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Effect of some medicinal herbs and spices from Enugu and Kaduna States, Nigeria, on *in-vitro* rumen fermentation and methanogenesis

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Abstract

A study was carried out to determine the effect of medicinal plants from Enugu (En) and Kaduna (Kd) States on in-vitro rumen fermentation and methanogenesis. Twelve medicinal plants comprising herbs and spices were collected from En and Kd States, respectively. The chemical composition and cell wall component (NDF, ADF and hemicellulose) of the plants were determined. The samples were evaluated in an in-vitro study at an inclusion level of 10 mg/ 200 mg substrate. The experiment was laid out in a factorial arrangement and executed as a randomized complete block design with incubation done in batches. At the completion of the 24-hour incubation period, total gas volume (GV), dry matter digestibility (DMD), fermentation efficiency (FE) and CH_4 were determined while organic matter digestibility (OMD), short chain fatty acids (SCFA) and metabolisable energy (ME) were estimated from established equations. The chemical composition indicated range values of 72.82 (Allium sativum, En) - 95.73% (Aloe barbadense, Kd) for DM, 7.01 (Aframomum melegueta, En) – 30.59% (Allium sativum, En) for CP, 8.94 (Allium sativum, Kd) – 81.41% (Cymbopogon citratus, En) for NDF, 3.58 (Allium sativum, En) – 63.64% (Xylopia aethiopica, En) for ADF, 1.79 (Allium cepa, Kd) - 48.77 % (Aframomum melegueta, En) for hemicellulose, 2.64 (Aframomum melegueta, En) – 22.51% (Ageratum conyzoides, Kd) and 77.49 (Ageratum conyzoides, Kd) – 97.36% (Aframomum melegueta, En) for ash and OM, respectively. The effect of location on chemical composition varied among the plant types with significantly higher ($P < 0.05$) levels of CP in most of the Kd plants. The range for GV, DMD, CH_4 percentage, CH_4 reduction, FE, OMD, SCFA and ME were 85.00 (Ageratum conyzoides, Kd) - 149.49 mL/g DM (Aloe barbadense, Kd), 525.50 (Sida acuta, Kd) - 764.50 g/Kg DM (Aframomum melegueta, En), 45.75 (Allium sativum, En) - 72.59% (Aframomum melegueta, Kd), 2.36 (Allium cepa, Kd) - 32.86% (Zingiber officinale, Kd), 4.23 (Sida acuta, Kd) - 8.12 (Ageratum conyzoides, Kd), 45.83 (Ageratum conyzoides, Kd) – 57.18% (Cymbopogon citratus, Enugu; Aloe barbadense, Kd), 0.35 (Ageratum conyzoides, Kd) - 0.66 mmol/200 mg DM (Cymbopogon citratus, En) and 5.18 (Ageratum conyzoides, Kd) – 6.93 MJ/Kg DM (Cymbopogon citratus, En; Aloe barbadense, Kd) respectively. Allium sativum bulbs, Zingiber officinale rhizomes, Cymbopogon citratus and Sida acuta (both locations) reduced CH_4 production without adversely affecting the desired fermentation parameters indicating that the medicinal plants have methane reducing potential.

Keywords: Medicinal herbs, spices, in-vitro, rumen fermentation and methanogenesis

Introduction

Methane is one of the greenhouse gases linked to climate change, which is adversely affecting agricultural production as well as human lives due to the occurrence of natural disasters across the planet. The Intergovernmental panel on Climate Change (IPCC, 2007) predicts that by 2010 the temperature of the earth's atmosphere may have risen by 1.8 – 3.9° C. FAO (2006) reports that a less than 1° C increase in the earth's atmospheric temperature over the last century has resulted in floods, forest fires and storms across the globe. Methane accounts for about 14% of human induced GHG production (Forster *et al*., 2007) and a

6-10% loss of gross energy intake via rumen fermentation (Eckard *et al.*, 2010). In Nigeria it is estimated that ruminant livestock have a methane output of 59 kg/animal/year (FAO, 2010). With global population increasing at an annual rate of 1.3% (UNDESA, 2013) there is expected to be a corresponding increase in the level of agricultural production to meet their nutritional requirements. Consequently, livestock numbers are also expected to increase thus resulting in higher methane output from ruminant livestock except proactive steps are taken to increase the efficiency of rumen fermentation by reducing losses to methane production. Although several options have been explored over the years to address this emerging challenge there is now a preference for strategies that are safe for humans and livestock. Indigenous medicinal plants and spices which are often underutilized have prospects in this regard due to their content of bioactive compounds such as essential oils, tannins, saponins, flavonoids and alkaloids (Adu *et al.*, 2013; Belewu *et al.*, 2009; Doherty *et al.*, 2010). These bioactive compounds have been reported to have antimicrobial activity (Belewu *et al.*, 2009; Garba and Okeniyi, 2012; Uzeh and Oguntosin, 2013). It is therefore pertinent to access the level to which these medicinal plants can reduce methane production in the rumen as well as influence other fermentation parameters. Thus this study was conducted to evaluate the effect of some herbs and spices from Enugu and Kaduna States of Nigeria on *in-vitro* methanogenesis and fermentation parameters.

Materials and methods

Sample collection, identification and preparation

Medicinal plants and spices in Enugu and Kaduna States were catalogued and thereafter collected using purposeful sampling technique. Identification was done using ethnobotanical in literature. Local residents in both locations also assisted with the identification. The samples collected were classified as herbs and spices. The samples were air dried, milled and stored in air tight plastic containers prior to the determination of chemical composition and the *in-vitro* fermentation study.

Substrate preparation

The substrate (CP = 11.80; OM = 83.59; NDF = 71.87; ADF = 34.82; CF = 19.92) comprised 30% legume hay (*Centrosema molle*), 30% grass hay (*Panicum maximum*) and 40% concentrate.

