

The Potential of Green Fodder as a Stimulant for Volatile Fatty Acid (VFA) Production and Methane Emission Reduction in Male Etawa Crossbred Dairy Goats

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Article History: 25-353 Received: 16-Dec-25 Revised: 12-Jan-26 Accepted: 14-Jan-26 Online First: 10-Feb-26

ABSTRACT

The low nutrient utilisation efficiency in Etawa crossbred goats (ECG) reduces production performance and increases methane emissions from rumen fermentation. Therefore, innovative feed technologies such as green fodder are essential to enhance productivity by stimulating volatile fatty acid (VFA) production while mitigating greenhouse gas emissions. This study aimed to evaluate the role of green fodder as a stimulant of volatile fatty acid (VFA) production and as a strategy to mitigate methane emissions in Male Etawa crossbred dairy goat. The experiment consisted of three dietary treatments: T0 = Male Etawa crossbred goat (ECG) fed 30% concentrate (as-fed basis) + 70% Napier grass (as-fed basis); T1 = Male Etawa crossbred goat (ECG) fed 30% concentrate (as-fed basis) + 35% Napier grass (as-fed basis) + 35% maize green fodder; and T2 = Male Etawa crossbred goat (ECG) fed 30% concentrate (as-fed basis) + 35% Napier grass (as-fed basis) + 35% mung bean green fodder. The nutritional evaluation from seeds to green fodder indicated an increase in crude protein (CP), crude fiber (CF), and ash contents, accompanied by a slight decrease in total digestible nutrients (TDN) for both plant species (maize and mung bean). Maize green fodder exhibited the highest TDN content, whereas mung bean green fodder showed the highest crude protein content. The results of the study showed that the T2 treatment (giving green mung bean green fodder) could increase the production of volatile fatty acids (VFA), especially acetic acid and propionic acid when compared with the control diet (T0). Compared with the control diet (T0), male ECG fed green fodder at 50% of the total forage proportion produced lower methane. The conclusions of this study indicate that mung bean green fodder has the potential to increase VFA production and reduce methane emissions.

Keywords: Etawa Crossbred Goats, Green Fodder, Methane, Volatile Fatty Acid (VFA).

INTRODUCTION

Milk production of the Etawa crossbred dairy goat in Indonesia remains relatively low and exhibits considerable variability. Observations reported by Andayani et al. (2023) indicated that the daily milk yield of Etawa crossbred goats (ECG) reached only 288.3±14.7g/head, but could be increased to 823.9±37.9g/head through zinc (Zn) mineral supplementation in the diet. In fact, with the implementation of proper management practices and adequate feeding strategies, milk production of ECG has the potential to reach 2,050-3,075g/head/day, equivalent to 2-3 L/head (Gultom et al. 2025). The low milk yield is

primarily attributed to suboptimal feed quality, resulting in inefficient nutrient utilization for both carcass and milk production.

Nutrients that are not optimally converted into animal products or body reserves are ultimately lost in the form of methane (CH₄) gas as a by-product of rumen fermentation. Methane emissions not only reflect low feed energy utilization efficiency but also contribute to environmental pollution (Daning et al. 2020). In Indonesia, ECGs are generally raised as dual-purpose livestock, serving as milk producers in females and as a source of carcass in both males and females; therefore, improving production efficiency is a crucial aspect.

Cite This Article as: Wulandari S, Respati AN, Kusuma SB, Bahariawan A, Vernando N and Sakinah NB, 2026. The potential of green fodder as a stimulant for Volatile Fatty Acid (VFA) production and methane emission reduction in male etawa crossbred dairy goats. *International Journal of Veterinary Science* 15(3): 784-790. <https://doi.org/10.47278/journal.ijvs/2026.021>

Methane is the second most significant greenhouse gas contributing to global warming after carbon dioxide, with a global warming potential approximately 28 times greater than that of CO₂ on a 100-year scale (Akpasi et al. 2024). In addition to its environmental impact, methane emissions represent a loss of dietary energy that could otherwise be utilized for productive processes. It is estimated that approximately 2–14% of the gross energy intake of ruminants is lost as methane, indicating that methane mitigation strategies have the potential to enhance energy efficiency and livestock productivity (Paya et al. 2024). Therefore, it is necessary to develop innovative feed technologies capable of enhancing production performance while simultaneously reducing methane emissions.

