In-Vitro study of some medicinal plant in Edo State for reducing methanogenesis in ruminant

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Abstract

The objective of this work was to evaluate the in vitro gas production characteristics of some medicinal plants and their effect on methanogenesis in ruminant animals. Medicinal plants like Azadiracta indica, Sida acuta Alstonia boneei, Newbouldia laevis among others were incubated in vitro with maize as substrate. The proximate composition of the test samples was determined prior to the in vitro study. Newbouldia laevis root, Sida acuta root, and Alstonia boneei root had high percentage crude protein (CP) of 8.75%. The values of ADF and NDF of the samples varied significantly with the ADF values ranging from 12% for Sida acuta leaves to 52% for A. indica, stem. While the NDF values were between 30% for E. Heterophylla and 71% for S. acuta roots. There was significant variation in the gas production among all the plant samples. However, Psidium guajava stem produced the highest volume of gas. Among the medicinal plants studied, Azadiracta indica stem, Astoniaboneei leaves, and Newbouldia laevis root had methane(%) reduction potential of 53.56%, 49.06%, and 41.50% respectively.

This result indicates that medicinal plants have the potential to mitigate methanogenesis in ruminants.

Introduction

Livestock production faces a number of challenges including pressure from the public to be good environmental stewards and adopt welfare-friendly practices. They often implement practices beyond those required from a regulatory standpoint to meet the demand of consumers. Ruminant livestock have been recognized as major contributors to greenhouse gases (Steinfeld *et al*., 2006). Similarly, livestock account for mainly 80% of all emission from the Agricultural sector (Steinfeld *et al*., 2006). There are three major greenhouse gases, which are carbondioxide, methane and nitrous oxide. Methane is a potent greenhouse gas and its emission from livestock is one of the major significant contributors towards the accumulation of this gas in the environment which contributes to global warming. The global warming potential of methane is 21-times that of CO_2over 100 years (UNFCCC, 2007). Methanogenesis also known, as biomethanation is the process by which microbes known as methanogens-organisms capable of producing methane produces methane in the rumen of ruminants.

Methane emissions from ruminants reduce the efficiency of nutrient utilization; manipulation of rumen microbial ecosystem for reducing methane emission by ruminants to improve their performance is one of the most important goals for animal nutritionists. The rumen is a diverse and unique microbial ecosystem comprised of bacteria, protozoa and fungi. In therumen, hydrogen is produce during anaerobic fermentation of nutrients. This hydrogen can be used during the synthesis of volatile fatty acids (VFA's) and microbial protein synthesis. The excess hydrogen from NADH is eliminated primarily by the formation of methane produced by methanogens. Reduction of methane in ruminant livestock is a top priority for researchers across the globe. Several

methods to reduce methane emissions from the rumen have been developed. These methods include processing of feeds, altering the type of ration, supplementation of unsaturated fatty acids (Johnson and Johnson 1995), defaunation (Van Nevel and Demeyer 1996), organic acids (Asanuma *et al*., 1999), halogenated methane analogues (Haque, 2001), ionophores (Kobayashi *et al*.,1992), microbial feed additives (Mutsvangwa *et al* 1992), non ionic surfactants (Lee and Ha 2003), sulphates (Kamra *et al* 2004) and herbal products (Patra *et al* 2006). The use of herbal preparations is a natural alternative to antibiotic use in animal nutrition. Plant secondary metabolites have been shown to modulate ruminal fermentation to improve nutrient utilization in ruminants (Hristov *et al*., 1999). These compounds possess antimicrobial activity that is highly specific, which raises their possibility to target methanogens.

Research Objectives

The objectives were to determining the effect of;

medicinal plants on methanogenesis *in vitro.*

medicinal plants on *in vitro* dry matter and organic matter digestibility as well as short chain fatty acid production.

Materials and Methods

Collection and Identification of Plant Materials

Multi-stage sampling technique was used in the collection of fresh plant parts such as the leaves, stems, roots, barks fruits and seeds of medicinal plants and was carefully identified by their local, common and botanical names, as well as their uses.

Chemical Analysis

Crude protein, Ash, and Dry matter were carried out according to the procedure of AOAC (1995) Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and hemicelluloses were determined using the method described by Van Soest *et al.* (1991).

