



Research article

Effect of diet and MC4R genotype on nutrient digestibility and nitrogen balance in Kacang goats

Kustantinah¹, Arie Riska², Panjono³, Asih Kurniawati¹ and Tety Hartatik^{4*}

¹Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

²Graduate School of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

³Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

⁴Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Abstract

This study aims to determine the effect of the relationship between feed treatment and Melanocortin 4 receptor (MC4R) genotypes on nutrient digestibility and nitrogen balance in Kacang goats. Blood samples were collected from 20 Kacang goats, and genomic DNA was extracted by using a Geneaid isolation kit. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype blood samples at the SNP position g.1079C/T cut by the *KpnI* enzyme. A 2 × 3 factorial design with two diet factors (diets 1 and 2) and three genotypes (homozygotes CC and TT and heterozygote CT) was used. The diet 1 and 2 groups comprised 4 goats with the TT genotype, 4 goats with the CT genotype and 2 goats with the CC genotype. Diet 1 contained an additional 400 g of concentrate with 11.25% crude protein (CP) and 55.88% total digestible nutrient (TDN) and diet 2 contained a total mixed ration containing 12.46% CP and 67.92% TDN. Results demonstrated that diet and MC4R genotype had a nonsignificant interactive effect ($P>0.05$) on nutrient digestibility, N balance and blood composition. However, feed factor shown a significant result ($P<0.05$) on EEI, NDFI, DM, CP, EE, NDF, DM, CP, EE and NDF on TT genotype (P0) with the highest level than CC and CT genotype (P1). The P0 goat group absorbs a lot of nitrogen in the body, however the P1 goat group is able to utilize the nutrient efficiently in the body. Kacang goats with the TT genotype can be selected as livestock that excel in utilizing nutrients in feed.

Keywords: Blood, Digestibility, Kacang goat, MC4R, Nitrogen balance

Corresponding author: Tety Hartatik, Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia. Phone number: +6285789641261 E-mail: tety@ugm.ac.id

Article history: received manuscript: 2 January 2024,
revised manuscript: 3 March 2024,
accepted manuscript: 27 March 2024,
published online: 22 April 2024,

Academic editor: Korakot Nganvongpanit

INTRODUCTION

Goats are widely distributed in the Special Region of Yogyakarta, which includes the Gunungkidul District. Kacang goat, a local Indonesian goat species, is raised in rural areas by using a traditional system wherein animals are left to graze on their own such that production levels remain relatively low (Adiwinarti et al., 2016). The feed is intended to fulfil the maintenance of goats. Furthermore, if these needs are fulfilled, it can be utilized for goat productivity. Improving the feed is done to unlock the potential of goats based on the nutrients absorbed in their bodies. Goat productivity is a qualitative attribute that can be observed by using selection, a process wherein certain individuals are selected and bred in a population with the aim of increasing production for the next generation (Maylinda et al., 2015).

On the other hand, the genetic potential inherited from parents also plays a role in determining livestock excellence in digesting nutrients from feed. Consequently, numerous recent studies have explored the interaction between feed and genes regarding specific parameters one gene, in particular, serves as a marker for the growth traits of ruminant livestock: the MC4R gene. This gene generates synthetic leptin in adipose tissue. It is crucial for regulating feed intake, body weight, and energy balance (Polini et al., 2016). The genotyping of goat genotypes based on the effect of MC4R on ADG helps support efforts to increase Kacang goat productivity. All livestock that are identified by their genotype serve as selection material to determine whether they belong to the homozygous or heterozygous genotype, which is capable of demonstrating excellence in digesting nutrients from feed.

In several other studies, SNPs are used as selection material, similar to what was done in the research from Zou et al. (2014) knowing ADG and meat quality in sheep, feed consumption, body weight, and ADG in yak (Cai et al., 2015); backfat thickness and marbling score in Korean cattle (Seong et al., 2012); and sexual desire behavior, feed intake, final weight in rabbits (Fontanesi et al., 2013; El-Sabrou, 2017; El-Sabrou and Soliman, 2018) and ADG in Bligon goat (Latifah et al., 2017). The novelty of this research lies in utilizing the obtained genotypes as markers for the selection of Kacang goats. The use of factorial tests aims to determine the interaction between two factors: feed and genotypes, specifically regarding nutrient digestibility parameters and nitrogen balance. Given the limited information available on the potential of Kacang goats as local livestock, this study is intriguing and warrants further observation to preserve genetic originality and prevent contamination.

