Synergistic effects of *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* leaves on weight gain and predicted enteric methane emission in sheep

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Abstract

The digestive tract of ruminants though unique in the utilisation of low quality feed materials also emits methane, a potent greenhouse gas with a global warming potential of 25 times that of carbon-dioxide. Climate smart livestock production necessitated the use of browse leaves with the potential to inhibit methanogens and protozoa activity in the rumen and reduce methane emission. Thus, sixteen forest type ram lambs (13.94 ±1.02) were fed *Albizzia lebbek* (AL)+*Moringa oleifera* (MO)+*Millettia thonningii* (MT), AL+MO, AL+MT and MO+MT for twelve weeks. Data collected were feed intake, digestible energy, weight gain, energy loss, nitrogen loss and methane emission by sheep. Rumen methane production (MJ/d) was estimated using a model equation: Methane = 8.25 + 0.07 x Metabolisable Energy Intake. Sheep fed AL+MO had the highest ($p<0.0001$) average daily gain whilst those fed AL+MO+MT recorded the lowest ($p<0.0001$). Sheep fed AL+MO+MT emitted the lowest ($p<0.0001$) methane and those fed AL+MO emitted the highest ($p<0.0001$) methane. The dry matter intake, digestible energy, energy intake, faecal energy losses, average daily gain, feed conversion efficiency, faecal nitrogen losses, urinal nitrogen losses and methane production were in the range of 588.9-651.5g/d, 15.1-16.7 MJ/kg DM, 17.3-18 MJ/kg DM, 1.26-2.56 MJ/kg DM, 69.94-83.33 g/d, 8.66-11.10 g/d, 60.43-88.01g/animal/d, 11.22-16.99 g/animal/d and 3-5.34 MJ/d respectively. This study demonstrates the synergic action of browse leaves as a climate smart approach in reducing methane production and improving the productivity of sheep. Furthermore, this feeding strategy promotes uniform utilization of browse species leading to the sustainable use of preferred browse species as no one browse species will be heavily utilised.

Keywords: climate smart livestock production, condensed tannins, energy losses, nitrogen losses, rumen

Introduction

Ruminants occupy a unique position in the food chain because of their special digestive system which converts otherwise inedible plant materials into nutritious feed. This unique digestive tract however emits methane during feed fermentation which is a potent greenhouse gas that contributes to climate change (Scholtz et al., 2013; Timpong-Jones et al., 2023). Methane produced in the rumen accounted for thirty-nine percent of worldwide greenhouse gas from livestock production (Gerber et al., 2013). The predictable effects that climate change has on agriculture includes temperature and precipitation fluctuations which negatively influence the existing agricultural systems. For instance, global warming of 1.5 °C was modelled to cause an average rise in temperature above the pre-industrial baseline in Botswana of 2.2 °C and 2.0 °C in Namibia (Maure et al., 2018). These predictions, if actualized, would reduce forage yield and quality (Lee et al., 2017). In reality, increased temperatures and reduced amounts of rainfall are already being experienced in Southern Africa and the productivity of arable lands and rangelands are declining (Batisani and Yarnal, 2010).

Ruminants emit methane through enteric fermentation during digestion of forages. Methane represents a loss of carbon from the rumen and therefore an unproductive use of dietary energy (Goel and Makkar, 2012). Several studies therefore sought for alternate approaches to reduce methane emission while maintaining productivity. A promising method for minimizing methane release from livestock is by enhancing feed use efficiency and productivity (Hristov et al., 2013). Such improvement will be good for the environment and will also boost the profit margins of farmers (Yisehak et al., 2014). Browse leaves such as *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* have been fed solely to sheep and there was reduction in methane emission and improvement in weight gain compared to urea treated rice straw in Ghana (Sarkwa et al., 2020). Additionally, Sarkwa et al. (2023) reported that *Albizzia lebbek*, *Moringa oleifera* and *Millettia thonningii* contained condensed tannins that inhibited the activities of protozoa and methanogens. There are two modes of action of tannins on enteric methane production: a direct influence on methanogens in the rumen and an indirect effect on the production of hydrogen (Tavendale et al, 2005; Martin et al., 2010). These tannins cause death of cells by forming a complex with steroid in the protozoa cell membrane (Cheeke, 1999).

