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Effect of substituting concentrate mix with *Cajanus cajan* leaf on growth performance traits and carcass components of yearling rams and its potential in mitigating methane production

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Abstract

The main challenges in ruminant production are to reduce feeding cost, and to improve products quality with minimum impact on the environment. The use of unconventional feedstuffs may contribute to decrease feeding cost and environmental impact. A study was conducted to investigate the supplementation effect of *Cajanus cajan* leaves (CCL) on the growth performance and carcass characteristics of yearling rams and its association with CH_4 reduction *in vitro*. Thirty yearling rams with an initial body weight of 15.1 ± 0.68 kg were randomly allocated into five treatment diets with six rams each. A basal diet was prepared to contain 300 g/head/d concentrate mix (CM) for the control group (T1) and treatment (T) diets were formulated by replacing the CM with CCL at 5 % (T2), 10 % (T3), 15 % (T4) and 20 % (T5). Data were collected on feed intake, body weight, carcass and CH_4 production from 24h *in vitro* gas production (GP). The dOM and ME were estimated from 24h GP. The analysed ash, ether extract and crude protein contents of the CCL were 126, 43 and 240 g kg^{-1} DM, respectively. The feed intake, body weight gain, feed efficiency and carcass components were not affected by treatment diets. The 24h GP (ml g^{-1} DM) was significantly higher for T1 and T2 diets than that of T4 and T5. The lowest CH_4 was obtained from T5 and differed significantly with that of T1 and T2. The ME and dOM values in T1 and T2 diets were higher than those of T4 and T5. The supplementation of CCL considerably reduced the CH_4 production across treatment diets without affecting the voluntary feed intake, weight gain and carcass components suggesting its potential as alternative supplement to poor quality forages while keeping CH_4 production at a minimum level.

Keywords: body weight, feed intake, carcass, methane production, pigeon pea, yearling ram

Abbreviations

CCL = *Cajanus cajan* leaves; CH_4 = methane; CM = concentrate mix; GP= gas production; dOM = digestible organic matter; ME = metabolisable energy

1 Introduction

Livestock play a significant role in rural livelihoods and the economies of developing countries. They are providers of income and employment for the communities of many developing nations by transforming inedible plants or waste into human food (FAO, 2012; Mottet and Tempio, 2017). On the other hand, farm animals particularly ruminants produce methane as part of their digestive process, which involves microbial (enteric) fermentation (Steinfeld *et al.*, 2006). Livestock and environment interactions in developing countries can be both positive and negative. Because of the high yield gaps in most of these production systems, increasing the efficiency of the livestock sector through sustainable intensification practices presents a real opportunity where research and development can contribute to provide more sustainable solutions (Dumont *et al.*, 2018 and Xie *et al.*, 2019).

In many tropical countries, the productivity of ruminants is limited by the low nitrogen and high fibre content of native pastures and crop residues, which form the basis of the diet in these regions (Berhanu *et al.*, 2019; De Angelis *et al.*,

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2021). In most tropical countries, there are potential plants that may be particularly useful as feed supplements to the typical low-quality animal diets particularly during the dry season (Melesse *et al.*, 2012, 2017, 2018, 2019; Debela and Tolera, 2013). The use of concentrates as supplements to poor quality forage has been reported to improve the intake and digestibility of roughages (Nurfeta, 2010). Nevertheless, the supplementation of ruminants with concentrate mixtures is not affordable under smallholder livestock production settings. It would be thus justifiable to search for alternative feed resources that are easily accessible to smallholder farming communities. *Cajanus cajan* might be among these potential plants, which is an important multi-purpose shrub legume that grows throughout the tropics and subtropics for its edible legume grain that is used as a human food. It is the preferred pulse crop in dry land areas where it is intercropped or grown in mixed cropping systems with cereals or other short duration annuals (Pramod *et al.*, 2013; Kumar *et al.*, 2022). Melesse *et al.*, (2019) screened some local plant materials for their nutritive value along with possible low CH_4 production and from which leaves of *C. cajan* was identified a potential candidate for supplementation of low quality forages with possible effect on methane production reduction.

Cajanus cajan leaves also have been used as feed additives or supplementary feed for ruminants particularly for goats and sheep (Abebe and Tamir, 2016; Dida *et al.*, 2019). Supplementation of low-quality feeds with leguminous forage like *C. cajan* in ruminant diets can potentially be considered for use to offset limitations associated with low feed quality in systems where livestock are increasingly becoming dependent on low-quality roughages (Buthelezi *et al.*, 2022). Moreover, leaves of these plants are rich in protein and are well-suited for feeding small ruminants (Melesse *et al.*, 2019; Mekonen *et al.*, 2022). The use of these forage browses could help to ease some of the problems associated with low feed quality and partly address the major problems of long-term sustainability of crop-livestock systems in Ethiopia. Moreover, *C. cajan* leaves are rich in protein and are well-suited for feeding small ruminants (Melesse *et al.*, 2019; Mekonen *et al.*, 2022). Melesse *et al.*, (2019) reported that leaves of *C. cajan* as potential candidates in mitigating methane production. Meanwhile, in Ethiopia, the information on utilisation of *C. cajan* as to feed consumption, growth performance and carcass yield in livestock feeding and its significance and role in relation to methane reduction is very scanty. Therefore, this study was conducted to investigate the effects of partially substituting of the concentrate mix with dried *C. cajan* leaf on feed intake, weight gain and carcass characteristics of yearling rams and its effect on methane emission reduction *in vitro*.

