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Nutrient Digestibility, N Balance, Performance, and Blood Parameters of Kacang Goats Differing in GDF9 Genotype Fed Different Sources of Dietary Fiber

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ABSTRACT

This study aims to determine the impact of different GDF9 genotypes on feed intake, nutrient digestibility, and nitrogen balance in Kacang goats by examining various metabolic processes. Twenty-nine Kacang goats were genotyped using PCR-RFLP DNA at position g.3855A/C of the GDF9 gene in exon 2. A 2 × 2 factorial design with two factors, namely, diet type (diets 1 and 2) and genotype (homozygote AA and heterozygote AC), was adopted. Diet 1 group comprised 11 goats with the AA genotype and 4 goats with the AC genotype, and the diet 2 group consisted of 9 goats with the AA genotype and 5 goats with the AC genotype. Both diets had the same protein content (iso-protein) and consisted of free-choice Napier grass (*Pennisetum purpureum*). Diet 1 had an additional 400 g of concentrate containing 11.25% crude protein (CP) and 55.86% total digestible nutrients (TDN). Diet 2 was a total mixed ration (TMR) containing 12.46% crude protein and 67.92% TDN. Results demonstrated a significant interaction ($p<0.05$) effect between diet and genotype on crude protein (CP) digestibility, neutral detergent fiber (NDF) digestibility, fecal nitrogen (N feces), urinary nitrogen (N urine), nitrogen retention (%), and blood urea nitrogen (BUN). However, no significant interaction effect was observed on the consumption of dry matter (DM), CP, crude fiber (CF), nitrogen free extract (NFE), and NDF and the digestibility of DM, CF, and NFE. TMR resulted in a 9% higher consumption of NDF, higher nutrient digestibility, improved nitrogen balance, average daily gain (ADG), and elevated glucose levels compared with diet 1. Kacang goats with the AC genotype exhibited better CP digestibility and increased nitrogen intake than those with the AA genotype. In conclusion, the GDF9 genotype influences the nutrient digestibility in Kacang goats, and those with the AC genotype utilize feed nutrients more efficiently than those with the AA genotype. The AC genotype resulting from the GDF9 gene mutation at position g.3855A>C can be used for genomic marker selection of high-quality Kacang goats in nutrient digestibility.

Keywords: GDF9; genotype interaction; Kacang goats; nutrient digestibility

INTRODUCTION

Kacang goats represent a domestic goat breed known for their advantages in husbandry efficiency, resistance to drought, tropical diseases, and weather-related stress (Khalil *et al.*, 2019). Compared with other imported goat breeds, Kacang goats are relatively smaller in size but exhibit superior disease resistance, excellent adaptability, higher carcass percentages (44%–51%), and moderate prolificacy (Mulyono, 2008). Therefore, these traits are prioritized for genetic improvement in Kacang goats. With the proliferation of Indonesia's population, the meat supply must meet the demand of the increasing population. Hence, increasing the meat production becomes a priority. For ruminant animals such as goats, achieving rapid genetic progress through traditional breeding methods is challenging and time-consuming. Using DNA markers to enhance traits through genomic marker-assisted selection is a more effective and efficient approach to genetic improvement compared with traditional breeding methods, which rely on phenotype selection rather than molecular-level selection (Pedersen *et al.*, 2009). This approach helps obtain genetically superior traits of economic importance, such as prolificacy regulated by growth differentiation factor 9 (GDF9) (Feng *et al.*, 2011; Ahlawat *et al.*, 2016; Celikeloglu *et al.*, 2021).

GDF9 belongs to the transforming growth factor-beta family that plays a crucial role in supporting follicular growth, stimulating granulosa cell development, regulating ovulation rates and DNA synthesis, and increasing the number of live-born offspring (Feng

et al., 2011; Ahlawat *et al.*, 2016). Ahlawat *et al*. (2016), Celikeloglu *et al*. (2021), and Hartatik *et al*. (2023) showed that mutations in the exon 2 of the GDF9 gene in several goat breeds (Bligon, Jining Grey, Xinong Saanen, Guanzhong, and Boer) result in three genotypes: AC (heterozygous genotype with high fertility), AA (homozygous genotype with low fertility), and CC (recessive). Understanding the correlation between GDF9 gene variations and livestock prolificacy may accelerate the improvement in Kacang goats. However, current studies only focus on single nucleotide polymorphism (SNP) and its impact on prolific traits. Nutrition also influences livestock performance (Webb *et al.*, 2004), and inadequate feeding can adversely affect production and reproductive performance (Grazul-Bilska *et al.*, 2012). Feng *et al*. (2011) researched GDF9 gene polymorphism and demonstrated that AC-genotyped animals had superior prolificacy compared with AA-genotyped ones.