MATERIALS AND METHODS

Ethical Committee and Research Location

This study was conducted in compliance with regulations based on the Code of Ethics No. 0124/EC-FKH/Eks./2022 issued by the Faculty of Veterinary Medicine, Universitas Gadjah Mada. The research location was a cage of Gama Sumber Rezeki Women Farmers Group, Wonolagi Village, Gunungkidul, Yogyakarta Special Region, Indonesia.

Blood Sampling

Blood samples were collected from 20 goats with an average body weight of 16 into 19 kg. As much as ± 3 mL of blood was collected from female Kacang goats through the jugular vein, inserted into ethylenediaminetetraacetate tubes stored in a cool box with ice packs, and transported to the animal breeding laboratory of the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.



DNA Isolation and Amplification

Genomic DNA was extracted from blood samples by using gSYNC™ DNA Extraction Kit (Geneaid, New Taipei, Taiwan). A 200 μ L blood sample was placed into a 1.5 mL tube, added with 20 μ L of proteinase K, and homogenized. Subsequently, the solution was incubated for five min at 60°C. Next, the solution was added with as much as 200 μ L of GSB buffer and mixed until homogenized. It was then incubated for five min at 60°C and mixed every two min until homogeneous. Subsequently, the solution was added with 200 μ L of absolute ethanol and mixed again for 10 s until homogeneous. The homogeneous mixture was transferred to a GS column and centrifuged at 10,000 rpm for two min. A fresh tube was used in lieu of the centrifuged solution, and the column was discarded. Next, the mixture was added with 400 μ L of W1 buffer, and the mixture was centrifuged for 30 s at 10,000 rpm. Subsequently, the liquid was discarded, and the wash buffer was centrifuged for another 30 s. The liquid was then disposed of and centrifuged again for 3 min at the same speed. Next, the liquid was discarded, and the column was replaced with a 1.5 μ L tube. The mixture was then added with 150 μ L of elution buffer and centrifuged for 30 s at 10,000 rpm. DNA isolation results were visualized by 1% agarose gel electrophoresis at 100 V for 20 min (Latifah et al., 2017).

DNA was amplified through PCR with a 25 μ L total reaction volume. The reaction volume comprised 9.5 μ L of double-distilled water (DDW), 12.5 μ L of PCR kit, 0.5 μ L of forward and reverse primers, and 2 μ L of DNA (54 ng). The forward primer 5'-TCGGGGTCTTGTTCATCAT-3' and reverse primer 5'-CAAGACTGGGCACTGCTTCA-3' flanked the MC4R gene. PCR was performed with a 3 min initial denaturation stage at 94°C followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 59.75°C, extension for 30 s at 72°C, and a final extension cycle for 10 min at 72°C. Electrophoresis was performed with 1.5% agarose gel.

PCR-RFLP

The *KpnI* restriction enzyme was used to cut PCR products. The reaction volume had a total volume of 9 μ L in a 0.6 mL tube and contained 4 μ L of the PCR product, 3.3 μ L of DDW, 1.5 μ L of buffer 1.1, and 0.2 μ L of the *KpnI* enzyme. The mixtures were incubated in a multiheater at 37°C for 3 h and electrophoresed on a 3% agarose gel for 60 min at 50 V.

Experimental Design and Diets

Twenty Kacang goats were housed in individual cages with feed and water containers. Water was given *ad libitum*, and feed was provided twice a day at 7:00 a.m. and 3:00 p.m. The adaptation period was 14 days, and the rearing period was 60 days. Feed consumption data were collected during the total collection period of 15 days. Kacang goats were divided into two diet groups, with both diets including three genotypes: TT, CT, and CC. Diets each comprised four TT goats, four CT goats, and two CC goats.

Diet 1 consisted of 60% *Pennisetum purpureum* and 40% concentrate, whereas diet 2 was a total mixed ration (TMR) with *Ipomea aquatica* as the fiber source. Feed samples were analyzed to determine the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), extract ether (EE), and crude fiber (CF) by using the proximate analysis method (AOAC, 2005).

