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Association of Genetic Polymorphism TP53INP1 Gene with Mineral Content in Javanese Thin-Tailed Sheep

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ABSTRACT

The Tumor Protein P53 Inducible Nuclear Protein 1 (TP53INP1) gene is expected to be a crucial candidate for mineral content. This research aimed to analyse the genotype polymorphism and investigate its association with the TP53INP1 gene and sheep mineral content. To analyse gene polymorphisms and conduct an association study, 30 rams of Javanese thin-tailed sheep were utilised. The NlaIII restriction enzyme was applied to investigate the genotype polymorphism of the TP53INP1 gene through Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Regarding the association with analysing TP53INP1, a General Linear Model (GLM) was used. The findings indicate that the TP53INP1 gene shows polymorphism, revealing three distinct genotypes identified by the results: AA, AG, and GG. The genotypes were detected using the Hardy-Weinberg Equilibrium (HWE) principle. According to the association analysis, a significant association ($P < 0.05$) was found between the TP53INP1 gene and mineral content, particularly iron (Fe). A higher mineral content was associated with the GG genotype, whereas a lower mineral content was related to the AA genotype. The TP53INP1 could be the candidate gene for sheep's mineral content.

Keywords: TP53INP1, Indonesian sheep, mineral content, PCR-RFLP

INTRODUCTION

In Indonesia, sheep meat is a primary source of protein. There are several breeds of sheep in Indonesia that serve as a source of protein. One of the most essential sheep breeds in Indonesia is the Javanese thin-tailed sheep (Sujarwanta et al., 2024; Listyarini et al., 2022). Javanese thin-tailed sheep is a small, primarily white animal that occasionally has black patches around its snout and eyes (Edey, 1983). Javanese thin-tailed sheep is the common sheep of West and Central Java (Tiesnamurti et al., 2023; Tiesnamurti & Shiddieqy, 2022). In Indonesia, a developing nation with a sizable population, these sheep play a crucial role as a source of protein. Therefore, it is essential to understand their nutritional value and genetic composition.

Improving the nutritional value of sheep meat will help the industry meet consumers' demand for sheep products (Pethick et al., 2011). Consumers desire lean, palatable, and nutritious sheep meat, which influences their decision to repurchase (Pethick et al., 2006). Sheep meat has been reported to have high nutrient levels, essential for human health (Pannier et al., 2010).

Genetic improvement programs can play a significant role in enhancing the quality of sheep meat. However, determining the essential nutritional value component traits that require measurement on an ongoing and cost-efficient basis will be a complex task (Pethick et al., 2011). Mineral content shows moderate heritability in sheep, indicating that it is possible to alter mineral content through selection (Mortimer et al., 2014).

The TP53INP1 gene is expected to be a candidate that plays a crucial role in regulating mineral content. The p53 protein regulates DNA metabolism, angiogenesis, cellular differentiation, the immune response, apoptosis, senescence, and the cell cycle. TP53INP1 is reduced in cancers of various organs, functioning as a tumour suppressor (Shibuya et al., 2010; Gironella et al., 2007; Jiang et al., 2006). The p53-regulated protein TP53INP1 influences mitochondrial function and possibly the redox state, which can impact various metabolic processes and contribute to mineral metabolism. Given its role in mitophagy (the elimination of damaged mitochondria) and redox regulation, TP53INP1 may have an impact on the intake and use of minerals, especially those involved in oxidative

phosphorylation inside the mitochondria (Dinh et al., 2021; Simabuco et al., 2018).

Enhancing the nutritional value of sheep meat is necessary because of several key consumer-focused characteristics that will contribute to the prospective value offering of red meat products. These include the need for products to be health-enhancing, meaning they ought to be rich in essential nutrients such as fatty acids, minerals, and vitamins that align with a nutritious diet (Pethick et al., 2021). The identification and association between the TP53INP3 gene and mineral content remain unclear in Javanese thin-tailed sheep. This study aimed to investigate the genotype polymorphism and the association of the TP53INP1 gene with mineral content in sheep.

MATERIALS AND METHODS

Sampling

To identify gene polymorphism, a total of 30 longissimus dorsi samples consisting of Javanese thin-tailed sheep were collected from the rams that had a weight range of 25 to 30 kg and were aged 12 months. The longissimus dorsi muscle was extracted for up to 100 g for mineral content analysis and DNA extraction. The samples were frozen at -20 °C for analysis of mineral content and DNA extraction.

Analysis of Mineral Content

An analysis of mineral content was conducted on longissimus dorsi samples weighing up to 50 grams. The mineral content of each sample was quantified utilising the IK method. LP-04.10-LT-1.0. Measurements of mineral content included iron (Fe), zinc (Zn), potassium (K), and selenium (Se).

