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Research article

Nutrient intake, nutrient digestibility, growth performance, and blood parameters of Kacang goats with *GDF9* genotype

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Abstract

This study aims to determine the different growth differentiation factor 9 (*GDF9*) genotypes in Kacang goats and their influence on the animals' nutrient intake, digestibility, digestible nutrients, performance, and blood chemical composition. Twenty-five Kacang goats underwent PCR-RFLP using the *Msp*I enzyme to determine their genotype. Genotyping results showed that 16 Kacang goats had the AA genotype, whereas 9 had the AC genotype. Following a completely randomized design in a 2 × 2 factorial pattern, the goats were divided into two diet groups (consisting of both genotypes): Diet 1 consisted of Elephant grass (*Pennisetum purpureum*) supplemented ad libitum and 400 g of concentrate, and Diet 2 was a total mixed ration. No significant interaction effect ($P > 0.05$) between diet and genotype on nutrient intake, digestible nutrients dry matter (DM), organic matter (OM), crude protein (CP), nitrogen free extract (NFE), neutral detergent fiber (NDF), and nitrogen of neutral detergent fiber (N-NDF), and nutrient digestibility (DM, OM, NFE, NDF, and N-NDF). However, a significant interaction effect between diet and genotype ($P < 0.05$) was observed for CP digestibility and blood urea nitrogen (BUN) levels. Diet 1 reduced OM consumption by 15.16%, NFE by 26.87%, and digestible NFE nutrients by 31.29% compared with Diet 2. The average daily gain and relative daily gain (%) were lower in Diet 1 compared to Diet 2. The goats with the AC genotype exhibited higher CP digestibility and had the lowest BUN levels compared with those with the AA genotype. This study concluded that providing high-quality feed to goats results in their good performance, and Kacang goats with the AC genotype demonstrates more optimal CP digestibility and performance compared with those with the AA genotype.

Keywords: Digestibility, *GDF9* gene, Genotype and Nutrient interaction, Kacang goat, Nutrient intake

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INTRODUCTION

Indonesia is rich in genetic resources, including goat genetic resources. According to the Global Animal Genetic Resources Bank, goats are categorized as a risk (FAO, 2016). The Kacang goat is a domestic goat breed that has advantages in terms of maintenance efficiency, resilience to drought, tropical diseases, and weather-related stress (Khalil et al., 2019). To protect the genetic resources of Kacang goats as native Indonesian livestock, legalization has been established through Minister of Agriculture's decree No. 2840/KPTS/LB,430/8/2012 (Kementan, 2012). In this context, it is important to conduct selection or genetic improvement of livestock to protect the population and select animals with superior performance. Recently, advances in molecular genetics have provided information on how single nucleotide polymorphism (SNP) genomic markers affect livestock production and reproduction, such as the genotype differences of the *GDF9* gene associated with prolificacy traits (Feng et al., 2011; Ahlawat et al., 2016; Celikeloglu et al., 2021).

Advancements in molecular genetics have provided insights into the effect of single nucleotide polymorphisms (SNPs) in the genome on the production and reproduction of livestock, including the role of the growth differentiation factor 9 (*GDF9*) gene in regulating prolificacy traits (Feng et al., 2011; Ahlawat et al., 2016; Celikeloglu et al., 2021). Ahlawat et al. (2016), Celikeloglu et al. (2021), and Hartatik et al. (2023) demonstrated 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 distinct genotypes: AC, which is a heterozygous genotype associated with high fertility, and AA, which is a homozygous genotype associated with low fertility, and CC genotypes which is a homozygous genotype associated with high fertility.

Studies on the genes responsible for milk production indicated that a goat's diet influences its gene expression and nutrient digestion (Alves et al., 2021; Novo et al., 2021). Several researchers (Silva et al., 2011; Bonanno et al., 2013; Avondo et al., 2019; Schmidely and Bahloul, 2022) revealed that goats with heterozygous genotypes efficiently utilize protein and exhibit a good response to dietary energy, resulting in increased production.