Feed, Fecal and Urine Collection

Individual cages for each Kacang goat had feed, fecal, and urine collection containers from which samples were collected for 15 days. Samples of feed and excreted feces were collected. As much as 300 g of the total feces excreted in a day were sun-dried and placed on newspaper that had been baked overnight. The sample was then further dried in an oven at 55°C until its weight was constant. As much as 10 g of the total feces excreted in a day was collected. Feed and fecal

samples were analyzed by using the method described by the Official Analytical Chemists Association (AOAC, 2000). NDF was analyzed in accordance with the method of Van Soest et al. (1991). Urine samples were treated with three drops of H₂SO₄ before sampling, and as much as 50 mL of the total excreted urine was sampled and analyzed for N content by using the Kjeldahl method.

Variables Observed

Chemical composition of feed including; feed samples and feed residues, which will be tested for digestibility of DM, OM, CP, EE, and CF, while fecal and urine are tested to determine N retained in the body as well as measuring net N use (NNU), digested N/consumed N, and biological value (BV).

Feed consumption

DM, OM, CP, EE, and CF were used to measure feed intake. Feed intake was calculated by using the formula reported by Tillman et al. (1998):

Nutrient intake (g) = nutrient in the provided feed (g) – nutrient in the residual feed (g).

Nutrient digestibility

Nutrient digestibility was calculated by using the formula of Tillman et al. (1998):

$$\text{Digestibility (\%)} = \frac{\text{Amount of digested nutrients (in DM)}}{\text{Amount of nutrients consumed (in DM)}} \times 100\%$$

N balance

N consumption in g/head/day and metabolic body weight were measured before N balance was measured. N balance determines the amount of N retained. The following formulas are used to calculate N consumption, N balance, NNU, N Digestibility, and BV:

$$\text{N intake (g/head/day)} = (A \times B) - (a \times b),$$

$$\text{N intake (g/kg BW}^{0.75}\text{/day)} = \frac{\text{N intake (g/head/day)}}{\text{kg metabolic BW}}$$

Information:

A = Feed N content (%)

B = DM feeding (g/head/day)

a = Residual feed N content (%)

b = DM feed residues (g/head/day)

$$\text{Fecal N excretion (g/kg BW}^{0.75}\text{/day)} = \frac{\text{Fecal N excretion (g/head/day)}}{\text{kg metabolic BW}}$$

$$\text{N retention (N) (g/head)} = \{\text{N intake} - (\text{N in feces} + \text{N in urine})\},$$

$$\text{N retention (g/kg BW}^{0.75}\text{/day)} = \frac{\text{N retention (g/head/day)}}{\text{metabolic BW in kg}}$$

$$\text{NNU (\%)} = \frac{\text{N retention (g/head/day)}}{\text{N intake (g/head/day)}}$$

$$\text{N Digestibility (\%)} = \frac{\text{Digested N (g/head/day)}}{\text{N intake (g/head/day)}} \times 100$$

$$\text{BV (\%)} = \frac{\text{Retained N (g/head/day)}}{\text{Digested N (g/head/day)}}$$

Statistical analysis

The effect of feed and MC4R genotype on nutrient digestibility, N balance, and blood composition was analyzed by using a 2×3 factorial completely randomized design (CRD) test with two types of diet (diet 1 and diet 2) and three genotypes (CT, TT, and CC). The CRD test was conducted by using SPSS software version 16.00 (SPSS, the USA) with a significance level of 5%. The mathematical model used is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk},$$

where Y is the observation of the feed factor at level i , factor b at level j , and replication at level k ; μ is the overall mean; α is the feed effect of sample i ; β is the genotype effect of sample j ; and ϵ is the random error.

RESULTS

Genotyping

The result of photo electrophoresis after the method of PCR-RFLP which is cut by the restriction *KpnI* enzyme to produce thick and thin bands. The identification of the genotype resulted in three genotypes results for the MC4R genotype, which had two alleles, namely, C and T, and three genotypes, namely, the homozygotes TT and CC and the heterozygote CT.