One potential alternative in feed technology is green fodder, a soilless cultivation technique for forage that utilizes water solutions enriched with essential nutrients. Green fodder generally contains adequate levels of nutrients and fiber to support rumen fermentation. The primary products of carbohydrate fermentation in the rumen are volatile fatty acids (VFAs), consisting of acetate ($\pm 65\%$), propionate ($\pm 20\%$), butyrate ($\pm 10\%$) and valerate ($\pm 5\%$) (Daning et al. 2020). VFAs serve as the main energy source for ruminants and are readily absorbed into the bloodstream to be utilized in metabolic processes.

Propionate plays a critical role as the main precursor for hepatic gluconeogenesis, thereby supporting lactose synthesis and milk production, while acetate primarily contributes to milk fat synthesis (Adekeye 2022). Park et al. (2024) reported that increased forage intake enhances the proportion of acetate derived from pyruvate, whereas increased propionate concentrations associated with higher feed intake contribute to body energy reserves. Moreover, increased propionate production contributes to methane reduction by competing with methanogenesis pathways for hydrogen utilization in the rumen (Park et al. 2024).

The chemical composition of green fodder has been reported to be highly suitable for ruminant nutrition and to exert positive effects on nutritional status, productivity, and the sustainability of livestock production systems. Supplementation with green fodder has been shown to improve milk quality, growth rate and feed efficiency (Vastolo & Cutrignelli 2025). Previous studies by the authors indicated that optimal fodder production for maize and sorghum was achieved when harvested at 10 days of growth (Wulandari et al. 2023). Based on these considerations, the present study aimed to evaluate the role of green fodder as a stimulant of VFA production and as a strategy for reducing methane emissions in male Etawa crossbred dairy goats.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were conducted in compliance with standard operating protocols and were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia, under registration number: 161/EC-FKH/int./2025.

Green fodder production

Seed preparation commenced with soaking 500g of

seeds overnight; any buoyant seeds were discarded and not utilized for cultivation. Subsequently, the seeds were immersed in an antifungal solution for 20 minutes. The growth containers consisted of plastic trays with perforated bottoms, which were placed on each rack of the planting installation. Before sowing, the gutters were treated with a 10% bleach solution for 15 minutes to minimize fungal growth and subsequently rinsed with clean water.

The prepared seeds were evenly distributed in a thin layer across plastic trays (dimensions: 54cm×34cm×8.5cm) and placed into the racks of planting installation. The racks were tightly covered with black plastic sheets and sprayed with water three times daily. This blackout period was maintained for three days until germination occurred. Thereafter, the plants were grown uncovered until harvest, with AB mix fertilization applied via foliar spray on days 5, 7 and 9. AB mix is a combination of inorganic fertilizer solutions consisting of two separate components: Solution A, which contains macronutrients such as calcium (Ca) and iron (Fe), and Solution B, which contains essential micronutrients including iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (Cl). The fodder was harvested 10 days post-planting, and representative samples (approximately 100g per sample) were collected for laboratory analysis.

Evaluation of partial volatile fatty acid (VFA) profiles (acetate, propionate, and butyrate), ammonia (NH₃), and protozoa populations in Etawa crossbred goat (ECG) fed green fodder

The feeding trial for the Etawa Crossbred Goats (ECG) was conducted over a period of eight weeks, which included a two-week dietary adaptation phase. Subsequently, the goats were maintained with green fodder dosages administered according to the assigned treatments. The basal diet consisted of concentrates and forage, with the nutrient composition detailed in Table 1. Rumen fluid was collected during the final week of the experimental period for data analysis. Throughout the study, drinking water was provided ad libitum.

Table 1: Feedstuff and nutrient composition of the experimental diets

Item	Treatments		
	T0	T1	T2
Ingredient (% of the feed)			
- Concentrate	30	30	30
- Elephant grass	70	35	35
- Green fodder maize	-	35	-
- Green fodder mung beans	-	-	35
Nutrient composition of experimental diet (% of Dry matter)			
- Crude Protein (%)	15.28	16.18	18.12
- Crude Fiber (%)	20.13	17.87	17.91
- TDN (%)	66.09	69.72	68.36

The research consists of 3 treatments, namely:

- T0= Male Etawa Crossbred Goats (ECG) fed a diet consisting of 30% concentrate and 70% Napier grass (as-fed basis).
- T1= Male Etawa Crossbred Goats (ECG) fed a diet consisting of 30% concentrate, 35% Napier grass, and 35% maize green fodder (as-fed basis).

