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In vitro Estimation of Gas and Methane Production from Locally Available Feed Stuffs and Rumen Content

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Abstract

Enteric fermentation is a major source of greenhouse gases emission from ruminants. Among other factors, the nature of the feed consumed by ruminants determines the amount of gas produced. *In vitro* gas production techniques simulate the rumen fermentation process and they have been used to evaluate the gas production potential of feedstuffs. Thus, an *in vitro* based gas production technique was employed to estimate overall gas and methane production potential of 12 commonly available local feedstuffs in and around Mekelle, and rumen content inoculum collected from Abergelle export abattoir. Both feed and rumen content substrates were inoculated with strained rumen fluid from slaughtered cattle and an equal amount of tap water was added, then follow-up was made for 24 hrs to measure gas production. A sequence of gas measurement was taken at 3, 6, 9, 12, 15 and 24 hrs post incubation by observing the calibrated syringes. The gas production showed a declining trend as the time of incubation increased. In feed substrates, a relatively high amount of gas production was observed at 6 hrs of incubation (9.27±1.92) followed by 3 hrs (7.03±1.71). The overall mean gas and methane produced from rumen content samples was higher at 12 hrs of incubation (6.57±15.15) and (2.57±5.92), respectively. The mean gas produced from each of the feed substrates showed a statistically significant variation (p=0.0012), where there was a relatively high amount of gas production in wheat bran (48.33±18.35) followed by commercial alfalfa hay (42.50±26.61). On the contrary, pods of *Faidherbia (F.) albida* scored the least gas production (0.83±2.04) followed by molasses (4.17± 10.21). The incubation time effect on total gas and methane production was apparent at 6 hrs being the maximum, (p=0.0097). The mean gas production of young animals was higher (30.73±21.99) than that of adult animals (18.00±20.18). Feed manipulation is one strategy to mitigate emission of greenhouse gases from ruminants. Thus, it is recommended to supplement ruminant feed with seeds of *F. albida* and molasses to reduce the emission of gases as the gas production from these feed sources is low. Further experiments should also be conducted using *in vitro* and *in-vivo* techniques to better understand the emission potential of locally available feedstuffs.

Keywords: Cattle; Feed; Gas production; In-vitro; Methane; Rumen content

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1. INTRODUCTION

Ruminants convert poor quality roughages into high quality, human edible foods through the production of volatile fatty acids (VFA) as a main source of energy, but with concomitant carbon dioxide (CO_2) and methane (CH_4) production. Methane produced in the rumen represents 2–12% of feed gross energy loss and contributes to greenhouse gas (GHG) emissions (11–17%) globally (Beauchemin et al., 2009; Goel and Makkar, 2012; Lee et al., 2003). Depending on their size and dry matter intake, sheep and goats produce, on average, 10–16 while cattle produce 16–60 kg CH_4/head/year (Hristov et al., 2013).

Among the ruminant animals, cattle contribute the most towards greenhouse effect through methane emission followed by sheep, and goats, respectively (Charmley et al., 2008). Most of the methane that accumulates in the rumen is expelled via the mouth through eructation and breathing which results in an increased temperature of the

earth. Ruminants have unique digestive systems bearing on a symbiotic relationship between bacteria, archaea, protozoa, and fungi within the rumen environment. Plant polymers such as cellulose, hemicellulose, and lignin components are degraded by hydrolytic exoenzymes released or exposed by these microbes and subsequently converted by fermentation into short chain fatty acids (SCFAs) - namely, acetate, propionate, and butyrate, which are consumed as nutrients by the ruminants. Thus, ruminants can utilize plant material more effectively and conserve more of its energy than other herbivores, which is due to the presence of a dense and diverse microbial environment in the rumen that carryout these metabolic activities (Henderson et al., 2015; Kolte et al., 2017). The benefit of ruminants taking advantage of otherwise inaccessible plant materials offsets the negative effects of GHG production. However, due to the increasing ruminant population to satisfy the milk and meat demand, there is a change in the diet of animals, which could play for the emission of GHGs from the sector. Dietary components and changes cause shifts in the rumen microbial ecology that can play a role in animal health and productivity. Cattle with high feed efficiencies produce less methane gas than those with low feed efficiencies (Zhou and Hernandez-Sanabria, 2009). Thus, efficient balancing of rations helps to minimize the methane production.

