Can feeding dried browse leaves to sheep reduce methane emissions and improve weight gain?

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Abstract

It is important to evaluate whether a specific browse leaf may be able to reduce methane emissions as well as improve weight gain before recommending it to farmers. Therefore, the objective of this study was to determine performance, methane emission and amplification of methanogens and protozoa of sheep fed four different dried browse leaves. Sixteen forest-type ram lambs of 14.38±0.51 kg average weight were used. The animals were fed four dried browse leaves [*Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT)] for six weeks. Genomic DNAs were extracted from methanogen and protozoa strains obtained from rumen fluid and sheep faeces using Quick-DNA™ Fecal/Soil Microbe Miniprep Kit. Sheep fed AL, MO and MT responded by gaining weight but those fed GS lost weight with time. All four browse leaves fed produced low methane and the trend of methane emission was relatively stable. Detecting methanogens and protozoa in the rumen fluid and faeces of sheep is an indication that the browse leaves were able to inhibit the activities of methanogens and protozoa in the rumen and eliminated them through faeces. Therefore, AL, MO and MT can improve weight gain and reduce methane emission while GS can minimise methane emission but cannot improve weight gain. This study highlights the contribution of natural resources to climate-smart approaches in sheep production and reduction of enteric methane production hence minimising the effects of climate change.

Keywords: Amplification, Methane Emission, Methanogens, Polymerase Chain Reaction, Protozoa, Sheep

Introduction

Methane production is a natural by-product of anaerobic respiration and its production serves as the principal electron sink within the rumen. Methane production represents a significant loss of dietary energy and thus reducing enteric methane production may also improve feed efficiency (Beauchemin et al., 2007; Beauchemin, 2015; Hristov et al., 2015). It has been reported that the formation of methane in the rumen causes a loss of digestible energy of about 8-14 % (Cottle et al., 2011; Audsley and Wilkinson, 2014; Millen et al., 2016) which could have been used for productive purposes.

Methane is a more powerful greenhouse gas (GHG) than carbon dioxide, with a global warming potential of 84 times greater on a 20-year duration (Balcombe et al. 2018; IPCC, 2021). However, methane has a shorter atmospheric existence than carbon dioxide (Balcombe et al. 2018; IPCC, 2021). There is,

therefore, an incentive to reduce the effects of climate change by targeting methane to achieve faster outcomes than it would be for carbon dioxide. The livestock sector contributes substantially to climate change, forming 14.5 % of GHG emissions induced by humans (Gerber et al., 2013). According to the United States of America's Environmental Protection Agency (EPA), agriculture is responsible for 9 % to 10 % of total GHG emissions (Dillon et al., 2021). Livestock contributes less than 4 % of direct emissions excluding GHG emissions from fuel and production of feed (Tedeschi, 2022).

Scientists have been looking for ways to reduce methane emissions. A promising method for reducing methane release from livestock is by enhancing the efficiency and productivity of livestock production (Hristov et al., 2013). Greater improvement of livestock production will benefit the environment and will also enhance the profit of farmers (Yisehak et al., 2014). Such improvement include feeding management of ruminants which is sustainable and has been found to include the use of feeding resources from rangelands such as browse leaves. Browse leaves are abundant in Ghana, high in nutritional quality, maintain their nutritional quality regardless of the season and can, therefore, be used for methane mitigation throughout the year (Adogla-Bessa et al 2012; Sarkwa et al., 2020a; 2020b and 2021).

According to Grainger et al. (2009), the use of pasture supplemented with grain to reduce methane production resulted in a thirty percent reduction in methane emission but a ten percent reduction in milk production. In addition, the use of grain as a strategy for reducing methane yield is not morally justifiable in Sub-Saharan Africa and is not economically viable. It is therefore important to evaluate whether leaves from a specific browse plant will be able to reduce methane emissions and improve weight gain before recommending it to farmers. Additionally, it is necessary to minimise enteric methane emissions by using available feed resources to meet production objectives and improve the profit of farmers amidst the problems created by global warming (Sahoo et al. 2021; Sarkar et al. 2021). Therefore, the objective of this study was to determine growth performance, methane emission and amplification of methanogens and protozoa of sheep fed dried leaves from four different browse plants.

