PARTIAL SUBSTITUTION OF CONCENTRATE MIX WITH DRIED *Leucaena leucocephala* LEAF REDUCED *in vitro* METHANE PRODUCTION IN RAMS WITHOUT AFFECTING THE NUTRIENT INTAKE AND PERFORMANCE TRAITS †

[SUSTITUCIÓN PARCIAL DE ALIMENTO CONCENTRADO POR HOJA SECA DE *Leucaena leucocephala* REDUCE LA PRODUCCIÓN *in vitro* DE METANO EN CARNEROS SIN AFECTAR LA INGESTA DE NUTRIENTES Y DESEMPEÑO PRODUCTIVO]

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SUMMARY

Background. Through *in vitro* studies, dried *Leucaena leucocephala* leaf (DLL) was identified as potential candidate in mitigating enteric methane (CH_4) emission. Nevertheless, its efficiency as suitable feed for sheep has not been determined in Ethiopia**. Objective.** To assess the suitability of replacing the concentrate mix (CM) with DLL on voluntary intake, growth performance in rams and *in vitro* CH_4 production. **Methodology.** Thirty yearling rams were first stratified according to their initial body weight similarities and then individuals from each stratum were randomly assigned to five treatment diets with six rams each. The control diet contained CM with 346 g/head/d (T1), and treatment diets replacing the CM in the control diet with DLL at a rate of 5% (T2), 10% (T3), 15% (T4) and 20% (T5) with the corresponding CM to DLL mixture ratio of 346:0, 329:17, 311:35, 294:52 and 277:69 g/head/day. Grass hay was provided *ad libitum* to all rams. Data were collected on fed intake and body weight. Methane (CH_4) production was determined along with 24h *in vitro* gas production (GP). Digestible organic matter (DOM) and Metabolizable energy (ME) were estimated from 24h GP. **Results.** The contents of ash, crude protein (CP), neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and acid detergent lignin were higher in DLL than in the control diet while it contained the lowest EE value. The DLL had the highest Ca and K values as compared to the control diet. The feed intake, live weight and weight gain did not differ ($P>0.05$) among rams supplemented with various levels of DLL. The total intake of CP increased across treatment diets and was significantly higher for T3, T4 and T5 than T1 and T2 diets. None of the supplementation levels of *L. leucocephala* affected the total intake of DM, NDFom and ADFom. There was a linear reduction ($p<0.05$) of *in vitro* CH_4 production as the levels of DLL in the diet were increased. It was lowest in T4 and T5 diets being significantly different from those of T1 and T2. The T5 diet showed the lowest values and differed ($p<0.05$) from those of T1, T2 and T3. The ME and DOM values were higher ($p<0.05$) in T1 and T2 diets than that of T4 and T5. No difference in ME and DOM values were observed between T1, T2 and T3 diets as well as among T4 and T5 diets. **Implications.** The current findings suggest that conventional CM could be replaced with DLL up to 20% as alternative protein source in ruminant nutrition in tropical and sub-tropical regions. **Conclusion**. The replacement of CM with DLL significantly reduced the *in vitro* CH_4 production across treatment diets without affecting the voluntary intake and growth performance parameters in rams.

Keywords: live weight; nutrient intake; concentrate mix; enteric methane; *Leucaena leucocephala* leaf; rams.

RESUMEN

Antecedentes. A través de estudios *in vitro*, se identificó la hoja seca de *Leucaena leucocephala* (DLL) como candidata potencial para mitigar la emisión de metano entérico (CH4). Sin embargo, su eficiencia como alimento adecuado para ovejas no ha sido determinada en Etiopía. **Objetivo.** Evaluar la idoneidad de la sustitución de la mezcla de concentrados (CM) por DLL sobre el consumo voluntario, el crecimiento de carneros y la producción de CH4 *in vitro.* **Metodología.** Treinta carneros de un año se estratificaron primero de acuerdo con sus similitudes de peso corporal inicial y luego los individuos de cada estrato se asignaron al azar a cinco dietas de tratamiento con seis carneros cada uno. La dieta control a base de CM se asignó a 346 g/cabeza/d (T1), y las dietas de tratamiento reemplazan la CM en