Globally, there has been a major increase in GHGs since the preindustrial era. According to Pachauri et al. (2014), total anthropogenic global greenhouse gas emissions in 2010 were 49 ± 4.5 giga tone (Gt) CO2-eq, with the livestock sector accounting for around 7.1 Gt CO2-eq per year (Gerber et al., 2013). Methane is the main GHG in most ruminant production systems. Methane from manure and enteric fermentation accounts for 40–70% of total GHG emissions in beef production systems and 48–75% of the total GHG emissions on dairy farms. Emissions of CO_2 are typically 5–10% of total GHG emissions, and the remaining emissions (20–55% beef; 15–47% dairy) are N_2O. Livestock CH_4 enteric fermentation accounts for 18–22% of anthropogenic and 11–13% global emissions, respectively (Beauchemin et al., 2009). Methane has 28 times more global warming potential than CO_2 (Gerber et al., 2013; IPCC, 2014). The main substrates that enable methanogens to produce CH_4 are low carbon substrates such as formate, pyruvate, methylamine, acetate, and CO_2. These account for about 17-37 % of anthropogenic CH_4 emission (Sejian et al., 2011).

Fibrous feeds, including crop residues, agro-industrial by-products and natural pasture or native grass, of low digestibility, constitute the major proportion of feeds available to most ruminants under smallholder production systems in the developing countries (Wanapat et al., 2009). In Ethiopia, ruminants are fed on fibrous feeds, mainly crop residues that are poor in digestibility and the production of methane in such feeds is often very high. Ruminal methane production results in the inefficient conversion of potentially energy yielding substrates into a form that cannot be conserved by the host (Afazeli et al., 2014). Therefore, reducing rumen methane production has the potential to improve the efficiency of nutrient utilization and protects the environment from warming due to GHGs, mainly methane. There are a number of factors that affect *in vitro* fermentation of feeds and could cause intra- or inter-laboratory differences. These are mainly associated with the nature of rumen fluid inoculum, the breed of animal and its physiological condition, the time of collection of rumen fluid relative to feeding time and the time elapsed between rumen fluid sampling and inoculation (Robinson et al., 1999).

In vitro gas production using rumen fluid as an inoculum and with that of substrate incubations increases during early incubation and is maintained or even reduced slightly during prolonged incubation. This is predicted because the gas production rate in batch condition is directly equal to specific growth of methanogenic bacteria (Nopharatana et al., 2007). The high concentration of ammonia nitrogen produced is toxic to anaerobes, which might decrease the efficiency of the digestion and upset the process which results in low methane production (Chen et al., 2008).

There is no sufficient report on the assessment of *in vitro* methane production from locally available feedstuffs and that of rumen fluid as an inoculum in Ethiopia. Thus, the present study was undertaken to investigate *in vitro* gas and methane production from selected locally available feedstuffs and rumen content inoculum and to determine the possible application of least methane producing feedstuffs in ruminant diets.

2. METHODOLOGY

2.1. Study Design

This experimental study was conducted at Mekelle University, College of Veterinary Sciences Nutrition Laboratory from November 2018 to April 2019 to evaluate *in vitro* gas and methane production of 12 feed substrates and rumen content inoculum.

2.2. Substrates Used in *In vitro* Incubation

The fermentation was evaluated using samples of 12 feeds: six forages and roughages (grass hay, wheat straw, commercial alfalfa hay, green alfalfa, pods and leaves of *Faidherbia (F.) albida*; three concentrates (wheat bran, nug seed cake and molasses) and their combinations (grass hay + green alfalfa, grass hay +

wheat bran, and grass hay + wheat bran + nug cake + molasses). The feedstuffs were collected from Aynalem, while *F. albida* leaves and pods were collected from Abreha-We-Atsbeha village. The forages, roughages and the nug-seed cake were finely grounded to ease fermentation. One sample of each feed and the combinations above were inoculated individually.

2.3. Rumen Fluid Collection

Rumen fluid was collected from slaughtered cattle at Abergelle International Abattoir. Immediately after evisceration, the rumen was removed from the gastrointestinal tract of each cattle and a small opening was made, and the rumen content was mixed thoroughly. A great deal of attention was paid to keep the condition strictly anaerobic. The rumen fluid for estimation of methane from feeds was strained via a double layer muslin cloth, collected via a pre-warmed thermos flask (permuz), and transported to the laboratory immediately. Whereas, the rumen content used for direct inoculation was collected and incubated separately. For this, seventy rumen fluid samples were collected from the slaughtered animals in the abattoir. In the laboratory, the unstrained inoculum was used for direct inoculation to assess *in vitro* gas production via rumen microbes. All the animals slaughtered during the abattoir visit were males, and animals in the age range of 2-5 years were considered as young and animals above 5 years of age were classified as adult (Nicholson and Butterworth, 1986).