- T2= Male Etawa Crossbred Goats (ECG) fed a diet consisting of 30% concentrate, 35% Napier grass and 35% mung bean green fodder (as-fed basis).

Rumen fluid was collected using the rumen sonde method, a non-invasive and safe technique for the animals. To prevent post-procedural bloat, each male Etawa Crossbred Goat (ECG) was orally administered approximately 15mL of coconut oil (Wulandari et al. 2024).

Methane Gas (CH₄) measurement

Methane emissions were measured by collecting the gas produced by each animal housed in a methane chamber, following the protocol described by (Ortega et al. 2020). Measurements were recorded hourly over 24 hours to establish the kinetic curve of methane gas production. Total methane production for the male ECGs was determined based on the peak production time derived from the average of the three treatments (T0, T1 and T2).

Observed parameters

- Nutritional Evaluation of Seeds and Green Fodder
Samples of maize seeds, maize green fodder, mung bean seeds and mung bean green fodder were collected for nutrient quality testing. The analysis included proximate composition, as well as Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) concentrations.

- Rumen Profile Evaluation

The collected rumen fluid samples were analyzed for pH, partial Volatile Fatty Acid (VFA) concentrations (acetate, propionate, and butyrate), and ammonia (NH₃) levels. Additionally, protozoa populations were quantified according to the method of (Wulandari et al. 2024).

- Methane Emission Reduction

Methane reduction was evaluated based on: a) the kinetics of methane production monitored hourly for 24 hours and b) methane production measured at the peak production time identified from the kinetic curves across all treatments.

Data Analysis

- Nutritional Analysis

Nutritional quality data of seeds and green fodder were analysed by two-way analysis of variance (Two-Way ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($P < 0.05$), using SPSS version 24.

- Rumen Profile Evaluation

Data were analyzed using a one-way completely randomized design with three treatments and four replications. Each male ECG was considered an experimental unit. Treatment means were compared using Duncan's Multiple Range Test (DMRT) in SPSS version 24.

- Methane emission reduction in ECG supplemented with green fodder

Data were analyzed using a one-way Completely Randomized Design (CRD) with three treatments and three replications, in which each goat served as an experimental unit. Differences among treatment means were further analyzed using Duncan's Multiple Range Test (DMRT) with SPSS version 24.

RESULTS AND DISCUSSION

Nutritional evaluation from seed to green fodder

Based on the observations presented in Fig. 1, there was a notable shift in nutrient composition as the seeds transitioned into green fodder harvested at 10 days post-planting ($P < 0.05$). An upward trend was observed in both plant species from seed to green fodder regarding crude protein (CP), fiber fractions, including crude fiber (CF), Neutral Detergent Fiber (NDF), and Acid Detergent Fiber (ADF), and ash content. Conversely, a decrease in nutrient content from seed to green fodder was recorded for Total Digestible Nutrients (TDN).

The increase in crude protein (CP) content and the concomitant decrease in total digestible nutrients (TDN) in fodder compared to its seeds are influenced by the reduction in the carbohydrate fraction percentage utilized during germination and growth. Furthermore, there is a trend toward higher CP content as harvest age increases (Rayani et al. 2021). Wulandari et al. (2025) stated that carbohydrate reserves in seeds are primarily stored as starch or nitrogen-free extract (NFE). During the growth process, NFE is consumed as an energy source for metabolic activities, leading to a decline in NFE levels, while other organic matter components, such as CP, subsequently increase.