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la dieta control por DLL a razón de 5% (T2), 10% (T3), 15% (T4) y 20% (T5) con la correspondiente relación de mezcla de CM a DLL de 346:0, 329:17, 311:35, 294:52 y 277:69 g/cabeza/día. Se proporcionó heno de pasto *ad libitum* a todos los tratamientos. Se recogieron datos sobre la ingesta de alimentos y el peso corporal. La producción de metano (CH4) se determinó a partir de la producción de gas (GP) *in vitro* durante 24 horas. La Materia Orgánica Digestible (DOM) y la Energía Metabolizable (ME) se estimaron a partir de 24h GP. **Resultados.** Los contenidos de ceniza, proteína cruda (PC), fibra detergente neutra (NDFom), fibra detergente ácida (ADFom) y lignina detergente ácida fueron más altos en DLL que en la dieta control, mientras que contenía el valor más bajo de EE. El DLL tuvo los valores más altos de Ca y K en comparación con la dieta de control. El consumo de alimento, el peso vivo y la ganancia de peso no difirieron (P>0.05) entre los carneros suplementados con varios niveles de DLL. La ingesta total de PC aumentó en las dietas con DLL y fue significativamente mayor para las dietas T3, T4 y T5 que para las dietas T1 y T2. Ninguno de los niveles de suplementación de *L. leucocephala* afectó la ingesta total de MS, NDFom y ADFom. Hubo una reducción lineal (p<0,05) de la producción de CH4 *in vitro* a medida que aumentaban los niveles de DLL en la dieta. Fue más bajo en las dietas T4 y T5, siendo significativamente diferente de las de T1 y T2. La dieta T5 presentó los valores más bajos y difirió (p<0.05) de las de T1, T2 y T3. Los valores de EM y DOM fueron mayores (p<0.05) en las dietas T1 y T2 que en las dietas T4 y T5. No se observaron diferencias en los valores de ME y DOM entre las dietas T1, T2 y T3, así como entre las dietas T4 y T5. **Implicaciones**. Los hallazgos actuales sugieren que el CM convencional podría reemplazarse con DLL hasta en un 20 % como fuente de proteína alternativa en la nutrición de rumiantes en regiones tropicales y subtropicales. **Conclusión.** El reemplazo de CM con DLL redujo significativamente la producción de CH4 *in vitro* en las dietas de tratamiento sin afectar la ingesta voluntaria y los parámetros de desempeño del crecimiento en los carneros.
Palabras clave: peso vivo; ingesta de nutrientes; alimento concentrado; metano entérico; hoja de *Leucaena leucocephala*; carneros

INTRODUCTION

Supplementing basal feeds that are deficient in major nutrients with grain concentrate generally improves the performance of farm animals particularly sheep and goats. However, due to their inaccessibility and high cost, the use of such supplements is usually limited under the smallholder production systems. One way of improving the utilization of poor-quality feed is through supplementation with leaves of multipurpose tree species (Gebregiorgis *et al.*, 2012; Melesse *et al.*, 2015), which are affordable and easily accessible by the individual smallholder farmers. One of such feed could be leaves of *Leucaena leucocephala*, which is cheaper and readily available than any of the ingredients of a concentrate mix.

Recent studies have indicated that the *L. leucocephala* leaves contained high levels of crude protein (233 g/kg, Mohammadabadi, T. and Jolazadeh, 2017, 229 g/kg; 237 g/kg, Mataveia *et al.*, 2019; 245 g/kg, Melesse *et al.*, 2019). It has been further reported that the leaves are a good source of essential amino acids such as isoleucine, leucine and limiting amino acids such as lysine and methionine as compared with other multipurpose tree foliages (Melesse *et al.*, 2019).

Several researchers have recommended various supplementation levels of *L. leucocephala* leaf in ruminants. For example, Kang *et al.* (2012) recommended *L. leucocephala* leaf meal as alternative protein source in the diets of swamp buffalo along with increased apparent digestibility. Balogun and Otchere (1995) have recommended inclusion of *L. leucocephala* leaves upto 40% (on DM basis) in the diet of rams. On the other hand, Harun *et al.* (2017) recommended 25% of *L. leucocephala* leaves in the diet of goats. Recently, Montoya-Flores *et al.* (2020) reported that the inclusion of dried *L. leucocephala* leaf upto 12% (on DM basis) enhanced digestible crude protein and reduced daily production of enteric methane without adversely affecting the dry matter intake, rumen microbial population, and fermentation parameters and suggested this level as optimum for crossbred heifers of *B. taurus* and *B. indicus*.

Nevertheless, in Ethiopia, there is little information on the effect of *L. leucocephala* leaves on the feed consumption, growth performances, and enteric methane production of rams. This study was thus conducted to investigate the effect of replacing concentrate mix with graded levels of dried *L. leucocephala* leaves on feed intake, live weight of yearling rams, and *in vitro* methane production.

MATERIALS AND METHODS

Preparation of experimental rations

Branches of *L. leucocephala* with leaves were harvested from available trees regardless of the age of the trees at the end of the rainy season. Branches were spread on a plastic sheet for air-drying in an area protected from direct sun light to prevent loss of vitamins and other volatile nutrients. During the process of drying, the leaves were detached from branches and directly collected from the plastic sheet. The dried *L. leucocephala* leaves (hereafter referred to as DLL) were chopped using mortar and piston to reduce its particle size, packed in bags of 100 kg and stored in cool place until used. As shown in Table 1, 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% as feed basis, respectively. Before being mixed with the other ingredients of the CM, the maize grain was grinded using a feed miller (type AWILA, Germany) with an adjustable particle size. The CM was formulated to contain crude protein (CP) and metabolizable energy (ME) to meet the optimum recommendation of NRC (2007) for intensive feeding (i.e., 17 % CP and 9 MJ (2150 kcal) ME/kg DM). Before commencing the trial, the DLL was mixed manually with the CM to prepare the experimental diets. Mixing the DLL with CM is essential for the increased palatability of the leaf. Grass hay was bought from a private farm and hand chopped into the size of 3 to 5 cm and offered separately.