Sample/substrate mixture

200 mg of the substrate with 10% inclusion of the medicinal plant and spices respectively was weighed into previously numbered and weighed incubation bags which were then sealed and inserted into numbered 100 mL plastic syringes The 100 ml syringes were used in line with the protocol of Fievez *et al.* (2005).

Rumen liquor collection and buffer preparation

Rumen liquor was collected from six West African dwarf goats at the University of Benin Teaching and Research farm. The goats were confined and fed concentrate alongside Guinea grass (*Panicum maximum*) and served water *ad-libitum*. The liquor was collected early in the morning prior to feeding via stomach tube into a pre-warmed thermos flask. The liquor was strained through four layers of cheese cloth and mixed with the buffer solution in a ratio of 1:2. (Tilley and Terry, 1963; Menke *et al.*, 1979) This mixture (inoculum) was dispensed into beakers which were placed in a water bath maintained at 39° C and flushed with CO_2 to provide anaerobic conditions and keep the microorganisms alive.

In-vitro fermentation of samples

30 mL of the inoculum was introduced into

each of the syringes containing the incubation bags. The syringes containing only inoculum served as the blank while those with bags containing only the substrate served as control. The volume of gas produced was read at six hour intervals for the 24 hour incubation period. Upon completion of the incubation period the syringes were prepared for methane determination. Methane production at the end of the incubation period was determined by injecting 4 mL of 40% sodium hydroxide (NaOH) into each incubation syringe with agitation. The volume of gas after the movement of plunger ceased was recorded as the methane produced. The incubation bags were removed from the syringes, washed under running tap water and thereafter oven dried to constant weight to determine the dry matter digestibility (DMD). Metabolisable energy (ME), organic matter digestibility (OMD %) and short chain fatty acid (SCFA) were estimated with established equations. The former were established with the equations of Menke and Steingass (1988) while the latter was with that formulated by Getachew *et al.* (1999).

Chemical analysis

Organic matter, crude protein, crude fiber and ash content of the substrate, spices and herbs were determined using the official procedure of AOAC (2000) while the Van Soest *et al.* (1991) method was used to determine the cell wall components i.e. acid detergent fiber (ADF), neutral detergent fiber (NDF) and hemicellulose.

Data analysis

Data collected were analyzed using ANOVA in a completely randomized design, following the procedure of SAS (2014). Separation of significant differences between the means was done using Duncan New Multiple Range Test of the SAS (2014) software.

Results

Chemical composition of Panicum maximum used for the study

Table 1 shows the chemical composition of the *Panicum maximum* fed to the WAD goats used in this study prior to rumen liquor collection. The DM, CP, NDF, ADF and HEM were 29.05, 7.71, 59.00, 37.00 and 22.00 % respectively.

Proximate composition of concentrate used for the study

The DM, CP, CF, EE, ash and NFE respectively of the concentrate were 91.40, 15.82, 4.41, 7.51, 6.34 and 57.32 % as shown in Table 1.

Chemical composition of herbs and spices used for the study

The chemical composition of the herbs and spices in the present study is presented in Tables 2 and 3. The DM, CP, NDF, ADF, hemicellulose, ash and OM among the samples ranged from 72.82 (*Allium sativum*, En) - 95.73% (*Aloe barbadense*, Kd), 7.01 (*Aframomum melegueta*, En) – 30.59% (*Allium sativum*, En), 8.94 (*Allium sativum*, Kd) – 81.41% (*Cymbopogon citratus*, En), 3.58 (*Allium sativum*, En) – 63.64% (*Xylopia aethiopica*, En), 1.79 (*Allium cepa*, Kd) - 48.77 % (*Aframomum melegueta*, En), 2.64 (*Aframomum melegueta*, En) – 22.51% (*Ageratum conyzoides*, Kd) and 77.49 (*Ageratum conyzoides*, Kd) – 97.36% (*Aframomum melegueta*, En) respectively.

Effect of spices on *in-vitro* gas production and fermentation parameters

The GV, OMD, SCFA, FE and ME values obtained for the spices in this study as presented in Table 4 were similar to those obtained for the control indicating that there was no significant effect of the spice treatments. There were varying responses in the DMD due to the spice treatments. There were no significant differences among the Enugu spices while for Kaduna spices *Aframomum melegueta* significantly increased the DMD. Although there was a significant increase in CH_4 production (7.99 %) by *Aframomum melegueta* (Kaduna), non-significant CH_4 reduction was observed in any of the spice treatments.

Effect of herbs on in-vitro gas production and fermentation parameters

There were no significant differences between the herbs for *in-vitro* gas production and other fermentation characteristics as shown in Table 5. All the herbs reduced CH4 production to varied levels except *Ageratum conyzoides* (Enugu) and *Tridax procumbens* (Enugu).

Table 1: Chemical composition (g/100 g DM) of *Panicum maximum* and concentrate fed to WAD goats used for the study

Composition	*Panicum maximum*	Concentrate
Dry Matter	29.05	91.40
Crude Protein	7.71	15.82
Crude fibre	ND	4.41
Ether extract	5.00	7.51
Ash	9.00	6.34
Nitrogen free extract	ND[1]	57.32
Neutral detergent fibre	59.0	ND
Acid detergent fibre	37.0	ND
Hemicellulose	22.0	ND