The increasing height of green fodder during germination correlates with the accumulation of cellulose, hemicellulose, and lignin, as plant cells are constructed from cell walls composed of these structural carbohydrates, which raises the crude fiber value. Zhang et al. (2022) affirmed that the plant wall structure consists of cellulose, hemicellulose, and lignin. The elevation in ash content is attributed to the application of nutrient solutions, whereby minerals are retained within the plant tissue alongside water absorption. Additionally, the germination process enhances phytase enzyme activity, which liberates mineral bonds from organic compounds, thereby increasing the measurable ash content (Sriagtula et al. 2021).

pH value, Partial volatile fatty acid and Ammonia concentration

The rumen pH measurements of male Etawa Crossbred Goats (ECG) supplemented with maize green fodder (T1) and mung bean green fodder (T2) showed no significant differences compared to the control group (T0), which received a basal diet of concentrate and forage (Table 2). This indicates that the green fodder is safe for livestock consumption. According to Wulandari et al. (2024) the degree of acidity (pH) of rumen fluid serves as an indicator of ongoing bioprocess activities in the rumen; for optimal function, a pH range of 6–7 is acceptable for ruminants. The rumen pH is a critical factor controlling various digestive functions, reflecting the distribution of rumen microbes, feed digestibility, and overall animal health (Faniyi et al. 2025).

The results of the present study showed that treatment T2 (mung bean green fodder) significantly increased volatile fatty acid (VFA) production, with a more pronounced increase in propionate (C3) and butyrate (C4) (Table 2). This response is related to the high crude protein (CP) content of mung bean green fodder, which reached 40.7% (Fig. 1). A higher dietary CP level may enhance

Table 2: pH value, Partial volatile fatty acid, and Ammonia concentration

Treatments	pH	C ₂ Acetic acid	C ₃ Propionic acid	C ₄ Butyric acid	NH ₃ Ammonia
T0	6.33±0.20	69.07±2.87	54.72±6.57 ^b	8.37±1.63 ^{ab}	7.53±0.58 ^a
T1	6.03±0.05	61.72±14.59	39.16±5.17 ^c	4.03±0.84 ^a	4.49±0.89 ^b
T2	6.26±0.05	80.70±9.42	74.74±4.10 ^a	11.04±3.49 ^b	7.34±1.09 ^a
Mean	6.21 ^{ns} ±0.17	70.50 ^{ns} ±12.09	56.20±16.13	7.81±3.64	6.46±1.66

Different superscripts in the same column mean significant ($P < 0.05$), ns= non-significant.

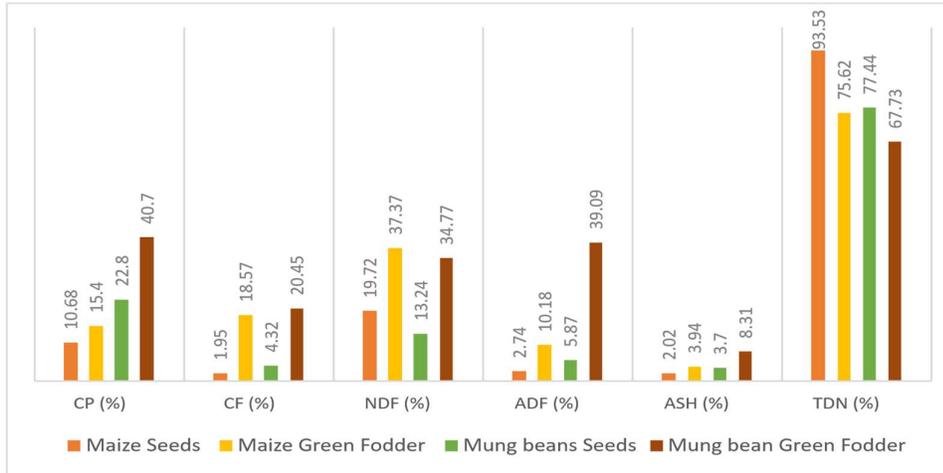


Fig. 1: Nutrient content of seeds and green fodder. CP=Crude Protein; EE=ekstrak Ether; CF=Crude Fiber; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; ASH=Abu; TDN= Total Digestible Nutrient.