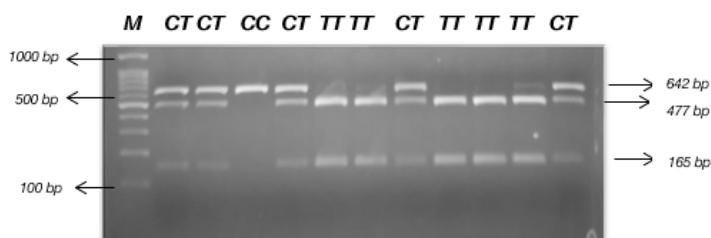


Figure 1 Electrophoresis pattern of the g.1079C/T locus in the Kacang goat MC4R gene revealing three genotypes (CC = 642 bp; CT = 165, 477, and 642 bp; and TT = 165 and 477 bp), M: Marker 1 Kb; Agarose 3%; 50 V until 1 h.

Feed Intake and Nutrient Digestibility

The effects of the interaction between feed and MC4R genotype on feed intake are listed in Table 2. Dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI), extract-ether intake (EEI), crude fibre intake (CFI) and neutral detergent fibre intake (NDFI) had nonsignificant results ($P>0.05$). However, on feed parameters EEI and NDFI gave significant results ($P<0.05$). EEI and NDFI in Kacang goat treatment P0 genotype TT showed the highest results while the lowest in the treatment P1 genotype CC. EEI was highest in P0TT which was 0.81 g/kg BW^{0.75}/day and lowest in P1CC which was 0.37 g/kg BW^{0.75}/day. Furthermore, NDF consumption was highest in P0TT at 20.34 g/kg BW^{0.75}/day and lowest in P1CC at 16.11 g/kg BW^{0.75}/day.

The interaction between feed and MC4R genotypes on nutrient digestibility did not show significant differences ($P>0.05$), however the feed factor showed significant results in the parameters of DM, CP, EE and NDF. The digestibility of DM, CP, EE and NDF in goats treated with P0 genotype TT was highest while in the treatment of P1 genotype CC was lowest. The highest DM digestibility in P0TT was 72.76% and the lowest in P1CC was 56.44%, the highest CP digestibility in P0TT was 73.79% and the lowest in P1CC was 62.50%, the highest EE digestibility in P0TT was 80.24% and the lowest in P1CC was 31.11% and the highest NDF digestibility in P0TT was 66.04% and the lowest in P1CC was 26.78%.



Table 1 Proximate analysis of feed treatments P0 and P1

Feed (%)	DM	OM	CP	EE	CF	NFE	TDN	NDF
P0	38.62	77.89	11.25	1.26	24.96	40.43	55.86	32.48
P1	66.67	89.20	12.46	1.04	21.74	53.96	67.92	28.97

Description: P0 = control feed treatment (60% forage + 40% concentrate) and P1 = first treatment (TMR).

Table 2 Effect of the association between feed and MC4R genotypes on feed intake and nutrient digestibility

Feed intake (g/kg BW ^{0.75} /day)									
Parameter	P0			P1			Sig.		
	TT	CT	CC	TT	CT	CC	A	B	C
	N = 4	N = 4	N = 2	N = 4	N = 4	N = 2			
DMI	62.61±7.70	59.42±7.88	61.33±5.98	60.13±5.48	62.20±7.31	57.72±8.09	ns	ns	ns
OMI	51.95±6.27	50.28±6.88	50.99±4.97	53.74±4.89	55.72±6.40	51.79±7.21	ns	ns	ns
CPI	7.17±0.89	6.86±0.94	7.01±0.60	7.14±0.66	7.41±0.88	6.87±0.98	ns	ns	ns
EEI	0.81±0.09	0.75 ±0.10	0.78±0.05	0.39±0.03	0.39±0.04	0.37±0.03	***	ns	ns
CFI	15.12±1.88	14.43±1.89	14.86±1.77	13.12±1.19	13.58±1.52	12.69±1.65	ns	ns	ns
NDFI	20.34±2.54	19.27±2.54	19.95±1.87	17.24±1.48	17.55±1.48	16.11±2.14	**	ns	ns
Nutrient digestibility (%)									
Parameter	P0			P1			Sig.		
	TT	CT	CC	TT	CT	CC	A	B	C
	N = 4	N = 4	N = 2	N = 4	N = 4	N = 2			
DM	72.76±0.39	65.60± 8.53	70.10±3.83	62.03±10.73	69.05±4.31	56.44±2.96	*	ns	ns
OM	73.50±0.53	66.97 ±8.18	71.27±3.61	65.87±10.16	72.34±3.76	60.67±2.39	ns	ns	ns
CP	73.79±1.12	72.78±3.60	73.46±5.46	70.82±7.15	71.59±3.22	62.50±0.32	*	ns	ns
EE	80.24±2.70	79.24±5.37	78.87±6.08	55.69±7.97	53.25±15.83	31.11 ±6.19	***	ns	ns
CF	72.91±0.98	65.54±12.51	69.32±4.38	60.05±13.07	68.68±4.79	55.48±0.48	ns	ns	ns
NDF	66.04±1.51	54.98±11.76	62.81±4.53	40.63±18.76	48.82±9.09	26.78±5.55	***	ns	ns