DNA Extraction and Amplification

The Geneaid gSYNC DNA Extraction Kit was applied to extract genomic DNA from longissimus dorsi samples. In MEGA 6.0, two pairs of primers (5'-CCAGATTAGCCATGCAGTTC-3' and 5'-ATAGATGTCACCAGGAACGC-3') were designed, and the profile was reviewed utilising Primer Stat. The PCR reaction was performed in a 15 µL volume, incorporating 1 µL of DNA, 7.5 µL of MyTaq Red Mix, 0.2 µL of primers, and 6.1 µL of ddH_2O. The ESCO GeneAmp PCR system was used for TP53INP1 gene fragment amplification under the following conditions: denaturing at 94 °C for 1 min, then 35 cycles of 10 s at 94 °C, 15 s at 60 °C, and 15 s at 72 °C, followed by a final elongation of 1 min at 72 °C. A 1.5 per cent electrophoresis gel was used to visualise the DNA amplicon. The genotyping was performed using PCR-RFLP. The restriction of the NlaIII enzymes and the DNA amplification product were incubated at 37 °C for approximately 4 hours. PCR-RFLP products were checked in a 2% agarose gel.

Analysis of Data

Calculations were performed to determine genotype frequencies, allele frequencies, and the Hardy-Weinberg equilibrium value after acquiring genotypes using the PCR-RFLP method (Nei & Kumar, 2000). The statistical test was calculated in SAS version 9.2. A fixed-effects model was applied using PROC GLM to analyse the influence of genotype (ANOVA).

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

Y_{ijk} is the mineral content, µ is the overall mean, then genotype is the fixed effect of the i-th genotype AA, AG, and GG, and e_{ij} is the residual error.

RESULT AND DISCUSSION

A Single Nucleotide Polymorphism of the TP53INP1 Gene

The TP53INP1 gene (g.81913043 A>G) fragment length of 457 bp was successfully amplified (Figure 1). Three genotypes were discovered, labelled as AA, AG, and GG (Figure 2). NlaIII restriction enzyme successfully digested the 457 bp fragment. The fragment sizes of the digested PCR products for the AA genotype were 319, 124, and 14 bp; 443, 319, 124, and 14 bp for the AG genotype; and 443, 14 bp for the GG genotype.

In this study, the frequencies of the AA, AG, and GG were 0.70, 0.27, and 0.03, respectively (Table 1). The AA genotype was more common than the AG and GG genotypes. This finding was similar to a previous result examined with another gene (AHSG gene) in Indonesian sheep (Munyaneza et al., 2019). TP53INP1 gene (g.81913043 A>G) was detected in Hardy-Weinberg Equilibrium. Without any external disturbance in a population, the genotype frequency should remain steady across generations, and HWE should be in equilibrium (Edward, 2008). The frequencies of genotype and allele of the TP53INP1 gene (g.81913043 A>G) are presented in Table 1.