ND[1] = Not Determined

Discussion

The values for the chemical components of *Panicum maximum* are comparable with the range of 26.00 - 38.49, 5.20 -9.36, 40.30 – 70.54, 26.20 – 39.14 and 14.10 – 22.00 % for DM, CP, NDF, ADF and HEM respectively reported by several authors (Bamikole and Babayemi, 2004; Ajayi and Babayemi, 2008; Odedire and Babayemi, 2008; Babayemi, 2009). The CP content met the critical limit of 7% recommended by ARC (1980) for tropical livestock for proper functioning of the rumen below which there will be a decline in performance. Norton (1994) however suggested a minimum CP of 8% as the limit for optimal ruminal activity. The digestibility of the grass due to its NDF content is likely to be adversely affected as it has been reported (McDonald *et al*., 1995; Gillespie, 1998) that NDF is inversely related to plant digestibility. The CP, CF, EE, Ash and NFE respectively of the concentrate fed in this study are within the range of 13.5-16.80, 3.40 -6.00, 6.60 -8.60, 4.80 – 6.40 and 56.50 – 62.40 % reported by Omajasola and Kayode (2015). The CP of the concentrate was higher than the minimum protein requirement of 10 – 12 % recommended by ARC (1985) for ruminants. This indicates that the concentrate could aid in ameliorating dietary protein deficiency in a grass – concentrate mixture for ruminant livestock. The CP content for majority of the herbs in this study were within the range of 8.90–24.90 % reported for herbs (Ahamefule *et al*., 2006; Sultan *et al*., 2009; Agunbiade *et al*., 2012; Midau *et al*., 2015). However, the values reported for *Ageratum conyzoides* (Kaduna) were higher than what was reported (15.67 %) by Agunbiade *et al*. (2012). The values for *Sida acuta* and *Tridax procumbens* (Kaduna) were also higher than those reported (20.65 %, 17.80 %) by Ahamefule *et al*. (2006). The NDF content of the herbs except *Aloe barbadense* (Kaduna) and *Cymbopogon citratus* (Enugu) were within the range (34.50 - 75.00 %) reported (Ahamefule *et al*., 2006) for herbs. The ADF values were comparable with what was reported (32.40–57.40 %) by Ahamefule *et al*. (2006) except for *Aloe barbadense* (Kaduna) and *Sida acuta*. Most of the herbs had hemicellulose values which were lower than what was reported in literature (Sultan *et al*., 2009). The ash content was comparable to those reported by Ahamefule *et al*. (2006) and Midau *et al*. (2015).

Table 2: Chemical composition of medicinal herbs used for the study (% DM)

		Herbs (H)							Significance		
Variable	Location (L)	*Aloe barbadense*	*Ageratum conyzoides*	*Cymbopogon citratus*	*Euphorbia heterophylla*	*Sida acuta*	*Tridax procumbens*	SEM	H	L	H*L
DM	Enugu	86.17^{ab}_{A}	82.19^{b}_{B}	90.18^{a}_{A}	80.46^{b}_{A}	83.27^{b}_{A}	84.56^{ab}_{A}	0.91	***	***	+
	Kaduna	95.73^{a}_{A}	88.96^{b}_{A}	90.18^{b}_{A}	84.81^{c}_{A}	89.32^{b}_{A}	87.32^{bc}_{A}	0.54			
	SEM	1.40	0.33	0.82	1.60	2.09	0.66				
CP	Enugu	15.04^{d}_{A}	20.74^{b}_{B}	7.41^{f}_{B}	22.07^{a}_{B}	18.08^{c}_{B}	10.51^{e}_{B}	0.02	***	***	***
	Kaduna	9.03^{f}_{B}	29.92^{a}_{A}	14.12^{e}_{A}	22.49^{c}_{A}	28.27^{b}_{A}	19.64^{d}_{A}	0.01			
	SEM	0.04	0.01	0.04	0.03	0.01	0.02				
NDF	Enugu	42.69^{e}_{A}	57.30^{c}_{A}	81.41^{a}_{A}	43.74^{e}_{B}	63.41^{b}_{A}	50.80^{d}_{A}	0.72	***	***	***
	Kaduna	32.97^{d}_{A}	47.98^{bc}_{B}	74.61^{a}_{A}	56.18^{b}_{A}	42.77^{c}_{B}	50.03^{bc}_{A}	1.46			
	SEM	2.68	0.59	2.31	1.01	3.05	0.78				
ADF	Enugu	36.06^{d}_{A}	41.37^{c}_{A}	58.51^{a}_{A}	32.80^{e}_{B}	20.33^{f}_{A}	47.42^{b}_{A}	0.41	***	***	***
	Kaduna	28.43^{c}_{B}	30.22^{c}_{B}	42.65^{a}_{B}	34.66^{b}_{A}	15.48^{d}_{A}	44.62^{a}_{A}	0.60			
	SEM	1.01	1.37	0.81	0.53	0.92	0.53				
HEM	Enugu	6.63^{de}_{A}	15.93^{c}_{A}	22.90^{b}_{A}	10.94^{d}_{B}	43.08^{a}_{A}	3.38^{e}_{A}	0.73	***	+	***
	Kaduna	4.54^{d}_{A}	17.76^{c}_{A}	31.96^{a}_{A}	21.52^{bc}_{A}	27.29^{ab}_{B}	5.41^{d}_{A}	1.39			
	SEM	1.75	1.96	2.42	1.08	2.57	1.26				
ASH	Enugu	13.50^{c}_{A}	16.93^{b}_{B}	10.45^{d}_{A}	13.14^{cd}_{A}	11.49^{cd}_{A}	20.32^{a}_{A}	0.45	***	+	**
	Kaduna	10.57^{d}_{A}	22.51^{a}_{A}	11.60^{cd}_{A}	14.79^{bc}_{A}	11.06^{cd}_{A}	17.59^{b}_{A}	0.61			
	SEM	0.80	0.39	0.79	1.11	1.20	1.05				
OM	Enugu	86.50^{b}_{A}	86.07^{c}_{A}	89.55^{a}_{A}	86.86^{ab}_{A}	88.51^{ab}_{A}	79.68^{d}_{A}	0.45	***	+	**
	Kaduna	89.43^{a}_{A}	77.49^{d}_{B}	88.40^{ab}_{A}	85.21^{bc}_{A}	88.94^{ab}_{A}	82.41^{c}_{A}	0.61			
	SEM	0.80	0.39	0.79	1.11	1.20	1.05				