The impact of *GDF9* genotype variations on feed nutrient consumption and digestion has not been previously reported and represents a novel aspect of this study. This knowledge gap has sparked an interest to investigate the relationship between the *GDF9* genotype variants and nutrient consumption of goats to verify whether the *GDF9* genotype affects feed nutrient consumption and digestion. This study aims to identify the relationship between the *GDF9* genotypes and diet of Kacang goats in terms of nutrient consumption, nutrient digestion, livestock performance, and blood chemical profiles.

MATERIALS AND METHODS

Ethical Committee and Experiment Location

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

Genotype Identification

Blood Sampling

Blood samples were collected from 25 Kacang goats with an average weight of 19.37 kg and initial age 1.5 - 2 year. Approximately 5 mL of blood plasma was

collected from the jugular vein of each goat and then added with 0.5 mL of ethylene diamine tetra acetate (EDTA, 0.5 M, pH = 8) as an anticoagulant. The blood samples were then transported in a double-walled freezer box with ice packs to the Genetics and Animal Breeding Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia and stored at –20°C until genomic DNA isolation was performed.

DNA Extraction

In brief, 200 µL of blood samples were placed in 1.5 mL tubes and mixed with 20 µL of proteinase K until well blended. The mixtures were incubated at 60°C for 5 minutes. Afterward, 200 µL of gel sample buffer (gSYNC^{TM} DNA Extraction Kit Protocol, Geneaid Biotech Ltd.) was added to each solution and homogenized by shaking. The solutions were then incubated for 5 minutes at 60°C with intermittent shaking every 2 minutes, followed by the addition of 200 µL of pure ethanol. The solutions were mixed, homogenized, and transferred to GD columns (collection tubes). The columns were then centrifuged for 2 minutes at 10,000 rpm. After centrifugation, the solutions were discarded, and the columns were replaced. Centrifugation was continued at 10.000 rpm for 30 seconds after the addition of 400 µL of buffer W1 (wash buffer, 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA, 50% v/v Ethanol (pure). The discarded liquid was centrifuged for 3 minutes at 10,000 rpm. The liquid was discarded, and the pellet was transferred to a 1.5 mL tube. Afterward, 200 µL of elution buffer was added, and the tube was centrifuged for 30 seconds at 10,000 rpm.

DNA Amplification

The isolated DNA samples were amplified using the polymerase chain reaction (PCR) method. The total PCR reaction volume was 25 µL, which consisted of 2.0 µL of genomic DNA, 0.5 µL each of forward and reverse primers, 12.5 µL of 2× Eco Taq PCR Supermix (+dye), and 9.5 µL of ddH_2O.

The specific target gene *GDF9* in the samples was amplified by PCR using the forward primer 5′-CTCCTCTTGAGCCTCTGGTG-3′ and reverse primer 5′-TCCAGTTGTCCCACT TCAGC-3′ (Genebank Accession No: EF446168.2). The PCR reaction was carried out using thermal cycling on a PEQLAB Primus 25 thermal cycler (Germany). The cycling included an initial denaturation at 94°C for 1 minute, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and an extension at 72°C for 1 minute. The final step was an extension at 72°C for 5 minutes. Electrophoresis was performed using 1.5% agarose gel stained with ethidium bromide to detect the amplification products.

Genotyping Using PCR-RFLP Analysis with *Msp*I Enzyme

The PCR products were digested with the restriction enzyme *Msp*I using a 15 µL of mixture consisting of 8.5 µL of ddH_2O, 1 µL of 10× FastDigest Green Buffer, 5 µL of PCR product (DNA), and 0.5 µL of *Msp*I restriction enzyme. These materials were mixed in a 0.6 mL microtube and then incubated on a hotplate at 60°C for 60 minutes. The mixture was then inactivated on the hotplate at 80°C for 20 minutes. The PCR-RFLP products were electrophoresed on a 3% agarose gel for 30 minutes at 100 volts to separate the DNA fragments.