2.4. *In vitro* Incubation

In vitro Gas Production Technique (IVGPT) was carried out using different feed substrates to measure the production of gas following standard protocols (Akinfemi et al., 2009; Amlan, 2016; Blümmel and Ørskov, 1993) with some modification. A 60 ml syringe based IVGPT was used for overall gas and methane determination. Fresh rumen fluid collected from slaughtered cattle was used for IVGPT. The rumen fluid used for testing feed substrates was prepared by mixing rumen fluid from donor animals to get as many different rumen microbes as possible. From each feed substrate, 50 grams was inoculated with 500 ml strained rumen fluid in 1000 ml black bottle. For each feedstuff six replica were prepared except one feedstuff (commercial alfalfa hay) with four replicas due to shortage of sample.

Rumen content of 250 grams from each sample was mixed with equal amount of tap water and inoculated in 2000 ml black bottle. Then the inoculated feed substrates and rumen contents were incubated in a controlled space under anaerobic condition (using wax as sealant) at a temperature of 39^{o}C. A 60-watt power lamp was used to provide heat to maintain the rumen temperature and a follow-up was made until 24 hr while measuring the gas produced (Akinfemi et al., 2009).

2.5. *In vitro* Gas Production and Methane Estimation

The gas produced was measured from *in vitro* fermentation of feed substrates using filtered rumen fluid. The amount of gas produced depends on the amount of substrate fermented and the amount and molar proportions of the VFA produced (Davies et al., 2000). The gas produced was measured for evaluation of the interaction between forage and/or roughage, concentrate diets by incubating forage and/or roughages, and concentrate diets separately and in combination.

Starting from 3hrs of incubation up to the stoppage of gas production, the volume of the fermentation gas produced was recorded using the calibrated scale on the plastic syringes. The CH_4 gas produced due to fermentation of each substrate inoculated with strained rumen fluid was estimated as a percentage of the total gas produced from each inoculation by taking the average percentage of CH_4 as reported by Mengistu (2017) and Melesse et al. (2017) which is about 17.4% and methane to total gas ratio (CH_4:gas production=0.18:1), respectively. Whereas, in rumen content inoculum the amount of methane emitted was estimated by taking FAO report which stated that enteric fermentation of cattle constitutes about 39.1% CH_4 (Gerber et al., 2013). The incubation was stopped after 24 hrs. by decanting the syringe and bottle contents. Thus, CH_4 production in ml was calculated as CH_4 volume (ml) =CH_4% × total gas produced (ml) in 24 hrs. (Akinfemi et al., 2009; Melesse et al., 2017).

2.6. Data Management and Analysis

The data obtained from the IVGP experiment was entered into Microsoft Excel spreadsheet and analyzed statistically using one-way analysis of variance (ANOVA) to compare the means within groups. A univariate regression analysis was also performed using STATA version 12 software (StataCorp, 2011) to see the level of association in gas production between the different feedstuffs. To consider a result to be statistically significant, 95% CI and p-value < 0.05 was considered.

3. RESULT

3.1. Gas Production from Feed Substrates

The amount of aggregate gas production measured from tested feed types varied numerically with time of incubation, although it was not statistically significant in most of the cases (Table 1). A relatively high amount of gas production was observed at 6 hrs of incubation (9.27±1.92) followed by 3 hrs of incubation (7.03±1.71), and the least gas production (1.73±6.34) was recorded at 15 and above hrs of incubation.

3.2. Effect of Feed Substrates on *In vitro* Gas Production

The amount of cumulative gas measured from each of feed substrates showed a statistically significant variation (p=0.0012) among themselves where there was a relatively high amount of gas production in wheat bran (48.33±18.35) followed by commercial alfalfa hay (42.50±26.61). On the contrary, pods of *F. albida* scored the least gas production (0.83±2.04) followed by molasses (4.17± 10.21) (Table 2). In most feeds, the minimum gas produced was 0 ml and the maximum gas produced reached up to 70 ml. Similarly, the estimated methane produced was higher from wheat bran (8.41±3.19) and commercial alfalfa hay (7.40±4.11).