Acquisition and management of experimental animals

Thirty yearling rams with initial average body weight of 20.0±1.31 kg were purchased from local market. Upon arrival, all rams were ear tagged and acclimatized to the experimental environment for two weeks. They were housed in individual pens with concrete floor of 1.5 × 1.0 m dimensions, fitted with individual feeders and drinkers. Pens were cleaned on daily basis while watering and feeding troughs were checked twice a day. During the acclimatization period, the rams were treated with half bolus (150 g/sheep) of Albendazole 300 g against internal parasites and with Ivermectin 5 g tablets (ecto-endo-parasiticide) against external parasites. Moreover, rams were injected with Oxytetracycline 10% at a dose of 2 ml/10 kg of live weight for the treatment of infectious diseases caused by a variety of gram-positive and gram-negative microorganisms (including *Mycoplasma pneumonia*, *Pasteurella pestis*, *Escherichia coli*, *Haemophilus influenza* and *Diplococcus pneumonia*). All treatments were given according to the dosage recommended by the manufacturers and adjusting the same with respect to the body weight of the rams.

Experimental design and treatment diets

At the end of the acclimatization period, the rams with initial average body weight of 20.0±1.31 kg were first stratified according to their initial body weight similarity and then individuals from each stratum were randomly allocated into five treatment diets with six rams each in a completely randomized design. The treatment diets contained 346 g CM, (control diet, T1), and treatment diets replacing the CM of the control diet with DLL at a rate of 5% (T2), 10% (T3), 15% (T4) and 20% (T5). Accordingly, the CM to DLL mixture was offered with a ratio of 346:0, 329:17, 311:35, 294:52 and 277:69 g/head/day to T1, T2, T3, T4 and T5 treatments, respectively. The CM/DLL mixture was offered to the rams as a total, in order to maintain homogeneity in particle size and particle type among rations. All the experimental rams had *ad libitum* access to grass hay and clean water. The supply of grass was measured daily and adjustments were made if the refusal was less than 10% of the offered. The experiment lasted for 91 days exclusive of the acclimatization period.

Data collection procedures

Feed intake and body weight

The body weight taken at the start of the experiment was considered as initial body weight for individual animals. The CM/DLL mixture was offered per treatment twice a day in equal portions at 08:00 and 17:00 h. A measured amount of CM/DLL and hay was offered separately and the refusal was collected and weighed in the next morning. Feed intake of CM/DLL and hay was then determined by difference between amounts of feed offered and refused. Moreover, the individual nutrient intake (DM, CP, NDF, etc.) from hay, CM and DLL was computed based on their proximate composition and the corresponding total intake.

Table 1. Proportion of ingredients used in the concentrate mix (CM) and their analyzed proximate compositions.

Ingredients	Proportion in CM (%)	Proximate compositions (g/kg DM)					
		Ash	Crude protein	Ether extract	aNDFom	ADFom	ADL
Maize	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	-	-	-	-	-	-

aNDFom = neutral detergent fiber without residual ash after amylase treatment; ADFom = acid detergent fiber without residual ash; ADL = acid detergent lignin

To monitor body weight change, body weights were recorded every 14 days early in the morning before feed was offered. At the end of the experiment, all rams were weighed individually in the morning before feeding, and this was taken as final body weight. Total body weight gain was then calculated by subtracting the initial body weight from the final. Feed conversion ratio (FCR) was calculated as a ratio of total feed intake to total weight gain.

***In vitro* gas and methane production protocols**

The *in vitro* gas and methane production was carried out at the Institute of Animal Science, University of Hohenheim, Germany according to the procedure of VDLUFA official method (VDLUFA, 2007) Menke and Steingass (1988) as described by Melesse *et al.* (2019). About 120 mg of feed sample was weighed and transferred into 100 ml calibrated glass syringes, fitted with white Vaseline lubricated glass-made plungers. A mixture of rumen fluid was used which was collected from two rumen fistulated Jersey cows fed a total mixed ration consisting of 12% maize silage, 38% grass silage, 18% hay, 30% concentrate mixture and 2% mineral mixture on DM basis. The cows were in their third lactation and approximately 5 years old. The mean body weight was 450 kg.