Nutrient Utilization in Goats with Different Genotypes

Animals, Experimental Design, and Diets

Twenty-five Kacang goats were divided into two different diet groups, each consisting of two genotypes, namely, AA and AC. The diet group 1 comprised 8 goats with AA genotype (six female and 2 male) and 4 goats with AC genotype (two female and two male), and the diet group 2 consisted of 8 goats with AA genotype (six female and 2 male), and 5 goats with AC genotype (three female and two male). These goats were raised in three phases: a feed adaptation phase for 14 days, a

feed treatment phase for 46 days, and a data collection phase (feed, feces, urine, and blood) for 15 days.

Both diet treatments had the same protein content (iso protein). The fiber component of Diet 1 was Elephant grass (*Pennisetum purpureum*) 60%/DM plus 400 g of concentrate, and Diet 2 was a complete feed (total mixed ration, TMR) with dried *Ipomoea aquatica* as the fiber source. According to the National Research Council (NRC, 2007), this diet was designed to achieve a daily weight gain of 100 g with a crude protein content of 12% and TDN 55%. The goats were fed twice a day at 07:00 and 16:00. The research began with the injection of ivermectin as a preventive measure against parasites and vitamin AD3E to prevent vitamin deficiencies, reduce stress in the animals, and improve feed conversion. The chemical composition and proportions of diets 1 and 2 (TMR) are listed in Table 1.

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

Item	DM (%)	Chemical composition (%DM)							Percentage (%)	
		OM	Ash	CP	EE	CF	NFE[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	10.8	12.46	1.04	21.74	53.96	67.92	-	-

DM=dry matter; OM=organic matter; CP=crude protein; EE=ether extract; CF= crude fiber; NFE=nitrogen free extract; TDN = total digestible nutrient.
[1]NFE = 100 – (% CP+% CF+% EE+% ash).
[2]TDN = 5.31 + 0.412 CP% + 0.249 CF% + 1.444 EE% + 0.937 NFE% (Moran, 2005)

Sample and Data Collection

Feed intake and nutrient digestibility

The body weights of the goats were measured at the beginning and end of their individual maintenance phase. During the 15-day data collection period to determine nutrient digestibility, feed samples, refusal feed, and feces were collected from each goat using the total collection method. The samples were then dried at 55°C, ground (particle size 1 mm using a Cyclotech Mill, Tecator, US), and analyzed for dry matter (DM), ash, ether extract (EE), crude protein (CP), and crude fiber (CF) in accordance with the protocols set by the Official Analytical Chemists Association (AOAC, 2000). NDF was analyzed using Van Soest et al. (1991) method. Fecal nitrogen of neutral detergent fiber (N-NDF). The total nitrogen levels in feces consist of nitrogen from undigested feed and metabolic nitrogen, as highlighted by (Barboza et al., 2009). In order to assess feed quality resulting from undigested feed nitrogen, we opted for N-NDF as an indicator. This choice was made because the

NDF rinse method removes metabolic nitrogen from the samples, which is influenced by microbial digestion in the rumen, nitrogen recycled in saliva, and cells from the digestive systems of animals (Barboza et al., 2009).

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 a final pH of 3 to 4 and prevent nitrogen (N) loss. Approximately 50 mL of the total urine volume was collected, frozen, and analyzed using the AOAC method to measure the total N content (AOAC, 2000). As a result, N-NDF provides the most direct measure of feed nitrogen in fecal samples. The digestibility (%) and digested nutrient *(g/d/BW^{0,75})* of each nutrient was measured by using the following formula:

$$\textit{Nutrient digestibility (\%)} = \frac{\text{nutrient intake (g/d)} - \text{nutrient output(g/d)}}{\text{nutrient intake(g/d)}} \times 100$$

$$\textit{Digested nutrient (g/d/BW}^{0,75}\textit{)} = \frac{\{\text{nutrient intake(nutrients)(g/d)} - \text{nutrient output(g/d)}\}}{\text{Body Weight Metabolic (kg BW}^{0.75}\text{)}}$$