Ruminant farmers may likely accept and practice any methane emission mitigation strategy if the intervention is simple, not cumbersome, familiar and not likely to negatively affect their output, but rather improve animal productivity. It is against this background that, this study explored the use of diets formulated from the combination of dried leaves of three different browse species as feed for sheep.

The objective of this study was therefore to determine performance parameters and estimate methane emission after feeding sheep on diets formulated from the combinations of dried leaves of the three different browse species.

Materials And Methods

Study Location

The study was carried out at the Livestock and Poultry Research Centre, University of Ghana, Legon (5˚68'N, 0˚10'W). Total annual rainfall ranges from 508 mm to 743 mm. Rainfall is bimodal in pattern, with the major and minor rains in June-August and September-October respectively. Temperature varies between 32.22 ºC and 34.49 ºC (Sarkwa et al., 2020).

Browse species and experimental diets

Albizzia lebbek (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) leaves were harvested from mature trees in rangelands within the coastal savannah zone and sun dried for 48 hours. There were four diets namely: AL+MO+MT (Control), AL+MO, AL+MT and MO+MT. The diets were mixed in equal proportions based on the constituents of a particular diet.

Animals and management

Prior to the commencement of the study, sixteen forest type (Djallonke) ram lambs of average weight 13.94 kg±1.02 were kept in individual pens (2 m x 1.5 m) and were treated against ectoparasites (sprayed with cypermetrin: 12% pour on; Hebei New Century Pharmaceutical Company Limited, China) and endoparasites (albendazole: Oral suspension 10%; Hebei New Century Pharmaceutical Company

Limited, China). At the commencement of the study, the sixteen forest type (Djallonke) ram lambs were put into four groups with four animals per group and each group randomly allocated to a treatment (diet). The feeding trial lasted for 12 weeks preceded by a 14-day adaptation period to the pens, faecal bags and diets. Live weights were measured every fourteen days after fasting for twelve hours. Individual feed intake were recorded daily. Water was provided *ad libitum* to all animals throughout the study. Animals were fitted with faecal bags for faeces collection. Faeces were collected once every two days for 24 hours (08.00 till the next day 08.00) to estimate *in vivo* digestibility and rumen methane emissions. Urine was collected for nitrogen loss estimation according to the method of Yeboah et al. (2017).

Laboratory analyses

Samples of the three dried browse leaves were ground and mixed in equal proportions for the various combinations before analyses. Proximate, fibre components and condensed tannins (CT) were evaluated using the methods of AOAC (2016), Goering and Van Soest (1970) and butanol-HCl method as described by Iqbal et al. (2011) respectively. Energy was determined by using bomb calorimeter (Parr 6100 Calorimeter, Wagtech International). Feed samples, feed refusals, and faecal outputs were analysed for DM by drying at 55^{o}C in an oven. Organic matter (OM) was calculated as DM less the residual ash obtained after combustion for 6 hours at 550 ^{o}C. Thus, OM in the feed and faeces, dry matter intake (DMI) and Digestible Organic Matter in Dry Matter (DOMD) were estimated.

Intake, digestibility, nitrogen and energy loss estimations

Nutrient intake was estimated based on the difference between total nutrient in feed and nutrient content of refusals. Digestibility coefficients were calculated as: [(kg of nutrient ingested-kg of nutrient excreted) / (kg of nutrient ingested)] x 100. Dry matter intake (DMI) and digestible organic matter in dry matter (DOMD) values were fitted into Ministry of Agriculture, Food and Fishery (MAFF) (1984) equation to estimate metabolisable energy intake (MEI) for each diet as follows:

$$\text{MEI} = \text{DOMD} \times 0.15 \times \text{DMI}$$

Digestible energy (DE) was calculated by deducting faecal energy losses (FEL) from energy intake (EI) according to Sun et al. (2012). Average daily gain was calculated as total weight gained divided by number of days fed per animal. Feed conversion efficiency was estimated as total weight gained (TW) divided by total feed consumed (FC) per animal multiplied by 100 (TW/FC * 100).

