

METHANE EMISSION OF GOATS FED FIFTEEN DIETS: ON-FARM OBSERVATIONS

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↳Supporting Information

ABSTRACT: It is suggested that the measurement of methane production from enteric fermentation must be done under situations similar to that of typical farming methods. It is against this background that this study measured methane emission from goats on a farm to ascertain the real situation on most farms. The objective of this study was to measure performance and methane emission from goats fed Ghanaian ruminant diets comprising of basal diets supplemented with browse leaves and to determine the effects of temperature and humidity on methane emission. Ten West African dwarf goats (5 males and 5 females; average weight 14 kg ±1.01) were fed fifteen Ghanaian ruminant diets for four months. Each diet was randomly fed twice in 24 hours for 2 days in a month. Methane emission, temperature and humidity were measured using handheld gas methane detector. Completely randomized design was used. Dry matter intake (DMI) was lowest ($P<0.05$) when cassava (*Manihot esculenta*) peels were fed and highest ($P<0.05$) when plantain peels were supplemented with *Moringa oleifera*. Weight gain, DMI and methane emission from manure increased with time. The highest enteric methane emission was recorded ($P<0.05$) when untreated rice straw (749 ppm) was fed and the lowest was recorded ($P<0.05$) when *Moringa oleifera* leaves (313 ppm) were fed. High environmental temperature favored low methane emission and high humidity was associated with high methane emission. In conclusion, feeding browse leaves alone and browse supplementation with basal diets resulted in lower methane emission than feeding basal diets alone. Moderate weight gains were recorded. High environmental temperature was inversely related to methane emission and high environmental humidity was directly related to methane emission. It is recommended that, browse leaves be incorporated in the feed of ruminants, especially when environmental temperatures are low and humidity is high.

Keywords: Basal diets, Browse leaves, Dry matter intake, Humidity, Temperature, Weight gain.

INTRODUCTION

Greenhouse gas emission is one of the drivers of Climate change (EPA, 2017). Agricultural activities are major sources of atmospheric greenhouse gas emissions, forming about thirty percent of the global anthropogenic emissions (Vermeulen et al., 2012; Rosenstock et al., 2016). Animal agriculture is a significant producer of greenhouse gases, forming about 14.5% of global emissions (Gerber et al., 2013a; Kristiansen et al., 2020) and 29.7% of the total Agricultural greenhouse gas emissions in Sub-Saharan Africa (FAOSTAT, 2024). The worldwide annual methane emission from ruminants is estimated to range between 80 and 95 million tons (Patra, 2014). The process of enteric fermentation contributes more than 90% of methane emissions from livestock (FAO, 2019) and forms 40% of the agricultural greenhouse gas emissions (Tubiello et al., 2013). This forms a major source of greenhouse gas emissions from the agricultural system (Steinfeld et al., 2006; Palangi et al., 2022). Methane represents 20% of the global anthropogenic greenhouse gas emission that causes global warming (Nisbet et al., 2016).

Methane is a potent greenhouse gas, next to carbon dioxide regarding its contribution to global warming (Martin et al., 2008; Olivier et al., 2018; IPCC, 2021). The United States Environmental Protection Agency (EPA) states that "methane is a powerful greenhouse gas, with a global warming potential more than 25 times greater than that of carbon dioxide over a 100-year time horizon" (EPA, 2017). Methane has a Global Warming Potential (GWP) of 85 times more than that of carbon dioxide over a 20-year time horizon, although carbon dioxide has thousands of years atmospheric lifetime but methane disappears in about 10-15 years (IPCC, 2021).

The rapid disappearance of methane and its high contribution to atmospheric temperature makes it a primary focus to curtail in an effective and timely manner in terms of climate change (Verde et al., 2023). According to the report of the International Energy Agency, reduction in methane emissions is one of the most effective interventions that should be included in economic terms, to rapidly decrease the rate of global warming and contribute immensely to activities to minimize the rise in global temperature (IEA, 2021).

Methane emission is a loss of 2 to 12 % of dietary energy to the ruminant, depending on the composition of diet and intake level (McGin et al., 2011; Goel and Makkar, 2012). Broucek (2014) suggested that the measurement of methane production from enteric fermentation must be done under situations similar to that of typical farming methods. It is against this background that this study measured methane emission from goats on a farm to ascertain the real situation on most farms. Also, methane emission in goats fed common ruminant diets have not been extensively studied in Ghana. The objective of this study was to measure performance and methane emission from goats fed Ghanaian ruminant diets and to determine the effects of temperature and humidity on methane emission.

