

## The digestibility, ruminal fermentation and methane product of *Cajanus cajan* forage as a concentrate substitute in goats

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### ABSTRACT

Sixteen local goats (9.3 kg bodyweight) were assigned to different groups based on a 21-day completely randomized design and fed with *Cajanus cajan* forage. The objectives of the study were to evaluate digestibility, ruminal fermentation and estimation of methane production of *C. cajan* forage as a concentrate substitute in goats. Four treatments were used in the feeding trial: T0CC = 100% concentrate; T25CC = 75% concentrate + 25% *C. cajan* forage; T75CC = 25% concentrate + 75% *C. cajan* forage; and T100CC = 100% *C. cajan* forage. Ruminal fluid was collected during the last week of the experiment after feeding and used to determine pH, ammonia, partial VFA, and methane gas production. Dry matter intake, nutrient digestibility, and total body weight gain were not significantly different ( $P>0.05$ ) between goats fed with T0CC and T25CC. The addition of *C. cajan* forage to the diet did not significantly ( $P>0.05$ ) affect pH, ammonia content, and total VFA but influenced acetate, propionate, butyrate, AP, and estimated CH<sub>4</sub> produced. Hence, *C. cajan* forage may replace 25% of concentrates in a rice straw-based diet for goats.

**Keywords:** *Cajanus cajan*, Digestibility, Goats, Methane production

### INTRODUCTION

Feed is the most expensive single-cost item in livestock production and accounts for more than 70% of the total cost. Most farmers prefer cheap and easily accessible feed sources to maximize their profits. In developing a low-cost feed, the nutritional composition of forage and the needs of animals should be considered. Such information can be used to optimize the utilization of available feedstuff sources, such as forages. *Cajanus cajan* is one of the most common forages used for ruminants, since it is easy to grow in the tropics and subtropical areas. The *C. cajan* forage production, ranges from 2.93 t h<sup>-1</sup> to 4.45 t

h<sup>-1</sup> (Mekonen *et al.*, 2022a; Tenakwa *et al.*, 2022; Buthelezi *et al.*, 2022), which is higher compared to other forages, such as *Moringa* (Santos *et al.*, 2021), *Macroptilium* and *Lablab* (Ratnawaty and Chuzaemi, 2013).

Moreover, the crude protein content in *C. cajan* forage is equivalent to that in alfalfa and concentrates (Buthelezi *et al.*, 2019). With its high production and protein content, *C. cajan* forage can be used as a replacement to concentrates. Several studies used *C. cajan* leaves as a supplementary feed for ruminants (Adebisi *et al.*, 2019; Dida *et al.*, 2019; and Mekonen *et al.*, 2022b). It can also be utilized at up to 50% level in a pellet of complete diets for growing goats

(Reddy *et al.*, 2012). However, studies on the use of *C. cajan* forage as a concentrate substitute for goats remain limited.

It is known well that digestibility refers to the extent to which a feed can be digested and absorbed by the digestive system of livestock (Merten and Grant, 2020). In this context, digestibility is a measure of digestive efficiency, which involves the breakdown of feed into nutrients that can be absorbed and utilized by the animal (Chojnacka *et al.*, 2021). Furthermore, rumen fermentation is closely linked to livestock productivity. In the rumen, microorganisms such as bacteria, protozoa and fungi break down feed components that are difficult to digest, such as cellulose fiber and hemicellulose, into products that are more easily digested by animals (Cholewińska *et al.*, 2020). The results of rumen microbial fermentation are Volatile Fatty Acids (VFAs), such as acetate, propionate and butyrate, which are the main energy source for ruminants. Further, gases, such as methane and carbon dioxide (Liang *et al.*, 2022).

Methane production is an important aspect that must be managed to increase feed efficiency, reduce energy losses and minimize the environmental impact of livestock activity (Kumari *et al.*, 2020). Continued efforts in research and management practices are expected to reduce methane production while maintaining or increasing livestock productivity. Therefore, the present experiment aimed to evaluate the digestibility, ruminal fermentation and estimation of methane production of *Cajanus cajan* forage as a concentrate substitute in goats.