Description: N = sum of data, TT, CT and CC = genotype, A = feed treatment factor, B = genotype factor, C = feed × genotypes, N= sum of goats, genotyping, and feed treatment associated with parameters (DM, OM, CP, EE, CF and NDF), * = significant ($P<0.05$), **=significant ($P<0.01$), ***= significant ($P<0.001$), ns=nonsignificant ($P>0.05$), and ^{a,b} =different superscripts in the same line indicate differences.

Nitrogen Balance

The interaction between feed and MC4R genotype on nitrogen balance consisted of N intake, N fecal, N urine, N retention (metabolic body weight), net nitrogen utilization (NNU), N digestibility, and biological value (BV). Feed factors had significant effects on the N feces (g/day), N feces metabolic, N urine, N digestibility and BV in Kacang goats ($P<0.05$). Nitrogen excretion in feces in grams (g) and body weight metabolic (BWM) was highest in the P1 treatment group of CC genotype goats at 4.62 g and 0.33 g/kg BW^{0.75}. However, excretion of N in urine was highest in the P0 genotype CC treatment goat group at 1.61 g while the lowest in the P1CT treatment at 0.83 g. Then, N digestibility was highest in the P1 genotype CC treatment goat group at 4.62 g and 0.33 g/kg BW^{0.75}. Then, the highest N digestibility was found in P0TT at 73.79% while the lowest in P1CC at 62.50%. The highest biological value was shown in P1CT which was 91.05% and the lowest in P0TT which was 83.76%.

Table 3 The Association between feed and MC4R genotype on nitrogen balance

Parameter	P0			P1			Sig.		
	TT N=4	CT N=4	CC N=2	TT N=4	CT N=4	CC N=2			
N Intake (g/day)	12.19±0.90	13.73±1.40	13.50±3.06	15.93±1.77	13.39±2.56	14.23±2.15	ns	ns	ns
N Intake (g/kg BW ^{0.75})	1.14±0.14	1.09±0.15	1.12±0.09	1.14±0.10	1.18±0.14	1.09±0.15	ns	ns	ns
N Feses (g/day)	3.19±0.24	3.72±0.22	3.50±0.07	4.62±1.12	3.79±0.75	5.33±0.85	**	ns	ns
N Feses (g/kg BW ^{0.75})	0.30±0.04	0.29±0.02	0.29±0.03	0.33±0.10	0.33±0.02	0.41±0.06	*	ns	ns
N Urine (g/day)	1.46±0.25	1.52±0.21	1.61±0.85	1.02±0.14	0.83±0.08	1.10±0.20	***	ns	ns
N retention (g/kg BW ^{0.75})	0.71±0.90	0.68±0.12	0.69±0.08	0.73±0.07	0.77±0.13	0.60±0.11	ns	ns	ns
NNU (%)	61.79±0.67	61.72±3.96	61.92±1.72	64.34±6.65	65.22±4.06	54.53±2.35	ns	ns	ns
N digestibility (%)	73.79±1.12	72.78±3.60	73.46±5.46	70.82±7.15	71.59±3.22	62.50±0.32	*	ns	ns
BV (%)	83.76±2.09	84.77±2.22	84.43±3.93	90.82±1.14	91.05±1.84	87.26±4.21	***	ns	ns

Description: TT, CT, and CC = genotype, N = sum of goats, BWM = body weight metabolic, factor A (feed treatment), factor B (genotype), factor C (feed \times genotype), genotype and feed treatments associated with parameters (N intake, fecal N, and urinary N) and N intake, NNU = net nitrogen utilization, BV = biological value, *** = significant ($P<0.001$), ** = significant ($P<0.01$), * = significant ($P<0.05$) and ns = nonsignificant ($P>0.05$).