Materials and methods

Study location

The study was carried out at the Livestock and Poultry Research Centre , University of Ghana, Legon in the Coastal Savannah of Ghana. Livestock and Poultry Research Centre is geographically located at approximately 5˚68' North latitude and 0˚10' West latitude. Rainfall varies from 508 mm to 743 mm per annum. Rainfall is bimodal in pattern, with the major rains in March to July and minor rains in September to November. Temperature and humidity range between 32.22 ºC and 34.49 ºC and 36.60 % and 73.73 % respectively (Sarkwa et al., 2020).

Collection and processing of the leaves

The browse leaves were harvested from the rangelands within the Coastal Savannah zone of Ghana. Branches were looped from the trees and leaves were taken from the branches and sun- dried for 48 hours. Dried leaves were packed in sacks and stored under a well-

ventilated shed before feeding.

Chemical analysis

Dry matter (DM), ash and crude protein (CP) were determined using the methods of A.O.A.C. (2016). Acid detergent fibre (ADF), Neutral detergent fibre (NDF) and lignin were evaluated using the procedure of Goering and Van Soest (1970). Condensed tannins (CT) were assessed by the method as outlined by Iqbal et al. (2011) and validated by Sarkwa et al. (2023a).

Feeding and animal management

The weights of sixteen forest-type ram lambs (14.38 kg±0.51) were measured to determine weight gains for six weeks after a two- week acclimatization period. The animals were kept in individual pens as described by Sarkwa et al. (2023b). Allocation of animals to pens was through a completely randomised design. Before the experiment began, the animals were treated to control 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) as outlined by Sarkwa et al. (2023b). The sixteen ram lambs were put into four groups with four animals per group (replicates). Each group was randomly assigned to a treatment (experimental diet). There was a two-week pen, hand feeding, drinking from bowls and faecal bags adaptation period. The animals were fed four dried browse species [*Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT)] for six weeks to evaluate feed intake, weight gain, methane emission and rumen microbes (methanogens and protozoa). Animals were fed (3 kg) three times daily (Morning: 1 kg, afternoon: 1 kg and evening: 1kg). Animals had free access to water during the study. Feed intake was measured by weighing the feed refusal and deducting it from the feed offered. Live weight was measured every two weeks after starving the animals for half day. Animals were fitted with faecal bags for collection of the faeces for microbial evaluation and the estimation of MEI. On the final day of the experiment, one sheep from each of the treatments was selected on random basis and slaughtered to obtain rumen fluid for microbial evaluation as described by Martinele et al. (2014), Sangkhom et al. (2017) and Sarkwa et al. (2023a).

Methane emission estimation

Methane production from the four dried browse leaves was measured using the model equation by Mills et al. (2003), which uses metabolizable energy intake (MEI).

Methane (MJ/day) = 8.25 + 0.07 x MEI (MJ/day)

The above equation requires that MEI be determined and fitted into the equation to obtain methane emission.

Determination of metabolizable energy intake (MEI)

Samples of feed refusals, faecal outputs and feeds were analysed for DM by drying them in an oven at 55 ^{o}C. Organic matter (OM) was estimated as DM less the residual ash obtained after ashing at 550 ^{o}C for 6 hours. Organic matter in the feed and faeces, DMI and Digestible Organic Matter in Dry Matter (DOMD) were estimated (MAFF, 1984). The values were fitted into the equation by the Ministry of Agriculture, Food and Fishery

(MAFF) (1984) to calculate MEI for each diet as follows:

DOMD=100(OMI-FOM)/OMI (MAFF, 1984)

MEI= DOMD x 0.15 x DMI

The above-mentioned model equation for methane estimation was developed by Mills et al., (2003) based on a significant amount of data obtained from the respiratory chamber method, which is considered the standard for measuring methane. Additionally, Mills et al. (2003) developed three model equations but this was chosen for the current study because it involves measuring digestibility, energy and feed intakes which are very crucial factors influencing methane production. This is one of the model equations recommended by USDA (2014). It is adaptable to different diets and intake levels. Browse species have the potential to reduce enteric methanogenesis in ruminants but the challenge in Africa is the nonexistence of suitable equipment such as respiration chambers and a sniffer (Sultan and Konca, 2022) and hence, the use of model equation in this study.