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 10 mL of blood was taken from the jugular vein of each goat. Blood samples were collected 4 hours after feeding and stored in 12 mL EDTA containers. The blood samples were centrifuged at 1,500 g for 10 minutes using a tabletop centrifuge (Table Top Centrifuge PLC-02, United States) to separate the plasma. The separated plasma was then stored at –20°C and subsequently analyzed for blood urea nitrogen (BUN), phosphates, phosphorus, blood glucose (GLU), and calcium (Ca). Blood analysis was performed automatically using a UV-visible spectrophotometer (Microlab 200: Merck Vital Scientific, Netherlands). The relative daily gain of goats was measured by using the following formula:

$$\text{Relative daily gain (RDG)} = \frac{\text{average daily gain (g/d)}}{\text{initial weight (g)}} \times 100\%$$

Statistical Analysis

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

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

where *yijk* represents the observation value for each individual, μ is the overall mean, *αi* represents the influence of the diet factor, *βj* is the influence of the genotype factor, *αβ(ij)* is the interaction between the two factors, and *εij* is the residual error (variation between replicates for each treatment).

RESULTS

Genotype Identification

The genotype identification of the livestock was performed using the *Msp*I enzyme digestion method. The *Msp*I enzyme recognizes the SNP at position g.3855A>C at the C'CGG cleavage site. The AA genotype (homozygous) was identified when the PCR-RFLP product was visualized using electrophoresis, resulting in a single fragment of 456 bp. Meanwhile, the AC genotype (heterozygous) produced fragments of 149, 307, and 456 bp. The electrophoresis results of the amplified DNA sequences are shown in Figure 1.

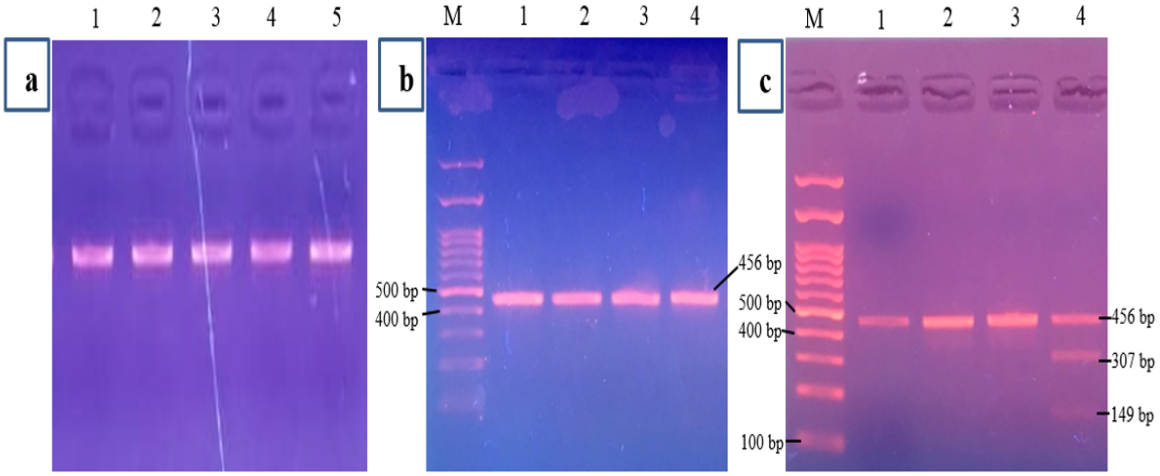

Figure 1 Product size of PCR and PCR-RFLP in the growth differentiation factor 9 (*GDF9*) target gene of Kacang goats. Note: (a) DNA extraction from Kacang goats; (b) PCR product of *GDF9* gene target; (c) PCR-RFLP *GDF9* gene with *Msp*I enzyme. (M = Marker 100 bp, AA= genotype homozygote, AC= genotype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