2 Materials and methods

2.1 Preparation of experimental rations

The fresh leaves of *C. cajan* were harvested from trees regardless of age. The leaves were then spread on a plastic sheet and air-dried under shade to preserve the nutritional quality (Buthelezi *et al.*, 2022). Regular turning of the leaves was done to facilitate the drying process and prevent growth of molds. The dried leaves were then ground using locally available materials to produce the *C. cajan* leaf (hereafter referred to as CCL) and packed in bags of 100 kg and stored in cool place until used. A concentrate mix (CM) was prepared from wheat bran, maize, linseed cake, and salt with the proportions of 40, 40, 19.5 and 0.5 %, respectively as feed basis (Table 1). The CM was formulated to contain CP and energy to meet the maintenance requirements of sheep as recommended by Flint (2005) for intensive feeding (i.e., 17 % CP and 9 MJ ME kg^{-1} DM). Before the start of the trial, the CCL was mixed with the CM to formulate experimental diets according to the experimental design. Natural grass hay was bought from a private farm and hand chopped and offered separately.

Table 1: *Proportion of the feed ingredients used in the concentrate mix (CM) and their analysed proximate compositions.*

Ingredients	*Prop. in CM (%)*	*Proximate compositions (g kg^{-1} DM)*					
		Ash	*CP*	*EE*	*NDF*	*ADF*	*ADL*
Maize /Corn	40	18.0	87.0	72.0	98.0	39.0	19.0
Wheat bran	40	46.0	160	52.0	331	118	32.0
Line seed cake	19.5	84.0	280	130	231	159	60.0
Salt	0.5	-	-	-	-	-	-

Prop. = proportion; CP = crude protein; EE = Ether extract; NDF = neutral detergent fibre on organic matter basis after amylase treatment; ADF = acid detergent fibre on organic matter basis; ADL = acid detergent lignin

2.2 Experimental design and treatment diets

Thirty yearling Arsi-bale local yearling rams with similar initial average body weight were purchased from local market, ear tagged and allowed to acclimatize to the experimental environment for two weeks. At the end of the adaptation period, all rams were blocked according to their body weights similarities and then, individual animals from each block were randomly assigned to the individual pens. Each pen has a dimension of 1.2 x 0.7 m and was fitted with individual feeders and watering troughs.

The control diet contained 300 g CM, (T1), and treatment diets were prepared by replacing the CM of the control diet with CCL at a rate of 5 % (T2), 10 % (T3), 15 % (T4) and 20 % (T5). Accordingly, the ratio of CM : CCL mixture offered to T1, T2, T3, T4 and T5 treatment groups were 300 : 0, 285 : 15, 270 : 30, 255 : 45 and 240 : 60 g/head/day, respectively (Table 2). The concentrate/CCL mixture was offered in combination twice a day in equal portions at 08:00 and 17:00 h while the natural grass hay was provided ad libitum to all treatment diets. The supply of natural grass hay was measured, and an adjustment was made if the refusal was less than 10 % of the offered. The experiment lasted 91 days exclusive of the 15 days acclimatisation.

Table 2: *Layout of the experimental design.*

Variables	*T1*	*T2*	*T3*	*T4*	*T5*
Yearling rams (n)	6	6	6	6	6
Concentrate mix*	300	285	270	255	240
Cajanus cajan leaves*	0	15	30	45	60
Grass hay	*adlib*†	*adlib*	*adlib*	*adlib*	*adlib*

* in g head^{-1}day^{-1}; † *ad libitum*

2.3 Data collection procedures

2.3.1 Feed intake and body weight

The individual body weight was taken at the start of the experiment and considered as initial body weight. The body weight was then recorded every fortnight early in the morning before feed was offered. At the end of the experiment, the individual body weight of all rams was taken in the morning before feeding and was considered as final body weight. Total body weight gain was then calculated by subtracting the initial body weight from that of the final. The amount of daily feed offered was weighed while feed refusal was collected the following morning before feed was offered. Feed intake was determined by difference between amounts of feed offered and refused. Feed conversion ratio (FCR) was calculated as a ratio of total feed intake to total weight gain.