MATERIALS AND METHODS

Study location

The study was carried out on Sakyi & Abban Farms at East Legon Hills, Accra (5° 43' 27.4" N 0° 05' 52.2 W) in the Coastal Savannah zone of Ghana. Total rainfall ranges from 508 mm to 743 mm per annum. Rainfall pattern is bimodal, with the major rains between May to August and minor rains in September- November. Temperature varies between 30 °C and 34 °C and relative humidity is from 53 % to 73 % (Sarkwa et al., 2020a).

Chemical analysis

Dry matter (DM), crude protein (CP) and ash were carried out using the methods of AOAC (2016). Fibre components were determined using the procedure of Goering and Van Soest (1970) and condensed tannins (CT) by butanol-HCl method as outlined by Iqbal et al. (2011) and validated by Sarkwa et al. (2023a).

Animal management and feeding

Ten West African dwarf goats (5 males and 5 females; average weight 14 kg ±1.01; two years old) were fed fifteen Ghanaian ruminant diets for four months. The goats were kept in a communal housing pen (60 m x 5 m). The goats were treated against ectoparasites and endoparasites before the start of the experiment as carried out by Sarkwa et al. (2023b). The fifteen diets were untreated rice straw (URS), urea treated rice straw (UT), plantain peels (PP), cassava peels (CP), *Moringa oleifera* (MO), *Albizia lebbek* (AL), *Leucaena leucocephala* (LL), *Millettia thonningii* (MT), yam peels (YP), *Mangifera indica* (MG), plantain leaves (PL), cassava leaves (CL), MO+PP, AL+PP and MO+CP.

Each diet was fed twice in 24 hours (day) and each of the fifteen diets was fed for two consecutive days in a month in no specific order or randomly. The feeding was carried out for four months. The fifteen diets were given separately to each of ten goats for 2 days and methane gas was measured for each feed. All the basal diets were cut into pieces of about 4cm in length. Untreated rice straw and urea treated rice straw were prepared as described by Sarkwa et al. (2021). Before the commencement of the experiment, each of the experimental diets was offered to the goats for 24 hours. All ten goats were offered 20 kg of experimental diets daily (10 kg in the morning and 10 kg in the afternoon). The quantity of browse leaves (AL, LL, MG, MO and MT) fed as supplement (MO+CP, MO+PP, AL+PP and AL+LL+PP) was 1000 g. However, goats fed sole basal diets or browse leaves were offered 2000 g of feed. In supplementing with browse leaves, each goat was offered 100 g of the browse leaves and 1900 g basal diet. Water was offered on *ad libitum* basis. Feed intakes were recorded daily by subtracting feed offered from feed residual or leftover. Weight gains were determined by weighing every month after starving the goats for 12 hours.

Methane emission, temperature and humidity measurements

Methane emission, temperature and humidity were measured daily using hand-held gas methane detector (GASTiger 2000, Stark Instrument Company, China). Enteric methane emission from each diet was measured after 12 hours of feeding a particular diet to the goats. Enteric methane emission was measured from goats by restraining them individually and about 30 meters away from the other goats. Then, the methane detector was placed very close to the mouth of the goats. This is because it has been reported that about 95 to 99 % of enteric methane is released through the mouth (Olijhoek and Lund, 2017). Manure from the goats excreta were heaped under a mango tree on the farm and methane emission was measured monthly.

Statistical analysis

Completely randomized design was used. Data obtained were subjected to analysis of variance using GenStat-2009 version 12.1 (GenStat, 2009) in accordance with the model below:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Which Y_{ij} is the response variable such as feed intake, feed leftover, total feed offered and enteric methane emission; μ was the overall mean; T_i is the experimental diets (15 diets); E_{ij} is the residual error.

Student Newman-Keuls (SNK) test was used to separate significant ($P < 0.05$) means. Differences in monthly enteric methane emission, effect of sex on methane emission, monthly methane emission from manure and monthly feed intake were determined using error bars.

RESULTS

Chemical composition

The dry matter, crude protein, ash, neutral detergent fibre, acid detergent fibre and lignin of the experimental diets ranged between 840-946 g/kg, 31-330 g/kg DM, 50-200 g/kg DM, 202-620 g/kg DM, 175-548 g/kg DM and 105-201 g/kg DM respectively (Table 1). The condensed tannins content of the browse leaves were from 1.9 g/kg DM to 6.9 g/kg DM (Table 1).