Evaluation of protozoa and methanogens

DNA extraction of protozoa and methanogen strains from rumen fluid and faeces

Protozoa and methanogens obtained from faeces and rumen fluid of sheep as described by Sarkwa et al. (2023a) were incubated for 24 hours at 37°C. Genomic DNAs were extracted using Quick-DNA™ Faecal/Soil Microbe Miniprep Kit (Zymo Research, USA) and used as a template for PCR amplifications.

Polymerase chain reaction (PCR) amplification of protozoa strains

Ciliate protozoan-specific PCR primers (Table 1) were used for the amplification of the 18S rRNA gene of protozoa (Sylvester et al., 2004; Sarkwa et al., 2023a). Polymerase chain reaction amplification was carried out as outlined by Sarkwa et al. (2023a). The formation and size of the PCR products were determined by electrophoresis in a 1.5 % agarose gel and stained with ethidium bromide (Karnati et al., 2003; Sarkwa et al., 2023a).

Polymerase chain reaction (PCR) amplification of methanogen strains

Methanogen-specific primers (Table 1) were used for PCR amplification (Wright and Pimm, 2003). The AccuPower® HotStart PCR PreMix kit (Bioneer, Inc. USA) was used in performing the PCR amplification. Polymerase chain reaction amplification was done as described by Sarkwa et al. (2023a). The formation and size of the PCR products were checked by electrophoresis in a 1.5 % agarose gel and stained with ethidium

TABLE 1

PCR primers and sequence used for the protozoa and methanogens strains

Primers	Sequence (5'-3')	References
P.SSU-54f	CAYGTCTAAGTATAAATAACTAC	Sylvester et al.(2004)
P.SSU-1747r	CTCTAGGTGATWWGRTTTAC	Sylvester et al.(2004)
Met86f	GCTCAGTAACACGTGG	Wright and Pimm (2003)
Met1340r	CGGTGTGTGCAAGGAG	Wright and Pimm (2003)

NB: P.SSU-54f and P.SSU-1747r were for protozoa; Met86f and Met1340r were for methanogens

bromide.

Statistical analysis

The values obtained for dry matter intake, initial body weight, final body weight and methane emission were analysed as a completely randomised design. The data were subjected to ANOVA using GenStat (2009) version 12.1 according to the model as follows:

$$Yij = \mu + Bi + Eij$$

Yij was the response variable such as feed intakes, initial weight, final weight and methane emission; μ is the overall mean; Bi is the different browse leaves; Eij is the residual error. Least significant difference (LSD) was used to separate significant means ($p<0.05$).

Results

The chemical composition of the experimental diets is presented in Table 2. The dry matter (DM), crude protein (CP), ash, condensed tannins (CT), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin ranges between 866 and 900 g/kg, 234 and 287 g/kg DM, 74.7 and 157 g/kg DM, 0.9 and 1.2 g/kg DM, 202 and 534 g/kg DM, 205 and 453 g/kg DM and 105 and 139 g/kg DM respectively (Table 2).

Table 3 shows the performance and methane emission of sheep fed four different dried leaves from browse plants. Feed intake was lowest ($p<0.05$) in sheep fed *Gliricidia sepium* (GS) and the highest ($p<0.05$) was recorded in sheep fed *Moringa oleifera* (MO). The feed intakes of *Albizzia lebbek* (AL) and *Millettia thonningii* (MT) differed ($p<0.05$) from each other (Table 3). The initial and final body weights ranged from 11.80 to 15.62 kg ($p<0.05$) and 8.63 to 15.80 kg ($p<0.05$). Methane emission was highest in sheep offered *Albizzia lebbek* (AL) and lowest in sheep offered *Millettia thonningii* (MT) but were not significantly different ($p>0.05$) from each other (Table 3).