Feed Intake and Nutrient Intake

No significant interaction effect ($P > 0.05$) between diet and genotype was found for the nutrient consumption variables (DM, OM, CP, NFE, TDN, and N-NDF). The provision of different diets significantly affected ($P < 0.05$) OM consumption and had a highly significant effect ($P < 0.01$) on NFE, NDF, and N-NDF consumption but did not significantly influence DM and CP consumption. The Kacang goats fed with Diet 2 showed higher OM, NFE, and N-NDF consumption than those fed with Diet 1, and the highest NDF consumption was observed in the goats fed with Diet 1. Meanwhile, the difference in livestock genotype did not have any significant effect ($P > 0.05$) on nutrient consumption (DM, OM, CP, NFE, TDN, and N-NDF). No interaction effects between diet and genotype for nutrient consumptions in Kacang goats are presented in Table 2.

Table 2 Effect interaction between diet and genotype on the nutrient intake of Kacang goats.

Item	Diet 1		Diet 2		SEM	p-Values		
	AA	AC	AA	AC		Diet	Genotype	Diet × Genotype
	Nutrient intake (g/kg BW$^{0.75}$)							
DMI	60.16	59.42	61.73	59.27	1.07	0.764	0.502	0.719
OMI	49.88	49.17	55.24	53.20	1.06	0.035	0.516	0.751
CPI	6.84	6.68	7.36	7.05	0.13	0.114	0.391	0.782
NFEI^{x}	27.94	27.32	34.03	32.71	0.82	0.000	0.446	0.782
NDFI	19.48	19.25	17.44	16.82	0.37	0.003	0.523	0.771
N-NDFI	3.37	3.31	3.90	3.75	0.08	0.002	0.468	0.753

AA = genotype homozygote, AC = genotype heterozygote, DMI=dry matter intake; OMI= organic matter intake; CPI=crude protein intake; NFEI=nitrogen free extract intake; NDFI= neutral detergent fiber intake; N-NDFI= nitrogen of neutral detergent fiber intake;
NFEI^{x} = 100 – (% CP+% CF+% EE+% ash).

Nutrient Digestibility and Digested Nutrients

A significant interaction effect ($P < 0.05$) between diet and genotype was observed for CP digestibility. Therefore, the difference in diet significantly affected

(P < 0.05) NDF and N-NDF digestibility and had a highly significant effect (P < 0.01) on EE digestibility. For the digestible nutrient parameters, the difference in diet had a highly significant effect (P < 0.01) on NFE and NDF. However, the difference in genotype did not significantly affect (P > 0.05) nutrient digestibility and digestible nutrients DM, OM, CP, NFE, TDN, and N-NDF. Diet 2 resulted in higher level of digestible NFE nutrients compared with Diet 1. Data on the interaction effect between diet and genotype for nutrient digestibility (%) and digestible nutrients (g/kg $BW^{0.75}$) in Kacang goats are presented in Table 3.

Table 3 Effect interaction between diet and genotype on nutrient digestibility and digested nutrient in Kacang goats.

Item	Diet 1		Diet 2		SEM	p-Values		
	AA	AC	AA	AC		Diet	Genotype	Diet × Genotype
	-------- Digestibility (%) --------							
DM	69.09	65.49	63.37	69.89	1.47	0.831	0.636	0.112
OM	69.82	66.03	67.03	73.18	1.36	0.447	0.679	0.092
CP	73.04^{ab}	72.70^{ab}	68.08^{a}	76.11^{b}	1.04	0.688	0.056	0.039
NFEx	69.01	65.25	68.71	74.37	1.41	0.142	0.746	0.118
NDF	61.18	54.95	41.06	52.58	2.64	0.023	0.569	0.066
N-NDF	76.12	74.73	62.57	68.51	2.00	0.012	0.536	0.322
	Digested nutrient (g/kg $BW^{0.75}$)							
DM	41.57	39.07	39.35	41.52	1.26	0.967	0.953	0.410
OM	34.83	32.60	37.21	39.00	1.15	0.079	0.928	0.408
CP	5.00	4.87	5.04	5.37	0.13	0.350	0.742	0.425
NFEx	19.26	17.91	23.51	24.39	0.84	0.001	0.872	0.453
NDF	11.92	10.65	7.24	8.88	0.59	0.003	0.851	0.150
N-NDF	2.56	2.48	2.46	2.59	0.09	0.986	0.890	0.582