Faecal nitrogen losses (FNL), urinary nitrogen losses (UNL) and faecal energy losses (FEL) were estimated by firstly averaging the total faecal output per day or total urine output per day for each animal. Secondly, procedure of AOAC (2016) was used to estimate N content in the faeces and urine and bomb calorimeter was used to determine energy in the faeces. The amount of N or energy in the urine or faeces determined was used to estimate the amount of N or energy loss in the average of total amount of faeces or urine per animal.

Methane emission estimation

Rumen methane emission was estimated using a model equation by Mills et al. (2003) that uses Metabolisable energy intake (MEI). This model is recommended by USDA (2014). The model is as follows:

$$\text{Methane (MJ/day)} = 8.25 + 0.07 \times \text{MEI (MJ/day)}$$

The model equation for methane estimation was developed based on large amount of data collected from the respiratory chamber method, which is considered as the standard for measuring methane. Mills et al. (2003) developed three model equations but this was chosen for the current study because it involved measuring digestibilities, energy and feed intakes which are very important factors influencing methane production. According to USDA (2014), this model equation is adaptable to various diets and intake levels.

Statistical analysis

Data obtained on chemical composition were analysed as completely randomised design and subjected to ANOVA using GenStat for windows version 12.1 (VSN International Ltd, 2009) according to the statistical model below:

$$Yij = \mu + Ti + Eij$$

Yij is the response variable such as DM, CP, ash, Condensed tannins, NDF, ADF, Cellulose and Lignin

μ is the overall mean; Ti is the different browse leaves; Eij is the residual error.

Significant difference in the means were separated using Student Newman Keuls Test. Time-series data of methane yield estimate was subjected to restricted maximum likelihood (REML) to calculate repeated measures using the statement " repeated" within Proc Mixed procedure of SAS (2002-2012) to calculate variances and covariances (Holand, 2006). The compound symmetry (CS) structure was used for within sample variance and covariance structure for these data. The model for methane yield included diets as well as interaction between diets and time. Animal identity and initial methane yield was also used in the random effect statement. To tease out the nature of trends in time series parameters, the effect of time was broken down into polynomial contrasts. Time was dropped from the class statement, making it a continuous variable and then included three times on the model statement to construct non-orthogonal polynomial items (Wolfinger and Chang, 1998).

Results

Diet composition, intake and growth performance

Chemical composition of diets used are presented in Table 1 whilst Table 2 shows intake, digestibility and growth data. The range of crude protein, ash, acid detergent fibre, neutral detergent fibre, cellulose and lignin contents of the diets were 885.4 - 894.9 g/kg, 266.2-304 g/kg DM, 94.6-135.6 g/kg DM, 261.4 -356.9 g/kg DM, 287.2- 477.5 g/kg DM, 124.3-198.1 g/kg DM and 123.9-161.7 g/kg DM respectively (Table 1). Condensed tannins levels were from 1.07 g/kg DM to 1.20 g/kg DM (Table 1). The highest ($p<0.0001$) dry matter intake (DMI) was recorded for sheep fed MO+MT whilst the lowest ($p<0.0001$) was for AL+ MO+MT (Table 2). Energy intake (EI) was highest ($p<0.0001$) for sheep fed AL+MT whilst the lowest ($p<0.0001$) was recorded for AL+ MO but did not differ ($p>0.0001$) from AL+MO+MT and MO+MT (Table 2). Digestible energy (DE) was lowest ($p<0.0001$) for sheep offered AL+ MO+MT and highest ($p<0.0001$) for AL+MT (Table 2). Diet AL+MO had the highest ($p<0.0001$) average daily gain (ADG) and animals consuming it were the most ($p<0.0001$) efficient feed