The dry matter intake revealed that the intake

TABLE 2

Chemical Composition of diets (g/kg DM)

Diets	DM(g/kg)	CP	Ash	CT	NDF	ADF	Lignin
AL	900^{d}	287^{b}	74.7^{a}	1.2	453^{c}	453^{d}	192^{c}
GS	866^{a}	288^{b}	115^{c}	0.9	325^{b}	257^{b}	139^{b}
MO	873^{b}	330^{c}	157^{d}	1.0	202^{a}	205^{a}	105^{a}
MT	894^{c}	234^{a}	109^{b}	1.1	534^{d}	391^{c}	139^{b}
P values	p<0.001	p<0.001	p<0.001	p<0.064	p<0.001	p<0.001	p<0.001

TABLE 3

Dry matter intake (DMI), performance and methane emission of sheep fed four different dried browse leaves

Diets	DMI (g/d)	Initial weight (Kg)	Final weight (Kg)	Methane emission(MJ/d)
AL	433.10^{b}	11.80^{a}	13.83^{b}	11.23
GS	219.87^{a}	12.63^{b}	8.63^{a}	8.66
MO	496.14^{d}	14.25^{c}	16.00^{c}	9.75
MT	481.45^{c}	15.62^{d}	15.80^{c}	7.38
P values	p<0.001	p<0.001	p<0.001	p<0.132

Means in the same column with different superscripts are significantly different ($p<0.05$)

of sheep fed GS declined from the first week till the sixth week whilst the rest of the sheep fed the other three browse leaves showed some improvements over the six-week duration (Figure 1). Sheep fed GS lost weight from the beginning till the sixth week, whilst the rest initially lost weight for two weeks, after which gains were recorded as shown in Figure 2. Methane emissions from sheep fed AL and MO were relatively stable in the sense that the initial levels were similar to the level at the end of the six weeks, but MT was more stable than AL, meaning that methane emission for MT was almost the same throughout the experiment (Figure 3). However, sheep fed GS and MO recorded higher methane emissions in the beginning than at the end of the experiment (Figure 3).

Rumen and faecal methanogens and protozoa

Molecular identification of isolates

Amplification of the 18S rRNA of methanogens using the Met86F/Met1340R species-specific primer pair produced PCR products of about 1100-base pair (bp) for all the methanogen strains for rumen fluid (lanes: I, II, III & IV) (Figure 4). Also, Met86F/Met1340R species-specific primer pair generated PCR products of approximately 1100-bp for four methanogen strains (lanes: i, ii, iii and iv) obtained from faeces (Figure 5). Additionally, the Met86F/Met1340R species-specific primer pair

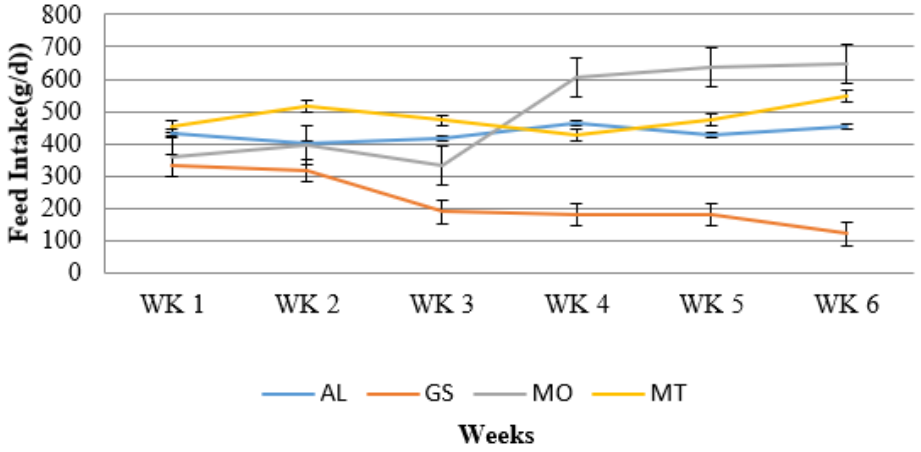

Figure 1 Dry matter intake of the dried *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) fed for six weeks