There was a relative difference in gas percentage among feed substrates. In about 50% of the nug seed cake and wheat bran samples the gas produced ranged from 21-60 ml and 30-60 ml, respectively (Figure 1). About 50% of the samples of *F. albida* leaves produced 20-50 ml. In mixed substrates (grass hay, molasses, wheat bran and nug seed cake), the gas produced in about 50% of the samples was about 0-26 ml.

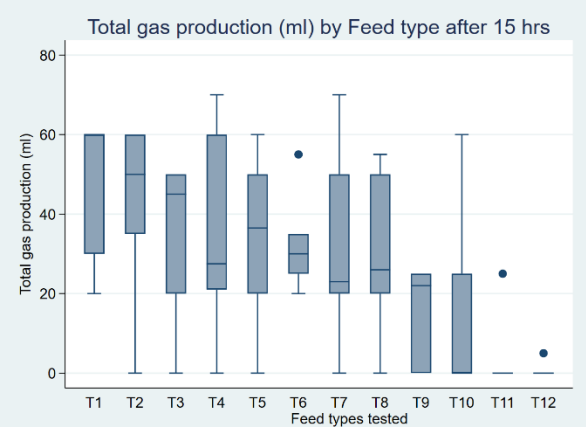

Figure 1: The effect of substrate on percentage of gas production

Feed substrates incubated for 3 hrs gave less gas production than that of 6 hr but higher than the gas produced at 9 hrs. The incubation time effect on total gas and methane production was apparent at 6 hrs being the maximum. After 15 hrs, gas production showed a significant reduction and then gas production stopped. There was higher mean gas production, 48 ml, in wheat bran followed by commercial alfalfa hay, 43 ml and the least mean gas production was recorded in pods of *F. albida*, 0.83 ml (Figure 2). Among mixed formulations, mixes of grass hay, wheat bran, molasses and nug seed cake produced lesser gas, 13 ml followed by grass hay and green alfalfa mixes, 15 ml. The univariate analysis result with crude odds ratio indicated statistically significant variation in gas production at 6 hrs (p=0.034), 12 hrs (p=0.034), and 15 and above hrs (p=0.029) incubation for the different feedstuffs (Table 3). The gas produced at 6 hrs was 1.2 times higher than gas produced at 3 hrs. However, the gas produced at 12 and 15 hrs was 0.67 and 0.38 times lower than that of the 3 hr gas production.

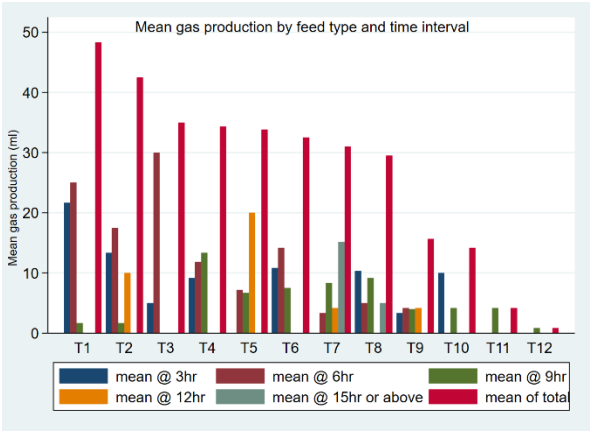

Figure 2: Effect of duration of in vitro incubation on gas production

Additionally, the gas production of the remaining feedstuffs was compared with the gas production of wheat bran, the highest gas producer, using a univariate analysis. The gas produced in molasses and seeds of *F. albida*, respectively was 0.04 and 0.03 times lower than that of gas produced from wheat bran. The odds of commercial alfalfa hay, leaf of *F. albida*, nug seed cake, grass hay-green alfalfa combination, and wheat straw were similar with that of wheat bran (OR = 1.0), which implies that there was no statistically significant difference in the mean gas production between these feedstuffs (Table 4)

Table 1. Aggregate gas production from tested feed types

	Gas production (ml)				Estimated CH_4 (ml) Mean±SD
Inoculation time	**Mean±SD**	**95% CI**	**Min**	**Max**	
3 hrs.	7.03±1.71	3.623-10.434	0	60	1.22±0.30
6 hrs.	9.27±1.92	5.443-13.010	0	60	1.60±0.33
9 hrs.	5.27±1.56	2.159-8.383	0	60	0.91±0.27
12 hrs.	3.29±1.37	0.548-6.023	0	60	0.57±0.24
15 hrs. & above	1.73±0.76	0.216-3.241	0	30	0.30±0.13
Total	26.59±2.76	21.080-32.091	0	70	4.63±0.48