DISCUSSION

Identification of the genotype of the MC4R gene was successfully digested using the PCR-RFLP technique which was electrophoresed for 1 hour with a voltage of 50 V. The TT genotype produced two bands with sizes of 165 and 477 bp, the CT genotype produced three bands with a size of 165, 477 and 642 bp and the CC genotype produced one band with a size of 642 bp. Literature from Latifah et al. (2020) reported the results of genotyping MC4R gene in Bligon goat showing two genotype variations CC and CT. In this research, the genotypes were plotted into feed treatments with each of six variations, namely; P0TT; P0CT; P0CC; P1TT; P1CT; P1CC. From the results of the grouping of Kacang goats, it can be seen that the influence of the two factors on the observed nutrient consumption and digestibility parameters includes; DM, OM, CP, EE, CF and NDF and nitrogen balance. The interaction between feed and MC4R genotype on nutrient consumption did not show significant results ($P>0.05$). However, EE consumption on feed factors showed significant results in P0TT goats which were higher than P1TT. This is because fresh forage consumption was given to the P0 group, so this resulted in dominant EE and NDF consumption values in the P0 treatment group. Research by Diwi et al. (2020) reported that giving complete feed consisting of concentrate, corn and king grass to FH cross-breed dairy cows had better PK and LK digestibility values than complete feed without giving fresh forage. In line with the increase in crude fat, NDF consumption was also higher, this is because NDF is a fiber component found in plant cells so that giving fiber feed (as fed) caused higher LK and NDF consumption in treatment P0 compared to P1 which was only fed TMR. However, the energy required by microbes to digest the fiber fraction components will be greater to digest cellulose, hemicellulose and lignin (Wahyono et al., 2019).

Feed digestibility is influenced by the type and composition of feed as well as the speed of feed flow (Kustantinah et al., 2012). The interaction between feed and genotype on nutrient digestibility parameters did not have a significant effect ($P>0.05$). However, feed factors showed a significant influence ($P<0.05$) on the digestibility of DM, CP, EE and NDF. Treatment P0 has a higher level of digestibility compared to treatment P1. This is in line with the level of livestock consumption in the P0 treatment, which can then be defined as that the Kacang goat P0 group has

an incoming nutrient intake that is directly proportional to the digestibility of the nutrients absorbed in the body. It can be categorized as P0 feed having better digestibility compared to P1 feed. According to Suardin et al. (2014) that the higher the digestibility value of a feed ingredient, the higher the quality of the feed. The digestibility of DM and CP in treatment P0 with a percentage of 40% concentrate and 60% elephant grass was higher when compared to TMR-based P1 feed. This is in line with research reported by Tulung et al. (2020) The use of 50% concentrate with king grass forage can increase DM digestibility in PO cattle. Protein is useful for rumen microbes in their body's protein synthesis besides requiring ATP as an energy source for chemical reactions to occur. High protein is expected to increase the amount of protein retained in the body so that it can be utilized by livestock to meet basic living and production needs (Gultom et al. 2016; Soetanto, 2019). The digestibility of EE and NDF was higher in the P0 treatment because the P1 feed was not given fibrous feed (as fed), so this increased the level of NDF digestibility. In treatment P0, NDF in homozygous (TT) goats was higher compared to goats of the CC and CT genotypes because forage contributed fiber. The nutritional content of *P. purpureum* was reported by Dumadi et al. (2021) includes 20.49% DM, 12.76% ash, 11.23% CP, 2.42% EE, 31.56% CF, 0.40% calcium, 0.32% P, and 70.33% NDF. The presence of NDF, especially in greenery, is an important nutritional indicator for ruminant livestock. Its important role has been widely studied, showing its significance in stimulating saliva secretion, increasing rumination, maintaining rumen health and ensuring optimal production performance (Lechartier et al. 2010; Maltz et al. 2013).