Samples were incubated in a rotary incubator for 24 hours and four independent runs were performed for each feed material. The methane (CH_4) level of the total gas in the syringes was analysed as described by Melesse *et al.* (2019). Briefly, after 24 h incubation of the samples, the gas production (GP) was recorded and the incubation liquid was decanted carefully, while leaving the gas inside the syringes. The CH_4 level of the total gas in the syringes was then analysed using an infrared methane analyser (Pronova Analysentechnik, Berlin, Germany) calibrated with a reference gas (13.0 vol % CH_4, Westfalen AG, Münster, Germany). Syringes were connected directly to the analyser and a minimum amount of 20 ml of gas was injected 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 the blank syringes. The proportion of CH_4 to total gas production (GP) from 24 h sample incubation was determined using the following formula:

$$\mathrm{CH_4} = \frac{\mathrm{CH_4\ in\ sample}}{\mathrm{24\ hr\ GP}} * 100$$

Moreover, the GP of the feed samples was corrected using the blanks for estimation of metabolizable energy (ME) and digestible organic matter (DOM) by applying the following equation (Menke and Steingass, 1988):

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

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

where GP (ml/200 mg DM), CP (g/kg DM), XL (g/kg DM), and XA (g/kg DM) are gas production, crude protein, crude fat and crude ash, respectively.

Chemical analysis

Analyses of proximate nutrients and fiber 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 analyzed 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 fiber (aNDFom) was assayed without residual ash after amylase treatment and acid detergent fiber (ADFom) without residual ash (methods 6.5.1 and 6.5.2, respectively). Acid detergent lignin (ADL) was analyzed according to method 6.5.3. The cellulose and hemicellulose were computed as ADFom minus ADL, and aNDFom minus ADFom, respectively. Non-fiber carbohydrate (NFC) was calculated as 100-(NDF + CP + crude fat + ash) according to NRC (2001).

Minerals [Calcium (Ca), Phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), copper (Cu), and manganese (Mn)] were determined according to methods 10 and 11 of VDLUFA (2007) using an Inductively Coupled Plasma spectrometer (ICP-OES). All analyses were run in duplicate and averaged.

Statistical analysis

Data on intake of nutrients, body weight, weight gain and FCR were analyzed using the GLM procedure to account for the missing data. Data on *in vitro* gas and CH_4 productions as well as OMD and ME contents were subjected to one-way ANOVA. Mean comparisons were performed using Tukey's Studentized Range (HSD) test. All the statistical analyses were performed using the Statistical Analysis System, SAS (SAS, 2012, Ver. 9.4) by fitting treatment diets as an independent variable. The data were analyzed using the following statistical model.

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

Y_{ij} = individual values of the dependent variables (nutrient 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 the dependant 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 variation in the response of individual animals.

RESULTS

Nutrient compositions of experimental diets and *L. leucocephala* leaf

As shown in Table 2, DLL had relatively high ash and CP contents. This has resulted in increased levels of these nutrients across treatment diets. Similarly, the contents of NDFom, ADFom and ADL increased with increasing substitution levels of CM with that of DLL. On the other hand, the EE, cellulose and NFC contents consistently reduced with increased substitution of DLL. The grass hay contain high structural carbohydrates concentrations with low CP and NFC. The contents of CP, NDFom, ADFom and ADL in DLL were considerably high when compared to that of the control diet.

The concentration of major and trace minerals is shown in Table 3. The DLL had the highest calcium (Ca) and potassium (K) values as compared to the control diet. However, it contained lower values for phosphorous (P), copper (Cu) and iron (Fe). The Ca content considerably increased with increased levels of supplementation of the treatment diets while the concentrations of P, sodium (Na), Cu, Fe and manganese (Mn) consistently reduced with increasing levels of substitution of CM with DLL. On the other hand, the contents of magnesium (Mg) and K remained unchanged across the treatment diets. The concentration of trace minerals in DLL was generally low while that of Ca and K in DLL was relatively high.

Live weight, weight gain and intake of nutrients

As shown in Table 4, no significant differences were observed in feed consumption, live weight and weight gain among the rams fed with treatment diets containing different supplementation levels of DLL. However, there was a significant effect of replacing CM with DLL on FCR being significantly ($p<0.05$) higher in rams fed with T3 than those reared on the other treatment diets.

As shown in Table 5, the intake of nutrients from hay did not differ among treatment diets. However, the nutrient intake from the CM and DLL significantly differed between treatments. The intake of all nutrients from CM significantly ($p<0.001$) reduced with increased supplementation of DLL while it consistently increased for DLL. The total intake of CP increased across treatment diets and was significantly higher for T3, T4 and T5 than T1 and T2 diets. However, the EE intake significantly reduced across treatment diets.