nitrogen availability in the rumen, thereby supporting the growth and activity of rumen microbial populations, particularly fermentative bacteria, which subsequently promotes fermentation processes and the production of partial VFAs. Nevertheless, the increase in acetate concentration observed in treatment T2 remained statistically non-significant compared with the other treatments (T0 and T1). Kim et al. (2024) reported that germination during green fodder production increases the proportion of free sugars and their degradability in the rumen. These free sugars are preferentially utilized by rumen microorganisms that produce propionate and butyrate. In contrast, acetate synthesis is primarily associated with the activity of fibrolytic microorganisms that require diets rich in structural fiber (Park et al. 2024). Therefore, the relatively low fiber content of green fodder can suppress the proliferation of fibrolytic bacteria, which are the dominant acetate producers, resulting in a relative increase in propionate and butyrate concentrations. Golbotteh et al. (2024) also reported that acetate is mainly synthesized from the fermentation of fibrous feeds fractions, whereas propionate production mainly originates from the fermentation of non-fibrous carbohydrates. Consequently, the type and quality of the diet strongly influence both the abundance and composition of rumen microbial populations (Arya et al. 2024).

In contrast, a similar increasing trend in partial VFAs was not observed in treatment T1 (maize green fodder). This may be attributed to the lower CP content of maize green fodder, which was approximately 15.4%, considerably lower than that of mung bean green fodder (40.7%) (Fig. 1). Insufficient dietary CP can restrict rumen microbial growth, leading to a reduction in overall fermentation activity (Zhu et al. 2022). The lower NH₃ concentration observed in the rumen fluid of T1 indicate that ammonia was more efficiently utilized by microorganisms for microbial protein synthesis rather than

being accumulated as free ammonia. As reported by Wulandari et al. (2024) affirmed that ammonia levels in rumen fluid indicate the processes of protein degradation (remodelling) and microbial protein synthesis.

VFAs are essential energy sources that play a vital role in body maintenance and milk synthesis in lactating ECGs. Arya et al. (2024) reported that VFAs are metabolized into alternative energy sources supporting physiological functions such as maintenance, growth, pregnancy, and lactation. Acetate is crucial for milk fat synthesis, while propionate serves as an energy source for body weight gain and is essential for lactose production in the mammary system. Butyrate undergoes metabolism in the liver, where it is converted into ketone bodies, which serve as an important energy source for fatty acid synthesis, skeletal muscle support, and tissue maintenance.

Kinetics and production of methane

The kinetic patterns of methane production generally showed an upward trend following the morning feeding (approximately 06:30 to 07:00). Subsequently, a steady decline in methane emissions was observed until midday, around 12:30. Methane production then rose again, reaching its maximum peak following the afternoon feeding (approximately 16:00 to 16:30) (Fig. 2). Methane production across all treatments increased within one hour following the morning feeding of the Etawa Crossbred Goats (ECG). This elevation is attributed to the initiation of the fermentation process as feed enters the rumen.

Fermentation produces volatile fatty acids (VFAs) and hydrogen (SH₂S), the latter of which is utilized by methanogens to synthesize methane. Donatelli and Chang (2024) stated that dietary protein, carbohydrates, and lipids are converted into intermediates, specifically VFAs, H₂, and CO₂ through hydrolysis, acidogenesis, and acetogenesis, which are subsequently transformed into methane via methanogenesis.