Collection of rumen liquor

Rumen liquor was collected from goats at the ruminant unit of the University of Benin Teaching and Research Farm, Ugbowo Campus, Benin City. The collection was via stomach tube. The liquor was collected in the early hours of the morning into a pre warmed flask prior to feeding the animals. The flask containing the rumen liquor was taken to the laboratory where it was strained through four layers of cheese cloth. The strained liquor was mixed with a buffer solution in a ratio of 1:2. This mixture was put in a water bath and gassed with CO_2 to maintain anaerobic condition and a temperature of 39°C to keep the microorganisms alive

Buffer Preparation

The buffer was prepared a day before rumen liquor collection and maintained at a pH of 6.2 (Navaro-villa *et al.,* 2011) and temperature of 39^{o}C. The buffer used was consisting of the following reagents: Na_2HPO_4. $12H_2O$ 1.985g/l, KH_2PO_4 1.302g/l, $MgCl_2.6H_2O$ 0.105g /l, NH_4HCO_3 1.407g /l, $NaHCO_3$ 5.418g /l, NaOH 0.100g/l.

***In Vitro* Fermentation of Samples**

The *in vitro* incubation was carried out using 120 ml calibrated syringes containing the inoculums (Rumen liquor: buffer, 1:2). 200 mg of substrate was weighed into nylon bags for the incubation at 39^{o}C with 30 ml of inoculums. The bags were placed inside the syringes before the inoculum was introduced into the syringes. The syringes were fitted with silicon tube and clipped before placing them in the incubator at 39°C. The syringes containing only inoculum served as the blank while the syringes containing bags with only the substrate served as the control. The time for the commencement of incubation was noted and the syringes were monitored at three hour intervals for the next 24 hours. For each incubation time, the head space of the syringes was measured and recorded. At 24 hours of incubation, the final readings were taken and the syringes put on ice to stop further gas production.

Estimation of Methane Production by the Injection of NaOH
Methane content in fermentation gas was determined by Injecting 4mL of 40% NaOH solution into the syringes

Statistical analysis

Data collected during chemical analysis and at different incubation period were analyzed using two ways ANOVA according to the procedure of SAS (2004) and separation of means was done using Duncan New Multiple Range Test for multiple means and least significant difference (LSD) for pair mean comparison in the same SAS (2004) software.
Complete Randomised design was used in this experimental work.

Results and Discussion

Chemical Composition of some medicinal plants

Table 1: Chemical composition (%) of some medicinal plants in Edo State

Samples	CP	ASH	NDF	ADF	HEMI	OM
A. Indica stem	31.50^{a}	6.72^{ab}	62.40^{c}	52.20^{a}	10.23^{e}	93.28^{bc}
A. boneei leaves	21.00^{b}	6.40^{ab}	36.50^{f}	31.95^{c}	4.58^{f}	93.60^{bc}
C.papaya seed	14.85^{c}	6.49^{ab}	34.45^{g}	32.00^{c}	2.45^{f}	93.52^{bc}
S. acuta root	9.63^{d}	7.16^{ab}	71.40^{a}	39.00^{b}	32.40^{b}	92.85^{bc}
N. laevis root	9.63^{d}	5.27^{c}	47.15^{d}	31.50^{c}	15.65^{d}	94.74^{a}
S.acuta leaves	7.18^{e}	7.20^{a}	37.15^{e}	12.75^{f}	24.40^{c}	92.80^{c}
E.heterophylla	6.13^{e}	6.00^{bc}	30.93^{h}	17.70^{e}	13.23^{d}	94.00^{ab}
C. dactylon	6.13^{e}	7.10^{ab}	67.40^{b}	25.10^{d}	42.37^{a}	92.90^{bc}
SEM	0.41	0.35	5.13	0.84	0.85	0.35

abcdefgh = means along the same column with the same alphabet are not significantly different ($p > 0.05$)