Table 1 - Chemical Composition of Diets (g/kg DM)

Diets	DM(g/kg)	CP	Ash	NDF	ADF	Lignin	CT
AL	880	287	74.7	453	350	192	3.2
LL	840	271	102	300	176	139	6.9
MO	873	330	157	202	205	105	3.0
MT	894	234	109	534	391	139	3.1
Cassava Peels	946	31	73	363	274	201	-
UT	916	101	200	552	520	180	-
UNRS	936	66.8	173	620	548	191	-
Plantain Peels	900	80.7	143	371	270	199	-
Cassava leaves	879	120	83	354	250	125	1.9
Mango leaves	890	187	113	364	241	128	2.7
Yam Peels	889	90	50	370	380	160	-

AL:- *Albizia lebbek*, LL: *Leucaena leucocephala*, MO: *Moringa oleifera*, MT: *Milletia thonningii*, UT: Urea treated rice straw, UNRS: Untreated rice straw, CP: Crude Protein, NDF: Neutral Detergent fibre, ADF: Acid detergent fibre, CT: Condensed tannins.

Dry matter intake, feed leftover, total feed offered and methane emission of goats fed different diets

Dry matter intake was lowest ($P < 0.05$) in goats fed cassava peels (CP) but was not different ($P > 0.05$) from goats fed untreated rice (UNRS), plantain peels (PP) and yam peels (YP) (Table 2). Goats fed PP supplemented with *Moringa oleifera* (MO) recorded the highest ($P < 0.05$) DMI but did not differ ($P > 0.05$) from all the browse supplemented diets and solely fed browse leaves apart from goats fed urea treated rice straw (UT) (Table 2). Feed leftover and total feed offered on dry matter basis were in the range of 783 to 1418 g/d ($P < 0.05$) and 1566 to 1884 g/d ($P < 0.05$) respectively (Table 2).

Enteric methane emission was highest ($P < 0.05$) in goats fed UNRS but was not different ($P > 0.05$) from goats fed CP. The lowest ($P < 0.05$) enteric methane emission was observed in goats fed MO but did not differ ($P > 0.05$) from those fed *Albizia lebbek* (AL) and *Milletia thonningii* (MT) (Table 2). Goats fed MO+CP, MO+PP and AL+PP did not differ ($P > 0.05$) from each other in enteric methane emission. Goats fed AL+ *Leucaena leucocephala* (LL)+PP and cassava leaves were not different ($P > 0.05$) in terms of enteric methane emission but were higher ($P < 0.05$) than those fed LL and *Mangifera indica* which differed ($P < 0.05$) from each other (Table 2).

Table 2 - Dry matter intake, feed leftover, total feed offered and methane emission of goats fed different diets

Feeds	Dry Matter Intake (g/d)	Feed Leftover on DM basis (g/d)	Total Feed offered on DM basis (g/d)	Methane Emission (ppm)
Untreated Rice straw	454 ^c	1418 ^a	1872 ^b	749 ^a
Urea treated rice straw	608 ^b	1224 ^c	1832 ^c	517 ^{de}
Cassava peels (CP)	448 ^c	1310 ^b	1758 ^f	719 ^{ab}
Plantain peels (PP)	450 ^c	1350 ^b	1800 ^d	721 ^a
Yam peels	481 ^c	1297 ^b	1778 ^e	660 ^{abc}
<i>Moringa oleifera</i> (MO) + CP	830 ^a	1054 ^d	1884 ^a	589 ^{def}
<i>Moringa oleifera</i> (MO) + PP	839 ^a	958 ^e	1797 ^d	607 ^{def}
<i>Albizia lebbek</i> (AL) + PP	831 ^a	967 ^e	1798 ^d	596 ^{def}
AL + <i>Leucaena leucocephala</i> (LL) + PP	835 ^a	961 ^e	1796 ^d	560 ^{bd}
<i>Albizia lebbek</i> (AL)	817 ^a	943 ^e	1760 ^f	370 ^g
<i>Mangifera indica</i>	783 ^a	783 ^f	1566 ^h	426 ^{efg}
Cassava leaves (CL)	799 ^a	959 ^e	1758 ^f	555 ^{bd}
<i>Leucaena leucocephala</i> (LL)	811 ^a	949 ^e	1760 ^f	408 ^{fg}
<i>Milletia thonningii</i> (MT)	811 ^a	977 ^e	1788 ^{de}	387 ^g
<i>Moringa oleifera</i> (MO)	820 ^a	926 ^e	1746 ^g	313 ^g
SEM	±19.52	±24.86	±4.68	±45.11
P-values	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Means in the same column with different superscripts are different ($P < 0.05$); SEM: Standard error of means.