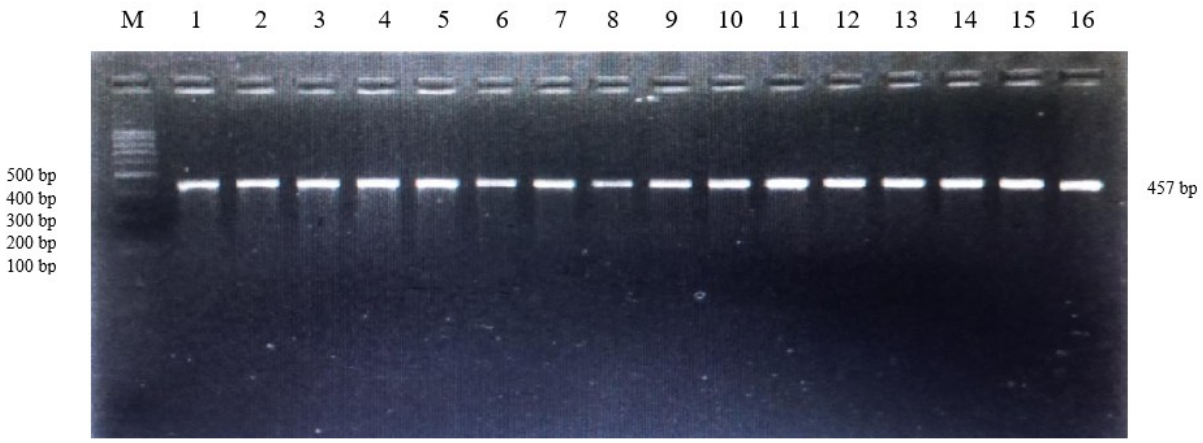

Figure 1. The TP53INP1 gene amplification result

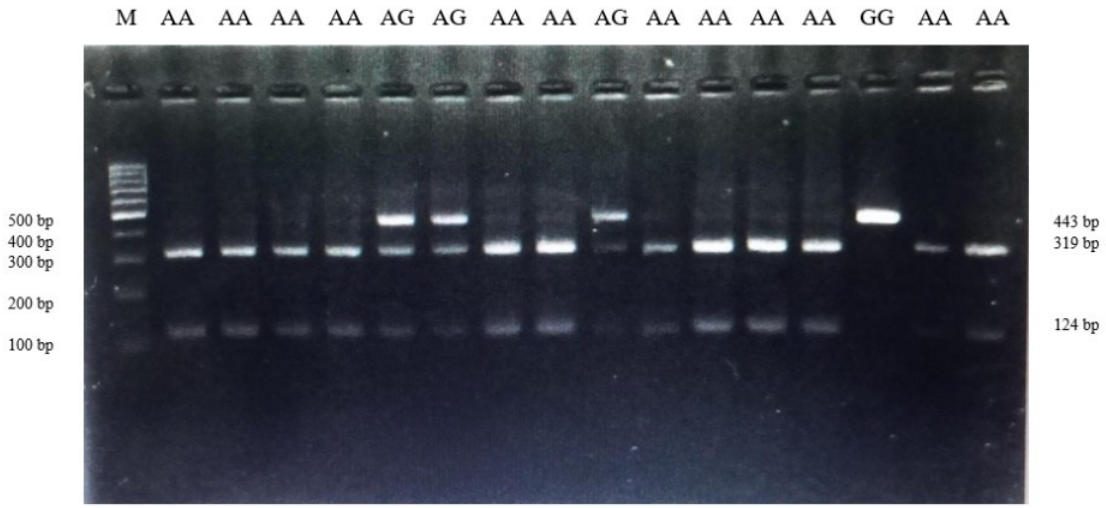

Figure 2. The TP53INP1 gene genotyping result

Table 1. The frequencies of the TP53INP1 genotype and allele

Sample	N	Genotype frequency			Allele frequency		Chi-quadrat (χ^2)
		AA	AG	GG	A	G	
Javanese thin-tailed sheep	30	0.70 (21)	0.27 (8)	0.03 (1)	0.83	0.17	0.048

Association between TP53INP1 Gene Polymorphisms and Mineral Content

Association analysis revealed that the TP53INP1 gene (g.81913043 A>G) was generally significantly ($P<0.05$) associated with mineral content, in particular iron (Fe). Sheep with the GG genotype were associated with higher mineral content, whereas AA was associated with lower mineral content (Table 2). Iron (Fe) is an essential component of haemoglobin in blood, which helps reduce the risk of anaemia (Baba et al., 2021). Iron (Fe) is critical for cellular homeostasis. Iron (Fe) is required in heme fraction production, which contributes to the formation of various proteins involved in cell energy generation, oxygen transport, and detoxification (Andrews, 2005; Grotto, 2008). Iron (Fe) is an essential nutrient, and studies indicate that iron (Fe) supplementation decreases the occurrence of abomasal bloat in lambs. Furthermore, iron (Fe)

insufficiency has been related to pica, which increases the absorption of *Eimeria* oocysts. *Eimeria* spp. Causes coccidiosis in sheep, a severe infection that results in decreased welfare and economic losses (Odden et al., 2018).

Table 2. Association of the TP53INP1 gene polymorphism with mineral content

Mineral Content (mg/100 g muscle)	Genotype (μ ± S.D)		
	AA (n=21)	AG (n=8)	GG (n=1)
Iron (Fe)	17.43 ± 6.05[b]	19.85 ± 3.40[b]	61.27 ± *[a]
Zinc (Zn)	27.40 ± 8.80	27.26 ± 9.34	21.67 ± *
Potassium (K)	3245.98 ± 1206.26	2213.10 ± 886.64	2017.83 ± *
Selenium (Se)	7.13 ± 1.97	7.99 ± 1.38	6.42 ± *

Notation: [a,b] means in the line with different superscripts represent differences significantly (P<0.05). Numbers in parentheses represent the total number of samples with the defined genotype (Duncan's test).

CONCLUSIONS

The TP53INP1 gene (g.81913043 A>G) is polymorphic in Javanese thin-tailed sheep. According to an association analysis, the TP53INP1 gene is significantly associated with mineral content, in particular iron (Fe). The GG genotype was associated with higher mineral content, whereas the AA genotype was associated with lower mineral content. These findings suggest that an SNP in the TP53INP1 gene (g.81913043 A>G) might contribute to the selection of high mineral content of sheep meat.

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