Determining whether these two genotypes have different nutrient digestibility abilities is essential to maximize their expression. One SNP at position g.3855A>C in exon two produces AA and AC genotypes. To verify whether the GDF9 genotype affects nutrient utilization, this study explores the relationship between the GDF9 genotype and diet regarding the feed intake, nutrient digestibility, nitrogen balance, livestock performance, and blood metabolites of Kacang goats. The effect of feeding with Napier grass (*Pennisetum purpureum*) ad libitum combined with either 400 g of additional concentrate containing 11.25% CP and 55.86% total digestible nutrients (TDN) (diet 1) as per farmers' practice or total mixed ration (TMR) containing 12.46% CP and 67.92% TDN (diet 2) on the nutrient digestibility in each livestock genotype is also evaluated. The findings will provide insights into livestock markers for superior performance based on nutrient digestibility. Therefore, this study aims to investigate the impact of different GDF9 genotypes on nutrient digestibility, which is yet to be reported and is a novelty of this research.

MATERIALS AND METHODS

Ethics Committee and Experiment Location

All actions involving animals in this research were conducted with the approval of the Animal Care and Utilization Committee of the Faculty of Veterinary Medicine, Gadjah Mada University (FKH UGM), following protocol No. 0124/EC-FKH/Eks./2022. This study was conducted in the "KWT Gama Sumber Rejeki" Women Farmers Group located in Wonolagi Village, Gunungkidul, The Special Region of Yogyakarta, Indonesia, for 11 weeks from August to October 2022.

Genotype Identification

Blood samples. Blood (±5 mL) was collected from the jugular vein of 29 Kacang goats (average weight, 21.0 ± 2.5 kg) and added with 0.5 mL of ethylene diamine tetra acetate (EDTA) as an anticoagulant (EDTA, 0.5 M, pH= 8). The blood samples were then transported to the laboratory using a double-walled refrigerator with ice packs and stored at −20 °C before genomic DNA isolation.

DNA extraction. DNA was extracted from the blood samples of Kacang goats using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan) at the Genetics and Animal Breeding Laboratory, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta, Indonesia. In brief, 200 µL of blood samples and 20 µL of proteinase K were transferred to a 1.5 mL tube, and the mixture was homogenized thoroughly. The samples were then incubated at 60 °C for five minutes. Each incubated solution was added with 200 µL of GSB buffer and then homogenized by shaking. The solution was further incubated at 60 °C for five minutes, shaking every two minutes. The solution was agitated after being added with 200 µL of absolute ethanol. The homogenized solution was transferred to a GD column and centrifuged for two minutes at 10.000 rpm. After centrifugation, the solution was discarded, and the column was replaced. After adding 400 µL of W1 buffer, the solution was centrifuged at 10.000 rpm for 30 seconds. The supernatant was discarded, and the pellet was transferred to a 1.5 mL tube. After adding 200 µL of elution buffer, the tube was centrifuged for 30 seconds at 10.000 rpm to elute the DNA (Latifah *et al.*, 2018).

DNA amplification. The isolated DNA products were amplified by polymerase chain reaction (PCR). The PCR reaction had a total volume of 25 µL consisting of 2.0 µL of genomic DNA, 0.5 µL of forward and reverse primer, 12.5 µL of 124 2 × Eco Taq PCR Supermix (+dye), and 9.5 µL of ddH_2O.

The GDF9 gene-specific target in the sample was amplified by PCR using the forward primer 5'-CTC CTC TTG AGC CTC TGG TG-3' and the reverse primer 5'-TCC AGT TGT CCC ACT TCA GC-3' (Chairunissa *et al.*, 2022). The reactions were conducted using a thermal cycle (PEQLAB Primus 25 advanced, Germany) with a pre-denaturation temperature of 94 °C for one minute, followed by 35 cycles of denaturation at 94 °C for one minute, annealing at 57 °C for one minute, and an extension at 72 °C for one minute. The final step was an extension at 72 °C for five minutes. Electrophoresis was performed on a 1.5% agarose gel stained with ethidium bromide to detect the products.

Genotyping using PCR-RFLP with MspI enzyme. The PCR products were digested with MspI restriction enzyme as follows: a 15 µL mixture consisting of 8.5 µL of ddH_2O, 1 µL of 10X Fast-Digest Green Buffer, 5 µL of PCR product (DNA), and 0.5 µL of MspI restriction enzyme was prepared in a 0.6 mL microtube and incubated on a hotplate for 60 minutes at 60 °C. The mixture was then inactivated on the hotplate for 20 minutes at 80 °C. The RFLP PCR products were electrophoresed on a 3% agarose gel for 30 minutes at 100 volts to separate the fragmented DNA fragments. Genotype frequency (Equations A and b), allele frequency (Equation C), observed heterozygosity (Equation D), and expected

heterozygosity (Equation E) were calculated following the method of Nei & Kumar (2000):