Monthly dry matter intake of the diets can be seen in Figure 1. In general, there was improvement in dry matter intake with time (Figure 1). Dry matter intake in the first month had the lowest and the fourth month had the highest in all the 15 diets (Figure 1). Monthly intake of all diets were not different from each other except UNTRS (Figure 1). Intake of goats fed UNTRS for the first and second months were not different but the first month was different from the the third and fourth months. Intake for the second and third months was not different from each other according to the error bars (Figure 1).

On the contrary, enteric methane emission decreased with time (Figure 2). The first month recorded the highest enteric methane emission while the fourth month recorded the lowest enteric methane emission (Figure 2). Enteric methane emission in goats fed UT in the fourth month was lower than the rest which was not different. Goats fed MO+CP, AL+PP and MO recorded enteric methane emission higher in the first month than the fourth month which did not differ from the other two months (Figure 2). Enteric methane emission from goats fed AL was highest in the first month but was not different from the second month. Goats fed AL recorded the lowest enteric methane emission in the fourth month but was not different from the third month. The highest methane emission in goats fed AL was recorded in the first month but the other three months were not different. In goats fed LL, enteric methane emission was lowest in the fourth month and was different from the other three months (Figure 2). In the first month, enteric methane emission from goats fed AL+LL+PP recorded the highest but was not different from the second month. Enteric methane emission from goats fed AL+LL+PP was lowest in the fourth month but not different from the third month according to the error bars (Figure 2).

Figure 3 shows enteric methane emission of males and females fed the experimental diets. Males recorded higher enteric methane emission but was not different from that of females (Figure 3). Methane emission from manure increased with time (Figure 4). The first month recorded the lowest methane emission from manure but it did not differ from the second month. The fourth month recorded the highest but it was not different from the third month (Figure 4).

Figure 5 shows an inverse relationship between methane and temperature: methane emission decreases with increase in temperature. Methane emission had direct relationship with humidity (Figure 6). Thus, methane emission increases with increase in humidity (Figure 6). There was improvement in weight gain with time (Figure 7). The fourth month recorded the highest weight gain of goats fed the experimental diets but it was not different from the second and third months (Figure 7). The first month recorded the lowest weight gain (Figure 7).

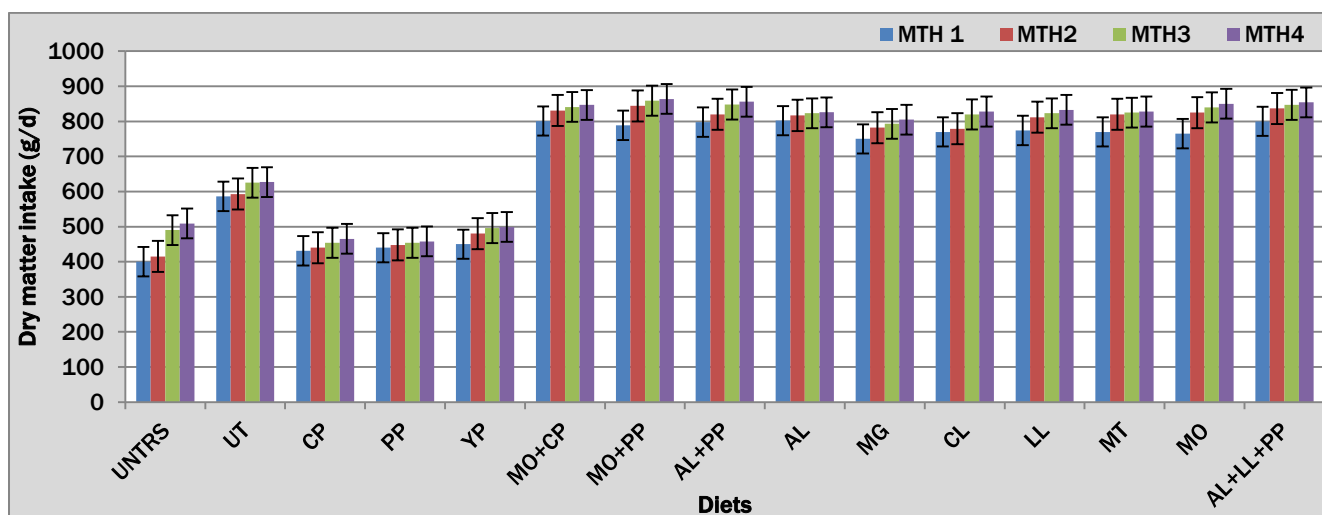


Figure 1 - Monthly dry matter intake of goats fed 15 diets.