^{a,b} Means of herbs with different superscripts across the rows within a specific location for each variable are significantly diferent (P < 0.05)

$_{A,B}$ Means of location with different subscripts along the columns within a specific variable for each herb aresignificantly different (P < 0.05)

**= P< 0.01, ***= P< 0.001, + = Tended to be significant (P > 0.05 - 0.10)

DM= Dry matter, CP = Crude protein, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, HEM= Hemicellulose, OM = Organic matter, SEM = Standard Error of the Mean

Table 3: Chemical composition of spices used for the study (% DM)

[a,b] Means of spices with different superscripts across the parametric rows within a specific location for each variable are significantly different (P < 0.05)

Variable	Location (L)	Spices (S) *Allium cepa*	*Aframomum melegueta*	*Allium sativum*	*Piper nigrum*	*Xylopia aethiopica*	*Zingiber officinale*	SEM	Significance S	L	S*L
DM	Enugu	77.28^{c}_{A}	89.38^{a}_{A}	72.82^{d}_{B}	82.44^{b}_{B}	88.77^{a}_{A}	88.33^{a}_{A}	0.41	***	***	***
	Kaduna	77.75^{d}_{A}	87.74^{b}_{A}	82.06^{c}_{A}	89.32^{ab}_{A}	91.93^{a}_{A}	88.70^{b}_{A}	0.48			
	SEM	0.45	0.38	0.92	0.34	1.22	0.90				
CP	Enugu	19.35^{b}_{A}	7.01^{f}_{B}	30.59^{a}_{A}	12.42^{c}_{B}	11.73^{e}_{A}	11.94^{d}_{B}	0.02	***	***	***
	Kaduna	15.80^{c}_{B}	12.67^{e}_{A}	24.46^{a}_{B}	19.04^{b}_{A}	10.76^{f}_{B}	12.79^{d}_{A}	0.02			
	SEM	0.06	0.02	0.02	0.01	0.00	0.03				
NDF	Enugu	10.56^{d}_{A}	67.40^{a}_{A}	13.94^{d}_{A}	28.88^{c}_{B}	69.89^{a}_{A}	43.32^{b}_{A}	1.03	***	+	**
	Kaduna	9.94^{d}_{A}	69.77^{a}_{A}	8.94^{d}_{A}	53.80^{bc}_{A}	61.27^{ab}_{B}	43.01^{c}_{A}	2.10			
	SEM	1.01	2.51	1.48	1.33	0.43	6.15				
ADF	Enugu	5.28^{d}_{B}	18.63^{b}_{A}	3.58^{d}_{A}	16.06^{bc}_{B}	63.64^{a}_{A}	11.98^{c}_{A}	0.77	***	**	***
	Kaduna	8.15^{d}_{A}	24.75^{c}_{A}	3.89^{e}_{A}	41.10^{b}_{A}	50.80^{a}_{B}	5.68^{de}_{B}	0.56			
	SEM	0.00	1.57	0.39	1.58	1.70	0.37				
HEM	Enugu	5.28^{c}_{A}	48.77^{a}_{A}	10.26^{c}_{A}	12.82^{c}_{A}	6.25^{c}_{A}	31.34^{b}_{A}	1.52	***	+	+
	Kaduna	1.79^{b}_{A}	45.02^{a}_{A}	5.05^{b}_{A}	12.70^{b}_{A}	10.47^{b}_{A}	37.33^{a}_{A}	2.31			
	SEM	1.01	4.03	1.32	2.44	2.05	6.29				
ASH	Enugu	2.93^{b}_{A}	2.64^{b}_{B}	5.45^{b}_{A}	9.91^{a}_{A}	2.75^{b}_{B}	11.53^{a}_{A}	0.55	***	+	***
	Kaduna	4.62^{b}_{A}	6.80^{ab}_{A}	6.18^{ab}_{A}	6.43^{ab}_{B}	7.78^{a}_{A}	6.10^{ab}_{A}	0.37			
	SEM	1.21	0.57	0.57	0.31	0.56	1.20				
OM	Enugu	97.07^{a}_{A}	97.36^{a}_{A}	94.55^{a}_{A}	90.09^{b}_{B}	97.25^{a}_{A}	88.47^{b}_{A}	0.55	***	+	***
	Kaduna	95.38^{a}_{A}	93.20^{a}_{A}	93.82^{ab}_{A}	93.57^{ab}_{A}	92.22^{b}_{B}	93.90^{ab}_{A}	0.37			
	SEM	1.21	0.57	0.57	0.31	0.56	1.20				

[A,B] Means of location with different subscripts along the parametric columns within a specific variable for each spice are significantly different (P < 0.05) NS = Not significant, **= P< 0.01, ***= P< 0.001, + = Tended to be significant (P > 0.05 - 0.10) . DM= Dry matter, CP = Crude protein, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, HEM= Hemicellulose, OM = Organic matter, SEM =Standard Error of the Mean

Table 4: Effect of spices and location on *in-vitro* methanogenesis and fermentation parameters