$\chi_{ii} = n_{ii} / N;$
$\chi_{ij} = n_{ij} / N;$
$\chi_i = (2n_{ii} + 2n_{ij}) / N;$
$H_o = n_{ij} / N;$
$H_o = 1 - \sum_{i=1}^{n} p_i^2$

where χ_{ii} was the frequency of the AiAi genotype (homozygote); χ_{ij} was the frequency of the A_iA_j genotype (heterozygote); χ_i was the frequency of the ith allele; n_{ii} was the number of individuals with A_iA_i genotype; n_{ij} was the number of individuals with A_iA_j genotype; H_o was the observed heterozygosity; H_e was the expected heterozygosity; and N was the number of samples.

Polymorphism information content (PIC) was calculated as follows (Botstein et *al.*, 1980):

$$PIC = 1 - \sum_{i=1} p_i^2 - \sum_{i=1} \sum_{j=i+1} 2p_i^2 p_j^2$$

where p_i was the allele frequency of A_i and p_j was the allele frequency of A_j. The Hardy–Weinberg equilibrium (HWE) was calculated as follows (Hartl & Clark, 1997):

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

where χ^2 was the chi-square; O was the total number of observations of genotype to I; and E was the total number of genotypes to expectations to i.

Diet and Feeding

After their genotypes were identified, the 29 Kacang goats were divided into two feeding groups consisting of the two genotypes, AA and AC. Feeding group 1 included 11 goats with the AA genotype and 4 goats with the AC genotype, and feeding group 2 consisted of 9 goats with the AA genotype and 5 goats with the AC genotype. The goats were raised in three phases: an adaptation phase for 14 days, a feeding treatment phase for 46 days, and a data and sample collection phase (feed, feces, urine, and blood) for 15 days.

Both diet treatments in this experiment consisted of two different sources of fiber, namely fresh Napier grass (*Pennisetum purpureum*) provided *ad libitum* with an additional 400 g of concentrate (diet 1) and total mixed ration (TMR) with dried *Ipomoea aquatica* as the fiber source (diet 2). These diets were designed following the recommendations of the National Research Council (NRC, 2007) to achieve an average daily gain of 100 g. Diet 1 contained 11.25% CP and 55.86% TDN, and diet 2 was a TMR containing 12.46% CP and 67.92% TDN (Table 1). The goats were fed twice a day at 07:00 and 16:00. The research began with administering ivermectin injection as a preventive measure against parasites and vitamin injections containing AD3E to prevent vitamin deficiencies, reduce stress, and improve feed conversion. The composition and chemical proportions of diets 1 (basal ration) and 2 (TMR) are shown in Table 1.

Sample Collection and Data Measurement

Feed intake and nutrient digestibility. Samples of feed, leftover feed, feces, and urine were collected throughout the trial to determine nutrient digestibility. During the last 15 days, samples were collected from each goat using the total collection method. The collected samples were dried at 55 °C, ground (using a Cyclotech Mill with a 1 mm screen, Tecator, US), and analyzed for dry matter (DM), ash, ether extract, and crude protein (CP) following the protocols established by the Association of Official Analytical Chemists (AOAC, 2000). NDF was analyzed according to Van Soest *et al*. (1991).

The digestion coefficient of each nutrient was measured using the following formula:

Digestibility (%)= {[dietary intake(nutrients)(g/d) - fecal output(g/d)] / dietary intake(g/d)} × 100

Table 1. Ingredient ratios and chemical compositions of experimental diets 1 and 2 (% DM basis) for Kacang goats

Item	DM (%)	Chemical composition (%DM)							Percentage (%)	
		OM	Ash	CP	EE	CF	NFC[1]	TDN[2]	Diet 1	Diet 2
Ingredients										
Corn husk	79.83	79.86	20.14	10.30	0.38	14.09	55.09	65.23	6	6
Manihot esculenta	75.96	98.08	1.92	2.54	0.64	2.39	92.51	94.56	7	9
Pollard	78.55	95.26	4.74	12.34	0.76	8.78	73.38	82.43	8	12
Palm oil cake	80.30	89.31	10.69	11.33	0.82	14.22	62.94	73.67	2	5
Soybean husk	72.33	94.43	5.57	12.21	3.65	22.79	55.78	73.55	8	8
Ipomoea aquatica	79.09	88.83	11.17	7.30	1.28	20.90	59.35	70.98	6	50
Soybean meal	81.24	69.34	30.66	33.52	0.83	2.70	32.29	51.25	3	10
Count (%DM)									40	100
Pennisetum purpureum	17.45	69.38	30.62	10.05	1.21	30.51	27.61	44.67	60	-
Total (%DM)									100	100
Ration										
Diet 1	38.62	77.89	22.11	11.25	1.26	24.96	40.42	55.86	-	-
Diet 2	66.67	89.20	10.80	12.46	1.04	21.74	53.96	67.92	-	-

Note: DM= dry matter, OM= organic matter, CP= crude protein, EE= ether extract, CF= crude fiber, NFC= non-fiber carbohydrate, TDN= total digestible nutrient. [1]NFC = 100– (% CP + % CF + % EE + % ash). [2]TDN = 5.31 + 0.412 CP% + 0.249 CF% + 1.444 EE% + 0.937 NFC% (Moran, 2005).