Chemical Composition of Some Medicinal Plant Samples

Wide variations existed in the chemical composition of the medicinal plants used in this study as shown in Table 1. The crude protein (CP) content of these medicinal plants varied significantly amongst the samples. The high value of CP in *N. laevis,* root *S. acuta* root and *A. boneei* root could be responsible for their ability to decrease NH_3 production from amino acids in rumen fluid *in vitro* (Wallace *et al*., 2002). Reducing the rate of ammonia production by targeting the (hyper ammonia-producing bacteria) HAP would benefit the animal by improving the efficiency of nitrogen utilization. The high increase in ruminal fermentation of some medicinal plants could be linked or characterized by ruminal digestibility of nutrients (OM, NDF, ADF and HEMICELLULOSE) with an optimum level of efficiency of microbial protein synthesis and this is linked to the finding of Christopher, (2012). In this study protein requirements are met with microbial protein produced during the fermentation of feed nutrient and might depend on the provision of dietary energy and protein in the right ratios and amounts (Christopher, 2012). This level of NDF has been linked to stimulation of fermentation that will produce ruminal gas with high proportion of CH_4 (Moss *et al*., 2000). On the other hand, this study found that OM was high in values and slight different was observed in all plant samples.

In Vitro Gas production at different Hours of incubation

Table 2: Effect of medicinal plants on the volume of gas (ml/120mg) produced at different incubation hours

	Incubation Hours							
Medicinal Plants	3h	6h	9h	12h	15h	18h	21h	24h
Control	2.75^{a}	6.25^{a}	9.50^{abc}	13.25^{bcdef}	17.75^{abcd}	19.25^{abcd}	21.75^{abcde}	22.75^{abc}
Spondia monbin seed	3.50^{a}	6.00^{a}	10.00^{abc}	12.50^{cdef}	14.00^{d}	17.75^{bcd}	20.00^{bcde}	21.25^{abc}
Spondia monbin leaves	3.50^{a}	6.50^{a}	10.50^{abc}	14.00^{abcdef}	17.25^{abcd}	18.25^{abcd}	21.00^{bcde}	21.75^{abc}
Carica papaya seed	3.50^{a}	5.75^{ab}	12.75^{a}	18.75^{a}	22.50^{a}	23.75^{abc}	25.75^{abc}	28.50^{ab}
Euphorbia hirta	3.50^{a}	6.00^{a}	9.50^{abc}	12.75^{bcdef}	17.00^{abcd}	20.75^{abcd}	22.00^{abcde}	24.75^{abc}
*Newbouldia laevis*bark	3.50^{a}	5.75^{ab}	8.50^{bc}	12.50^{cdef}	16.50^{abcd}	17.25^{bcd}	19.25^{bcde}	22.50$_{bcd}$
Alstonia boneei leaves	3.00^{a}	6.25^{a}	11.25^{ab}	12.50^{cdef}	15.00^{bcd}	16.50^{cd}	15.75^{c}	21.00^{bc}
Azadiracta indica stem	3.00^{a}	4.25^{abc}	10.25^{abc}	16.5^{abcd}	20.00^{abcd}	22.25^{abcd}	25.50^{abc}	28.00^{ab}
pidium guajava leaves	2.75^{a}	6.25^{a}	10.25^{abc}	13.75^{abcdef}	18.75^{abcd}	20.75^{abcd}	21.75^{abcde}	23.75^{abc}
Hura crepitans	2.75^{a}	5.75^{ab}	8.25^{bc}	11.50^{def}	14.50^{cd}	16.00^{d}	18.50^{cde}	21.25^{abc}
*Alstonia boneei*roots	2.75^{a}	4.50^{abc}	9.50^{abc}	14.25^{abcdef}	16.75^{abcd}	19.00^{abcd}	20.75^{bcde}	22.50^{abc}
Sida acuta root	2.75^{a}	5.75^{ab}	13.00^{a}	17.75^{ab}	21.00^{abc}	25.25^{a}	27.00^{ab}	29.50^{a}
Newbouldia laevis root	2.75^{a}	5.50^{ab}	11.00^{ab}	16.00^{abcde}	20.25^{abcd}	24.25^{ab}	28.75^{a}	29.50^{a}
Euphorbia heterophylla	2.75^{a}	5.75^{ab}	10.50^{abc}	16.75^{abc}	21.75^{ab}	24.25^{ab}	28.75^{a}	28.75^{ab}
Psidium guajava stem	2.50^{a}	3.25^{c}	6.75^{c}	10.75^{f}	14.5^{cd}	15.75^{d}	17.00^{de}	19.50^{c}
Aspilia Africana	2.50^{a}	5.50^{abc}	9.50^{abc}	13.75^{abcdef}	16.25^{abcd}	18.00^{abcd}	20.50^{bcde}	21.50^{abc}
*Azadiracta indica*bark	2.00^{a}	4.50^{abc}	8.75^{bc}	11.25^{ef}	15.50^{bcd}	17.75^{bcd}	20.25^{bcde}	26.75^{abc}
Sida acuta root	2.00^{a}	4.25^{abc}	8.25^{bc}	13.50^{bcdef}	16.75^{abcd}	18.25^{abcd}	20.00^{bcde}	20.75^{bc}
SEM =	0.52	0.69	1.13	1.51	1.99	2.17	2.26	2.39