SD= standard deviation, CI= confidence interval, Min= minimum value, Maximum value

Table 2. Cumulative gas production for each of the feed substrates

Feed types	**Total Gas production (ml)**				**Estimated CH_4 Mean±SD**
	Mean±SD	**Min**	**Max**	**P-value**	
Wheat bran (T1)	48.33±18.35	20	60		8.41±3.19
Commercial alfalfa hay (T2)	42.50±26.61	0	60		7.40±4.11
Leaf of *F. albida* (T3)	35.00±23.81	0	50		6.09±4.14
Nug seed cake (T4)	34.33±26.05	0	70		5.97±4.53
Grass hay & green alfalfa (T5)	33.83±23.07	0	60		5.89±4.01
Wheat straw (T6)	32.50±12.14	20	55		5.66±2.11
Grass hay (T7)	31.00±24.88	0	70	0.0012	5.39±4.33
Green alfalfa (T8)	29.50±20.43	0	55		5.13±3.55
Grass hay & Wheat bran (T9)	15.67±12.30	0	25		2.73±2.13
Grass hay and Concentrate (Wheat bran, Nuge Cake & Molasses (T10)	14.17±24.60	0	60		2.47±4.28
Molasses (T11)	4.17±10.21	0	25		0.73±1.78
Seed of *F. albida* (T12)	0.83±2.04	0	5		0.14±0.35

SD= standard deviation, Min= minimum value, Max=maximum value, CH_4= Methane

Table 3: *Comparison of gas production of substrates at different incubation times*

Inoculation time	**Mean±SD**	**Odds Ratio**	**p value**	**95% CI**
3 hrs.	7.03±1.71			
6 hrs.	9.27±1.92	1.20	0.034	1.01-1.40
9 hrs.	5.27±1.56	0.98	0.804	0.82-0.98
12 hrs.	3.29±1.37	0.67	0.034	0.47-0.98
15 hrs. & above	1.73±0.76	0.38	0.029	0.16-0.90

Table 4. Comparison of aggregate gas production between feedstuffs using adjusted Odds Ratio

Feed type	**Mean±SD**	**Adjusted OR**	**P value**	**95% CI**
Wheat bran (T1)	48.33±18.35			
Commercial alfalfa hay (T2)	42.50±26.61	1	1.000	0.50-20.83
Leaf of *F. albida* (T3)	35.00±23.81	1	1.000	0.05-20.83
Nug seed cake (T4)	34.33±26.05	1	1.000	0.05-20.83
Grass hay & green alfalfa (T5)	33.83±23.07	1	0.999	0.06-18.42
Wheat straw (T6)	32.50±12.14	1	0.998	0.04-21.02
Grass hay (T7)	31.00±24.88	0.71	0.081	0.03-17.45
Green alfalfa (T8)	29.50±20.43	0.67	0.067	0.03-6.20
Grass hay & Wheat bran (T9)	15.67±12.30	0.35	0.052	0.01-1.03
Grass hay and Concentrate (Wheat bran, Nuge Cake & Molasses (T10)	14.17±24.60	0.35	0.049	0.01-0.98
Molasses (T11)	4.17±10.21	0.04	0.008	0.00-0.83
Seed of *F. albida* (T12)	0.83±2.04	0.03	0.008	0.00-0.83

3.3. Gas Production from Rumen Content Inoculum

The overall mean gas and methane produced from rumen content samples, respectively was higher at 12 hrs of incubation (6.57±15.15) and (2.57±5.92). No gas production was recorded at the 3rd hr and then it showed an increasing trend until the 12 hrs of incubation. Some rumen content samples failed to produce gas and for that reason, the minimum value was 0 in all the incubation hrs. However, the maximum gas production was recorded after 15 hrs of incubation (64) (Table 5).

The overall gas production showed a statistically significant difference (p=0.014) between adult and young animals (Table 6). The mean gas production of young animals was higher (30.73±21.99) than that of adult animals (18.00±20.18). Furthermore, there was greater variation in percentage of gas production between groups where about 50% of the rumen samples from young and adult animals produced 12-58 ml and 0-26 ml, respectively (Figure 3). The methane produced was estimated using the reference from FAO and the mean methane production was 12.2±8.60 and 7.04±7.89 from adult and young animals, respectively.