In vitro gas and methane production

The production of *in vitro* GP and CH_4 as well as estimated parameters linearly and significantly reduced with increasing supplementation levels of DLL (Table 6). Accordingly, the values of GP and CH_4 were lowest in T4 and T5 diets being different ($p<0.05$) from those of T1 and T2. The T5 diet had the lowest value in both parameters being different ($p<0.05$) from T1, T2, and T3 treatment diets. Although not significant, the proportion of CH_4 to the total GP consistently reduced across treatment diets. The ME value was higher ($p<0.05$) in T1 and T2 diets than that of T4 and T5. No significant difference in ME was observed between T1, T2 and T3 diets as well as among T4 and T5 diets. Although the value of DOM in T1 was higher ($p<0.05$) than that of T4 and T5 diets, it was similar with T2 and T3 diets. The DOM value in T2 was also similar among T3 and T4 diets but was higher than that of T5. The DOM value did not differ among T4 and T5 diets. The DLL had the lowest GP, CH_4, CH_4:GP, and ME values being significantly different from all treatment diets. However, the DOM value of DLL was similar with that of T5.

Table 2. Analyzed proximate composition (g/kg DM) of grass hay, dried *Leucaena leucocephala* leaf (DLL), control (T1) and experimental diets (T2-T5).

Nutrients	T1	T2	T3	T4	T5	Gras hay	DLL
Ash	53.0	55.0	57.0	58.0	58.0	88.0	80.0
Crude protein	165	174	180	186	192	60.0	265
Ether extract	76.0	63.0	61.0	59.0	54.0	10.0	30.0
NDFom	267	270	281	275	279	682	368
ADFom	111	116	113	119	124	448	194
ADL	34.0	40.0	46.0	51.0	56.0	60.0	108
Cellulose	233	230	235	224	223	622	260
Hemicelluloses	156	154	168	156	155	234	174
NFC	438	436	419	417	411	160	248

aNDFom = neutral detergent fiber without residual ash after amylase treatment; ADFom = acid detergent fiber without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrate

Table 3. Analyzed mineral composition of grass hay, dried *Leucaena leucocephala* leaf (DLL), the control (T1) and experimental diets (T2-T5).

Minerals	T1	T2	T3	T4	T5	Hay	DLL
Major minerals (g/kg DM)							
Phosphorous	6.9	6.7	6.0	5.7	5.3	1.3	1.8
Calcium	1.6	1.6	3.8	4.6	5.7	6.0	16.0
Magnesium	3.2	3.1	3.2	3.3	3.3	2.6	3.8
Potassium	9.1	8.9	9.7	9.9	10.3	8.2	13.8
Sodium	3.0	2.4	1.8	1.7	1.5	1.6	0.1
Trace minerals (mg/kg DM)							
Copper	11.1	11.1	10.4	9.50	9.00	4.2	4.50
Iron	826	736	70.3	638	667	1869	240
Manganese	108	101	101	92.2	93.9	130	73.9

Table 4. Effect of substitution of concentrate mix with dried *Leucaena leucocephala* leaf on feed intake, live weight and gains and feed conversion efficiency of rams (kg/head).

Live weight and gain	T1	T2	T3	T4	T5	SEM	P-value
Initial weight	19.8	20.0	20.5	19.8	20.2	0.64	0.915
Final weight	26.2	26.4	25.5	26.1	26.5	0.74	0.893
Total weight gain	6.35	6.38	4.98	6.33	6.30	0.45	0.163
Daily gain (g/head)	69.8	70.1	54.7	69.6	69.2	7.05	0.470
Total feed intake	81.0	79.7	82.5	80.8	79.1	1.31	0.470
Daily feed intake (g/head)	889	876	906	888	869	14.5	0.645
FCR (kg feed/kg gain)	13.0 ^{b}	13.5^{b}	16.8^{a}	12.8 ^{b}	12.6 ^{b}	0.99	0.038

^{a,b} Means between level of supplementations with different superscript letters are significant

FCR = feed conversion ratio; SEM = standard error of the mean

Table 5. Intake of nutrients from hay, concentrate mix and dried *Leucaena leucocephala* leaf.