abcdefg = Means along the same column with the same alphabets are not significantly different (P> 0.05).

In Vitro Gas production at different Hours of incubation

Table 2. Shows the *in vitro* gas production at different hours of incubation for medicinal plants with hours ranging from 3 to 24 hours. Among samples incubated at 3hours of incubation, there was no significant difference (P> 0.05) in the total gas volume produced. At 6 hours of incubation there was no significant difference between gas production for the test samples except for *S. mombin* stem, *S. monbin* leaves, *E. hirta*, *A. boneei* leaves which had significantly (P>0.05) higher gas volumes than *P. guajava* leaves. At 9 hours of incubation a significant difference (P< 0.05) existed with *p. guajava stem* having the lowest value of gas production (mmol/g DM incubated) when compared to other plants.At the 12thhour similar observation was seen as in the 9thhour while at the 15thhour there was no significant difference (P>0.05) among plants except for *C. papaya* Seed and *S. mombin* seed showing slight difference with the former having the lowest and the latter having the highest mean value respectively. At 18 hours a significant difference (P<0.05) was observed in the volume of gas production for *P. guajava* stem, *S. acuta* leaves, *H. crepitans,* and *A. boneei* leaves when compared to others. *N. laevis*, *E. heterophylla, A. boneei* leaves were significantly different (P< 0.05) from other plants at the 21st hour of incubation. At the conclusion of the incubation period *N. laevis* and *S. acuta* leaves had the highest gas volumes which were significantly higher (P< 0.05) than *P. guajava* stem.

Some of the plants tested reduced methane productions (P < 0.001) in relation to the control and gas production, pH and fermentation efficiency were not affected (P < 0.05) by any of the plants. Some of the methane inhibitors may have adverse effects on ruminal metabolism or physiology, such as reducing digestibility (Beauchemin and McGinn2006). However,some plants decrease methane production and stimulate microbial metabolism, increasing digestibility of crude protein and cell wall constituents as well as yield of microbial biomass (Broudiscou *et al* 2002). Lack of effect on nutrient degradation *in vitro* with the inclusion of some plants accompanied by reduced methane

production has been reported by Sliwinski *et al.* (2002). In this work, some of the plant samples modified methane production and thus may have the potential to improve the ruminal fermentation profile.

Effect of medicinal plants on Post *In Vitro* Gas Production Parameters.

Table 3: Effect of medicinal plants on dry matter digestibility, methane gas and SCFA Production