AA = genotype homozygote, AC = genotype heterozygote, DM=dry matter; OM=organic matter; CP=crude protein; NFE=nitrogen free extract; NDF= neutral detergent fiber; N-NDF= nitrogen of neutral detergent fiber;
[x]NFE = 100 – (% CP+% CF+% EE+% ash).
[a,b,c]Different superscripts on the same line indicate significant differences (P < 0.05).

Performance and Blood Parameters of Goats

A significant interaction effect (P < 0.05) was found between diet and genotype for BUN levels. However, for variables such as ADG, relative daily gain, FCR, and blood chemical parameters (phosphate, phosphorus, protein, GLU, and Ca), no significant effect was observed (P > 0.05). The difference in diet had a highly significant effect (P < 0.01) on ADG, relative daily gain, and blood chemical parameters phosphate and phosphorus. Meanwhile, the difference in genotype had a highly significant effect (P < 0.01) on relative daily gain and a significant impact (P < 0.05) on FCR in Kacang goats. Data on the interaction effect between diet and genotype for the performance and blood parameters of Kacang goats are presented in Table 4.

DISCUSSION

PCR product electrophoresis showed a clear band with a size of 456 bp. This study used PCR as the initial step for all PCR-RFLP analyses. Genotyping analysis of the *GDF9* gene in Kacang goats was specifically performed on its exon 2. The *Msp*I restriction enzyme explicitly recognizes the sequence at position g.3855A>C in the exon 2 of the *GDF9* gene in Kacang goats. Previous studies successfully identified three genotypes, namely, AA, AC, and CC, in goats using the *Msp*I restriction enzyme (Feng et al., 2011; Ahlawat et al. 2016; Chairunissa et al., 2022; Hartatik et al., 2023). The genotypes of the goats were determined based on different fragment sizes after digestion by the restriction enzyme. The AA genotype

was characterized by the formation of a single band of 456 bp, and the AC genotype had band sizes of 456/307/149 bp, and the CC genotype had 307/149 bp. However, this study showed that only two genotypes (AA and AC) were found in Kacang goats.

Table 4 Effect interaction between diet and genotype on the performance and blood parameters of Kacang goats.

Item	Diet 1		Diet 2		SEM	p-Values		
	AA	AC	AA	AC		Diet	Genotype	Diet × Genotype
Performance								
Feed Intake (KgDM/day)	0.72	0.67	0.76	0.72	0.017	0.175	0.191	0.925
ADG (g)	88.34^{a}	107.57^{a}	119.88^{ab}	150.27^{b}	7.28	0.008	0.065	0.667
Relative DG (%)	0.33^{a}	0.45^{ab}	0.42^{ab}	0.52^{b}	0.02	0.041	0.010	0.833
FCR	8.23^{b}	6.25^{a}	6.65^{ab}	5.14^{a}	0.35	0.007	0.011	0.687
Blood parameter								
Urea	28.61^{a}	29.28^{ab}	36.13^{b}	25.80^{a}	1.33	0.399	0.052	0.029
Phosphate	4.81	5.32	6.14	6.08	0.20	0.008	0.537	0.440
Phosphor	1.57	1.74	2.00	1.98	0.07	0.008	0.527	0.434
Protein	7.26	6.95	7.12	6.75	0.15	0.601	0.292	0.937
Glucose	56.69	57.60	53.59	61.48	1.40	0.893	0.138	0.235
Calcium	24.40	17.77	20.27	29.59	2.31	0.435	0.783	0.114

AA = genotype homozygote; AC = genotype heterozygote; ADG= average daily gain; DG= daily gain; FCR= feed conversion ratio; ^{a,b} Different superscripts on the same line indicate significant differences ($P < 0.05$).