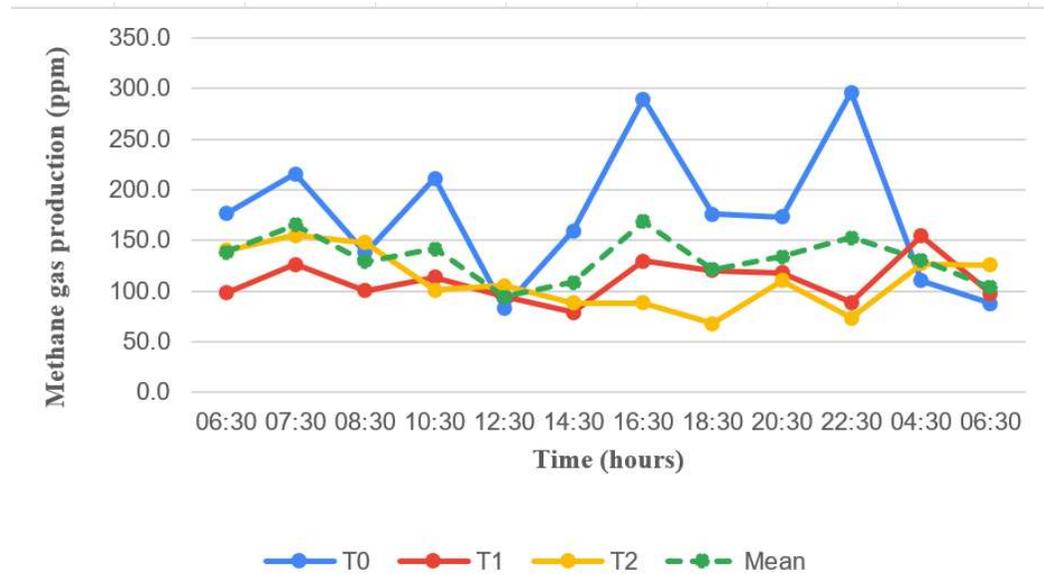


Fig. 2: Kinetics of methane for 24 hours.

Methane production declined at 12:30, approximately five hours post-feeding, reflecting the late post-prandial phase where most substrates have been fermented and the rumen environment enters a stable state. During this period, gas production tends to decrease several hours after the peak fermentation phase. This finding aligns with Islam and Wang (2023), who observed that VFA and methane production typically diminish 4 to 6 hours post-feeding. Following the afternoon feeding (15:30-16:00), a drastic increase in methane production occurred, with the daily peak values recorded at 16:30 and 22:30. The peaks during the evening and night were notably higher than those in the morning. This phenomenon is likely due to: 1) more intensive fermentation resulting from increased microbial activity in a stable rumen environment following daily activities; and 2) circadian rhythms, where rumination tends to peak during the evening and night (rest periods). Increased rumination produces significant amounts of saliva, which raises the rumen pH, creating a favorable environment for methanogenic microbes.

Hao et al. (2024) noted that ruminants exhibit a 'circadian rhythm' in rumination, which intensifies after late afternoon and peaks at night. Furthermore, slow-feeding-responsive microbiota may play a role in the evening and nighttime methane peaks. These microbiota, including the protozoal genus *Isotricha* and archaeal species *Group12 sp-ISO4-H5*, reach peak activity in the range of 12 to 24 hours post-feeding. Protozoa contribute to methane production through symbiotic relationships with methanogens (Dahlya et al. 2025). As shown in Fig. 2, treatments T1 (maize green fodder) and T2 (mung bean green fodder) suppressed the evening and night-time methane peaks compared to the control (T0). Consequently, the highest peak production time observed in T0 and the average of all three treatments (one-hour post-afternoon feeding) was utilized for the methane emission measurements presented in Table 3.

Table 3 illustrates that the substitution of Napier grass (*Pennisetum purpureum*) with either maize or mung bean green fodder at a level of 50% significantly reduced methane emissions. Specifically, emissions decreased from

\$159.70ppm \$31.90ppm to \$79.13ppm \$13.72ppm (a reduction of approximately 50.5%) for T1, and to \$88.16ppm \$44.89 ppm (approximately 44.8%) for T2. These findings indicate that incorporating green fodder as a replacement for Napier grass enhances energy utilization efficiency in Etawa Crossbred Goats (ECG).

Table 3: Methane gas production of male Etawa crossbred goats (ECG) fed with feed containing green fodder corn (T1) and green beans (T2)

Perlakuan	Methane gas production (ppm)
T0	159.70±31.90 ^a
T1	79.13±13.72 ^b
T2	88.16±44.89 ^b
Mean	109.00±47.60

Different superscripts in the same column mean significant ($P < 0.05$).

According to Saleem et al. (2025), lower methane emissions are desirable as they indicate more efficient energy utilization by the host animal. This efficiency is attributed to the propionate pathway, which utilizes hydrogen by reducing pyruvate to form propionate. This mechanism is further supported by the observed increase in ruminal propionic acid concentrations in ECGs supplemented with mung bean green fodder (Table 2).

Protozoa populations

Protozoa have been implicated in methane production, as methanogenic bacteria, particularly those associated with protozoa, consume H_2 to produce methane. Therefore, the decrease in protozoa numbers observed in our study may have contributed to the reduction in methane production (Saleem et al. 2025). In the present study, although no statistically significant differences were observed ($P > 0.05$), there was a discernible trend indicating that green fodder supplementation tended to suppress the rumen protozoa population when sampled 1-2 hours post-feeding (Fig. 3). This is likely because young green fodder (harvested at 10 days) undergoes rapid fermentation, leading to swift fluctuations in rumen pH. Such rapid pH changes can disrupt protozoal activity.