2.3.2 Carcass components

For the evaluation of carcass components, twenty rams were randomly selected (4 rams from each treatment), fasted overnight, weighed (here after referred as slaughter weight) and slaughtered following standard procedures. The weight of the following carcass components was then recorded: blood, head, skin, tail, neck, lumbar, foreleg, hind leg, thorax, neck, rib-eye area, abdominal fat, heart, liver, lung with trachea, gall bladder, spleen, testis with penis, kidneys, trotters, rumen (empty), and intestine (empty). Hot carcass weight was determined after the removal of the head, skin, blood, trotters and all other visceral organs. It included the tail, neck, thorax, lumbar, foreleg, hind leg and neck. The dressing percentage was calculated as proportion of hot carcass weight to the slaughter weight. The cross-sectional area of rib-eye muscle between the 12th and 13th ribs were traced on transparency paper from the right and left side and measured by using a planimeter. The average of the right and left cross-sectional areas was then taken as a rib-eye muscle area.

2.3.3 In vitro gas and methane production protocols

The *in vitro* gas and methane production were measured at the Institute of Animal Science, University of Hohenheim, Germany. Gas production (GP) was determined according to the procedure of VDLUFA official method (VDLUFA 2007, method 25.1) and Menke & Steingass (1988). About 120 mg of feed sample was weighed and transferred into 100 ml calibrated glass syringes, fitted with white Vaseline lubricated glass-made plungers. The buffered mineral solution was prepared and maintained in a water bath at 39 °C under continuous flushing with CO_2. A mixture of rumen fluid was collected from two rumen fistulated Jersey cows fed a total mixed ration as described by Tadesse *et al.* (2022). Three syringes with only buffered rumen fluid referred to as blank (rumen fluid without feed sample), three syringes with hay standard and three with concentrate standard with known GP were also included along with each run. Samples were incubated in a rotary incubator for 24 hours and four independent runs were performed for each feed material and standards.

2.4 Estimating metabolisable energy and digestible organic matter

After 24h incubation, the GP production of the feed samples was immediately recorded and it was corrected using the blanks and standards of hay and concentrate for estimation of metabolisable energy (ME) and digestible organic matter (dOM). The ME and dOM were determined according to Menke & Steingass (1988) by using the following formula:

$$\mathrm{ME}\ (\mathrm{MJ\,kg^{-1}\,DM}) = 1.68 + 0.1418 \times \mathrm{GP} + 0.0073 \times \mathrm{CP} + 0.0217 \times \mathrm{XL} - 0.0028\ \mathrm{XA}$$

$$\mathrm{dOM}\ (\%) = 14.88 + 0.889 \times \mathrm{GP} + 0.0448 \times \mathrm{CP} + 0.0651 \times \mathrm{XA}$$

Where GP, CP, XL, and XA are 24h GP, crude protein, crude fat and crude ash, respectively.

2.5 Determining the methane production

After recording the 24h GP, the incubation liquid was decanted carefully, while leaving the gas inside the syringes.

The methane (CH_4) level of the total gas in the syringes was then analysed using an infrared methane analyser as described by Melesse *et al.* (2019). Syringes were connected directly to the analyser and about 20 ml of gas was injected for about 20 s until the CH_4 concentration displayed was constant.

The CH_4 produced by each feed sample was corrected by the amount of CH_4 produced by blank syringes, the sample weight, and its DM concentration as well as by the factors of reference hay and concentrate feed which were included in each run as stated here above. In addition to the actual concentrations (ml g^{-1} DM), the ratio of the CH_4 to net gas production (NGP) was presented as a percentage of the net gas (pCH_4) in NGP as described by Melesse *et al.* (2018).

2.6 Chemical analysis

Analyses of proximate nutrients and fibre fractions were performed as outlined by Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA 2007) at the Institute of Animal Science, University of Hohenheim, Germany. The samples were analysed for dry matter (DM, method 3.1), ash (method 8.1), crude protein (CP, method 4.1.1) and petroleum ether extract (EE, method 5.1.1). Neutral detergent fibre (aNDFom) was assayed on organic matter basis after amylase treatment and acid detergent fibre (ADFom) on an organic matter basis (methods 6.5.1 and 6.5.2, respectively). Acid detergent lignin (ADL) was analysed according to (method 6.5.3.).

Minerals [calcium (Ca), phosphorous (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), copper (Cu), and manganese (Mn)] were determined according to the methods 10 and 11 of VDLUFA (2007) using an Inductively Coupled Plasma spectrometer (ICP-OES). All analyses were run in duplicate and averaged. If deviation between duplicates was below the level specified for each outcome, the analysis for each sample was repeated. All proximate and mineral analysis were conducted at the Institute of Animal Science, University of Hohenheim, Germany.

2.7 Statistical analysis

Results of proximate and mineral compositions were expressed as means for duplicate analysis of feed samples. Data on feed intake, body weight, carcass components, gas and CH_4 production, ME and dOM were subjected to one-way ANOVA using the Statistical Analysis System (SAS, 2012, Ver. 9.4) by fitting treatment diets as a single fixed factor. Mean comparisons were conducted using Tukey test and values were considered significant at $p < 0.05$. The following linear model summarizes the statistics used to analyse the data:

$$Y_{ij} = \mu + A_i + D_j/A_i + e_{ij}$$

Where:

Y_{ij} = individual values of the dependent variables (feed intake, body weight, etc.),

μ = overall mean of the response variable,

A_i = the fixed effect of the i^{th} treatment diet (i = 1, 2, 3, 4 and 5) on dependent variables,

D_j/A_i = the effect of the j^{th} animals (j =1, 2, 3, 4, 5, 6) within i^{th} treatment diets,

e_{ij} = random residual error.