TABLE 1

Chemical composition of combined browse diets

Component (g/kg DM)	Diets			
	AL+MO+MT	AL+MO	AL+MT	MO+MT
Dry matter	889.0^{a} ± 0.2	885.4^{a}±3.4	894.9^{a}±6.1	885.9^{a}±2.9
Crude protein	286.8^{b} ±0.9	304.0^{c}±18	266.2^{a}±19.7	286.4^{b}±0.5
Ash	117.2^{b}±1.48	127.3^{c}±8.62	94.6^{a}±4.08	135.6^{d}±6.92
Condensed tannins	1.20^{a}±0.32	1.13^{a}±0.013	1.20^{a}±0.57	1.07^{a}±0.76
Acid detergent fibre	301.5^{b}±16.25	261.4^{a}±56.35	351.2^{c}±33.45	356.9^{c}±39.15
Neutral detergent fibre	418.6^{c}±43	320.4^{b}±55.2	477.5^{d}±100.6	287.2^{a}±88.4
Cellulose	166.5^{b}±2.7	124.3^{a}±39.5	198.1^{c}±32.2	168.3^{b}±4.5
Lignin	143.2^{b}±0.97	147.9^{b}±3.73	161.7^{c}±17.53	123.9^{a}±20.27

Means in the same row with different letters differ significantly ($p < 0.05$)

AL-*Albizzia lebbek*, MO-*Moringa oleifera*, MT-*Millettia thonningii*

TABLE 2
Intake, growth and methane production of sheep fed combined browse leave diets

Parameter	Treatments					Treatment Effects
	AL+MO	AL+MO+MT	AL+MT	MO+MT	SEM	Significance
DMI(g/d)	623.20[b]	602.57[a]	635.52[c]	663.95[d]	7.630	***
Energy intake (MJ/kgDM)	18.00[a]	18.38[a]	20.51[b]	18.59[a]	0.5662	***
Digestible Energy (MJ/kgDM)	16.03[b]	15.38[a]	16.88[d]	16.42[c]	0.2180	***
Faecal Nitrogen Loss(g/anim/day)	60.32[a]	71.10[b]	87.94[d]	76.92[c]	0.984	***
Urinal Nitrogen Loss(g/anim/day)	11.73[a]	11.27[a]	11.50[a]	17.00[b]	0.305	***
Faecal Energy Loss (MJ/kg DM)	9.01[a]	10.12[b]	11.39[c]	10.42[b]	1.284	***
Final Liveweight (kg)	16.80[b]	15.88[a]	16.85[b]	18.52[c]	0.8721	***
FCE (%)	9.29[a]	10.39[a]	11.68[b]	10.74[a]	1.312	***
ADG (g/d)	84.33[b]	70.77[a]	71.39[a]	79.72[b]	7.003	***
Methane (MJ/d)	5.16[d]	3.18[a]	4.64[c]	3.82[b]	0.1753	***

***P<0.0001, AL-*Albizzia lebbek*, MO-*Moringa oleifera*, MT-*Millettia thonningii*;

converters (Table 2). The lowest (p<0.0001) ADG was recorded for animals consuming AL+ MO+MT but did not differ (p>0.0001) from AL+MT whilst the least (p<0.0001) efficient feed converters were animals fed AL+MT (Table 2). Regarding faecal nitrogen losses and faecal energy losses, animals fed AL+MT registered the highest (p<0.0001) and those fed AL+MO registered the lowest (p<0.0001) (Table 2). In the case of urinal nitrogen losses, sheep fed AL+MO+MT had the lowest (p<0.0001) but was not different (p>0.0001) from AL+MO and AL+MT and the highest (p<0.0001) was recorded by sheep fed MO+MT.

Trends of DMI and ADG are shown in Figures 1 and 2. In general, DMI improved over the duration of feeding (Figure 1). Sheep fed the combined browse leaves gained weight from the beginning to the end of the feeding period (Figure 2).