VAR	LOC. (L)	Spices (S)								Significance		
		ACE	AME	ASA	PNI	XAE	ZOF	CTR	SSEM	L	S	L*S
GV	EN	28.73^{a}_{A}	28.14^{a}_{A}	26.38^{a}_{A}	26.38^{a}_{A}	25.79^{a}_{A}	26.97^{a}_{A}	30.84^{a}_{A}	1.59	+	+	+
	KD	27.55^{a}_{A}	26.38^{a}_{A}	28.14^{a}_{A}	27.55^{a}_{A}	23.45^{a}_{A}	28.73^{a}_{A}	28.73^{a}_{A}	2.03			
	SEM	2.58	3.88	3.88	3.94	2.65	3.66	3.33				
DMDg	EN	682.75^{a}_{A}	667.00^{a}_{A}	606.75^{a}_{A}	641.50^{a}_{A}	569.75^{a}_{A}	618.50^{a}_{A}	635.17^{a}_{A}	22.87	+	+	+
	KD	602.75^{bc}_{A}	764.50^{a}_{A}	702.25^{ab}_{A}	529.50^{c}_{A}	578.75^{bc}_{A}	639.50^{abc}_{A}	614.00^{bc}_{A}	19.62			
	SEM	21.92	69.95	25.64	48.54	33.01	23.70	39.77				
GVg	EN	143.63^{a}_{A}	140.70^{a}_{A}	131.90^{a}_{A}	131.90^{a}_{A}	128.97^{a}_{A}	134.83^{a}_{A}	152.42^{a}_{A}	7.95	+	+	+
	KD	137.77^{a}_{A}	131.90^{a}_{A}	140.70^{a}_{A}	137.77^{a}_{A}	117.25^{a}_{A}	143.63^{a}_{A}	143.63^{a}_{A}	10.62			
	SEM	12.89	19.42	19.42	19.69	13.25	18.31	16.63				
CH_4g	EN	85.00^{a}_{A}	87.94^{a}_{A}	64.49^{a}_{A}	76.21^{a}_{A}	76.21^{a}_{A}	67.42^{a}_{A}	91.84^{a}_{A}	6.85	+	+	+
	KD	90.87^{a}_{A}	93.80^{a}_{A}	70.35^{a}_{A}	79.14^{a}_{A}	76.21^{a}_{A}	64.49^{a}_{A}	105.52^{a}_{A}	6.10			
	SEM	16.29	15.01	13.96	9.11	15.14	7.25	7.30				
CH_4p	EN	56.39^{a}_{A}	62.01^{a}_{A}	45.75^{a}_{A}	58.93^{a}_{A}	56.84^{a}_{A}	52.45^{a}_{A}	61.96^{a}_{B}	3.27	*	+	+
	KD	65.16^{abc}_{A}	72.59^{ab}_{A}	51.58^{bc}_{A}	62.60^{abc}_{A}	63.31^{abc}_{A}	45.23^{c}_{A}	76.29^{a}_{A}	3.00			
	SEM	6.94	5.92	4.96	8.11	7.25	4.58	3.32				
CH_4r	EN	14.35^{a}_{A}	6.83^{a}_{A}	30.72^{a}_{A}	12.00^{a}_{A}	13.84^{a}_{A}	22.47^{a}_{A}	0.00^{a}_{A}	5.59	+	+	+
	KD	2.36^{ab}_{A}	-7.99^{b}_{A}	23.78^{ab}_{A}	8.42^{ab}_{A}	4.54^{ab}_{A}	32.86^{a}_{A}	0.00^{a}_{A}	4.49			
	SEM	13.01	10.88	8.66	10.75	13.76	6.19	0.00				
FE	EN	5.02^{a}_{A}	4.94^{a}_{A}	4.97^{a}_{A}	5.27^{a}_{A}	4.66^{a}_{A}	4.97^{a}_{A}	4.39^{a}_{A}	0.34	+	+	+
	KD	4.51^{a}_{A}	6.91^{a}_{A}	5.54^{a}_{A}	4.52^{a}_{A}	5.26^{a}_{A}	4.99^{a}_{A}	4.63^{a}_{A}	0.50			
	SEM	0.51	1.27	0.77	0.90	0.64	0.79	0.46				
OMD	EN	56.15^{a}_{A}	55.64^{a}_{A}	54.09^{a}_{A}	54.09^{a}_{A}	53.57^{a}_{A}	54.60^{a}_{A}	57.70^{a}_{A}	1.40	+	+	+
	KD	52.12^{a}_{A}	54.09^{a}_{A}	55.64^{a}_{A}	55.12^{a}_{A}	51.51^{a}_{A}	56.15^{a}_{A}	56.15^{a}_{A}	1.87			
	SEM	2.27	3.42	3.42	3.46	2.33	3.22	2.93				
SCFA	EN	0.63^{a}_{A}	0.61^{a}_{A}	0.57^{a}_{A}	0.57^{a}_{A}	0.56^{a}_{A}	0.58^{a}_{A}	0.67^{a}_{A}	0.04	+	+	+
	KD	0.60^{a}_{A}	0.57^{a}_{A}	0.61^{a}_{A}	0.60^{a}_{A}	0.50^{a}_{A}	0.63^{a}_{A}	0.63^{a}_{A}	0.05			
	SEM	0.19	0.09	0.09	0.09	0.06	0.09	0.08				
ME	EN	6.77^{a}_{A}	6.69^{a}_{A}	6.46^{a}_{A}	6.46^{a}_{A}	6.38^{a}_{A}	6.53^{a}_{A}	7.01^{a}_{A}	0.22	+	+	+
	KD	6.61^{a}_{A}	6.46^{a}_{A}	6.69^{a}_{A}	6.69^{a}_{A}	6.06^{a}_{A}	6.77^{a}_{A}	6.77^{a}_{A}	0.29			
	SEM	0.35	0.53	0.53	0.54	0.36	0.50	0.45				

^{a,b} Means of spices with different superscripts across the parametric rows within specific locations are significantly different($P<0.05$)

$_{A,B}$ Means of location with different subscripts along the parametric columns within individual spices are significantly different($P<0.05$)

*= $P<0.05$, + = Tended to be significant ($P > 0.05$ - 0.10)

ACE = *Allium cepa*, AME = *Aframomum melegueta*, ASA = *Allium sativum*, PNI = *Piper nigrum*, XAE = *Xylopia aethiopica*, ZOF = *Zingiber officinale*, CTR = Control , GV= Gas volume (mL/200mg DM), DMDg = Dry matter digestibility (g/Kg DM), GVg = Gas volume (mL/g DM), CH_4g= Methane volume (mL/g DM), CH_4p= Methane (%), CH_4r = Methane reduction (%), FE=Fermentation efficiency, OMD=Organic matter digestibility (%), SCFA = Short chain fatty acid (mmol/200 mg DM), ME = Metabolisable energy (MJ/ Kg DM), SEM = Standard Error of the Mean, VAR = Variable, LOC = Location