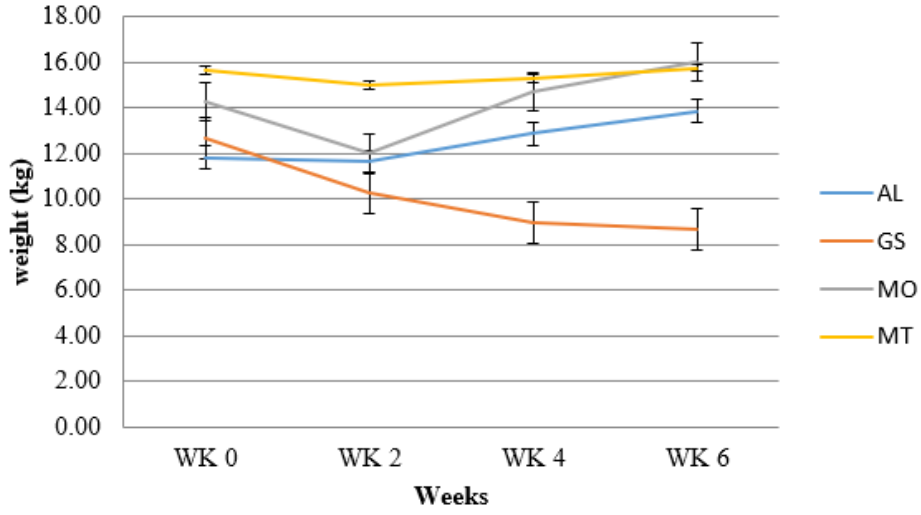

Figure 2 Weight gains of sheep fed dried *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) fed for six weeks

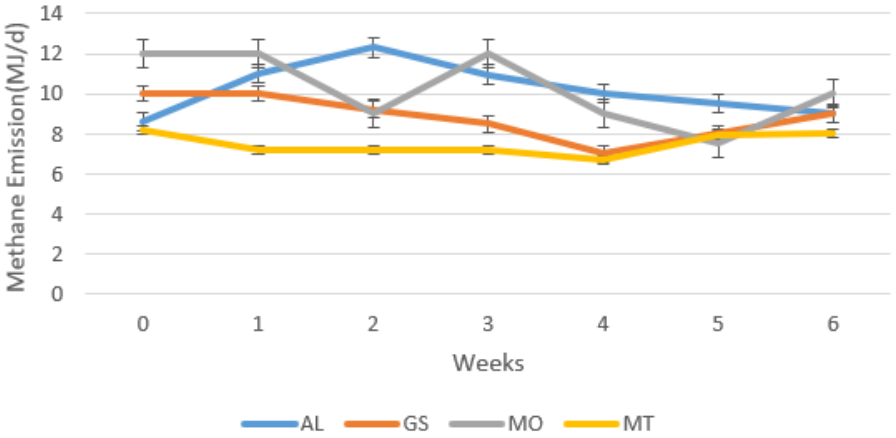

Figure 3 Methane emission of sheep fed dried *Albizzia lebbek* (AL), *Gliricidia sepium* (GS), *Moringa oleifera* (MO) and *Millettia thonningi*i (MT) fed for six weeks

amplified one PCR product at 750-bp (lanes: iv) and another two PCR products at 200-bp (lanes: i and iv) (Figure 4). However, the negative controls (-Ve) did not produce any PCR product in both reactions (Figures 4 and 5).