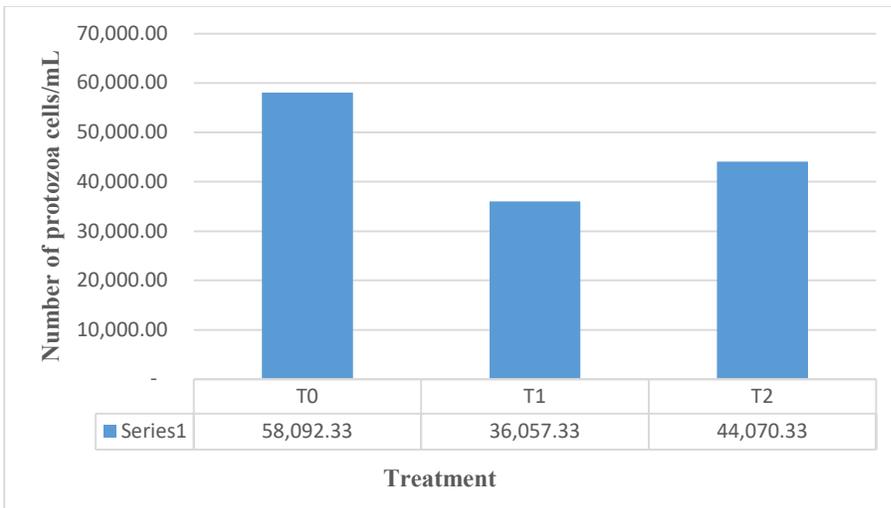


Fig. 3: The ruminal protozoa population (cells/mL) in male Etawa Crossbred goats (ECG) fed diets containing green corn fodder (T1) and green bean fodder (T2).

Alataş et al. (2022) stated that the presence of protozoa in the rumen is influenced by several factors, including the dry matter content and the pH of the rumen fluid. Furthermore, Norrapoke & Pongjongmit (2025) noted that variations in the rumen microbial ecology are highly dependent on the type of feed provided. The observed downward trend in the protozoa population contributes to methane mitigation. As affirmed by Saleem et al. (2025), Protozoa play a significant role in methanogenesis because methanogenic bacteria associated with protozoa utilize H₂ gas to produce methane. Consequently, the decrease in protozoa numbers found in this study likely contributed to the overall reduction in methane production.

Conclusion

The nutritional evaluation indicates that the transition from seeds to green fodder results in increased crude protein, crude fiber, and ash content, accompanied by a slight decrease in Total Digestible Nutrients (TDN) for both maize and mung bean. Maize green fodder exhibited the highest TDN content, while the highest protein content was observed in mung bean green fodder. The treatment of green mung bean green fodder could increase the production of volatile fatty acids (VFA). Compared to the control group (T0), male Etawa Crossbred Goats (ECG) supplemented with green fodder at a level of 50% of the total forage produced significantly lower methane emissions, with reductions of approximately 50.5% for maize green fodder and 44.8% for mung bean green fodder. Furthermore, protozoa populations showed a downward trend compared to those fed the conventional diet (T0). In conclusion, mung bean green fodder demonstrates significant potential to enhance Volatile Fatty Acid (VFA) production while concurrently mitigating methane emissions.

DECLARATIONS

Funding: This research was funded by the Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology, Indonesia (Fiscal Year 2025).

Acknowledgement: The authors gratefully acknowledge the Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology, Indonesia, for the financial support provided for this research. The authors also thank to the Center for Research and Community Service, Politeknik Negeri Jember, Indonesia, for facilitating this research.

Conflict of Interest: The authors declare that the present study had no conflict of interest.

Data Availability: The data sets generated and analysed during the present study are available within this article.

Ethics Statement: All experimental procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia (Approval No. 161/EC-FKH/int./2025), and conducted in accordance with established standard operating protocols.

Author's Contribution: Conceptualization: SW. Investigation: SBK. Writing original draft: SW. Writing – review, and editing: SBK, ANR. Tool making facilitator: AB, Data collection and validation: ANR, NV, NBS. Project administration and supervision: SW.

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