Nitrogen balance. The nitrogen (N) content of feeds, leftovers, feces, and urine was measured. The urine produced by the goats was collected daily, and its volume was measured. The urine was then treated with 10% H_2SO_4 to maintain the final pH within the range of 3–4 and prevent nitrogen loss. Approximately 50 mL of the total urine volume was taken, frozen, and later analyzed using the AOAC protocol to measure the total nitrogen (N) content (AOAC, 2000). Nitrogen balance was calculated as follows (Decandia *et al.*, 2000):

Nitrogen balance (%)= {[N ingested - (N feces + N urine)] / N ingested} × 100

Performance and blood metabolites. Each goat's weight was recorded at the beginning and end of each phase. On the last day of data collection, approximately 5 mL of blood samples were collected from the jugular vein of the goats 4 hours after feeding and stored in containers with 12 mg of EDTA. The blood samples were then centrifuged at a speed of 1500 g for 10 minutes using a tabletop centrifuge (Table Top Centrifuge PLC-02, United States) to separate the plasma. The separated plasma was stored at −20 °C until analysis for blood urea nitrogen (BUN), blood glucose (GLU), and calcium (Ca) concentrations. Blood analysis was performed automatically using a UV-visible spectrophotometer (Microlab 200: Merck Vital Scientific, Netherlands).

Statistical Analyses

This experiment employed a completely randomized design with a 2 × 2 factorial arrangement involving two factors: two types of diet (diets 1 and 2) and two genotypes (AA and AC). The data obtained from this experiment were analyzed using the Statistical Program for Social Science (SPSS) version 25 at a significance level of 5%. ANOVA was used as the statistical model:

$$yijk = \mu + \alpha i + \beta j + \alpha\beta(ij) + \varepsilon ij$$

where yijk represents the observation value of each individual, μ denotes the overall mean, αi represents the effect of the diet factor, βj represents the effect of the genotype factor, $\alpha\beta$(ij) signifies the interaction between these two factors, and εij denotes the residual error (variation among replicates for each treatment). Significant interactions were analyzed, followed by the Least Significant Difference test.

RESULTS

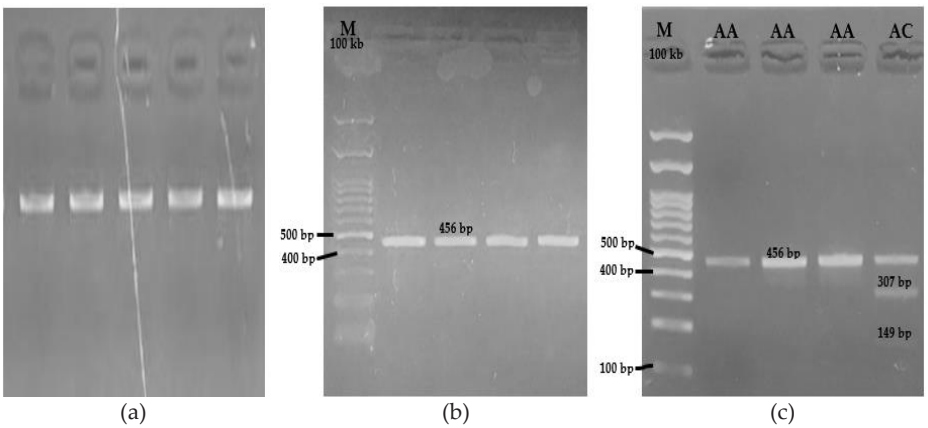
Genotype Identification

PCR amplification analysis and genotyping with PCR-RFLP. The results of DNA isolation, GDF9 gene PCR, and PCR-RFLP are shown in Figure 1. SNP g.3855A/C was used for genotyping 29 Kacang goats using PCR-RFLP (MspI enzyme with the C‖CGG restriction site). The AA genotype was determined as homozygous with a fragment size of 456 bp and the AC genotype as heterozygous with produced fragments of 149 bp, 307 bp, and 456 bp (Figure 1c).

Based on the above findings, Kacang goats have the highest frequency of the AA genotype at 0.69, followed by the AC genotype at 0.31, the A allele at 0.84, and the C allele at 0.16. The observed heterozygosity (H_o) is 0.31, the expected heterozygosity (He) is 0.26, and the PIC is 0.23. HWE test shows a chi-square (χ^2) value of 0.28.