Alluim sativum was the only spice which had CP values which was higher than the range (7.4 - 22.30 %) reported in literature for spices (Mepba and Nnoka, 2008; Khan and Choudry, 2010; Udofia and Alozie, 2015). The NDF values for *Allium cepa*, *Allium sativum* and *Aframomum melegueta*, *Xylopia aethiopica* were lower and higher respectively than the range of 28.00 – 55.20 % reported by Khan and Chaudhry (2010). The ADF values of *Allium cepa*, *Allium cepa*, *Zingiber officinale* were lower than 19.9 – 53.7 % reported by Khan and Chaudhry (2010). The values for *Aframomum melegueta* and *Xylopia aethiopica* (Kaduna) were comparable with this range while the ADF value of *Xylopia aethiopica* (Enugu) was

Table 5: Effect of location and medicinal herbs on *in-vitro* gas production and fermentation characteristics

		Herbs (H)								Significance		
VAR	LOC. (L)	ABA	ACO	CCI	EHE	SAC	TPR	CTR	SEM	H	L	H*L
GV	EN	21.69^{a}_{A}	17.59^{a}_{A}	29.90^{a}_{A}	29.31^{a}_{A}	21.69^{a}_{A}	21.69^{a}_{A}	30.48^{a}_{A}	1.67	+	+	+
	KD	29.90^{a}_{A}	17.00^{a}_{A}	25.21^{a}_{A}	23.45^{a}_{A}	26.38^{a}_{A}	28.14^{a}_{A}	28.73^{a}_{A}	2.18			
	SEM	4.90	3.70	2.33	4.40	2.86	2.94	3.33				
DMDg	EN	630.00^{a}_{A}	535.25^{a}_{A}	631.75^{a}_{A}	687.50^{a}_{A}	571.00^{a}_{A}	583.25^{a}_{A}	635.17^{a}_{A}	22.23	+	+	+
	KD	532.75^{a}_{A}	567.00^{a}_{A}	657.25^{a}_{A}	654.50^{a}_{A}	525.50^{a}_{A}	626.50^{a}_{A}	614.00^{a}_{A}	19.97			
	SEM	37.77	33.19	42.32	60.76	20.45	30.09	39.77				
GVg	EN	108.45^{a}_{A}	87.94^{a}_{A}	149.49^{a}_{A}	145.56^{a}_{A}	108.45^{a}_{A}	108.45^{a}_{A}	152.42^{a}_{A}	8.33	+	+	+
	KD	149.49^{a}_{A}	85.00^{a}_{A}	126.04^{a}_{A}	117.25^{a}_{A}	131.90^{a}_{A}	140.70^{a}_{A}	143.63^{a}_{A}	10.91			
	SEM	24.52	18.51	11.64	22.02	14.29	14.72	16.63				
CH_4g	EN	70.35^{a}_{A}	61.55^{a}_{A}	85.00^{a}_{A}	93.80^{a}_{A}	64.49^{a}_{A}	76.21^{a}_{A}	91.84^{a}_{A}	5.26	+	+	+
	KD	90.87^{ab}_{A}	52.76^{b}_{A}	73.28^{ab}_{A}	70.35^{ab}_{A}	70.35^{ab}_{A}	85.00^{ab}_{A}	76.29^{a}_{A}	6.73			
	SEM	12.16	12.32	9.87	13.82	7.05	15.10	7.30				
CH_4p	EN	63.54^{a}_{A}	72.08^{a}_{A}	56.25^{a}_{A}	64.30^{a}_{A}	62.50^{a}_{A}	71.55^{a}_{A}	61.96^{a}_{A}	2.62	+	+	+
	KD	66.49^{a}_{A}	61.67^{a}_{A}	58.21^{a}_{A}	58.59^{a}_{A}	52.92^{a}_{A}	59.17^{a}_{A}	76.29^{a}_{A}	3.07			
	SEM	5.73	5.01	4.19	4.20	3.81	9.42	3.32				
CH_4r	EN	4.18^{a}_{A}	-6.90^{a}_{A}	16.02^{a}_{A}	4.55^{a}_{A}	7.94^{a}_{A}	-6.50^{a}_{A}	0.00^{a}_{A}	3.99	+	+	+
	KD	2.58^{a}_{A}	8.65^{a}_{A}	13.07^{a}_{A}	12.45^{a}_{A}	20.88^{a}_{A}	12.05^{a}_{A}	0.00^{a}_{A}	4.43			
	SEM	8.97	7.33	8.19	7.07	5.44	13.94	0.00				
FE	EN	6.19^{a}_{A}	7.54^{a}_{A}	4.29^{a}_{A}	4.97^{a}_{A}	5.90^{a}_{A}	6.11^{a}_{A}	4.39^{a}_{A}	0.46	+	+	+
	KD	4.64^{a}_{A}	8.12^{a}_{A}	5.49^{a}_{A}	6.94^{a}_{A}	4.23^{a}_{A}	4.62^{a}_{A}	4.64^{a}_{A}	0.60			
	SEM	1.02	1.69	0.52	1.21	0.86	0.86	0.46				
OMD	EN	49.96^{a}_{A}	46.35^{a}_{A}	57.18^{a}_{A}	56.67^{a}_{A}	49.96^{a}_{A}	49.96^{a}_{A}	57.70^{a}_{A}	1.47	+	+	+
	KD	57.18^{a}_{A}	45.83^{a}_{A}	53.06^{a}_{A}	51.51^{a}_{A}	54.09^{a}_{A}	55.64^{a}_{A}	56.15^{a}_{A}	1.92			
	SEM	4.32	3.26	2.05	3.88	2.52	2.59	2.93				
SCFA	EN	0.46^{a}_{A}	0.36^{a}_{A}	0.66^{a}_{A}	0.64^{a}_{A}	0.46^{a}_{A}	0.46^{a}_{A}	0.67^{a}_{A}	0.04	+	+	+
	KD	0.65^{a}_{A}	0.35^{a}_{A}	0.54^{a}_{A}	0.50^{a}_{A}	0.57^{a}_{A}	0.61^{a}_{A}	0.63^{a}_{A}	0.05			
	SEM	0.12	0.09	0.06	0.11	0.07	0.07	0.08				
ME	EN	5.82^{a}_{A}	5.26^{a}_{A}	6.93^{a}_{A}	6.85^{a}_{A}	5.81^{a}_{A}	5.82^{a}_{A}	7.01^{a}_{A}	0.23	+	+	+
	KD	6.93^{a}_{A}	5.18^{a}_{A}	6.30^{a}_{A}	6.05^{a}_{A}	6.46^{a}_{A}	6.69^{a}_{A}	6.77^{a}_{A}	0.30			
	SEM	0.67	0.50	0.32	0.60	0.39	0.40	0.45				