The interaction of feed and genotype on nitrogen balance did not show a significant difference ($P>0.05$). However, the feed factor showed a significant difference ($P<0.05$), namely the parameters of fecal N excretion (g), fecal N (metabolic), and biological value (BV) in the P1 treatment group was higher than P0, while N urine and N digestibility in the P0 treatment were higher than P1. Saskara et al. (2015) stated that BV and NNU are determinants of protein quality. These two parameters indicate the proportion of feed protein consumed and absorbed which can be used by livestock to synthesize body protein. The real difference in N fecal and N urine can be caused by the different body metabolism of each individual livestock so this supports this data. It can be defined that a lot of nitrogen is absorbed in the goat group in the P0 treatment, however, the efficiency of absorption and use of nutrients is more utilized in the goat group in the P1 treatment. In this study, protein digestibility was higher in the P0 treatment, this is in line with the higher level of nitrogen digestibility compared to P1. Nuraini et al. (2017) The low protein quality found in the P1 treatment group will affect the resulting low nitrogen retention.

CONCLUSIONS

The interaction of feed and MC4R genotypes has not been able to show a significant level, however the feed factor can be defined as goats in the study having good consumption and digestibility when fed with a balance of forage and concentrates, so it is concluded that goats in the P0 treatment group of the TT genotype can be selected as superior livestock with better consumption and digestibility levels than CT and CC genotypes.

ACKNOWLEDGEMENTS

This research was supported by a grant from Indonesia Higher Education (Dikti) in the PTUPT program with contract number 2139/ UNI/DITLIT/DIT-LIT/PT/2021 and 1690/UNI/DITLIT/DIT-LIT/PT.01.03/2022. The authors are grateful to Siti Zubaidah, a laborer in Animal Nutrition, for helping with data collection and

to Retno Setyawati, a laborer in Animal Breeding and Reproduction at Gadjah Mada University.

AUTHOR CONTRIBUTIONS

Kustantinah: Designed and supervised the study and drafted the manuscript. Arie Riska: Executed the experiment, conducted laboratory analysis, analyzed the data, and drafted the manuscript. Tety Hartatik: Designed the research project, drafted and modified the manuscript. Asih Kurniawati and Panjono: Revised the manuscript. All authors read and agreed on the final manuscript.

CONFLICT OF INTEREST

All authors declare no competing interests.

REFERENCES

Adiwinarti, R., Kustantinah, Budisatria, I.G.S., Rusman, Indarto, E., 2016. Improving the performance of local Kacang goats using rumen undegradable protein feeds. *Asian J. Anim. Sci.* 10(4), 262-267.

AOAC, 2000. Official methods of analysis, 17th edition. Association of Official Analytical Chemist, Arlington, VA, USA.

AOAC, 2005. Official method of analysis of the Association of Official Analytical Chemist. 18th edition. AOAC International, USA.

Cai, X., Mipam, T.D., Zhao, F.F., Sun, L., 2015. SNPs detected in the yak MC4R gene and their association with growth traits. *Animal.* 9(7), 1097-1103.

Diwi N.Y. Polii, Waani, M.R., Pendong, A.F., 2020. Digestibility of crude protein and crude fat in dairy cows FH (Friesian Holstein) breed is given complete feed based on corn shells. *Zootec.* 40(2), 482-492.

Dumadi, E.H., Abdullah L., Sukria, H.A., 2021. Quality of napier grass forage (*Pennisetum purpureum*) with different growth type: quantitative review. *Asian. Australas. J. Anim. Sci.* 19(1), 6-13.

El-Sabrout, K., 2017. Associations between single nucleotide polymorphisms of melanocortin gene and sexual desire behavior in rabbit (*Oryctolagus cuniculus*). *J. Vet. Beh.* 19, 69-71.

El-Sabrout, K., Soliman, F., 2018. Association of single-nucleotide polymorphism of melanocortin gene with feed intake in rabbit (*Oryctolagus cuniculus*). *J. Anim. Physiol. Anim. Nutr.* 102, 564-567.

Fontanesi, L., Scotti, E., Cisarova, K., Battista, P.D., Dall'Olio, S., Fornasini, D., Frabetti, A., 2013. A missense mutation in the rabbit melanocortin 4 receptor (MC4R) gene is associated with finishing weight in a meat rabbit line. *J. Anim. Biotecl.* 24(4), 268-277.

Gultom, E.P., Wahyuni, T.H., Tafsin, M.R., 2016. Digestibility of crude fiber and crude protein of diets containing palm oil leaf sheaths with physical, biological, chemical and combination treatments in sheep. *J. Integr. Anim. Husb.* 4, 193-202.