Table 5. Cumulative total gas and methane production of rumen inoculum

Time interval	Gas production (ml)				Estimated CH_4
	Mean±SD	95% CI	Min	Max	Mean±SD
3 hrs.	0.00		0	0	0.00
6 hrs.	5.40±10.23	2.96-7.84	0	40	2.11±4.00
9 hrs.	6.30±14.40	2.87-9.73	0	60	2.46±5.63
12 hrs.	6.57±15.15	2.96-10.18	0	60	2.57±5.92
15 hrs & above	5.19±14.81	1.65-8.72	0	64	2.03±5.79
Total	23.46±21.77	18.27-28.65	0	64	4.08±3.79

Table 6: Effect of age of the animal on gas and CH_4 production of rumen content inoculum

Age category	Obs.	Gas production (ml)				Estimated CH_4 Mean±SD
		Mean±SD	P value	Min	Max	
Young	30	30.73±21.99	0.014	0	70	12.2±8.60
Adult	400	18.00±20.18		0	64	7.04±7.89

There was a significant difference in the gas production from direct rumen content inoculum with time variation between age groups. The gas produced at 6 hrs of incubation showed a statistically significant difference (p=0.031) between the rumen content samples from adult and young animals. However, the gas produced at other hrs of incubation did not show a statistically significant variation between the age groups (Table 7). The overall gas production was higher at 6 and 12 hrs of incubations (8.43±11.66) and (8.10±16.27), respectively for young animals. For adult animals, the higher production was at 9 hrs (5.88±14.83) and 12 hrs (5.43±14.35) of incubations, respectively.

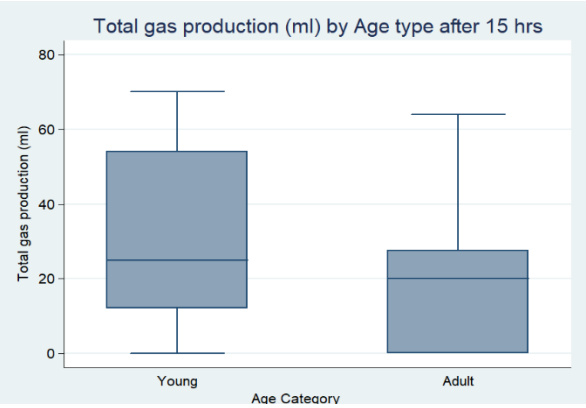

Figure 3: Percentile of gas production by age of animals

Table 7. Effect of time of inoculation on gas production

Age category	Incubation Time	Gas production (ml)				Estimated methane Mean±SD
		Mean±SD	Min	Max	P-value	
Young	3 hrs	0.00	0	0		0.00
Adult		0.00	0	0		0.00
Young	6 hrs.	8.43±11.66	0	40	0.031	3.30±4.56
Adult		3.13±8.46	0	35		1.22±3.31
Young	9 hrs.	6.87±14.01	0	60	0.778	2.69±5.48
Adult		5.88±14.83	0	60		2.30±5.80
Young	12 hrs.	8.10±16.27	0	50	0.469	3.17±6.36
Adult		5.43±14.35	0	60		2.12±5.61
Young	15 hrs & Above	7.33±17.60	0	60	0.297	2.87±6.88
Adult		3.58±12.31	0	64		1.40±4.81

4. DISCUSSION

The amount of cumulative gas production measured from each of the feedstuffs differed between substrate types and within each replica. This could be due to the difference in the digestibility characteristics of the incubated feedstuffs, the variation in rumen microbes (due to the difference in feed of the animal before slaughter), and the physiological condition of the animal. Moreover, the variation could be due to the time of collection of rumen fluid relative to feeding time, the time elapsed between rumen fluid sampling and inoculation, or the condition under which it was inoculated. Finally, differences could be explained by whether the temperature maintained like that of rumen environment or not and the maintenance of anaerobic environment (Gerber et al., 2013; Robinson et al., 1999).