## MATERIALS AND METHODS

### Feeding Experiment

The animal care committee of Universiti Putra Malaysia approved all animal procedures and use for scientific purpose. The experiment was conducted at the experimental farm of the Faculty of Agriculture, Universiti Putra Malaysia. Sixteen local goats with an average body weight of  $9.3 \pm \text{sd}$  kg bodyweight were assigned into different groups based on a 21-day completely randomized design. The goats were kept in individual pens that measured  $1.25 \text{ m}^2$  ( $1.25 \text{ m} \times 1.0 \text{ m}$ ). The animals were given *ad libitum* access to feed and water. The goats were fed with

*C. cajan* plant (leaves, petioles, and stems with 0.5 cm diameter). All diets consisted of concentrate/ *C. cajan* and rice straw ratio of 40:60. Four experimental treatments were established: T0CC = 100% concentrate; T25CC = 75% concentrate + 25% *C. cajan* forage; T75CC = 25% concentrate + 75% *C. cajan* forage; T100CC = 100% *C. cajan* forage. Feed was offered at 4% (DM basis) of body weight (BW) of the animal in equal portions twice a day (at 9:00 and 16:00). Feed refusal was collected daily before morning feeding. Ruminal fluid was collected on the last week of the experiment after feed and used to determine pH, ammonia, partial VFA, and methane gas produced.

### Digestibility Trial

The digestibility samples were collected during the last seven days of the study. Total faecal weight was collected daily before morning feeding and subsamples (10%). The pooled faecal samples were dried at 60°C for 48 hours prior to further analysis. Samples of feed and refusals during the total collection period were mixed thoroughly, and a composite sample for each animal was taken for analysis of chemical components. The apparent digestibility percentage of dry matter, organic matter, crude protein, ether extract, neutral detergent fibre, and acid detergent fibre was determined using the following formula:

$$\text{Digestibility (\%)} = [(\text{nutrient in feed} - \text{nutrient in faeces}) / \text{nutrient consumed in feed}] \times 100\%$$

### Chemical Analysis

All feed, refusal, and faeces samples were subjected to proximate analysis following the standard methods of AOAC (2012). The samples were dried in a forced air oven at 105 °C for 24 h to determine dry matter (DM) content. Crude protein content in feed and faeces was determined according to the Kjeldahl method using a Kjeltac Auto Analyzer (Tecator, Hoganas, Sweden). Ether Extract (EE) was evaluated in petroleum ether by using a Soxtec Auto Analyzer (Tecator). Ash content was measured by ashing the samples in a muffle furnace at 550 °C for 5h. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to an established method (Van Soest, 1991).

Table 1. Chemical composition of diets with different percentage of *C. cajan* forage for goats

Chemical composition (%)	Diets			
	T0CC	T25CC	T75CC	T100CC
Dry Matter (%)	85.9	85.1	83.7	83
Ash (%)	8.7	8.7	8.9	9.1
Neutral Detergent Fibre (%)	58.8	61.0	65	67.3
Acid Detergent Fibre (%)	38.2	39.8	43.0	44.5
Acid Detergent Lignin (%)	15	15.8	17.1	18.3
Crude Protein (%)	10.3	11.0	12.2	12.8
Ether Extract (%)	2.2	1.9	1.5	1.4
Condensed tannin (%)	-	1.41	4.30	5.63
ME (MJ kg <sup>-1</sup> DM)	10.36	9.95	9.10	8.50

T0CC = 100% concentrate; T25CC = 75% concentrate + 25% *C. cajan* forage; T75CC = 25% concentrate + 75% *C. cajan* forage; and T100CC = 100% *C. cajan* forage

### Ruminal Fermentation Measurement

Approximately 100 mL of ruminal fluid was collected from 16 goats using a stomach tubes. The pH of the rumen fluid was measured using a pH meter (Mettler-Toledo, Ltd., England). Ammonium was determined based on a previously reported method (Parsons and Howe, 1984) using a spectrophotometer (Labomed Inc., Culver City, CA). The VFA concentration was determined by gas chromatography (Agilent 69890N Series). Methane gas production was estimated using this formula (Moss et al., 2000):  $CH_4 = 0.45 C_2 - 0.275 C_3 + 0.40 C_4$ , where  $C_2$  is acetate,  $C_3$  is propionate, and  $C_4$  is butyrate.

### Statistical Analysis

A completely randomized design was used to evaluate digestibility and ruminal fermentation and estimate methane gas production in goats fed with *C. cajan* forage. The data were analyzed using a One-way ANOVA. SPSS version 20 software (2011).