Utilization of Nutrients in Kacang Goats with Different GDF9 Genotypes

Effect of feed intake and nutrient digestibility. No significant interaction effect between different types of dietary fiber and genotypes was observed on nutrient intake variables (DM, CP, CF, NFE, and NDF) and nutrient digestibility (DM, CF, and NFE). However, a significant interaction effect ($p<0.05$) between diet and genotype was found for CP and NDF digestibility. Differences in the type of dietary fiber of fresh Napier grass (*P. purpureum*) and *I. aquatica* had a significant effect ($p<0.01$) effect on nutrient intake (DM, CP, CF, NFE, and NDF) and digestibility variables but had no effect on NDF digestibility. Meanwhile, genotype differences did not affect nutrient intake (DM, CP, CF, NFE, and NDF) and digestibility variables (DM, CF, NFE, and NDF). The highest average values of nutrient intake and digestibility were found in the



(a) (b) (c)

Figure 1. Product size of PCR and PCR-RFLP in growth differentiation factor 9 (GDF9) target gene of Kacang goats. Note: (a) DNA extraction of Kacang goats; (b) PCR product of GDF9 gene target; (c) PCR-RFLP GDF9 gene with MspI enzyme. (M= Marker 100 bp, AA= genotype homozygote, AC= genotype heterozygote).

diet 2 group. Regarding the genotype factor, the AC genotype resulted in the highest CP digestibility (p<0.01) compared with the AA genotype. Data on the interaction effect between diet and genotype on nutrient intake and digestibility in Kacang goats are presented in Table 2.

Nitrogen balance. A significant interaction effect (p<0.05) between diet and genotype was found for N excretion variables (fecal N and urinary N) and N retention (%). For N intake, absorbed N (%), and N balance, no significant interaction effect between diet and genotype was found. The AC genotype in diet 2 produced the highest values for these variables. Data on the interaction effect between diet and genotype on N intake, N excretion, and N balance in Kacang goats are presented in Table 3.

Performance and blood metabolites of Kacang goats. A significant interaction effect (p<0.05) between diet and genotype was found for BUN levels but not for the variables of average daily gain (ADG), relative daily gain (RDG), feed conversion ratio (FCR), GLU, and Ca parameters. The difference in dietary fiber type of fresh Napier grass (*P. purpureum*) feed and *I.aquatica* diet had a significant effect (p<0.01) on ADG and RDG, with the highest average values recorded under diet 2. Meanwhile, genotype differences had a highly significant effect (p<0.01) on RDG and a significant effect (p<0.05) on ADG, FCR, and BUN levels. Data on the interaction effect between diet and genotype on performance and blood parameters in Kacang goats are presented in Table 4.

DISCUSSION

Genotype Identification

PCR product electrophoresis revealed a clear band with a size of 456 bp. According to Garibyan *et al*. (2013), the two main methods for visualizing PCR products are staining the amplified DNA products with reagents, such as ethidium bromide that intercalates between the double strands, or labeling them with PCR primers or nucleotides. In this study, PCR was used as the initial step for all PCR-RFLP analyses. Genotyping of the

Table 2. Nutrient intake and digestibility in Kacang goats with different diets and genotypes

Item	Diet 1		Diet 2		SE	p-values		
	AA	AC	AA	AC		1	2	3
Nutrient intake (g/kg $BW^{0.75}$)								
DM	42.870	45.110	57.100	61.250	1.736	***	NS	NS
CP	5.000	5.250	6.810	7.280	0.216	***	NS	NS
CF	8.961	9.878	12.469	13.493	0.411	***	NS	NS
NFC[x]	24.099	25.267	31.511	33.799	0.944	***	NS	NS
NDF	13.588	14.611	16.040	17.366	0.411	***	NS	NS
Digestibility (%)								
DM	54.015	49.266	59.957	66.842	1.772	***	NS	NS
CP	60.803[a]	61.408[a]	64.770[b]	73.736[b]	1.138	***	***	*
CF	48.433	41.898	59.536	66.076	2.413	***	NS	NS
NFC[x]	62.799	57.984	65.723	71.817	1.501	***	NS	NS
NDF	41.377	33.916	34.899	47.776	2.244	NS	NS	*

Note: AA= genotype homozygote; AC= genotype heterozygote; DM= dry matter; CP= crude protein; CF= crude fiber; NFC= nonfiber carbohydrate; NDF= neutral detergent fiber; 1= diet; 2= genotype; 3= diet × genotype; *p≤0.05; **p≤0.01; ***p≤0.001; NS= p>0.05
[x]NFC = 100–(% CP + % CF + % EE + % ash).
[a,b] Means in the same row with different superscripts differ significantly (p<0.05).