Untreated rice straw (UNTRS), urea treated rice (UT), cassava peels (CP), plantain peels (PP), yam peels (YP), cassava peels (CP), *Moringa oleifera* (MO), *Albizzia lebbek* (AL), *Mangifera indica* (MG), cassava leaves (CL), *Leucaena leucocephala* (LL) and *Milletia thonningii* (MT).

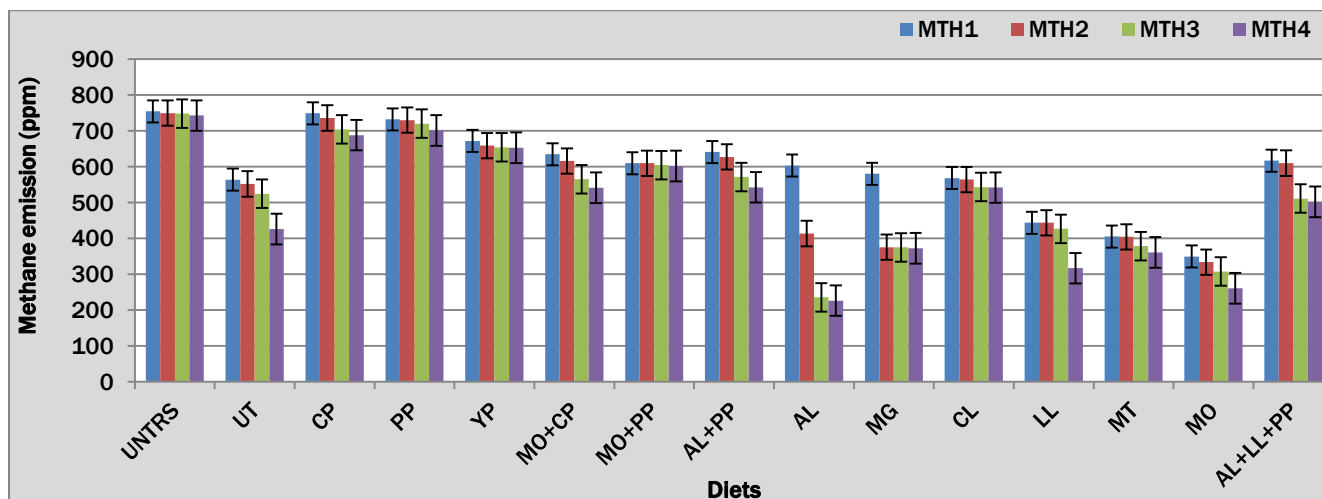


Figure 2 - Monthly enteric methane emission of goats fed 15 diets.

Untreated rice straw (UNTRS), urea treated rice (UT), cassava peels (CP), plantain peels (PP), yam peels (YP), cassava peels (CP), *Moringa oleifera* (MO), *Albizzia lebbek* (AL), *Mangifera indica* (MG), cassava leaves (CL), *Leucaena leucocephala* (LL) and *Milletia thonningii* (MT).