Nitrogen and Energy Losses

The faecal nitrogen losses (FNL), Urine nitrogen losses (UNL) and Faecal energy losses (FEL) of sheep fed combined browse diets ranged from 71.32 to 88.01 g/animal/day, 11.22 to 16.99 g/animal/day and 1.26 to 2.56

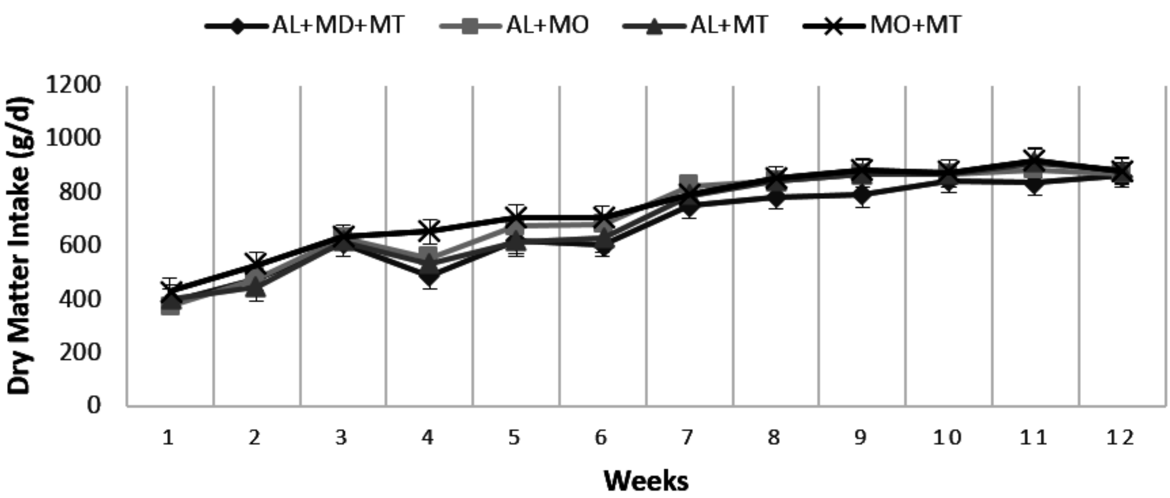

Figure 1 Trends of dry matter intake of sheep fed combination of *Albizzia lebbek* (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) browse for 12 weeks

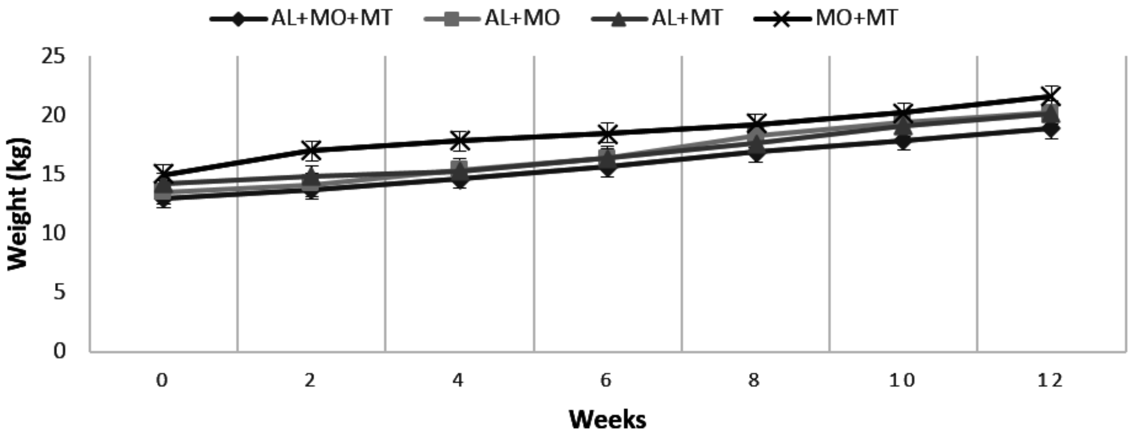

Figure 2 Trends of weight gain of sheep fed combination *Albizzia lebbek* (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) browse for 12 weeks