Table 3. N intake, N excretion, and N balance in Kacang goats with different diets and genotypes

Item	Diet 1		Diet 2		SE	p-values		
	AA	AC	AA	AC		1	2	3
	-------- g of N/metabolic BW --------							
N Intake	0.801	0.841	1.090	1.165	0.034	***	NS	NS
N excretion								
N Feces	0.314[a]	0.326[ab]	0.384[b]	0.305[a]	0.011	NS	NS	*
N Urine	0.150[c]	0.126[bc]	0.075[a]	0.107[b]	0.007	***	NS	**
N balance								
Absorbed N	0.487	0.515	0.706	0.860	0.030	***	*	NS
N balance	0.337	0.389	0.632	0.753	0.034	***	***	NS
N output	-------- % of N intake --------							
Absorbed	41.972	46.274	57.896	64.465	1.830	***	***	NS
Retained	69.136[a]	75.287[b]	89.332[c]	87.414[c]	1.829	***	NS	**

Note: AA= genotype homozygote; AC= genotype heterozygote; 1= diet; 2= genotype; 3=diet × genotype. *p ≤ 0.05, **p≤0.01, ***p≤0.001, NS= p>0.05.
[a,b,c] Means in the same row with different superscripts differ significantly (p<0.05).

Table 4. Performance and blood parameters in Kacang goats with different diets and genotypes

Item	Diet 1		Diet 2		SE	p-values		
	AA	AC	AA	AC		1	2	3
Performance								
ADG (g)	89.503	107.567	118.489	150.267	6.442	**	*	NS
RDG (%)	0.415	0.581	0.534	0.774	0.032	**	***	NS
FCR (gr/DM)	5.881	4.482	6.093	4.895	0.235	NS	*	NS
Blood parameter								
Urea	28.391[a]	29.275[a]	36.367[b]	25.800[a]	1.229	NS	*	*
Glucose	57.064	57.600	52.867	61.480	1.329	NS	NS	NS
Calcium	23.915	17.770	20.920	29.592	2.014	NS	NS	NS

Note: AA= genotype homozygote; AC= genotype heterozygote; 1= diet; 2= genotype; 3=diet × genotype. *p ≤ 0.05, **p≤0.01, ***p≤0.001, NS= p>0.05. ADG= average daily gain; RDG= relative daily gain; FCR= feed consumption ratio
[a,b,c] Means in the same row with different superscripts differ significantly (p<0.05).

GDF9 gene in Kacang goats was specifically performed on exon 2. The MspI restriction enzyme specifically recognizes the sequence at position g.3855 A>C located in the exon 2 of the GDF9 gene in Kacang goats.

Feng *et al*. (2011), Ahlawat *et al*. (2016), Chairunissa *et al*. (2022), and Hartatik *et al*. (2023) successfully identified three genotypes, namely, AA, AC, and CC, in goats using the MspI restriction enzyme. These genotypes were determined based on their different fragment sizes after digestion by the restriction enzyme: the AA genotype was characterized by the formation of a single band of 456 bp, the AC genotype with fragment sizes of 456/307/149 bp, and the CC genotype with the size of 307/149 bp. The results showed only two genotypes (AA and AC) were found in Kacang goats. In addition, the AA genotype had the highest frequency compared with the heterozygous AC genotype and A and C alleles, with frequencies of 0.84 and 0.16, respectively. Meanwhile, the CC genotype was not found, possibly due to the limited population of Kacang goats in this study.

According to the genotype data, Kacang goats exhibit polymorphism. Allele and genotype frequencies equal to or less than 0.99 indicate the presence of allele diversity within the population (polymorphic) (Nei & Kumar, 2000). This finding is reinforced by the observed heterogeneity (H_o) and expectation (H_e) values of 0.31 and 0.26, respectively, and PIC of 0.23 (Table 2). The results of the HWE test indicate that the genotype frequency of the GDF9 gene in the Kacang goat population is balanced. Therefore, the Kacang goat population in this study did not experience selection, mutation, migration, and inbreeding (Kardos *et al*., 2015). Further research on the association between diet and genotype regarding the utilization of feed nutrients must be conducted.

Nutrient Utilization in Kacang Goats with Different GDF9 Genotypes

Feed intake and nutrient digestibility. The use of Napier grass (*Pennisetum purpureum*) as a source of fiber feed for livestock is limited by its complex nutrients, such as protein, carbohydrates (Licona *et al*., 2022), and lignin that comprises 15% to 18% of DM. Using a large amount of Napier grass can reduce the nutrient digestibility of the feed. The average DM digestibility for diets 1 and 2 is 52.75% and 62.42%, respectively. This parameter affects the other nutrients (CP, CF, NFE, and NDF) constituting the DM.