^{a,b} Means of herbs with different superscripts across the parametric rows within specific locations are significantly different (P<0.05)

$_{A,B}$ Means of location with different subscripts along the parametric columns within individual herbs are significantly different(P<0.05)

+ = Tended to be significant (P > 0.05 - 0.10) ABA= *Aloe barbadense* ACO = *Ageratum conyzoides*, CCI= *Cymbopogon citratus*, EHE = *Euphorbia heterophylla*, SAC = *Sida acuta*, TPR = *Tridax procumbens*, CTR = Control, GV= Gas volume (mL/200mg DM), DMDg = Dry matter digestibility (g/Kg DM), GVg = Gas volume (mL/g DM), CH_4g= Methane volume (mL/g DM), CH_4p= Methane (%), CH_4r = Methane reduction (%), FE=Fermentation efficiency, OMD=Organic matter digestibility, SCFA = Short chain fatty acid (mmol/200 mg DM) ME= Metabolisable energy (MJ/Kg DM), SEM = Standard Error of the Mean,VAR = Variable,LOC=Location

higher. The ash values obtained from the spices were comparable with what was reported by Udofia and Alozie (2015).

The variations observed in the chemical composition of the experimental plant part(s) collected from Enugu and Kaduna states could be due to several factors which include plant species, part, leaf age, fertilizer application and others which vary with locations such as soil type, climate and rainfall (Ball *et al*., 2001; Njidda, 2010; Midau *et al*., 2015). The CP content of most of the herbs and spices collected from Kaduna were higher than their Enugu counterparts. This observation is in agreement with the reports by Rittner and Reed (1992) that plants in the Sahelian zone have higher nitrogen content than those found in the humid zone. Most of the herbs and spices in this study had CP values above the minimum protein requirement of 10 – 12 % recommended for ruminant livestock by ARC (1985) suggesting that they could be useful in meeting the dietary CP requirement of ruminant livestock. There were variable effects of location and plant species on the fiber content of the plant samples collected. The fiber content of plants is dependent on synergy between factors such as plant developmental stage, leaf to stem ratio, nutrient availability and climatic factors such as temperature, photoperiod and drought or water stress (Fulkerson *et al*., 2007). NDF gives an indication of the level of voluntary intake of forages. Thus as the NDF level increases, the voluntary intake is expected to decline (Coblentz and Hoffman, 2008). High temperature results in higher forage content of NDF and reduced fiber digestibility. The increase in temperature leads to increased microbial respiration. This in turn results in a rise in the level of NDF, ADF and lignin in plant tissues due to microbial oxidation of soluble sugars (Coblentz and Hoffman, 2008). Bakshi and Wadhwa (2004) stated that beyond 60 % NDF content of forage, voluntary DM intake is negatively affected. For Enugu alone *Sida acuta only exceeded this limit* while there was no plant for Kaduna only. While plants from both locations that exceeded the limit are *Aframomum melegueta, Xylopia aethiopica and Cymbopogon citratus*. The implication is that when these plants are to be included in ruminant diet techniques to improve their digestibility will also have to be considered. The level of ADF gives an indication of the digestibility and energy content of plant material. It has been established that the energy level of forages declines as the ADF level increases. Shroeder (2004) states that as the ADF content increases, forage digestibility decreases. The decrease in digestibility with increased ADF content was suggested to be due to the increased concentration of lignin. Van Soest (1967) also stated that as NDF level increases there is a decrease in the utilization of fiber. Hemicellulose content may also give an indication of how digestible forages are due to the fact that it may be highly digestible provided that it has not being lignified. Ball *et al.* (2007) classified forages with ADF values greater than 43.00 - 45.00 % as low quality forages. This is attributed to the fact that forages with higher ADF levels are expected to have lower digestibilities due to the aforementioned reasons.

Thus, the fiber components of forages may not be used in insolation from one another in accessing forage quality due to their individual significance. With these limits suggested from literature, the plant samples in this study may be classified on the basis of voluntary DM intake and digestibility by ruminant livestock into those that are expected to decline in one or both of the indices of forage quality. Thus, *Cymbopogon citratus* (Enugu), *Aframomum melegueta and Xylopia aethiopica* (both locations) may be classified as those with expected decrease in DM intake and digestibility. While *Cymbopogon citratus* (Kaduna) may be characterised with reduced voluntary DM intake by ruminant livestock. *Allium cepa* and *Allium sativum* were characterised with very low levels of fiber (NDF and ADF) which would necessitate a good fiber source with their inclusion in the diet of ruminant livestock. Significantly lowered GV with *Zingiber offinale* and *Allium sativum* has been reported (Bunglavan *et al*., 2010) at 50 mg/500 mg substrate. Conversely, higher GV with spice inclusion have been reported by several authors. Soroor and Moeini (2015) reported increased GV with *Zingiber officinale* at higher inclusion levels (30, 60 mg/ 200 mg substrate). Extracts of ginger have also been reported to increase GV (Patra, 2010 and Kim *et al*., 2012). Sallam *et al*. (2009) reported increased GV with methanol extracts (0.5, 1.0 and 1.5 mL/ 75 mL) of thyme, fennel, ginger and black seed. However, ginger and black seed at the highest inclusion level (1.5 mL/75 mL) decreased GV by 4.6 and 22.5 % respectively. These reports on various spice treatments are at variance with the observations in this study.