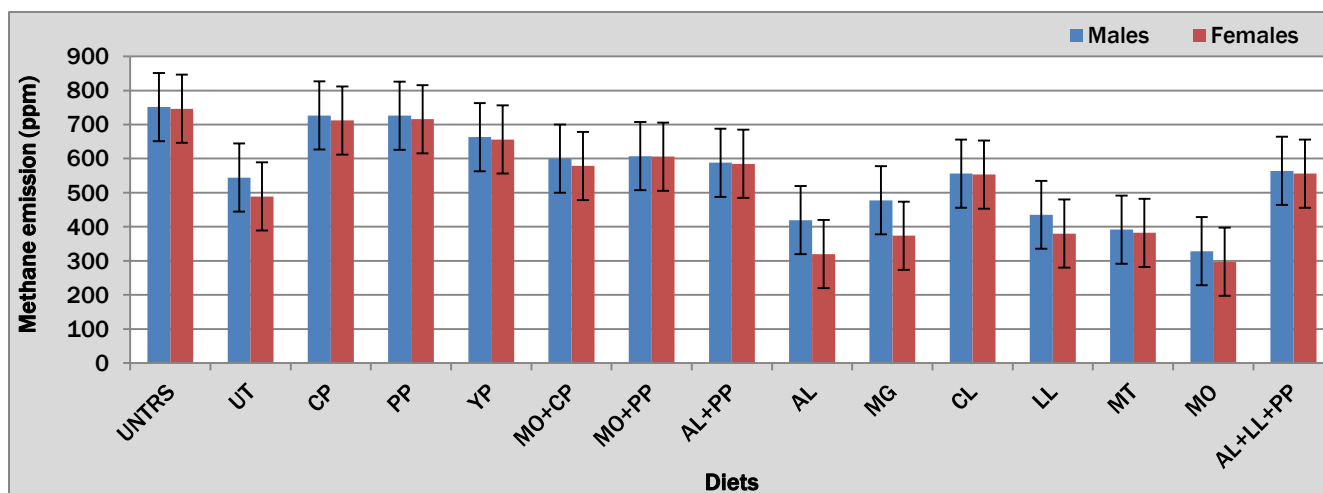


Figure 3 - Effect of sex on enteric methane emission of goats fed 15 diets.

Untreated rice straw (UNTRS), urea treated rice (UT), cassava peels (CP), plantain peels (PP), yam peels (YP), cassava peels (CP), *Moringa oleifera* (MO), *Albizzia lebbek* (AL), *Mangifera indica* (MG), cassava leaves (CL), *Leucaena leucocephala* (LL) and *Milletia thonningii* (MT)

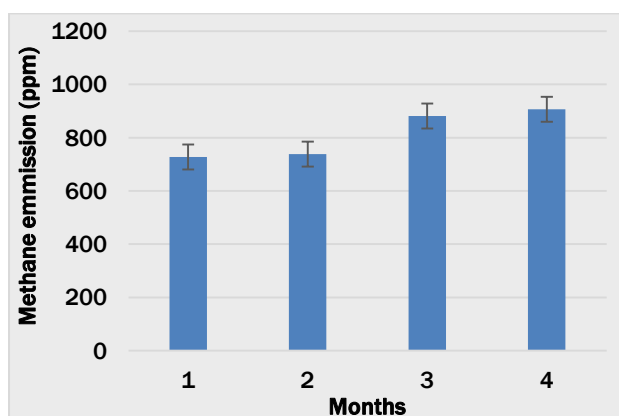


Figure 4 - Monthly methane emission from goats manure.

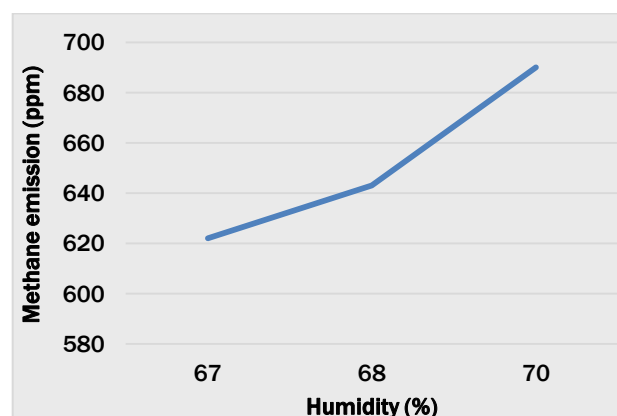


Figure 6 - Relationship between enteric methane emission and humidity.

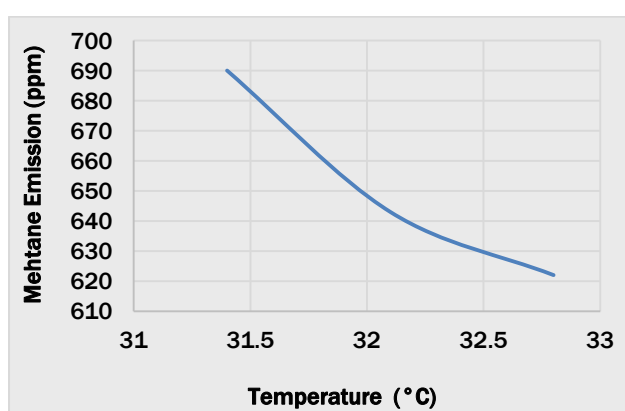


Figure 5 - Relationship between enteric methane emission and temperature.