Nutrient intake (g/head/day)	T1	T2	T3	T4	T5	SEM	P-value
Hay							
Dry matter	541	502	529	523	495	11.9	0.068
Crude protein	33.6	31.2	32.9	32.5	30.7	0.74	0.062
Ether extract	5.60	5.20	5.48	5.41	5.13	0.12	0.068
NDF	382	354	373	369	349	8.41	0.064
ADF	251	233	245	243	230	5.56	0.069
Concentrate mix							
Dry matter	332^{a}	316^{b}	298^{c}	282^{d}	266^{e}	0.01	<0.001
Crude protein	57.2^{a}	54.3^{b}	51.3^{c}	48.6^{d}	45.7^{e}	0.01	<0.001
Ether extract	26.3^{a}	25.0^{b}	23.6^{c}	22.4^{d}	21.1^{e}	0.01	<0.001
NDF	92.5^{a}	87.9^{b}	83.0^{c}	78.6^{d}	74.0^{e}	0.02	<0.001
ADF	38.5^{a}	36.5^{b}	34.5^{c}	32.7^{d}	30.8^{e}	0.08	<0.001
L. lecocephala leaves							
Dry matter	-	16.9^{d}	33.8^{c}	50.8^{b}	67.7^{a}	0.04	<0.001
Crude protein	-	4.60^{d}	9.2 ^{c}	13.8^{b}	18.4^{a}	0.02	<0.001
Ether extract	-	0.52^{d}	1.04^{c}	1.56^{b}	2.08^{a}	0.01	<0.001
NDF	-	6.37^{d}	12.7^{c}	19.1^{b}	25.5^{a}	0.01	<0.001
ADF	-	3.36^{d}	6.72^{c}	10.1^{b}	13.4^{a}	0.03	<0.001
Total intake							
Dry matter	873	834	861	856	839	11.4	0.134
Crude protein	90.7^{b}	90.1^{b}	93.4^{a}	94.9^{a}	95.5^{a}	0.70	<0.001
Ether extract	31.9^{a}	30.7^{b}	30.2^{c}	29.4^{d}	28.4^{e}	0.12	<0.001
NDF	474	449	469	467	456	8.01	0.197
ADF	290	273	287	285	279	5.32	0.189

$^{a-e}$ Means between level of supplementations with different superscript letters are significant

Table 6. ***In vitro*** **gas and methane productions, metabolizable energy and digestible organic matter contents of dried** ***Leucaena leucocephala*** **leaf (DLL), control (T1) and experimental diets (T2-T5).**

Parameters	T1	T2	T3	T4	T5	DLL	SEM	P-value
24 h gas production (ml/g DM)	285^{a}	274^{ab}	253^{ab}	237^{bc}	201^{c}	144^{d}	9.07	<0.001
Methane (ml/g DM)	48.8^{a}	47.5^{ab}	42.5^{ab}	40.0^{bc}	33.3^{c}	17.1^{d}	1.89	<0.001
Methane (% of total gas)	17.3^{a}	17.1^{a}	16.8^{a}	16.7^{a}	16.5^{a}	11.7^{b}	0.39	<0.001
ME (MJ/kg DM)	12.3^{a}	12.2^{a}	11.3^{ab}	10.9^{bc}	9.80^{c}	8.12^{d}	0.25	<0.001
Digestible organic matter (%)	76.9^{a}	74.5^{ab}	71.7^{ab}	69.2^{bc}	63.1^{cd}	57.6^{d}	1.62	<0.001

$^{a-d}$ Means between level of supplementations with different superscript letters are significant
ME = metabolizable energy; DM = dry matter

DISCUSSION

Nutrient compositions of experimental diets and *L. leucocephala* leaf

Proteinaceous legumes such as *L. leucocephala* are abundantly available in the tropics and considered as high-quality leguminous forage due to its high protein content with good amino acid profile (Melesse *et al*., 2019; De Angelis *et al*., 2021). The usage of these forages in the animal feed showed promising result in growth rate, dry matter digestibility and DM intake (Barros-Rodríguez *et al*., 2015). The contents of ash, CP, NDFom, ADFom and ADL increased with increasing substitution levels of CM with that of DLL, which are justified because the DLL is relatively rich in these nutrients. Similar findings were reported by Montoya-Flores *et al.* (2020) who observed a consistent increase of ADF and ADL due to increased inclusion levels of (120, 240, 360 g/kg DM) of dried leucaena leaf in the diets of crossbred heifers.

The CP content of DLL (265 g/kg DM) is more than sufficient to maintain optimum rumen ammonia N levels and improve protein supply at the duodenum (Jetana, 2017; Leketa *et al.*, 2019). However, DLL is relatively low in ME, EE and NFC, which suggests for the need of energy supplements that could be used to capture the excess rumen degradable protein and provide more microbial protein and ME to the animal, further increasing body weight gain or milk production. This approach has been tested in grazing cattle and in cut-and-carry systems in Australia and Indonesia. In both systems, body weight gain and milk production increased with the addition of supplements fermentable ME sources such as cereal grains and cassava, molasses, soybean hulls and pulps and rice or wheat bran, which are easily accessible by the smallholder farmers (Panjaitan *et al.*, 2014; Dahlanuddin *et al.*, 2018; Harper *et al.*, 2019). In the present study, findings suggest that supplementation of rams with DLL along with homemade concentrate mix can benefit small-scale farmers who cannot afford other expensive protein sources such as soybean and sunflower meals in improving the performance of their animals.