## RESULTS AND DISCUSSION

### Nutrient and Digestibility of Feed Intake and Body Weight Gain

Result on the body weight gain and digestibility of diets in goats are presented in Table 2. There was a significant increase ( $P < 0.05$ ) in the dry matter intake of goats fed with T75CC and T100CC compared to T0CC and T25CC, but no difference ( $P > 0.05$ ) between T75CC and T100CC or T0CC and T25CC. A high concentrate content in feed (T0CC and T25CC) less forage dry matter intake probably due to the nu-

trient requirement for the goats already met, as reported by Méndez *et al.* (2020). Furthermore, the high dry matter intake of goats fed with T75CC and T100CC could be due to the high forage content in feed since 75% and 100% of *C. cajan* was used to replace the concentrate feed, and the goats need to eat more to meet the nutrient required which leads to high dry matter intake (Khoury *et al.*, 2015).

High lignin content in feed is always associated with poor digestion, causing the ruminant to lack nutrients and in response to that they tend to consume more (Chand *et al.*, 2020). High lignin contents in T75CC and T100CC feeds which roughly 2% higher than those in T0CC and T25CC feeds (Table 1). The dry matter intake of goats fed with *C. cajan* in this study (table 2) was nearly identical to the study conducted by Barbosa *et al.* (2018), where goats fed with diets containing different forage to concentrate ratios (466–590 g/day). In contrast, in the present study, the dry matter intake of goats fed with *C. cajan* was higher than that of goats fed with different concentrate levels (292–424 g day<sup>-1</sup>) by Giger-Reverdin *et al.* (2014). The DMI is influenced by several factors, including ambient temperature, palatability, taste, physiological status, nutrient concentration, and feed form (Merten and Grant, 2020). The addition of *C. cajan* forage to substitute the concentrate feed affects the dry matter intake and body weight gain of goats. The highest body weight gain was found in T0CC treatment (1.2 kg), whereas the lowest body weight was recorded in T100CC (0.5 kg), however, T0CC did not differ from T25CC ( $P > 0.05$ ). The increase in goat body weight cor-

related with the increase in ADF and CP digestibility in this study which was supported by Zhu *et al.* (2020), who stated that increasing the digestibility of protein and fibre would increase the body weight gain of goats.

The addition of *C. cajan* forage to feed as a substitute for concentrate affected the digestibility values of DM, OM, ADF, NDF, ADL, and CP (Table 2); in particular, it decreased the nutrient digestibility. However, the digestibility values were not different ( $P>0.05$ ) between T0CC and T25CC. This is due to a good quality of *C. cajan* forage, so that substitution of this forage at the level of 25% did not decrease the feed digestibility (Table 2). *C. cajan* is recognized for its considerable protein content (Table 1); thus, at a substitution level of 25%, the digestibility of crude protein (CP) was predominantly remains comparable to the T0CC. Moreover, fibre digestion (NDF and ADF) remains effective in T25CC, since the additional fibre proportion is insufficient to impede the activity of rumen microorganisms for fibre-digesting enzymes. Conversely, the treatments of T50CC, T75CC and T100CC exhibited substantial variations ( $P<0.05$ ) in the digestibility of OM, DM, ADF, NDF, EE, and CP (Table 2). The disparities arise from increased fibre content, which is more challenging to digest, diminished energy availability from concentrates, and lowered microbial fer-

mentation efficiency. These variables adversely affect digestibility of these components, leading to substantial decreases as the quantity of forage in the diet escalates. Zentek and Boroojeni (2020) stated that feed digestibility is influenced by several factors, such as feeding level, physical form of feed ingredients, feed composition, crude fibre content in feed ingredients, processing of feed ingredients, and combination of feed ingredients. The dry matter digestibility in the present study was comparable to previous study (Adebisi *et al.*, 2020) which reported values of 40.56% to 60.46% for West African dwarf rams fed with *C. cajan*, while Omotoso *et al.* (2019), reported that the dry matter digestibility values of African dwarf goats fed with *C. cajan* were 54.5% to 72.68%. Furthermore, the digestibility values of NDF and ADF in *C. cajan* forage were higher than those of mulberry foliage (53.7% to 55.8%) (Yulistiani *et al.*, 2015). There was no difference in CP digestibility ( $P>0.05$ ) between T0CC and T25CC, but the value was different ( $P<0.05$ ) to T100CC. The highest value was 76.3% for T0CC, and the lowest value was 75% for T100CC. The protein digestibility in the present study was higher than that found by Dida *et al.* (2019), which reported a value of 67%. Differences in digestibility values in *C. cajan* forage organic material could be due to several factors, including plant type, harvest age, processing