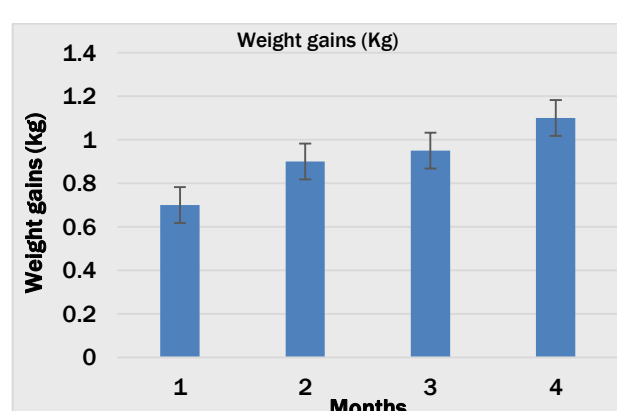


Figure 7 - Monthly weight gains of goats fed 15 diets.

DISCUSSION

Chemical composition

The high crude protein and ash, low condensed tannins, low to moderate fibre components of the browse leaves may have contributed to the gain in weight and lower methane emission recorded in this study and this is similar to earlier reports (Yisehak et al., 2014; Sarkwa et al., 2020a, 2020b; Adogla-Bessa et al., 2022). Also, Patra et al. (2007) and Jayanegara et al. (2012) reported that plant secondary substances such as tannins have inhibitory effects on

methanogenesis and this may be the reason why the browse diets recorded lower methane because they contained condensed tannins. The basal diets were high in fibre components and methane emission. This is in line with reports by Jayanegara et al. (2009) and Jin et al. (2012) that high ADF and NDF levels resulted in low digestibility and high formation of methane by altering short chain fatty acid proportion to acetate formation which yields more hydrogen.

Intake

Annan and Tuah (1999) reported intake of cassava peels between 255 to 347 g/d and for cassava peels supplemented with *Ficus exasperata* (browse leave) intake of 383-475 g/d. Similar trend of intakes were recorded in the present study but with higher values. Dry matter intakes of feeds containing different levels of condensed tannins were similar (Animut et al., 2008a). This is corroborated by the current study where the different browse leaves with different condensed tannins levels fed solely and as supplements had similar dry matter intake. This may be due to the fact that the condensed tannins levels of the browse leaves were also similar and low. The present study has confirmed an earlier study by Bhatta et al. (2002), that dietary condensed tannins levels below 60 g/kg did not reduce feed intake. In the present study, condensed tannins levels were low and there was high intake of the browse leaves as supplement compared to the basal diets.

Enteric and manure methane emission

Condensed tannins containing diets reduced enteric methane emission in goats irrespective of its content (Puchala et al., 2005 and 2012; Animut et al., 2008a). In this present study, sole browse leaves and their supplementation recorded lower enteric methane than the basal diets that did not contain condensed tannins and this is similar to earlier reports (Carula et al., 2005; Tavendale et al., 2005; Puchala et al., 2005 and 2012; Animut et al., 2008a). Condensed tannins from different sources had similar effects on enteric methane emission in goats, most likely by altering the activities of methanogens, although alteration in activities of bacteria and protozoa may also contribute to it (Animut et al., 2008b; Sarkwa et al., 2023a). This has been confirmed by the current study in which goats fed the different browse leaves emitted similar amount of enteric methane. Feeding two browse leaves as supplement recorded lower enteric methane than supplementing with one browse leaf. This has confirmed earlier reports that feeding combinations of diets resulted in lower enteric methane emission than feeding one diet (Naumann et al., 2015; Sarkwa et al., 2023b).

Forage size has immense effect on enteric methane emissions. Animals spend significant amount of their energy to the process of chewing (Gerber et al 2013b). Reduction of particle size of fodder mechanically helps to increase digestibility by enhancing accessibility of substrate to microbes, thereby reducing enteric methane emission and energy expenses and improving the passage rate of digesta and animal productivity (Hristov et al 2013). In the present study, the basal diets were all cut into pieces and this may have contributed to improve digestibility, feed intake and weight gain and lower enteric methane emission especially in the case of the urea treated rice straw. Browse feeding and reduction in the size of feed are good feeding practices that may have contributed to improve performance and lower enteric methane emission in this current study. This supports a report by Mayuni et al (2019) that greenhouses gases are reduced with better feeding practices.