In vitro gas production using rumen fluid as an inoculum and with that of substrate incubations increases during early incubation and is maintained or even reduced slightly during prolonged incubation. Gas and methane production of wheat bran after 15 hrs of *in vitro* incubation was higher. Wheat bran has a high content of nitrogen free extract (NFE) containing high amounts of easily fermentable carbohydrates (Lee et al., 2003). Whereas gas production and estimated CH_4 production of seed of *F. albida* was very little. Perhaps, *F. albida* has higher crude fibre content, which cannot be degraded easily. In addition, the gas production and estimated methane production from molasses and feeds with the addition of molasses was very minimum. This is due to the high starch content in molasses. It has been indicated that feeding diets with higher grain content, and other starch rich feeds are among the dietary CH_4 mitigation strategies as compared with feeding forage-based diets (Beauchemin et al., 2009; Dutreuil et al., 2014; Hatew et al., 2015; Johnson and Johnson, 1995). Starch fermentation promotes propionate production in the rumen and lowers ruminal pH, which inhibits the growth of rumen methanogens (Van Kessel and Russell, 1996). During the first 3 hrs of incubation wheat bran produced higher gas while grass hay and commercial alfalfa hay mixes showed higher methane production after 15 and 12 hrs, respectively which might be because of high contents of crude fibre in grass hay which can't be degraded quickly (Lee et al., 2003). The variations in gas and methane productions between the incubation ours and the feed stuffs could also be associated with the carbohydrate load in the rumen (Hatew et al., 2015; Tadesse, 2014).

At the beginning of incubation (3 hrs), gas production was less which might be due to the apparent inactivity (lag phase) in which the cells are adapting to a new environment (Bhatta et al., 2012; Mauricio et al., 2001; Robinson et al., 1999). However, on 6 and 9 hrs there were an increased amount of gas production which may be associated with the microbe's adaptation to the conditions or environment under which they are incubated. After 12 hrs, gas production showed a dramatic reduction and then gas production stopped and ends after 15 hrs. This may be due to the decline and death phase of microbes due to lack of favorable environment for their growth or due to the accumulation of ammonia (NH_4), which was released by protein degradation, combined with CO_2, methane substrate and resulted in less methane production. This is predicted because gas production rate in batch condition is directly equal to specific growth of methanogenic bacteria (Mauricio et al., 2001; Nopharatana et al., 2007). The high concentration of ammonia nitrogen produced is toxic to anaerobes, which might decrease the efficiency of the digestion and upset the process which results in low methane production (Chen et al., 2008).

In this study, the cumulative gas production from both feed and rumen content substrate inoculations was less than what are reported from other authors (Bhatta et al., 2012). This could be due to the difference in the methodology they used, source of rumen fluid (ruminal microbes), and duration of *in vitro* incubation. In gas and methane production of direct rumen content inoculum, there was a significant difference between adult and young animals. This is in line with the findings of Grandl et al. (2016) which states that methane production increasing with age in heifers (8–25 months) but decreasing in adult cows (4-10yrs). Liu et al. (2016) also reported that enteric methane production in heifers and adult cows, using the sulfur hexafluoride (SF_6) tracer technique, and showed an average of 35.1 ± 2.8 g/kg dry matter intake (DMI) for heifers which is higher than 27.2 ± 0.9 g/kg DMI for adults. This variation could be due to changes in microbial communities related to physiological changes along with age.

5. CONCLUSION

The feed substrates used in the present study were forages and roughages (grass hay, wheat straw, green alfalfa, commercial alfalfa hay, seed and leaf of *F. albida*); concentrates (wheat bran, nug seed cake and molasses) and their combinations (grass hay-green alfalfa, grass hay-wheat bran, and grass hay-wheat bran-molasses and nug seed cake). There was a difference in time and extent of degradability between feed substrates. Among all feed substrates, the fermentation of wheat bran was faster and contributes to higher gas production during the first *in vitro* incubation times (at 3 hrs). Leaf of *F. albida*, commercial alfalfa hay and grass hay were degraded at 6, 9 and after 15 hrs of incubation respectively, which all contribute significantly to a higher cumulative gas production. Seed of *F. albida* gave very low gas followed by molasses and mixed formulations containing molasses. Significant differences were observed between young and adult cattle and inoculum of young animals contributed higher gas and methane production. Thus, use of *F. albida* seeds and molasses as feed supplement, and use of mixed feeds has a potential for reducing total gas and methane emission from ruminants. In doing this, attention should be given to balance productivity (meat and milk) and methane emissions. Further experiments should also be conducted using *in vitro* and *in vivo* techniques to better understand the emission potential of locally

available feedstuffs, and for further selection of less methane emitting feeds and a combination of feed formulation. In addition, the impacts of other dietary mitigation strategies such as use ionophores, fats, high quality forages, and increased use of grains to mitigate excess methane should be evaluated under the existing production systems.

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