Nutrient intake and growth performances

In the current study, the feed intake was similar across treatment groups, which suggest that the voluntary intake of rams was not affected by the DLL supplementation. Similar findings were reported for sheep supplemented with different levels of Moringa pod (Melesse *et al.*, 2017). Leketa *et al.* (2019) reported that the body weight of dairy goats was not affected when fed a total mixed ration supplemented with leucaena leaf, which is also consistent with the current observation. The body weight gains of the non-supplemented and supplemented rams were similar with increasing levels of DLL, which could be associated with higher digestibility coefficients of CP. This observation concurs with that of Adegun and Aye (2013) and Melesse *et al.* (2017). The amino acid pattern of leucaena leaf is reported to be comparable with that of soya bean (Melesse *et al.*, 2019). These properties in leucaena could be further responsible for similar body weight and gain observed in rams supplemented with DLL. Supplementation with *L. leucacepha* leaves during the dry season had shown a positive effect on the growth rate and reproductive performance of goats (Mataveia *et al.*, 2019). The FCR did not vary (p>0.05) among T1, T2, T4 and T5 diets while it was significantly higher in T3 than the other treatments, which might be associated with health condition of rams reared on this diet.

While the total CP intake linearly increased ($P < 0.001$) with the inclusion of DLL, the intake of DM, NDF and ADF were not affected ($P > 0.05$). These findings are similar with those reported by Piñeiro-Vázquez *et al.* (2017) in heifers fed with low-quality forage supplemented with *L. leucocephala*. Intake of DM, CP, EE, NDF and ADF from leucaena leaf significantly increased while the intake of these nutrients decreased ($P<0.05$) which is associated with the amount of CM replaced by DLL. The increased total intake of CP with increasing levels of DLL also agrees with the reports of Gebregiorgis *et al.* (2012) and Melesse *et al.* (2015) who observed increased CP intake with increasing levels of Moringa leaf supplementation in small ruminants fed a basal diet of grass hay. The increased CP intake with increasing levels of DLL supplementation may possibly be due to the lower EE and higher CP contents of the leucaena leaf. Improved intake through dietary protein supplementation might be associated with increased N supply to the rumen microorganisms. Moreover, the digestibility of the protein in leucaena foliage has been reported to be 63% as measured *in vivo* (Barros-Rodríguez *et al.*, 2015).

Although not significant, the total intake of NDF and ADF slightly decreased with increased levels of DLL supplementation, which might have been influenced by the low amount of metabolizable energy contained in the DLL. The decrease of NDF and ADF might be also associated with low level of structural carbohydrates (NDF, ADF, cellulose and hemi-cellulose) in leucaena leaf and these results are in good agreement with those of Piñeiro-Vázquez *et al.* (2017). This observation further concurs with the findings of Montoya-Flores *et al.* (2020) who observed a consistent decrease of NDF consumption in heifers supplemented with leucaena leaf. However, these authors reported an increase of ADF consumption because of increased leucaena leaf in the diets of crossbred heifers. The reduction of EE with increasing supplementation of DLL is consistent with the findings of Montoya-Flores *et al.* (2020), who observed similar trends with increased levels of leucaena leaf supplementation to crossbred heifers. The decrease in EE intake might have been influenced by the low amount of fat contained in the DLL.

Soares *et al.* (2018) recommended that the leucaena foliage could safely be fed to growing Bali cattle as the sole component of the diet provided adequate biomass is available. On the other hand, other researchers reported that leucaena leaves cannot be used as sole feed in ruminant diets as the presence of mimosine and tannin compounds in the leaf could limit its utilization (Aye and Adegun, 2013; Barros-Rodríguez *et al.*, 2014). Moreover, the intake of leucaena foliage in high quantities may be limited due to the high level of nitrogen in the diet which may lead to a nutritional imbalance (protein-energy ratio), affecting microbial protein synthesis, and resulting in the formation of high levels of ammonia in blood which could affect the voluntary intake (Calsamiglia *et al.*, 2010).

Methane production, metabolizable energy and digestible organic matter

The GP, CH_4, DOM and ME values linearly reduced with increasing substitution levels of DLL. The values were particularly lowest in T5 and T4 diets. This could be due to the increased supplementation levels of DLL, which showed the lowest GP, CH_4, CH_4 to GP ratio, ME and DOM values as compared with those of the treatment diets. The low GP observed in DLL is consistent with the findings of Barros-Rodríguez *et al.* (2015) who reported low *in vitro* GP in diets containing leucaena leaf. This might be associated with the relatively low level of energy as well as high content of CP, NDF and ADF in the DLL which both could result in reduced gas production *in vitro* (Melesse *et al*., 2019). Suha Uslu *et al.* (2018) reported that NDF and ADF contents of legume plants were negatively correlated with gas production, ME and OMD. Moreover, the presence of condensed tannins in leucaena leaves could be responsible for the low gas production which could be related to reduced total fermentable fraction as reported by Albores-Moreno *et al.* (2019). This is because condensed tannins have the ability of modulating the enzymatic and microbial activity on organic matter, thereby causing a reduction in fermentation (Gunun *et al.*, 2018).