Kustantinah, 2012. Measuring the quality of cattle feed. PT Citra Aji Parama, Klaten.

Latifah, L., D., Maharani, D., Kustantinah, K., Hartatik, T., 2020. Polymorphism of MC4R gene associated with feed intake, nutrient digestibility, ADG and FCR at post-weaning in Bligon goats. *JITAA.* 45(3), 173-180.

Latifah, L., Priyadi, D., Maharani, D., Kustantinah, K., Hartatik, T., 2017. Genetic analysis using partial sequencing of Melanocortin 4 Receptor (MC4R) gene in Bligon goat. *Media. Peternakan.* 40(2), 71-77.

Lechartier, C., Peyraud, J.L., 2010. The effects of forage proportion and rapidly degradable dry matter from concentrate on ruminal digestion in dairy cows fed



corn silage-based diets with fixed neutral detergent fiber and starch contents. *J. Dairy. Sci.* 93, 666–681.

Maltz, E., Barbosa, L., Bueno, P., Scagion, L., Kaniyamattam, K., Greco, L., De Vries, A., Santos, J., 2013. Effect of feeding according to energy balance on performance, nutrient excretion, and feeding behavior of early lactation dairy cows. *J. Dairy. Sci.* 96, 5249–5266.

Maylinda, S., Susilowini, T.E., Surjowardojo, P., 2015. Genetic polymorphisms at the acaca locus and their relationship with production performance in ettawa peranakan goat sires. *Res. J. Life Sci.* 2(1), 16-22.

Nuraini., Djulardi, A and Trisna, A. 2017. Palm oil sludge fermented by using lignocellulolytic fungi as poultry diet. *International Jurnal of Poultry Science.* 16 (1), 6-10.

Polini, B., Maltese, P.E., Ciondolo, I.D., 2016. Prevalence of mutations in LEP, LEPR, and MC4R genes in individuals with severe obesity. *Genetic. Moleculer. Res.* 15(3), 201-209.

Saskara, I.M.T., Suryani, N.N., Astawa, I.P.A., 2015. The Effect of forage composition with different concentrate levels in Etawah crossbreed goat diets on nitrogen balance. *J. Trop. Anim. Sci.* 3(1), 176-188.

Seong, J., Dong, S.S., Kyung, D.P., Hak, K.L., Hong, S.K., 2012. Identification and analysis of MC4R polymorphisms and their association with economic traits of Korean cattle (Hanwoo). *J. Mol. Biol. Rep.* 39, 3597-3601.

Soetanto, H., 2019. Introduction to ruminant nutrition. UB Press, Malang.

Suardin, N., Sandiah., Aka, R., 2014. Digestibility of dry matter and organic matter of a mixture of mulato grass (*Brachiria* hybrid cv. Mulato) with different types of legumes using cow rumen fluid. *J. Trop. Anim. Husb. Sci. Tech.* 1(1), 16-22.

Tillman, A.D., Hartadi, H., Reksohadiprodjo, S., Prawiro Kusuma, S., Lebdosoekoekojo, S., 1998. Basic animal food science. Gadjah Mada University Press, Yogyakarta.

Van Soest, P.J., van, Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.

Wahyono, T., Jatmiko, E., Firsoni, S.N., Hardani, W., Yunita, E., 2019. Evaluation of nutrients and in vitro digestibility of several tropical field grass species in Indonesia. *J. Animal Science.* 17(2), 17-23.

Yohannis, L.R., Tulung, A.F., Pendong, Tulung, B., 2020. Evaluation of the biological value of complete feed based on corn tebon and mixed grass on the production performance of ongole crossbreed cattle (PO). *Zootec.* 40(1), 363-379.

Zuo, B., Liu, G., Peng, Y., Qian, H., Liu, J., Jiang, X., Mara, A., 2014. Melanocortin-4 receptor (MC4R) polymorphisms are associated with growth and meat quality traits in sheep. *J. Mol. Biol. Rep.* 41, 6967-6974.

How to cite this article:

Kustantinah, Arie Riska, Panjono, Asih Kurniawati and Tety Hartatik. Effect of diet and MC4R genotype on nutrient digestibility and N balance in Kacang goats. *Veterinary Integrative Sciences.* 2025; 23(1): e2025009-1-10.