The choice of using the Kacang goat species to evaluate the effects of *GDF9* gene polymorphism on nutrient intake, digestibility, and blood parameters lies on the results reported in a previous study (Feng et al., 2011; Ahlawat et al., 2016), where a polymorphism at this gene has been studied on goats. In particular, to the best of our knowledge, only a few studies have investigated the effects of *GDF9* genotypes in nutrient intake, digestibility, and blood parameters on different fiber source utilization in different *GDF9* genotypes in the goat species; this led us to deepen these results with a feeding trial to evaluate the possible interaction between dietary fiber source supply and the polymorphism at *GDF9* gene exon 2 in a Kacang goat for the first time. The diets in the present study were different sources of fiber inclusions to reach different sources of fiber to evaluate whether a *GDF9* polymorphism could act in different ways when sources of fiber availability were different.

Most of the studied parameters showed no interaction between genotype and diet. However, a significant interaction ($P < 0.05$) was found in CP digestibility and BUN. A significant interaction effect ($P < 0.05$) was observed between diet and genotype for CP digestibility, with the AC genotype on Diet 2 showing the best digestibility. The best interaction occurred when the AC genotype was combined with Diet 2. This result was attributed to the synergy between the optimal nutrient absorption capability of AC genotype for the feed and energy intake, influencing the amount of nutrients absorbed. This finding was supported by the lower NDF content in Diet 2 than in Diet 1 (Table 1). As mentioned by several authors (Avondo et al., 2019; Alves et al., 2021; Novo et al., 2021), a low NDF diet can increase feed intake and digestion, thus affecting the amount of nutrients absorbed. These findings could justify that livestock with heterozygous genotypes could exhibit enhanced fertility and the likelihood of twin births (Wang et al., 2019), suggesting

that goats with this genotype AC may have high nutritional requirements that result in optimal nutrient absorption.

This research shows that the nutrient digestibility efficiency of Diet 2 was higher than that of Diet 1. The inclusion of Elephant grass at 60% DM in Diet 1 contributed to an NDF fraction of 133.95 g out of the total feed intake. These reduced OM and NFE consumptions were 15.16% and 26.87% lower than those in Diet 2. This finding is consistent with those reported by Malik et al. (2020) and Galeano 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. This phenomenon was evident for Diet 1, which contained higher amounts of fiber components such as cellulose, hemicellulose, and lignin than Diet 2 (Vijay et al., 2016). In addition, using large quantities of Elephant grass can reduce the nutrient intake, i.e., OM, NFE, NDF, and N-NDF (Table 2).

To the best of our knowledge, only a few studies have investigated the effects of *GDF9* genotypes in nutrient intake, digestibility, and blood parameters. Dupont and Scaramuzzi (2016) reviewed that ovarian function is modulated by insulin and glucose systems, although gonadotropins, LH, and FSH are the primary regulators of terminal folliculogenesis. 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 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). These findings could justify that the AC genotype on Diet 2 exhibits the best CP and NDF digestibility. The high NFE content and low fiber in Diet 2 resulted in high energy production in ruminants through gluconeogenesis, starting from propionate, which constitutes the largest share of VFA (45–60%), is converted into glucose (Dupont and Scaramuzzi, 2016). Based on these findings, there is a connection between glucose and the *GDF9* gene, as noted by Al-Thuwaini (2020), who identified a strong correlation between *GDF9* polymorphisms and fertility disorders in diabetics. While no study has directly elucidated the link between the *GDF9* gene genotype and nutrient digestibility, it is plausible that the amino acid alterations resulting from mutations in exon positions, producing two genotypes (AA and AC), may impact amino acid expression, thus influencing the structure of the resulting protein (Ahlawat et al., 2016). This observation shows how the AC genotype influences CP digestibility and the glucose content acquired in the AC-Diet 2 combination.