A significant interaction effect (p<0.05) between diet and genotype was found for CP digestibility. The AC genotype on diet 2 exhibits the best CP digestibility due to the synergy between the capabilities of the AC gene in optimizing nutrient absorption from the feed and energy intake and subsequently influencing the quantity of absorbed nutrients and the low fiber content of diet 2. Several authors (Avondo *et al*., 2019; Alves *et al*., 2021; Novo *et al*., 2021) stated that a low-fiber diet could improve feed intake and digestibility, thus affecting the amount of absorbed nutrients. Wang *et al*. (2019) showed that livestock with heterozygous genotypes exhibit improved fertility and the likelihood of multiple births, resulting in their high nutritional requirements for reproduction.

In terms of feed, the different types of dietary fiber of fresh Napier grass (*P. purpureum*) and *I. aquatica* had a highly significant effect (p<0.01) on the digestibility of nutrients such as DM, CF, and NFE. The percentage of nutrient digestibility values was higher in diet 2 (p<0.01) than in diet 1, where the average digestibility values of DM, CF, and NFE for diets 1 and 2 were 52.75% and 62.42%; 46.69% and 61.87%; 61.52% and 67.90%, respectively. This finding shows that the nutrient digestibility efficiency of diet 2 was higher than that of diet 1. The inclusion of Napier grass (*Pennisetum purpureum*) at 60% of the DM in diet 1 led to an increase in the structural carbohydrate fraction in the feed. This finding is consistent with those reported by Malik *et al*. (2020) and Licona *et al*. (2022), who stated that the CF content in feed materials significantly affected DM digestibility or degradation. The higher the CF content, the lower the DM digestibility.

A significant interaction effect (p<0.05) between diet and genotype was found on NDF digestibility, where the AC-diet 2 combination produces the best digestibility. The best interaction occurred when the AC genotype was combined with diet 2. This finding is attributed to the synergy between the ability of the AC gene to absorb nutrients and energy intake optimally and the dietary support of diet 2 containing low fiber, thus influencing the quantity of absorbed nutrients.

This phenomenon is evidenced by the 9% lower NDF digestibility in diet 1 group compared with diet 2 group due to the content of fiber components (cellulose, hemicellulose, and lignin), which subsequently affects DM consumption (Vijay *et al.,* 2016). The inclusion of Napier grass (*Pennisetum purpureum*) in diet 1 contributed to more than half (72.16 g/kg) of the total NDF in the feed, resulting in a decrease in NFE content (122.75 g/kg) that consequently affected the feed digestibility.

Dupont & Scaramuzzi (2016) indicated that ovarian function is modulated by insulin and glucose systems, although gonadotropins, LH, and FSH are the primary regulators of terminal folliculogenesis. In the later stages of development, insulin acts as an external factor modulating gonadotropins. The high NFE content and low fiber in diet 2 produced high energy in ruminants through gluconeogenesis. Propionate, which constitutes the largest share of VFA (45%–60%), is converted into glucose (Dupont & Scaramuzzi, 2016). In female sheep, short-term increases in food energy availability during the last few days of the luteal phase can stimulate the final stage of folliculogenesis, leading to the increased ovulation rates and expression of the AC genotype (prolific) (Scaramuzzi *et al.,* 2006). This effect is associated with short-term increases in energy substrate availability from food (Teleni *et al.,* 1989), especially glucose (Gallet *et al.,* 2011; Scaramuzzi *et al.,* 2015). Based on these results, glucose and the GDF9 gene are related as reported by Al-Thuwaini (2020), who discovered a strong association between GDF9 polymorphisms and fertility disorders in women with diabetes. Although no study has directly explained the relationship between GDF9 gene genotype and nutrient digestibility, the amino acid changes resulting from mutations in exon positions that produce two genotypes (AA and AC) are likely to affect amino acid expression and thus influence the structure of the resulting protein (Ahlawat *et al.,* 2016). This phenomenon can be observed in how the AC genotype affects CP digestibility and the glucose content obtained in the AC-diet 2 combination.

Nitrogen utilization. No significant interaction effect between diet and genotype was found on N intake. However, the difference in the dietary fiber of fresh Napier grass (*P. purpureum*) and dried *I. aquatica* had a highly significant effect ($p<0.001$) on N intake. Diet 2 resulted in significantly higher N intake compared with diet 1. Both genotypes did not exhibit a significant influence on N intake.