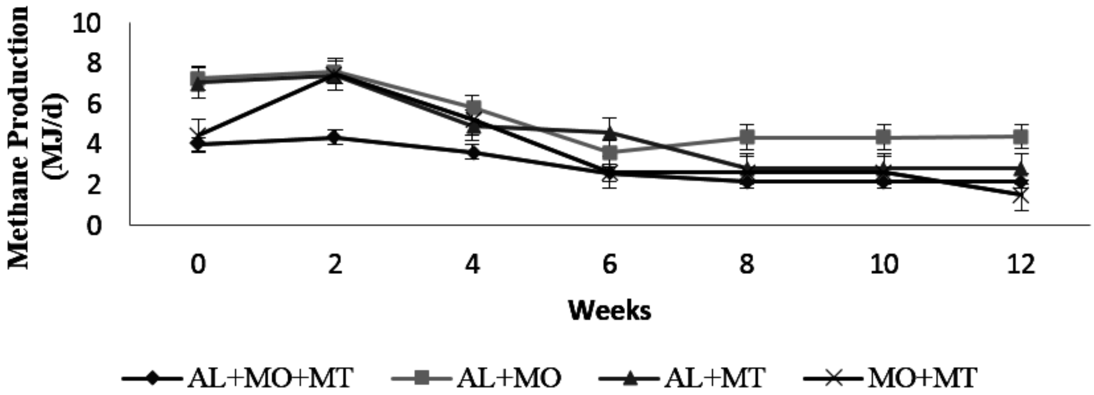

Figure 3 Trends of methane production of sheep fed combinations of *Albizzia lebbek* (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) for 12 weeks

MJ/kg DM respectively (Table 2). Sheep fed AL+MO, AL+MO+MT and AL+MT recorded the lowest ($p < 0.0001$) values for FNL, UNL and FEL respectively (Table 2).

Predicted Enteric Methane production
Estimated enteric methane production was lowest ($p<0.0001$) in sheep fed AL+ MO+MT whilst AL+MO had the highest ($p < 0.0001$) (Table 2). Trends for methane production are shown in Figure 3. Methane production was higher at the beginning than at the end of feeding for all browse leave combinations.
Table 3 shows statistical influence of the main effects and their interactions on performance indicators and methane production. Methane production was significant ($p<0.0001$) in the case of linear, cubic, quadratic and interaction between treatment and time. Quadratic was not significant in most of the parameters measured whilst linear was not significant only for faecal nitrogen losses (Table 3).

Discussion

The DMI recorded for the combinations of the browse leaves corroborates the results of Papachristou and Platis (2011) and Idan et al. (2023) that intake of combined browse leaves was superior to intake of sole browse leaves. However, the levels of intake recorded in this study were different from that reported by Papachristou and Platis (2011). The combinations of two browse leaves (equal proportions) recorded higher intakes than the combination of three browse leaves (equal proportions). The difference in the intake

TABLE 3

Statistical influence of the main effects and their interactions on performance indicators and methane production

Parameter	Orthogonal Contrast			Interaction effects
	Linear	Quadratic	Cubic	Treatment x Time
DMI	***	***	**	*
Energy intake	***	NS	***	***
Digestible Energy	***	NS	*	***
Faecal Energy Loss	**	*	*	*
Faecal Nitrogen Loss	NS	NS	*	***
Urinal Nitrogen Loss	**	NS	NS	***
Liveweight	***	NS	NS	NS
FCE	***	***	*	NS
ADG	***	*	NS	***
Methane	***	***	***	***

NS- not significant, * P<.005, ** P<.001, ***P<.0001

values may be due to differences in browse leaves used (Kermes oak, white mulberry and black locust were used by Papachristou and Platis 2011), the form of offering the browse leaves (dried or fresh; fresh leaves were used by Papachristou and Platis (2011) whilst the current study used dried form), preference levels of the individual browse leaves and the type of ruminant used in the feeding trial (Papachristou and Platis, 2011 used goats).

This study confirms the findings of Papachristou and Platis (2011) that consuming different kinds of browse leaves would enhance intake of feed and some mixtures would be better than others. Idan et al. (2023) reported higher growth rate in sheep fed a supplement made of the combination of two browse leaves than when one browse leave was used. The current study corroborates earlier studies (Papachristou and Platis, 2011;Sarkwa et al. 2020a; Idan et al., 2023) because feeding diets from a combination of browses resulted in higher weight gains than feeding sheep on one browse leave diets. The authors' finding supports the study outcome on nutrient utilization where sheep fed MO efficiently converted nutrients to body tissues than AL+MO+MT and MO+MT. Besides, sheep fed AL efficiently utilized more feed than those fed AL+MT. This may be explained by the poor utilization of MT diet by sheep which might have negatively compromised the positive attributes of AL and MO (negative associative effects) in the MO+MT and AL+MT combinations and thus lowering the efficiency of utilization. With legume and grass combinations, improved intake can be due to higher rate of degradation and passage through the rumen and a quick digestion of the soluble fraction of the legumes (Niderkorn and Baumont, 2009). In this study, each plant in the browse mix may have affected these attributes differently due to their chemical composition.