The low fiber content of Diet 2 can enhance NFE digestibility and nutrient CP digestion, influencing the goats performance. Goats performance depends on the interaction among genotype, nutrition, and physiological conditions (Santoso et al., 2006). Consequently, effective nutrition management must be tailored to the livestock's conditions (Junior et al., 2009). Regarding 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). Investigations 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 CP digestibility. Consequently, this phenomenon impacts the amount of nutrients absorbed.

This study demonstrated that goats with the AC genotype exhibited significantly higher average daily gain (ADG) ($P < 0.01$) and an improved feed conversion ratio (FCR) ($P < 0.05$) compared to those with the AA genotype. Moreover, blood urea nitrogen (BUN) levels in goats with the AA genotype fed Diet 2 were higher than in those with the AC genotype. While elevated BUN levels often signal high protein intake (Chimonyo et al., 2002; Kohn et al., 2005), they do not necessarily reflect increased crude protein (CP) digestibility. To explore these disparities, we analyzed the proportions of energy and fiber in each diet to assess potential links to microbial synthesis in the rumen. Our findings indicated that the energy content in both diets was relatively similar, with Diet 1 at 1390.67 Kcal/kg and Diet 2 at 1496.79 Kcal/kg. However, the smaller particle size in Diet 2 could lead to quicker transit through the digestive tract, potentially reducing rumen digestibility. This could lead to a rapid increase in ammonia production, which could then be absorbed into BUN, converted to urea in the liver, and eventually excreted through urine, suggesting inefficiency in livestock metabolism (McDonald et al., 2010).

The data also indicate that the AC genotype is more efficient in utilizing nutrients and has lower nitrogen excretion compared to the AA genotype, regardless of the diet type. The study revealed that goats with the AA genotype on Diet 1 had nitrogen excretion rates of 39.14% through urine and 18.74% through feces, while goats with the AC genotype on the same diet had rates of 38.76% through urine and 15.00% through feces. On Diet 2, nitrogen excretion through urine and feces for goats with the AA genotype was 35.19% and 6.86%, respectively, whereas for goats with the AC genotype, the rates were 26.21% and 9.21%.

These findings suggest that goats with the AC genotype are more effective in nutrient absorption, leading to reduced nitrogen excretion in both urine and feces (Joysowal et al., 2019; Valle et al., 2020). This supports earlier research Kand et al. (2021), which showed that nitrogen storage in the body increases with higher dietary protein intake. Our results are consistent with these conclusions, demonstrating that goats with the AC genotype exhibit better ADG and FCR compared to those with the AA genotype, with greater CP digestibility observed in goats with the AC genotype on Diet 2. These results imply that the AC genotype is better suited to utilize nitrogen for growth and performance, as evidenced by the lower BUN levels in the AC-Diet 2 group.

CONCLUSIONS

Genotype and diet had an interactive effect on CP digestibility and BUN levels. Diet 1 including 60% DM Elephant grass might reduce the OM consumption by 15.16%, NFE by 26.87%, and digestible NFE nutrients by 31.29% compared with Diet 2. Providing good-quality feed to Kacang goats results in a good performance, and Kacang goats with the AC genotype more optimally utilize feed nutrients than those with the AA genotype.

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AUTHOR CONTRIBUTIONS

Kustantinah: Designed and supervised the study and drafted the manuscript. Arif Irawan: Executed the experiment, conducted laboratory analysis, analyzed the data, and drafted the manuscript. Tety Hartatik: Designed and supervised the study, drafted and modified the manuscript. Sigit Bintara: revised the manuscript. Andriyani Astuti: Drafted and modified the manuscript. All authors read and agreed on the final manuscript.

CONFLICT OF INTEREST

All authors declare no competing interests while studying and writing the manuscript.

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