A significant interaction effect ($p<0.05$) between diet and genotype was observed for fecal N and urine N. The optimal interaction occurred when the AC genotype was combined with diet 2. The low fiber content of diet 2 can enhance NFE digestibility and nutrient CP digestion, thereby influencing livestock performance. Livestock performance depends on the interaction among genotype, nutrition, and physiological conditions (Santoso *et al.,* 2006). Therefore, effective nutrition management must be tailored to the livestock's conditions (Piola *et al.,* 2009). Among the nutritional impacts on sheep production, energy supplementation affects body weight gain, organ function, activity, cell renewal, nutrient utilization (Mahgoub *et al.,* 2000), and reproductive processes (Biehl *et al.,* 2011). Studies on silent mutations in the GDF9 gene at position g.3855 A > C showed that heterozygous genotypes (AC) have greater prolificacy than homozygous genotypes (AA) (Feng *et al.,* 2011). Although these mutations still code for the same amino acid (proline), the mutations located in exons are believed to affect protein expression, such as hormonal factors, infertility, and the resulting phenotype (Ma *et al.,* 2015; Qin *et al.,* 2015). Understanding the interaction between nutrition and genotype can help explain why goats with the AC genotype excel in nutrient digestibility and efficiently optimize nutrients to support their performance. This phenomenon is evidenced by the enhanced capabilities of goats with AC genotype to optimize nutrient absorption from feed and energy intake, resulting in low N excretion (in feces and urine). As a consequence, this phenomenon impacts the amount of absorbed N.

The excretion losses of urinary N and fecal N for the AA-genotyped goats fed with diet 1 were 39.14% and 18.74%, respectively, and those for the AC-genotyped goats fed with diet 1 were 38.76% and 15.00%, respectively. Meanwhile, the excretion losses of urinary N and fecal N for the AA-genotyped goats fed with diet 2 were 35.19% and 6.86%, respectively, and those for the AC-genotyped goats fed with diet 2 were 26.21% and 9.21%, respectively. These findings indicate the capacity of the goats with AC genotype to optimize nutrient absorption from feed and energy intake, resulting in their low N excretion (in feces and urine) under both diets and consequently affecting the absorbed N quantity (Joysowal *et al.,* 2019; Valle *et al.,* 2020). Kand *et al*. (2021) also showed that the variation in nitrogen stored in the body increases with dietary protein consumption.

The utilization of protein or nitrogen by goats increases proportionally and is influenced by the quality of the feed, such as low fiber content (Joysowal *et al.,* 2019). The efficiency of protein utilization in feed for livestock with the AC genotype increased with the decrease in fiber content in the diet, thereby enhancing the productivity. In this study, the 9% higher NDF digestibility in diet 2 group can explain the lower N retention in diet 1 group, and the AC-diet 2 treatment resulted in the highest N retention. N retention indicates the effectiveness of the dietary protein metabolic utilization by livestock for productive needs. In this study, the high N retention in the AC-diet 2 combination group led to the highest ADG in these animals.

Out of the 23 measurable data variables, more than 50% (13 data) were influenced by feed variations, indicating that the efficiency of goat production is affected by dietary differences. However, the AC-diet 2 combination resulted in the highest ADG, as supported by the high CP digestibility and low excretion, leading to high N retention. This result concludes that providing good feed results in improved goat performance.

Several studies on genetic superiority in dairy cattle showed that absorbed nutrients are allocated more toward production rather than milk fat addition (Gordon *et al.,* 1995). In the present research, the goats

with AC genotype showed higher daily weight gain, FCR ($p<0.05$), and RDG ($p<0.01$) than those with AA genotype. This finding indicates that the retained nitrogen is utilized for livestock performance, such as body weight growth, as evidenced by the lowest BUN level in the AC-diet 2 combination group.

A significant interaction effect between diet and genotype ($p<0.05$) was found for the N retention rate (%). Meanwhile, a high N balance ($p<0.01$) was observed in the goats with the AC genotype, indicating that the GDF9 genotype influences N retention for livestock products. Metabolizable nitrogen is usually calculated as the difference between digestible N and total N losses in urine and feces. The goats with genotypes AA and AC lost 30.31% and 25.35% of metabolizable nitrogen, respectively. Gordon *et al*. (1995) reported that nitrogen losses are influenced by genetic variations in livestock. Although the feed intake of goats with the AC genotype was higher than that of the goats with the AA genotype, the energy loss through urine and feces did not differ significantly between these two genotypes ($p<0.05$) under both diets. Therefore, the high production capacity of the goats with AC genotype improves their feed efficiency, as shown by the high N retention rate in the AC genotype for all diet treatments.

CONCLUSION

The interaction between diet and GDF9 genotype affects CP digestibility, NDF digestibility, fecal and urine N excretions, N retention (%), and blood urea levels. The GDF9 genotype influences the nutrient digestibility in Kacang goats, and those with the AC genotype utilize feed nutrients more efficiently than those with the AA genotype. The AC genotype resulting from the GDF9 gene mutation at position g.3855A>C can be used for genomic marker selection of high-quality Kacang goats in nutrient digestibility.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any organization or third party regarding the material discussed in this research.

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