In the current study, a significant positive correlation was observed between CH_4 production and ME content of the experimental rations (data not shown). Accordingly, as the ME concentration in the treatment diets decreased due to increased levels of DLL supplementation, the corresponding methane production values reduced. Yan *et al.* (2010) reported similar observations in which CH_4 was positively correlated with that of dietary ME concentration. These authors suggested that selecting of dairy cows with high-energy utilization efficiencies would offer an effective approach to reducing enteric CH_4 emission rates. Although no significance differences in feed intake were observed among treatments in the current study, Hammond *et al*. (2013) observed that CH_4 emissions from sheep and dairy cattle showed an inverse relationship between feed intake and CH_4 production, which suggests opportunities for reducing CH_4 emissions from feed eaten and per unit of animal production.

Mataveia *et al.* (2019) and Melesse *et al.* (2019) reported from 10 to 11 MJ/kg DM of ME in *L. leucocephala* leaves, which is higher than observed in the current study. The energy content of tree leaves is mainly dependent on the maturity of the leaf and season, which might be responsible for such large variations (Melesse *et al.*, 2012). As the age of the tree increases the contents of the structural carbohydrates will increase, which could reduce the gas production potentials of the leaf materials. This situation has been observed in the chemical composition of the DLL in the current study in which the NDF, ADF and ADL contents were higher than reported by Melesse *et al.* (2019). The season in which the leucaena leaf samples were collected could also affect the content of structured carbohydrates. Leaf samples collected during the dry season may contain more fibrous material being highly lignified than those harvested in the rainy season. Moreover, since leucaena leaves contain considerably higher content of tannin phenols (Melesse *et al.* (2019), this might have limited the *in vitro* GP and consequently, degradation of DM and NDF components as reported by Simbaya *et al.* (2020).

The replacement of CM with DLL considerably reduced the CH_4 production by 68% (48.8 in the T1 diet vs. 33.3 ml/g DM in T5). These observations are consistent with those of Montoya-Flores *et al.* (2020), who reported reduced CH_4 emission in crossbred heifers fed on increasing levels of leucaena leaf supplementation. Piñeiro-Vázquez *et al.* (2017) studied the effect of leucaena leaf supplementation to poor quality forage in heifers and concluded that its inclusion had reduced the energy losses in the form of methane emissions. The methane reducing ability of leucaena leaf might be associated with its anti-methanogenic properties principally due to the presence of condensed tannins (Patra *et al.*, 2010). Recently, Melesse *et al.* (2019) reported relatively higher level of soluble condensed tannin in leucaena leaf (23.1 g/kg DM). Previous *in vitro* studies reported even higher levels of condensed tannins in leucaena leaves ranging from 33 to 61 g/kg DM (Tan *et al.*, 2011; Soltan *et al.*, 2012).

Tannins in general appeared to protect protein from rumen microbial degradation and reduce methane production though effects between source (plant species) and experiments are still variable (Jayanegara *et al.*, 2011). Moreover, the leucaena leaves have been shown to improve the metabolic protein supply of ruminants due to their relatively high contents of protein as observed in the current study. Previous works have indicated that because of their protein binding capacity, the high protein content of leucaena leaves and the action of the condensed tannins works in synergy to prevent protein from ruminal degradation by yielding by-pass protein to small intestine (Soltan *et al.*, 2012; Barros-Rodríguez *et al.*, 2015). In addition, Dalzell *et al.* (2012) reported that leucaena leaf contains mimosine, which acts against the activities of gram-positive bacteria and fungi suggesting that this compound might be involved in the CH_4 mitigating properties of leucaena leaves. Furthermore, Soares *et al.* (2012) have suggested 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 allowing ruminants to reduce or block the effect of astringency, which could lead to a reduction of feed intake. It would be however worthwhile to note that in the current study, it is not only the CH_4 production reduced, but also the total gas production as well as the digestibility of organic matter was decreased especially in diets supplemented with higher levels of DLL. Thus, reduction of methane production by means of DLL supplementation is possible, but may impair the overall digestibility of nutrients. This calls for optimizing the level of leucaena leaf supplementations to overcome such problems.

CONCLUSIONS

The inclusion of DLL in the diet of rams significantly increased CP intake while reducing the *in vitro* methane production without affecting the voluntary intake and growth performance traits. It could thus effectively substitute the concentrate mix upto 20% by serving as alternative protein and mineral sources. However, the low energy content with high CP concentration in DLL suggests that easily accessible energy supplements could be used to capture the excess rumen degradable protein and provide more microbial protein and energy to the animal, particularly such practices are useful if it is used for fattening purposes. Smallholder farmers could be encouraged to grow leucaena tree in their homesteads or farms to take the advantage of using the leaf to supplement poor quality forages.

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Author contribution. A. Tadesse - Data curation, Conceptualization, Methodology, Writing-original draft., **A. Melesse** – Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing, Supervision., **M. Rodehutscord** – Writing – review & editing, Supervision

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