Amplification of the 18S rRNA of protozoa obtained from rumen fluid using the P.SSU-54F/P.SSU-1747R species-specific primer pair produced PCR products of about 1100-bp for one of the protozoa strain (lane: IV), one at 1000-bp (lane: III), but the remaining did not produce any PCR product (lanes: I,II)(Figure 6). On the contrary, using the

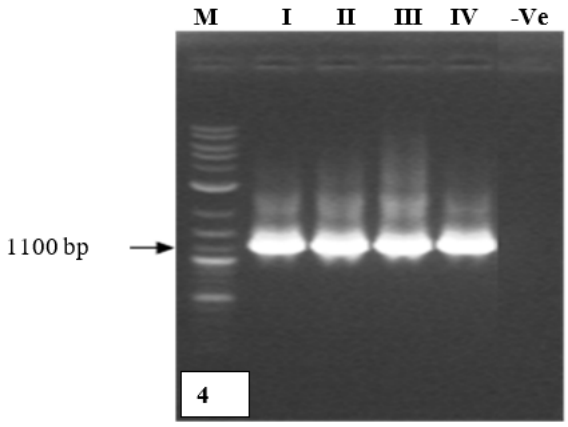

Figure 4 Methanogen strains (A) from rumen fluid (lanes – I= mALr, II=mMOrIII=mMTr & IV=mGSr) (M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); -Ve = Negative controls (nuclease-free PCR water)

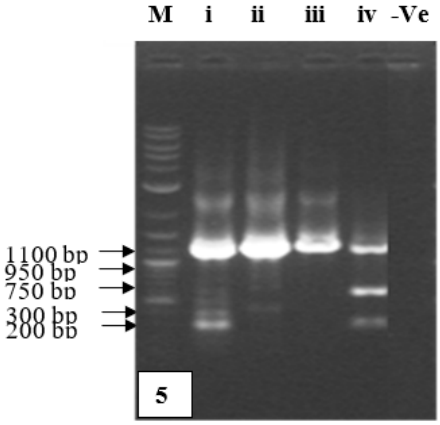

Figure 5 Amplified DNA fragments of methanogens strains from faeces (lanes – i=mALf, ii= mMOf, iii=mMTf & iv =mGSf) M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); -Ve = Negative controls (nuclease-free PCR water)

P.SSU-54F/P.SSU-1747R species-specific primer pair on faeces of sheep generated PCR products of approximately 200-bp for three protozoa strains (lanes: i, ii and iv), 750-bp for one protozoa strain (lane: ii) whilst lane C did not produce any PCR product (Figure 7). The negative controls (-Ve) did not produce any PCR product in both reactions (Figures 6 & 7).

Discussion

The chemical composition of the experimental diets used in this study were similar to those of several studies that revealed that the diets were able to improve weight gain and reduce methane emission either as a supplement or a sole diet (Sarkwa et al. 2020b and 2023b; Adogla-Bessa et al., 2022; Idan et al. 2023). The browse leaves contain low level of condensed tannins. The action of tannins on emission of methane is as a result of direct influence on methanogens in the rumen and indirectly by affecting the production of hydrogen and causing the death of cell by forming a complex with steroid in the protozoa cell membrane (Cheeke, 1999; Tavendale et al, 2005; Martin et al., 2010; Martinele et al., 2014; Sarkwa et al., 2023a).

The continued reduction in the intake of *Gliricidia sepium* (GS) may be due to the

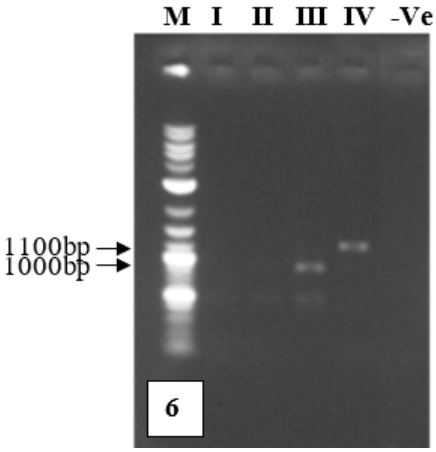

Figure 6 Amplified DNA fragments of protozoa strains from rumen fluid (lanes – I=pALr, II=pMOr, III=pMTr & IV=pGSr); M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); -Ve = Negative controls (nuclease-free PCR water)

