia Library. Indonesia.
- Sellmayer, A., U. Danesch and P.C. Weber. 1996. Effects of different polunsaturated fatty acids on growth-related early gene expression and cell growth. *Lipids.* 31:37–40.
- Sinha, D., A.K. Maurya, G. Abdi, M. Majeed, R. Agarwal, R. Mukherjee, S. Ganguly, R. Aziz and M. Bhatia,. 2023. Integrated Genomic Selection for Accelerating Breeding Programs of Climate-Smart Cereals. *Genes.* 1484:1-37.
- Soeparno, 2011. Nutritional Science and Meat Nutrition. UGM Press, Yogyakarta.
- Tachtsis, B., D. Camera and O. Lacham-Kaplan. 2018. Potential roles of n-3 PUFAs during skeletal muscle growth and regeneration. *Nutrients.* 10:1-20.
- Tallima, H. and R.E. Ridi. 2018. Arachidonic acid: Physiological roles and potential health benefits – A review. *J. Adv. Res.* 11:33–41.
- Thiessen, D. and B. Gregg. 1980. Human assortative mating and genetic equilibrium: An evolutionary perspective. *Ethol. Sociobiol.* 1:111–140.
- Vacca, G.M., V. Carcangiu, M.L. Dettori, M. Pazzola, M.C. Mura, S. Luridiana and G. Tilloca. 2008. Productive performance and meat quality of Mouflon×Sarda and Sarda×Sarda suckling lambs. *Meat Sci.* 80:326–334.
- Vaidya, H. and S. K. Cheema. 2014. Sea cucumber and blue mussel: new sources of phospholipid enriched omega-3 fatty acids with a potential role in 3T3-L1 adipocyte metabolism. *Food funct.* 5:3287–3295.
- Wang, L., Y. Huang, Y. Wang and T. Shan. 2021. Effects of Polyunsaturated Fatty Acids Supplementation on the Meat Quality of Pigs: A Meta-Analysis. *Front. Nutr.* 8:1–13.
- Waples, R. S., T. Antao and G. Luikart. 2014. Effects of Overlapping Generations on Linkage Disequilibrium Estimates of Effective Population Size. *Genetics.* 197:769–780.
- Wood, J. D.w and M. Enser. 1997. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Br. J. Nutr.* 78:49–60.
- Yalcintan, H., B. Ekiz, O. Kocak, N. Dogan, P.D. Akin and A. Yilmaz. 2017. Carcass and meat quality characteristics of lambs reared in different seasons. *Arch. Anim. Breed.* 60:225–233.