Table 2. Body weight gain and digestibility of diets substitute with different percentage *Cajanus cajan* forages in goats

Parameters	Treatments				S.E.M
	T0CC	T25CC	T75CC	T100CC	
Body weight (kg)					
Initial (kg)	9.2	9.4	8.9	9.5	1.96
Final (kg)	10.4	10.4	9.7	10	0.40
Total body weight gain (kg)	1.2 <sup>a</sup>	1.0 <sup>ab</sup>	0.8 <sup>ab</sup>	0.5 <sup>b</sup>	0.68
Dry matter intake (g day <sup>-1</sup> )	447 <sup>b</sup>	448 <sup>b</sup>	466 <sup>a</sup>	473 <sup>a</sup>	3.12
Digestibility (%)					
DM (%)	56.0 <sup>a</sup>	55.0 <sup>a</sup>	52.3 <sup>b</sup>	50.8 <sup>c</sup>	0.58
OM (%)	64.0 <sup>a</sup>	63.5 <sup>a</sup>	62 <sup>b</sup>	60.5 <sup>c</sup>	0.39
NDF (%)	60.5 <sup>a</sup>	59.8 <sup>a</sup>	58.5 <sup>b</sup>	57.2 <sup>b</sup>	0.34
ADF (%)	53.3 <sup>a</sup>	52.7 <sup>ab</sup>	52 <sup>b</sup>	51.5 <sup>b</sup>	0.22
CP (%)	76.3 <sup>a</sup>	76.0 <sup>ab</sup>	75.7 <sup>ab</sup>	75 <sup>b</sup>	0.19
EE (%)	66 <sup>a</sup>	65.5 <sup>b</sup>	64.7 <sup>b</sup>	64.5	0.20

<sup>a, b</sup> Mean values with different superscripts within the same rows are significantly different at  $P<0.05$ . SEM: Standard error of mean; T0CC: 100% concentrate; T25CC: 75% concentrate + 25% *C. cajan* forage; T75CC: 25% concentrate + 75% *C. cajan* forage; and T100CC: 100% *C. cajan* forage

method, and feed formulation (Tenakwa *et al.*, 2022).

### Measurement of pH, Ammonia, VFA, and CH<sub>4</sub> in Rumen Fluid

The pH and ammonia production were not significantly different ( $P>0.05$ ) among the treatments (Table 3). The pH of the rumen fluid ranged from 6.80 to 6.90, which is close to that reported by Chetan *et al.* (2017) that the pH of the rumen fluid of cattle given with a combination of concentrate, wheat straw, and pigeon pea straw was 6.66. The pH of the rumen fluid in the present study is within the range of normal ruminant physiological conditions (pH 6.2–7.2) (Radostits *et al.*, 2000). Furthermore, the ammonia production ranged from 5.65 mg dl<sup>-1</sup> to 5.75 mg dl<sup>-1</sup>, which is consistent with the report of Foster *et al.* (2009). The ammonia production of goat rumen fluid illustrates the amount of ration protein that is easily degraded by rumen microbes (Owen and Basalan, 2016). The ammonia concentration of 5.0 mg dl<sup>-1</sup> in rumen fluid is the minimum concentration at which rumen microbes can grow well, and the optimum ammonia concentration ranges between 8.5 and 30.0 mg dl<sup>-1</sup> or 6–21 mM (Gunun *et al.*, 2022). Ammonia production is affected by time after feeding; that is, maximum production is achieved 2 to 4 hours after feeding, depending on the protein source and whether the protein is easily degraded (Zurak *et al.*, 2023).