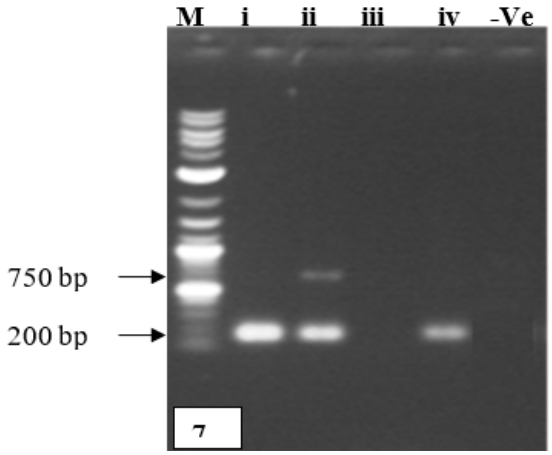

Figure 7 Amplified DNA fragments of protozoa strains from faeces (lanes – i =pALf, ii=pMOf, iii=pMTf & iv=pGSf); M = Molecular size marker, Quick-Load Purple 2-Log DNA Ladder (0.1-10.0 kb); -Ve = Negative controls (nuclease-free PCR water)

bitterness of the leaves or CT astringency. The low intake of dried GS leaves confirms a report of mean consumption of 12.9 g/ hour by Idan et al. (2020) in a preference study in which fresh leaves were used and GS was the least preferred browse leaves. The drying of GS may have improved its palatability to some extent such that, its intake in the current study was between 100-300 g/d. According to Carulla et al. (2005) dietary CT level of less than 50 g/kg DM did not reduce intake of forage. The CT level of GS was far less than 50 g/kg DM and hence the depressed intake may not have been caused by its CT level. However, CT astringency was reported by Landau et al. (2000) to depress forage intake. The CT astringency of GS may likely have been the cause of low feed intake by sheep. Other secondary metabolites which may also contribute to low feed intake were not measured. The intake of GS can be improved by pelleting it with other browse leaves as reported by Adjorlolo et al. (2020).

Feed intake is one of the most critical factors influencing the productivity and performance of ruminants (Ocak et al, 2006). Feed intake is predicted by the level of NDF in the diet (Timpong-Jones et al., 2015). When the feed intake of animals is low, performance swiftly decreases (Torres-Rodriguez et al., 1997). The current study aligned with the report by Torres-Rodriguez et al. (1997) regarding animals that were fed *Gliricidia sepium* that had low feed intake and lost weight. In the case of the animals that were fed *Albizzia lebbek*, *Millettia thonningii* and *Moringa oleifera*, their feed intake and weight gain were similar to earlier reports in Ghana, where browse leaves were fed as sole diets or supplements (Adjorlolo et al., 2020; Sarkwa et al, 2020b; Adogla-Bessa et al., 2022; Idan et al., 2023).

Feeding animals with non-tanniferous diets resulted in higher methane emission than feeding tannin-containing diets (Puchala et al., 2005, Molina-Botero et al., 2019; Sarkwa et al., 2020b). Methane production from animals fed leguminous forages was lower than that from animals consuming cereal-based diets (Fan et al., 2006; Patra, 2012; Min et al., 2020). These may be some of the reasons for obtaining low methane emission in this present study because the browse leaves contain condensed tannins (CT) and are leguminous forages. The combinations of CT and hydrolysable tannins have a higher antiprotozoal effect than hydrolysable tannins alone (Bhatta et al., 2009; Sarkwa et al., 2023a). The low methane emission estimated in the current study may be due to the use of whole browse leaves in feeding as compared to the use of CT extract from browse leaves. This may be because the whole browse leaves contain other anti-methanogenic substances beside CT such as hydrolysable tannins and saponins that have been reported to inhibit protozoa and methanogens activities in the rumen.