3 Results

3.1 Nutrient compositions

As presented in Table 3, dried CCL contained high CP as compared with that of different treatment diets. Comparatively high ash was observed for CCL and grass hay while the EE value was higher for the control diet. As the substitution levels of CCL increased, the corresponding values for ash, CP, NDF, ADF and ADL increased. However, as the substitution levels of CCL increased the EE content reduced.

Table 3: *Analysed proximate compositions (g kg^{-1} DM) of grass hay, dried* Cajanus cajan *leaf (CCL) and experimental diets containing different levels of CCL.*

Nutrient	*T1*	*T2*	*T3*	*T4*	*T5*	*Grass hay*	*CCL*
Ash	62	60	65	70	74	119	126
Crude protein	158	174	179	182	188	78	240
Ether extract	77	71	67	65	63	9.0	43.0
aNDFom	262	269	285	304	321	601	517
ADFom	108	125	156	175	194	391	495
ADL	34	48	59	70	81	57	245

Note: aNDFom = neutral detergent fibre on organic matter basis after amylase treatment; ADFom = acid detergent fibre on organic matter basis; ADL = acid detergent lignin; T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

Table 4: *Analysed mineral composition of experimental diets, grass hay and dried* Cajanus cajan *leaf (CCL) and experimental diets containing different levels of CCL.*

Minerals	*T1*	*T2*	*T3*	*T4*	*T5*	*Grass hay*	*CCL*
Major minerals (g kg^{-1} DM)							
Phosphorous	6.7	6.5	6.2	5.8	5.4	1.9	2.5
Calcium	1.6	2.7	3.3	4.3	5.2	7.6	14.5
Magnesium	3.1	3.2	3.2	3.2	3.2	3.7	3.6
Potassium	8.9	9.2	9.1	9.2	9.2	8.6	11.3
Sodium	5.4	2.2	2.4	2.0	1.5	5.0	0.2
Trace minerals (mg kg^{-1} DM)							
Copper	11.1	10.7	10.1	9.5	9.0	7.6	5.1
Iron	807	793	817	847	824	4400	963
Manganese	105	109	112	123	131	695	200

Note: T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

Table 5: *Effect of substitution of concentrate mix with dried* Cajanus cajan *leaf on feed intake, live weight, weight gains and feed conversion efficiency.*

Parameters	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*	*P-value*
Initial weight (kg)	15.7	15.3	15.1	14.9	14.6	0.678	0.794
Final weight (kg)	21.6	21.3	20.8	21.5	20.5	0.826	0.872
Total weight gain (kg)	5.88	6.00	5.77	6.60	6.00	0.384	0.653
Daily gain (g head^{-1})	71.8	73.1	70.3	80.5	72.8	4.686	0.656
Total feed intake (kg)	67.5	65.8	65.6	65.9	63.3	1.299	0.289
Daily feed intake (g head^{-1})	823	803	800	804	772	15.78	0.285
FCR (kg feed per kg gain)	11.6	11.3	11.8	10.1	10.7	0.706	0.482

Note: FCR = feed conversion ratio; SEM = standard error of the mean; T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

The lab analysis for mineral composition indicated that CCL was found to be rich in Ca and K (Table 4). Conversely, the CCL contained relatively low Cu, Fe and Mn concentrations. The Ca content consistently increased with increasing substitution levels of CCL. However, as the inclusion of CCL increased, the corresponding P values for experimental dies (T2-T5) slightly reduced. Comparatively higher values of Fe and Mn were recorded for grass hay than all treatment diets and CCL.

3.2 Live weight, nutrient intake, and feed utilisation

There was no significant difference between rams fed with different treatment diets for feed intake, live weight, weight gain and FCR (Table 5). Comparatively higher total and daily weight gain was observed for rams fed with T4 diet. The total and daily feed intake was similar for rams fed with T2, T3 and T4 diets except for those of T1 and T5 which had the highest and the lowest values, respectively. The FCR values were similar across T1, T2, and T3 diets while slightly lower FCR was observed for rams fed with T4 and T5 diets.

In general, no significant difference was observed for the intakes of DM, CP, EE, NDF and ADF from hay between rams fed with different treatment diets (Table 6). Nevertheless, the intake of nutrients from CM and CCL was ($p < 0.001$) affected by treatment diets. Accordingly, the DM and EE intake from CM showed significant and a smooth reduction trend with increasing substitution levels of CM with CCL. Conversely, the intake of ADF from CM increased ($p < 0.001$) as the substitution of CM with CCL increased. The CP intake from CM showed inconsistent trend but showed a decreasing trend in T4 and T5 diets. As expected, the intake of DM, CP, EE, NDF and ADF from CCL reduced ($p < 0.001$) with increasing substitution levels. A significant difference was further observed in total intake of CP and EE among treatment diets. The former increased while that of the latter reduced as substitution level of CM with CCL increased. The total intake of NDF and ADF was not consistent with increased substitutions levels of CM with CCL.