It has been suggested that nutritional strategies and management practices are traditional options by which enteric methane emission can be reduced in goats (Pragna et al., 2018). This is supported by the current study where browse leaves containing condensed tannins and urea treated rice straw recorded lower methane emission than the non tanniferous diets and untreated rice straw. Methane emission was higher initially than the later stages (Animut et al., 2008a; Sarkwa et al., 2023b). Similar trend was observed in this current study where enteric methane emission decreased with time. The reduction in enteric methane emission with time maybe due to decrease population and activities of protozoa and methanogens in the rumen due to regular feeding of tanniferous diets. A report showed that male ruminants generally have higher enteric methane (CH₄) emissions compared to females (Hegarty et al., 2007). This is in line with the current study and this may likely be due to differences in body size, metabolic rate and hormone levels between the sexes.

Methane from manure increased with time and this may be due to increase in the quantity. This study supports earlier report by Jabab (2023) who reported high methane emission from manure in the wet season due high quantity of manure as a result of high intake of feed. Methane emission from manure in intensively managed farms with manure storage system was higher than extensive system because it is mostly exposed to air (Knapp et al., 2014). Anaerobic digestion is a natural process in which the microorganisms consume organic matter under an oxygen-free environment and this result in greenhouse gas generation such as carbon dioxide and methane (Knapp et al., 2014). In the current study, manure was stored in a heap under a tree in the open or not stored under anaerobic conditions and therefore, additional generation of methane may have been minimal.

Methane emission, temperature and humidity

Enteric methane production is also influenced by environmental temperature (Nonaka et al., 2008). At high temperatures feed intake and rate of passage in the rumen becomes slow and this increases digestibility and decreases

methane production in the rumen (Kurihara et al., 1995; Kurihara, 1996; Bhatta et al., 2006; Nonaka et al., 2008). These findings are in line with present study because increased in temperature caused decreased in enteric methane emission. The current study recorded an increase in enteric methane emission as relative humidity increased which is in line with an earlier report by Hansen et al (2012). The findings of the current study reveal that, when temperatures are low and humidity is high, enteric methane emission will be high and therefore, more concerted effort will be needed to mitigate methane emission.

Weight gains

The results on weight gains in this current study are similar to earlier reports where browse leaves were fed solely (Sarkwa et al., 2020b) and as supplements (Adogla-Bessa et al., 2022; Idan et al., 2023a and 2023b). This may be due to the improvement in feed intake and reduction in methane emission with time. The improvement in feed intake and reduction in methane emission may imply that less feed energy is lost as a result of low methane production which may have made more energy available to the goats to use for weight gains. The results on weight gains in this current study corroborate earlier studies that enteric methane production is a loss of energy that is due to inefficient digestion in the rumen (Johnson and Johnson, 1995), which decrease metabolisable energy intake (MEI) by the animal (McGin et al., 2011; Goel and Makkar, 2012) and may potentially be used for meat production (Eckard et al., 2010).

CONCLUSION

Feeding browse leaves alone and browse supplementation with basal diets resulted in lower enteric methane emission than feeding basal diets alone. Moderate weight gains were recorded in goats fed the experimental diets. Feed intake, methane emission from manure and weight gains increased with time while enteric methane emission decreased with time. High environmental temperature resulted in low enteric methane emission and high environmental humidity favored high enteric methane emission. It is recommended that, regular incorporation of browse leaves in the feeding of ruminants should be encouraged especially when environmental temperatures are low and humidity is high. This will enhance climate smart and sustainable goat production and contribute to reduce the impact of climate change.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

FO Sarkwa, EC Timpong-Jones, T Adogla-Bessa and V Antwi contributed to the research, data analysis, and manuscript writing.

Conceptualization: FO Sarkwa and EC Timpong-Jones

Data curation: FO Sarkwa and V Antwi

Funding acquisition: FO Sarkwa, EC Timpong-Jones and T Adogla-Bessa

Methodology: FO Sarkwa and EC Timpong-Jones

Investigation, project administration, resources, software, analysis, visualization & writing original draft: FO Sarkwa

Supervision: FO Sarkwa and EC Timpong-Jones

Validation: FO Sarkwa and T Adogla-Bessa

Writing, review & editing: FO Sarkwa, EC Timpong-Jones and T Adogla-Bessa

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Ethical approval

Ethical clearance was sought from Ethics Committee of College of Basic and Applied Sciences, University of Ghana, Legon (ECBAS 032/21-22). The authors also complied with the ARRIVE guidelines.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

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