There are reports of varying DMD and OMD responses due to inclusion of spices as was observed in this study. Sirohi *et al*. (2012) reported DMD response with spice extract inclusion (1 mL/ 30 mL ruminal fluid) *in-vitro*. It was observed that methanol extracts of green chilli and *Myristica fragrans* increased and reduced DMD respectively. Increased OMD due to inclusion of ginger (Soroor and Moeini, 2015; Patra*,* 2010) and decreased OMD with methanol extracts of ginger and black seed at 1.5 mL/75 mL (Sallam *et al.,* 2009) has been reported. The Highest CH_4 reduction in this study is comparable to what was reported (21.00 %) at 30 mg/200 mg by Soroor and Moeini (2015) for *Zingiber officinale*. It is however lower than what was reported (62.04 %) for *Myristica fragrans* methanol extract (Sirohi *et al.,* 2012). However, the increase in CH_4 production observed in *Aframomum melegueta* (Kaduna) is not uncommon as Sallam *et al*. (2009) reported increased CH_4 production (55 -166 %) with inclusion of spice extracts. Kim *et al*. (2012) reported no significant effect of the inclusion of methanol extracts of *Allium sativum*, *Allium cepa* and *Zingiber officinale* on SCFA concentration *in-vitro* at inclusion level of 0.3 mL/30 mL buffered rumen fluid. The values for FE due to spice inclusion were higher than the theoretical range of 2.75 – 4.41 proposed by Blummel *et al.* (1997). Getachew *et al.* (2000) reported an FE range of 3.1 to 16.1 for feeds rich in tannin. *Zingiber officinale* and *Aframomum melegueta* have been reported to contain EO, tannins, saponins, flavonoids and terepenes (Jude and Nneka, 2012; Bhargava *et al.,* 2012). *Allium sativum* and *Xylopia aethiopica* have been reported to contain EO, with the former also containing saponins while tannins and saponins are present in the latter (Ameh *et al*., 2013; Omeh *et al*., 2014; Adesina *et al*., 2015).Gazuwa *et al*. (2013) reported that *Allium cepa* contains terpenes, flavonoids and alkaloids. These reports indicate the presence of EO and other PSM in spices. Hence the inhibition of methanogenesis may be attributed to presence of one or more of the PSM in the spices of the current study.

Ageratum conyzoides, *Sida acuta, Tridax procumbens* and *Aloe barbadense* have been reported to contain tannin and saponins (Jude *et al*., 2009; Adesuyi *et al*., 2012; Agunbiade *et al*., 2012; Rami *et al*., 2014; Nduche *et al*., 2015) while for *Euphorbia heterophylla*, saponins were not reported (Edeoga *et al*., 2005; James and Emmanuel, 2010). *Cymbopogon citratus* is reported to contain EO and alkaloids (Ogie-Odia *et al*., 2010; Salome *et al*., 2012; Ekpenyong *et al*., 2015; Uraku, 2015). Hence the reduction in CH_4 production by the herb treatments may be due to the presence of tannins and/or saponins or EO in the herbs. Tannins and saponins have been reported to reduce methanogenesis by inhibiting protozoa (indirect effect) and methanogens (Patra and Saxena, 2009; Mao *et al*., 2010; Patra, 2012). Significantly reduced digestibility, SCFA and GV have been attributed to the presence of tannin and saponin extracts respectively *in-vitro* (Santoso *et al*., 2007; Patra2010; Tan *et al*., 2011). The fact that these effects were not observed significantly in this study may be related to their concentration. Szumacher-Strabel *et al*. (2011) suggested that the low effect of tannin containing feed additives *in-vitro* may be due to their low concentration in the additives.

The source, type and level of tannins and saponins may be responsible for the different levels of inhibition of methane production and rumen fermentation associated with some medicinal plants (Mueller-Harvey, 2006; Gugliemelli *et al*., 2011; Jayanegara *et al*., 2014). Hence increasing the inclusion level of the additives may result in significant effects being observed. These herbs have the potential for improved DMD and other favourable responses *in vivo* although their effects were not significant in the present study. Wanapat *et al*. (2008) reported that lemon grass powder supplementation at 100 g/day DM to a basal diet of urea treated rice straw improved nutrient digestibility, rumen microbial population and microbial protein synthesis in beef cattle. Reduction in the quantity of CH_4 produced is a most desired characteristic of plant additives and forages which should not be at the detriment of desired fermentation parameters such as improved DMD, OMD, FE, SCFA and ME. From both locations *Allium sativum*, *Zingiber officinale, Cymbopogon citratus and Sida acuta* gave the highest CH_4 reductions without significant decline in the desired fermentation parameters in contrast with the control indicating that they have the potential of being able to safely mitigate CH_4 production in ruminant livestock.

Conclusion

Generally, *Allium sativum* bulbs, *Zingiber officinale* rhizome, *Cymbopogon citratus* and *Sida acuta* from both Enugu and Kaduna have shown potential for reducing ruminal methane production *in vitro*. The potential demonstrated was without compromising the desired fermentation parameters. The ability of these plants to sustain the reduction of rumen methane production *in vivo* should be considered for further study.

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