Medicinal Plant	SCFA (Mmmol)	CH4 (Ml/130mg)	CH4 (%)	CH4 Reduction (%)	DMD
Control	0.50^{abc}	10.75^{a}	47.99^{a}	0.00^{g}	83.27^{abc}
Sida acuta leaves	0.65^{a}	9.75^{ab}	34.05^{cde}	28.82^{bcde}	63.80^{def}
Aspilia Africana	0.47^{abc}	9.00^{abc}	43.58^{abc}	8.89^{efg}	58.27^{ef}
Psidium guajava stem	0.43^{c}	9.00^{abc}	45.74^{ab}	4.36^{fg}	87.692^{a}
Carica papaya seed	0.63^{ab}	8.75^{abcd}	31.24^{def}	34.69^{abcd}	71.54^{bcde}
Euphorbia heterophylla	0.63^{ab}	8.50^{bcd}	29.70^{def}	37.89^{abcd}	84.62^{ab}
Alstonia boneei root	0.50^{abc}	8.25^{bcde}	36.93^{bcd}	22.79^{cdef}	78.97^{abcd}
Azadiracta indica bark	0.59^{abc}	8.00^{bcde}	31.66^{def}	33.81^{abcd}	71.79^{bcde}
Newbouldia laevis root	0.65^{a}	8.00^{bcde}	27.98^{def}	41.50^{abc}	71.79^{bcde}
Jatropha curcas	0.51^{abc}	8.00^{bcde}	34.32^{cde}	28.25^{bcde}	70.77^{bcdef}
Hura crepitans	0.47^{abc}	7.75^{bcde}	37.65^{bcd}	21.28^{cdef}	68.21^{cdef}
Euphorbia hirta	0.55^{abc}	7.75^{bcde}	31.58^{def}	33.97^{abcd}	74.24^{abcd}
Psidium guajava leaves	0.52^{abc}	7.50^{bcde}	31.32^{def}	34.52^{abcd}	41.15^{g}
Spondias mombin	0.48^{abc}	7.50^{bcde}	35.53^{bcd}	25.71^{cde}	56.03^{f}
Sida acuta root	0.46^{bc}	7.25^{cde}	34.54^{cde}	27.78^{bcde}	75.64^{abcd}
Spondias mombin seed	0.47^{abc}	7.00^{cdef}	32.86^{cdef}	31.30^{bcd}	78.46^{abcd}
Newbouldia Laevis bark	0.05^{abc}	6.50^{def}	30.04^{def}	37.21^{abcd}	66.92^{def}
Azadiracta indica stem	0.62^{ab}	6.00^{ef}	22.22^{f}	53.56^{a}	69.78^{bcdef}
Alstonia boneei leaves	0.46^{bc}	5.00^{f}	24.36^{ef}	49.06^{ab}	75.89^{abcd}
SEM	0.68	0.05	3.27	6.36	4.07

abcdefg = Meansalong the same column with the same alphabet are not significantly different (P> 0.05)

Post *In Vitro* Gas Production Parameters of Medicinal Plants

Post *in vitro* gas production parameters of medicinal plants are presented in Table 3. It was observed in this study that medicinal plants which are low in percentage methane production were high in the short chain fatty acid (SCFA) production and observed to reduce methane production. This is in line with the utilization of dissolved H_2 for rumen fermentation modification (Pelchen and Peters, 1998). Production of high SCFA could be related to the use of large amount of the concentrate feed as reported by (Beauchemin and McGinn, 2005), and assumed to yield relatively less CH_4 per MJ of gross energy intake (Ferris *et al*., 1999).

This study shows that the high DMD value may be as a result of the rumen containing well adapted microbial population in order to make good utilization of the cellulose materials that can be later used by the animal (Bamikole, 2012). The inhibitory action of *Psidium guajava* leaves could have occurred due to presence of phytochemical constituents viz. alkaloids, saponins, steroidal rings and deoxy sugars. Furthermore, *Psidium guajava* leaves extract have shown antimicrobial activities (Elekwa *et al* 2009).

The VFA formation determines the amount of excess H_2 in the rumen which is converted to CH_4 by methanogenic bacteria (Bodas *et al*., 2012). In gas production or total VFA concentration, Reduction in CH_4 production have been mostly related to adverse effect on substrate degradation (Beauchemin and McGinn 2006)

Conclusion and Recommendation

The onus of salvaging mankind and the environment from the effects of climate change is on researchers, nutritionist and rumen microbiologist across the globe.The results obtained in this study indicate that medicinal plants have the potentials to reduce methanogenesis in ruminant.

There has been minimal adoption of practices to specifically reduce methane emission from livestock and to safeguard the environment particularly in developing countries. It is therefore recommended that inclusion of medicinal plants in ruminant livestock diet should be adopted as a strategy mitigating methanogenesis as it is generally regarded as safe.

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