Table 6: *Intake of nutrients from hay, concentrate mix and* Cajanus cajan *leaf in yearling rams (g head*$^{-1}$ *day*$^{-1}$*).*

Nutrient intake	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*	*P-value*
Hay							
Dry matter	510	490	487	490	460	15.7	0.289
Crude protein	40.8	39.2	39.0	39.3	36.9	1.26	0.292
Ether extract	4.71	4.53	4.50	4.53	4.25	0.15	0.291
NDF	314	302	300	303	284	9.65	0.293
ADF	205	197	195	197	185	6.33	0.295
Concentrate mix							
Dry matter	290^a	271^b	258^c	243^d	230^e	0.00	<.0001
Crude protein	47.4^c	49.6^a	48.3^b	46.4^d	45.1^e	0.00	<.0001
Ether extract	23.1^a	20.2^b	18.1^c	16.6^d	15.1^e	0.00	<.0001
NDF	78.6^a	76.7 b	77.0^{ab}	77.5^{ab}	77.0^{ab}	0.00	<.0001
ADF	32.4^e	35.6^d	42.1^c	44.6^b	46.6^a	0.00	<.0001
Cajanus cajan leaf							
Dry matter	-	14.5^d	29.0^c	43.5^b	57.9^a	0.00	<.0001
Crude protein	-	3.6^d	7.2^c	10.8^b	14.4^a	0.00	<.0001
Ether extract	-	0.65^d	1.29^c	1.94^b	2.58^a	0.00	<.0001
NDF	-	7.76^d	15.5^c	23.3^b	31.0^a	0.00	<.0001
ADF	-	6.68^d	13.4^c	20.0^b	26.7^a	0.00	<.0001
Total intake							
Dry matter	780	776	774	777	748	15.7	0.263
Crude protein	88.2^d	92.4^c	94.5^b	96.5^a	96.4^a	1.27	0.0005
Ether extract	27.8^a	25.4^b	23.9^c	23.1^c	22.0^d	0.14	<.0001
NDF	393	387	393	403	392	9.71	0.836
ADF	237^c	239^c	251^b	261^a	258^b	6.31	0.041

Note: $^{a-e}$ Means between treatment diets with different superscript letters are significant at $p < 0.05$. NDF = neutral detergent fibre; ADF = acid detergent fibre; T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

Table 7: *Commercial carcass components (kg) of yearling sheep fed with the control diet and diets containing different levels of* Cajanus cajan *leaf.*

Carcass components	*T1*	*T2*	*T3*	*T4*	*T5*	*SEM*	*P-value*
Slaughter weight	20.3	20.7	19.6	21.2	19.6	1.138	0.245
Hot carcass weight	7.65	7.90	7.85	7.95	7.40	0.625	0.640
Dressing (%)	37.5	38.0	40.0	37.6	37.9	2.023	0.999
Thorax	1.82	1.73	1.82	1.91	1.78	0.173	0.714
Foreleg	1.45	1.50	1.50	1.41	1.42	0.137	0.797
Hindleg	1.65	1.74	1.68	1.78	1.63	0.137	0.506
Lumbar	1.26	1.30	1.33	1.15	1.14	0.165	0.375
Neck	0.665	0.745	0.687	0.765	0.645	0.080	0.216
Liver	0.287	0.330	0.277	0.315	0.297	0.040	0.401
Heart	0.107	0.097	0.095	0.092	0.090	0.017	0.699
Rib-eye area (cm2)	22.6	18.1	24.0	21.9	17.7	1.828	0.102

Note: NDF = neutral detergent fibre; ADF = acid detergent fibre; T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

3.3 Carcass characteristics

There was no statistical difference between treatment diets for the investigated carcass components (Table 7). The average hot carcass weight was slightly highest in rams fed with T2 and T4 diets. Although not significant, the highest dressing percentage was observed in rams fed with T2 and

Table 8: *Commercial carcass components (kg) of yearling sheep fed with the control diet and diets containing different levels of* Cajanus cajan *leaf.*

Parameters	*T1*	*T2*	*T3*	*T4*	*T5*	*CCL*	*SEM*	*P-value*
24h GP (ml g^{-1} DM)	269^{a}	260^{ab}	242^{bc}	230^{cd}	219^{d}	75.3^{e}	6.33	<0.005
Methane (ml g^{-1} DM)	46.1^{a}	45.4^{a}	41.9^{ab}	40.8^{b}	38.6^{b}	12.9^{c}	1.42	0.005
Methane (% of NGP)	17.1^{a}	17.5^{a}	17.3^{a}	17.8^{a}	17.6^{a}	16.6^{b}	0.22	0.005
ME (MJ kg^{-1} DM)	9.58^{a}	9.31^{ab}	8.80^{bc}	8.45^{c}	8.13^{c}	4.19^{d}	0.18	<0.005
dOM (%)	63.6^{a}	62.3^{a}	59.1^{ab}	57.0^{b}	55.1^{b}	29.73^{c}	1.12	<0.005

Note: $^{a-e}$ Means between treatment diets with different superscript letters are significant at $p < 0.05$. GP = gas production; ME = metabolisable energy; dOM = digestible organic matter; NGP = net gas production; DM = dry matter; SEM = standard error of the mean; T1 control diet; T2 5 % CCL; T3 10 % CCL; T4 15 %CCL; T5 20 % CCL.