Genomic DNA of methanogens from rumen fluid and faeces produced a single band size of 1100 base pair in both cases in the current study. The base pair of the current study falls within the range of base pair (200-1100) reported by Sarkwa et al. (2023a). However, the values of the current study were higher than the reported 100-1000 base pair by Wright and Pimm (2003). According to Karnati et al. (2003), PCR amplification of the genomic DNA extracted from protozoa samples from the rumen fluid produced a band size of 1,360 base pair. However, in the current study PCR amplification produced multiple bands in the range of 200 to 1100 base pair which is lower

than the base pair reported by Karnati et al. (2003). The values (320-1100bp) reported by Sarkwa et al. (2023a) fall within the range of base pair recorded in the present study.

The difference in the size of base pair observed in this current study and reported studies may be due to the type and age of ruminant from which the rumen fluid was obtained and the types of feed fed to the ruminants. In the current study, rumen fluid was obtained from sheep fed browse leaves while in the reports by Karnati et al. (2003), rumen fluid was obtained from cattle fed concentrates and Sarkwa et al. (2023a) fed browse leaves and urea treated-rice straw to sheep.

All rumen fluid samples produced PCR products in both protozoa and methanogens. However, faecal samples obtained from sheep fed *Albizzia lebbek* and *Moringa oleifera* did not produce a PCR product in the case of methanogens. This may be an indication that *Albizzia lebbek* and *Moringa oleifera* diets have strong effect in limiting the activities of methanogens. Faecal samples obtained from sheep fed *Millettia thonningii* produced no PCR product in the case of protozoa. This may imply that *Millettia thonningii* may have a very strong negative effect on protozoa. This may be the reason why sheep fed *Millettia thonningii* recorded the lowest methane emission in this study.

Gliricidia sepium samples from rumen fluid and faeces produced PCR products for methanogens and protozoa. It may be possible that the *Gliricidia sepium* diet had moderate limiting effects on the activities of both protozoa and methanogens. Hence, it recorded slightly lower methane emission than *Albizzia lebbek* and *Moringa oleifera*. The present study has confirmed a finding of an earlier study by Martinele et al. (2014) that feeding *Gliricidia sepium* to lambs did not influence the density or diversity of rumen protozoa. Factors that influenced low methane output were variations in the density, population and activities of methanogens and protozoa (Animut et al., 2008; Mosoni et al., 2011).

A study by Goel et al. (2008) revealed that when there is inhibition of the population of protozoa, then the population of methanogen is also inhibited. It is likely that, the outflow of methanogens and protozoa from the rumen was moderate to high because whole browse leaves were fed to sheep not a component or an extract. This is because, at the same concentration, the use of browse leaf extract has lower antiprotozoal effect than unprocessed browse leaf (Goel et al. (2008).

Conclusion

Sheep fed *Albizzia lebbek* (AL), *Moringa oleifera* (MO) and *Millettia thonningii* (MT) gained weight but those fed *Gliricidia sepium* (GS) lost weight with time. All four browse leaves fed recorded low methane emission in sheep. Detecting methanogens and protozoa in the rumen fluid and faeces of sheep is an indication that the browse leaves were able to inhibit the activities of methanogens and protozoa in the rumen and eliminated them through faeces. Therefore, AL, MO and MT can improve weight gain and reduce methane emission while GS can minimise methane emission but cannot improve weight gain. This study highlights the contribution of natural resources to climate-smart approaches in sheep production and reduction of enteric methane production hence minimising the effects of climate change.

Ethical Clearance

Approval of research ethics was given by the University of Ghana- Institutional Animal Care and Use Committee, Legon (Protocol

number 2017-02-2R).

Conflict of interest

The authors have no conflict of interests.

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