With regards to ADG, combinations of AL, MO and MT was higher than sole browse leaves (AL, MO or MT) fed to sheep (Sarkwa et al., 2020a). On the average, the feeding of the combination of dried browse leaves to sheep resulted in 81 % more ADG, 4.2 % more feed efficiency and 16.7 % less methane compared to feeding of sole dried browse leaves (Sarkwa et al., 2020a). The energy intake (EI) and digestible energy (DE) recorded in this study were higher than the values reported by Sarkwa et al. (2020a) when the authors fed the same browse leaves solely. The low to moderate faecal nitrogen losses (FNL) recorded in this study may imply that the diets fed to sheep were low in tannins (see Table 1 for Condensed Tannins content) and therefore, majority of the protein were digested and absorbed in the small intestines which may be beneficial to the sheep. The FNL were higher than urinal nitrogen losses (UNL) and this may be because tannins in the browse leaves might have reacted with methanogens and protozoa (Saminathan et al., 2017) and contributed to eliminating them from the rumen through the faeces. This is because Saminathan et al. (2017) found that unfractionated and high molecular weight condensed tannins reduce protozoa population *in vitro*. The results of this study also suggest that diets made from a combination of browse leaves result in lower energy losses from excretion of faeces as compared to data from feeding sole browse leaves (Sarkwa et al., 2020a). Excretion of nitrogen from sheep production especially from urine is an important source of nitrate, ammonia and nitrous oxide responsible for ground water pollution and global warming (Zhao et al., 2016). Manipulating animal nutrition to shift nitrogen from urine to faeces, would be a strategy to mitigate N-losses to the environment. Zhao et al. (2016) found that improving feeding level and metabolisable energy concentration while decreasing nitrogen concentrations improved the efficiency of nitrogen utilization by shifting excretion of nitrogen into faeces rather than urine. Unlike the report by Zhao et al. (2016), this study may have utilised plant secondary metabolites like tannins to shift nitrogen from urine to faeces.

Feeding diets made from combinations of the browse leaves in this study produced lower methane than when sole browse leaves were fed to sheep. This is in line with the findings by Naumann et al. (2015) that the combination of browses produced lower methane than single browses. This study estimated that a diet containing a combination of three browse leaves resulted in the lowest methane production, and this may be due to the different mechanisms of each of the browse leaves to reduce methane emission, such as eliminating protozoa and methanogens in the rumen as a result of tannins (Jayanegara et al., 2015) and influencing the high production of propionate as end product of fermentation (Janssen, 2010). Perhaps browse plants produce other secondary metabolites and essential oils that might antagonise the methanogenic activities of the microbiome or act as hydrogen or electron acceptors to create a sink for hydrogen in the rumen. In addition, this feeding strategy promotes uniform utilization of browse species leading to the sustainable use and preservation of preferred browse species as no one browse species will be heavily utilised.

Conclusion

The combinations of dried leaves of three different browse species had high dry matter and crude protein, low condensed tannins and low to moderate fibre components. Feeding of the combination of the three dried browse leaves to sheep recorded high feed intakes, improvement in weight gain, low to moderate faecal and urinal nitrogen losses, low energy losses and low methane emission. Feeding a combination of these browse species will lessen the amount of rumen methane emission and thus contribute to the reduction of global greenhouse gas emission. Also, this feeding strategy will promote uniform utilization of browse species leading to the sustainable use and preservation of preferred browse species as no one browse species will be heavily utilised.

Ethical Clearance

The management of animals and all other practices were approved by Noguchi Institutional Animal Care and Use Committee of University of Ghana, Legon, Accra (Protocol number 2017-02-2R).

Competing Interest

The authors of this paper declared that they do not have any competing interest for this joint publication.

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