T3 diets. Rams fed with T4 diet had the highest values for thorax and hindleg. The highest rib-eye area (24.00 cm^2) was noted in rams fed with T3 followed by T1 and T4 diets while those reared in the T5 diet had the lowest value.

3.4 Methane production, metabolisable energy and digestible organic matter

Except for the percent methane, the *in vitro* analysis showed that all measured *in vitro* GP and CH$_4$ consistently and significantly reduced with increased substitution levels of CCL across the treatment diets (Table 8). The value of GP (ml g^{-1} DM) was lower ($p < 0.001$) for the T5 diet. T2 and T3 diets showed similar values for GP. Methane (ml g^{-1} DM) production was lower ($p < 0.001$) for T4 and T5 diets. Similar values of CH$_4$ (ml g^{-1} DM) were recorded for T1, T2 and T3 diets. As shown in Fig. 1, the CH$_4$ production consistently reduced as the levels of CCL increased across treatment diets. The GP and CH$_4$ productions were lowest in CCL. There was no difference between treatment diets for pCH$_4$ production. However, CCL showed lower ($p < 0.05$) pCH$_4$ production. The values of ME and dOM reduced ($p < 0.05$) with increasing levels of CM substitution with CCL being lower in T3, T4 and T5 diets as compared to those of T1 and T2. The CCL had the lowest GP, CH$_4$, ME and dOM values being different ($p < 0.005$) from all treatment diets. The dOM for CCL is far lower ($p < 0.005$) than the other experimental diets.

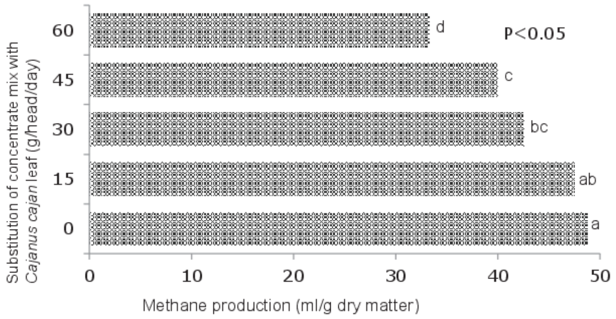

Fig. 1: *Trends of methane production with increased substitution levels of concentrate mix with* Cajanus cajan *leaf*

4 Discussion

4.1 Nutrient composition and intake

In the current study, the CCL contained high CP (240 g kg^{-1} DM) which had contributed to the uniform increase in CP value of the corresponding treatment levels as inclusion level of CCL increased. As the substitution level of CCL increased, the corresponding values for NDF, ADF and ADL increased which could be attributed to the increased substitution levels of CCL which contained relatively high structured carbohydrates. It is therefore essential to limit the substitution level of CCL in the concentrate mixture to avoid the possible negative effect on voluntary feed intake of animals that might lead to reduced productivity. It is apparent that the linseed cake was the main protein source in the control diet, which is also rich in crude fat. Thus, as the part of CM is replaced with CCL it became comparatively deficient in EE content. Buthelezi *et al.* (2022) reported the CP values ranging from 244 to 257 g kg^{-1} DM for three under shade air-dried *C. cajan* varieties which is in close agreement with the current observations. On the other hand, the CP content of CCL observed in the current study is higher than reported by Melesse *et al.* (2019) and Assegid *et al.* (2021). The former authors also reported lower NDF, ADF, ADL and ash contents while the latter found higher NDF than observed in the current study. In another study, Buthelezi *et al.* (2022) reported comparable NDF values for three CCL varieties. However, the same authors reported lower ADF and ADL values than observed in the current study. Such variations might be due to differences in the soil type, variety of the plant material, season, and age of the tree from which the leaves were collected.

The mineral composition for experimental feeds and feed composition in this experiment indicated that the CCL was found to be rich in Ca and K and this observation is consistent with the reports of Melesse *et al.* (2019) and Buthelezi *et al.* (2022). This may suggest the significance of CCL in the feeding of dairy animals for improved milk composition. Mineral calcium is also an essential mineral in poultry nutrition and CCL might be a good source for improved external egg qualities. As the inclusion levels of CCL increased, the corresponding P values for experimental diets slightly reduced. As phosphorus is one of the most important minerals for many metabolic processes in animals, its deficiency in young animal's diet may retard the growth and reproductive performances of livestock (Paterson *et al.*, 1996). It is thus worthwhile to be aware of the problem if CCL is considered as a sole feed resource to ruminants to avoid risk of P deficiencies. For well-balanced Ca and P sources, the recommended ratio of Ca : P in animal feed staffs is 1:2 to 2:1 (NRC, 2001). In the current study, the calculated Ca : P ratio for T3, T4 and T5 diets falls in the safe range. The Cu concentration in the current study is comparable to those reported by Buthelezi *et al.* (2022) for various varieties of *C. cajan* plants. However, the same authors reported much lower Fe and Mn concentrations than observed in the present study. The CCL is generally characterised by relatively lower trace mineral contents (Melesse *et al.*, 2019). Hence, attention is required in supplementing those trace minerals in the feeding scheme of animals especially when the CCL is used as a sole feed source in rural settings.