The high VFA value indicates the amount of organic matter in a ration that is easily degraded by rumen microbes (Bach *et al.*, 2005). The values of total VFA, acetate, propionate, and butyrate significantly differed among the treatments ( $P<0.05$ , Table 3). The VFA value at T0CC (99.32 mol 100 mol<sup>-1</sup>) was higher ( $P<0.05$ ) than that of T75CC (97.60 mol 100 mol<sup>-1</sup>) and T100CC (96.27 mol 100 mol<sup>-1</sup>) but was not significantly different ( $P>0.05$ ) from T25CC (99.25 mol 100 mol<sup>-1</sup>). The total VFA values in this study ranged from 96.27 mg dl<sup>-1</sup> to 99.60 mg dl<sup>-1</sup>, which is higher than that reported by Kang *et al.* (2019) for in vitro ruminal fermentation of pigeon pea (51.4 mol 100 mol<sup>-1</sup>). The total VFA concentration that can support microbial survival is 60–160 mM (Ma *et al.*, 2021). Differences in VFA concentration can be caused by several factors, such as feed type, consumption level, forage and concentrate ratio, feeding frequency, oil supplementation, and microbe type (Ibrahim *et al.*, 2021). Moreover, the estimated value of CH<sub>4</sub> were significantly different among the treatments (Table 3). The highest CH<sub>4</sub> production was found at T0CC (24.43 mmol 100 mol<sup>-1</sup>) whereas the lowest was recorded at T100CC (23.14 mmol 100 mol<sup>-1</sup>). As the level of *C. cajan* forage increased in treatments (from T0CC to T100CC), the percentage of condensed tannins also increased, from 0% in T0CC (100% concentrate) to 5.63% in T100CC (100% *C. cajan* forage). This rise in tannin concentration led to decreased me-

Table 3. Ruminal fluid pH, ammonia, and CH<sub>4</sub> estimation for substituting diets with increasing levels of *Cajanus cajan* forages in goats

Items	Treatments				S.E.M
	T0CC	T25CC	T75CC	T100CC	
pH	6.9	6.8	6.8	6.9	0.24
Ammonia (mg/dl)	5.75	5.75	5.65	5.65	0.02
Total VFA (mol/100mol)	98.25	98.13	97.70	97.38	0.34
Acetate	55.75 <sup>a</sup>	54.30 <sup>ab</sup>	54.0 <sup>b</sup>	53.67 <sup>c</sup>	0.21
Propionate	17.84 <sup>b</sup>	18.11 <sup>b</sup>	18.95 <sup>ab</sup>	19.70 <sup>a</sup>	0.21
Butyrate	10.62 <sup>c</sup>	10.80 <sup>bc</sup>	10.85 <sup>ab</sup>	11.02 <sup>a</sup>	0.05
A/P	3.12 <sup>a</sup>	3.00 <sup>b</sup>	2.85 <sup>c</sup>	2.72 <sup>d</sup>	0.41
CH <sub>4</sub> estimation (mol/100 mol)	24.43 <sup>a</sup>	23.77 <sup>b</sup>	23.42 <sup>c</sup>	23.14 <sup>d</sup>	0.13

<sup>a, b, c, d</sup> Mean values with different superscripts within the same rows are significantly different ( $P < 0.05$ ). SEM: Standard error of mean; T0CC: 100% concentrate; T25CC: 75% concentrate + 25% *C. cajan* forage; T75CC: 25% concentrate + 75% *C. cajan* forage; and T100CC: 100% *C. cajan* forage

thane production by disrupting the microbial populations responsible for methanogenesis. Vasta *et al.*, (2019) reported, condensed tannins have a selective inhibitory effect on rumen microbes, particularly those involved in fibre degradation, which are associated with methane production. By limiting the breakdown of fibrous material, tannins reduce the substrate available for methanogens, subsequently lowering methane output (Huyen *et al.*, 2016). Hence, *C. cajan* has the potential to reduce CH<sub>4</sub> production. Further, Cardoso-Gutierrez *et al.*, (2021), stated factors that influence CH<sub>4</sub> production in livestock include fibre and tannin contents in feed. The estimated CH<sub>4</sub> value in this study is lower than that (26.1 mmol 100 mol<sup>-1</sup>) found by Phe-satcha *et al.*, (2021) for beef cattle given with *Flemingia*. The propionate to acetate ratio also influences the rumen fermentation pattern and is determined by the concentrate-to-forage content of diets (Bhatta *et al.*, 2017).

## CONCLUSION

The result of the present study shows that *Cajanus cajan* forage could be used as a concentrate substitute in goats. Furthermore, *C. cajan* forage does not have a negative effect on nutrient digestibility, rumen fermentation and can reduce methane production. This study showed that *C. cajan* forage may substitute 25% of concentrates in rice straw-based diet for goats.

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## CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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