The intake of DM, CP, EE, NDF and ADF from hay were not significantly affected by treatment diets and these observations are consistent with those of Ephrem *et al.* (2015) who reported absence of significant effects among treatment groups of Washera lambs fed natural pasture hay supplemented with legume Lupinus angustifolius L. The intake of all nutrients from CCL significantly increased as the substitution level of CM with CCL increased which suggests the palatability of the leaf by the rams. The concentration rate of the anti-nutritive compounds in CCL does not limit animal performance (Buthelezi *et al.*, 2022). Moreover, microorganisms in the rumen of rams fed with increased levels of CCL supplementation might have benefited by receiving sufficient N for their growth and proper rumen function which have resulted in better degradation of the fiber and improvement in intake (Assegid *et al.*, 2021).

The total intake of CP increased with increased substitution levels of CCL which is consistent with the observations of Netsanet and Yonatan (2015) and Assgid *et al.* (2021). The latter authors reported a linear increase of total DM, OM, CP intake with increasing levels of pigeon pea foliage supplementation to Arisi-Bale goats. Improved total CP intake was reported in sheep supplemented with pigeon pea (CCL), cowpea and lablab compared with the non-supplemented group (Abebe and Tamir, 2016), which is similar with the current observations. The total intake of EE reduced with increasing substitution of CM with CCL. The protein source of CM used in the current work was line seed cake which is also rich in crude fat (130 g kg^{-1} DM; Table 1) and was reduced as the part of CM is replaced with CCL. Although not significant, the rams fed with the T5 diet had the lowest feed and DM intake compared with those of the control group. These observations are in good agreement with the reports of Mekonen *et al.* (2022) in which the feed intake of crossbred dairy cows has consistently decreased with increasing replacement of CM with pigeon pea leaves. This might be caused by the presence of anti-nutritional factors in CCL as reported by Diribsa *et al.* (2016), which might have limited the efficiency of feed utilisation. Melesse *et al.* (2019) have reported relatively high levels of anti-nutritional factors (more than 90 g kg^{-1} DM total phenols and higher than 95 g kg^{-1} DM) for CCL which could be a reason for declined trend of feed and DM intake as the level of CCL increased. This phenomenon has also been supported by the observations of Soares *et al.* (2012), who reported that ruminants possess proteins with a high content of amino acids such as proline in their saliva, which are more likely to bind with condensed tannin resulting in reduced feed intake.

4.2 *Growth performance and carcass traits*

Diribsa *et al.* (2016) reported a significant improvement of the growth performance traits of rams fed with a natural grass hay as a basal diet which was supplemented with CCL. This observation might have been caused by the supplementation of grass hay as a basal diet which had resulted in quick response of the sheep to CCL supplementation. In the current study, the CM has been partially replaced with CCL in which the former ration contains comparatively better nutrient composition than the CCL.

In the current study, the substitution of CM with *C. cajan* leaves did not affect live weight, weight gains and FCR of yearling rams which is consistent with those reported by Dida *et al.* (2019) for goats supplemented with CCL. These findings further agree with those of Mekonen *et al.* (2022) who reported a non-significant effect of CCL supplementation in crossbred dairy cattle. Haymanot *et al.* (2019) reported total body weight gain of 4.6 kg/head in sheep fed on a basal diet of natural grass hay supplemented with 320 g kg^{-1} DM of *C. cajan* leaf, which was lower than observed in the current study which ranged from 5.88 in T1 to 6.60 kg/head in T4. This might be associated with the age of the sheep used in their experiment. The reduced feed intake observed in rams fed with T5 diet might be associated with high contents of ADF and ADL with increased substitution levels of CCL which could have negatively influenced its palatability (Riaz *et al.*, 2014). Heuzé *et al.* (2016) have reported that although pigeon pea is protein-rich forage, its high fiber content (particularly ADF and lignin) may reduce its digestibility resulting in limited use of its potential.

The importance of supplementing leguminous browses like *C. cajan* on the improvement of the carcass performances has been reported by different researchers. For instance, Abebe and Tamir (2016), in their findings demonstrated a significant improvements of hot carcass weight, slaughter weight, dressing percentage and rib eye muscle area in the lambs fed with grass hay as basal diet and supplemented with legume forage leaf as compared to lambs that supplemented with only concentrate mix.

The hot carcass values and the dressing percentage obtained from the current study were consistent with the results of Abebe and Tamir (2016), who reported similar values for the Wollo sheep fed with a basal diet of grass hay which was supplemented with *C. cajan*, Vigna unguiculata and Lablab purpureus. Similar observations were also reported by Maciel *et al.* (2015) where sheep fed with cassava leaf did not show significant differences on the studied carcass and non-carcass characteristics among treatments. The dressing percentage in the current study ranged from 37.5 for rams reared in T1 to 40.0 for those fed with T3 diets. These values are in the range of those values reported for indigenous Ethiopian sheep (Ayele *et al.*, 2019). The highest rib-eye area (24.0 cm2) was noted in rams fed with T3 followed by T1 diets.

4.3 In vitro *gas and methane productions and estimated parameters*

Among the residues of the leguminous feed crops studied, Soliva *et al.* (2008) and Melesse *et al.* (2019) reported that *C. cajan* appeared to be a promising feed resource due to its low CH_4 production potential at concurrently high ruminal ammonia release. Berhanu *et al.* (2019), reported that CH_4 can be used as a potential indicator to determine the potential of a plant to mitigate methane production. In the current study, the 24h GP and actual CH_4 and calculated ME and dOM significantly ($p<0.05$) reduced with increased substitution levels of CCL across the treatment diets. The 24h GP reported by Meale *et al.* (2012) for *C. cajan* leaves collected from Australia and Ghana was higher than observed in the current study (114 vs 75.3 $\mathrm{ml\,g^{-1}}$ DM). This might be due to the effect of different factors including age of the plant and season in which leaves were collected, soil type, variety of the plant, and the way leaves were dried and processed (Melesse *et al.*, 2012).

As the inclusion level of CCL in CM increased, the *in vitro* CH_4 production decreased. The reduction in CH_4 production in *C. cajan* leaves might be associated with the highest content of condensed tannins which have a direct toxic effect on methanogens and have the potential to modify rumen fermentation resulting in reduced CH_4 production (Patra & Saxena 2011; Melesse *et al.*, 2019). Moreover, the low CH_4 in *C. cajan* leaf might be due to high concentration of phenolic compounds (Melesse *et al.*, 2019) and their antimicrobial properties as suggested by Kong *et al.* (2009). The reduction in CH_4 production from CCL might further be associated with the relatively low level of energy as well as high content of CP, NDF and ADF in the CCL which both could result in reduced GP in which the *in vitro* methane has been obtained (Melesse *et al.*, 2019). This positive scenario of CH_4 reduction suggest that CCL can be one locally available feed material for feeding animals in mixed-crop livestock production systems that could help in the effort of methane mitigation strategy in tropical and subtropical regions of the world. In addition to its methane reduction potential, the CCL has reliable adaptability in drought prone areas and hence, it could be advantageous of its cultivation and utilisation especially in arid and semi-arid areas of the world where it could be easily cultivated. However, it should be noted that the ME and dOM values have significantly reduced with increased substitution levels CCL which indicates its deficiency in energy density. Thus, increasing the substitution levels of the unconventional feeds may affect its palatability with possible determinantal effects on ME and dOM values specifically with high rates of inclusion as observed in rams fed with T5 of the current study. The ME (6.5 - 7.1 MJ/kg DM) values reported by Buthelezi *et al.* (2022) for three *C. cajan* varieties that were air dried under shade were higher than observed in the current study. Such variations might be caused by the variety of the plant, location, and season. With increased substitution levels of CCL, the values for NDF, ADF and ADL increased while that of GP, ME and dOM reduced. This is in good agreement with the results of Suhab Uslu *et al.* (2018) who reported a negative correlation of NDF and ADF with ME and dOM in legume plants.

5 Conclusion

This study aimed to investigate the value of *C. cajan* leaves as alternative feed resource in the ruminant nutrition and its potential in mitigating CH_4 production *in vitro*. Results showed that no significant effect was observed among treatments on feed intake, growth performance and carcass traits of yearling rams. Results further indicated that except for the $\%CH_4$, all measured *in vitro* GP and CH_4 consistently and significantly reduced with increased substitution levels of CCL across the treatment diets. However, ME and dOM values ($p < 0.05$) reduced with increasing substitution levels of *C. cajan* leaves being lower in T5, T4 and T3 diets than those of T1 and T2. Based on the current results, it has been proved the significance of *C. cajan* leaves in mitigating CH_4 production without affecting the voluntary feed intake and performances of yearling rams. The findings of the current study further suggest that the *C. cajan* leaves could be used as alternative supplement to poor quality tropical forages while keeping CH_4 emission at its minimum level. The *C. cajan* plant has a reliable adaptability in drought prone areas and hence it would be advantageous to grow it in arid and semi-arid regions of the world where it can be easily cultivated and utilised.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contribution

A. Tadesse - Data curation, Conceptualisation, Methodology, Writing-original draft.; A. Melesse – Conceptualisation, Formal statistical analysis, Funding acquisition, Writing – review & editing, Supervision; M. Rodehutscord – Writing – review & editing, Supervision; Natascha Titze – analysis and compilation of *in vitro* gas and methane production.

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