POTENTIALS OF *RAUVOLFIA VOMITORIA* LEAVES AS AN ANTIMETHANOGENIC FEED ADDITIVE FOR RUMINANTS

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ABSTRACT
Methane (CH4) mitigation potentials of Rauvolfia vomitoria leaves when used as phytogenic additive was evaluated using the in vitro gas technique. Substrate (200 mg) consisting of Megathyrsus maximus and concentrate in ratio 7: 3 was incorporated with R. vomitoria leaves at levels of 0, 1, 2 and 3% dry matter (DM) in a completely randomized design. Incubation was done in 100 mL glass syringes at 39oC for 48 h with total gas and CH4 production measured. Results showed that R. vomitoria leaves contained high saponin content (10.34%). Methane production was reduced (P < 0.05) at 2% inclusion while total gas production was not significantly affected with R. vomitoria leaves inclusion. It can be concluded that R. vomitoria leaves can be used as an additive at 2% inclusion in ruminant diet for reduced methanogenesis which has beneficial implication for efficient utilization of dietary energy.
Keywords: *in vitro*, methane, greenhouse gas, dietary energy, phytogenic additive

INTRODUCTION

Enteric methane production from ruminants represents an inefficient rumen fermentation process due to the associated dietary energy loss of 2 - 12% (Ahmed *et al*., 2021). This energy loss as methane, reduces feed utilization efficiency, ruminants' productivity and producers' profitability (Subepang *et al*., 2018; Lakamp *et al*., 2022). Furthermore, methane emission from ruminants poses a critical challenge to environmental sustainability through its significant contribution to greenhouse gas emission and climate change (Kroliczewska *et al*., 2023). Hence, several strategies have been adopted to alter rumen fermentation away from methane production. Dietary strategies with the use of anti-methanogenic substances which inhibit rumen methanogens and reduce substrate availability for methane production are considered as potentially effective for rumen methane mitigation (Smith *et al*., 2022). *Rauvolfia vomitoria* is a plant that has been identified with several bioactive compounds such as saponins, tannins, antraqionones, flavonoids, alkaloids, cardiac glycosides (Bassey *et al.*, 2014). These plant bioactive compounds have been linked with antimicrobial and anti-methanogenic properties. This study therefore, investigated the potentials of *R. vomitoria leaves* as a phytogenic additive for rumen methane mitigation.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Animal Nutrition Laboratory of the Federal University of Agriculture, Abeokuta. The area is located in the South-western zone of Nigeria and falls within latitude 7°13" N and longitude 3°26" E (Google Earth, 2023).

Collection and preparation of *Rauvolfia vomitoria*: *Rauvolfia vomitoria* leaves were harvested from Eguru village, Owode Egba in Ogun State, South-Western, Nigeria. The leaves were identified at the Forestry Department of University of Ibadan, Oyo State. Harvested leaves were screened to remove foreign materials and air-dried till a constant weight was attained. Thereafter, the leaves were milled through 1 mm sieve and sub-samples were taken for phytochemical analysis. The remaining samples were stored at room temperature in air-tight glass containers and kept away from sunlight for subsequent use as additive in an *in vitro* study.

In vitro gas production measurement

Experimental substrate consisted of *Megathyrsus maximus* and concentrate (Table 1) in ratio 7:3 on dry matter basis. *Rauvolfia vomitoria* leaves were used as additive in the substrate at varying levels of 0, 1, 2, 3% DM to make four treatments in a completely randomized design. The composition of experimental concentrate is presented in Table 1. Approximately 200 mg of the substrates, according

to the treatments, were incubated in 100 ml syringes filled with inoculum containing rumen fluid and medium in a ratio of 1:2 (v/v) to determine gas production following the procedure of Menke and Steingass (1988). The rumen liquor was collected from cattle immediately after slaughter. The incubation was done at 39°C over a period of 24 hours during which total gas production was measured at 3, 6, 12 and 24 h. At the end of the incubation period, methane production was determined following the procedure of Fievez *et al.* (2005).

Table 1: Chemical composition (% DM) of *Megathyrsus maximus* and concentrate used as substrate

Parameters	*Megathyrsus maximus*	Concentrate[1]
Dry matter[2]	36.00	91.00
Crude protein	9.15	14.47
Ether extract	3.00	7.72
Ash	6.85	8.93
Nitrogen free extract	32.06	45.93
Organic matter	93.15	91.07
Neutral detergent fibre	68.56	55.35
Acid detergent fibre	50.92	22.51
Acid detergent lignin	15.05	10.41
Hemicellulose	17.64	12.10
Cellulose	35.87	32.84
Metabolizable energy (MJ/kg DM)[2]	11.84	10.06

[1]Concentrate contained 44% wheat offal, 38% rice bran, 15% dried brewers' grain, 2% bonemeal and 1% common salt; [2]As-received basis; [3]Calculated according to De Boever *et al.* (1997).

Chemical analysis: Oven-dried and milled samples of *M. maximus and* concentrate were analyzed for proximate composition using the method of A.O.A.C. (2000) while the fibre fractions were determined according to Van Soest *et al.* (1991). The phytochemical composition of *R. vomitoria* leaves such as tannin, phenolic acid, oxalate, phytate, saponin and flavonoids were determined following standard procedures.

Statistical analysis: All data generated in the study were subjected to one-way analysis of variance (SAS, 2002). Significant differences were accepted at $P \leq 0.05$ and means were separated using Duncan Multiple Range Test (SAS, 2002).

RESULTS AND DISCUSSION

The phytochemical composition of *R. vomitoria* leaves is presented in Table 2. Results showed the presence of tannin, phenolic acid, oxalate, phytate, saponin and flavonoids in the leaves of *R. vomitoria* with a relatively high composition (10.34%) of saponin. Phytochemicals in plants have been reported to have strong biological activities, which include antimicrobial activities (Reddy *et al.,* 2020). Previous qualitative and quantitative studies have similarly reported the presence of tannin, saponin and flavonoids in *R. vomitoria* leaves although at varied concentrations (Ojo *et al*., 2012; Chinonye *et al*., 2022). The *in vitro* total gas and methane production in response to varying inclusion levels of *R. vomitoria* leaves is presented in Table 3. At the end of 24 h incubation period, total gas production was not altered ($P > 0.05$) at the varying inclusion levels of *R. vomitoria* leaves. *In vitro* gas production is correlated with rumen fermentation (Getachew *et al*., 1998), the similarity in total gas production implied that substrate fermentation was not altered with up to 3% inclusion of *R. vomitoria* leaves. Methane production per substrate degraded (ml/200 mg DM) and as a percentage of total gas volume (% total gas volume), reduced ($P < 0.05$) at 2 - 3% inclusion of *R. vomitoria* leaves. This suggests an inhibitory effect of *R. vomitoria* leaves on rumen methanogenesis. Methane-inhibiting effect of various phytogenic plants have been reported (Patra and Saxena, 2010; Aderinboye *et al.,* 2020). The methane reduction effect of *R. vomitoria* leaves was linked to the presence of high saponin content in the leaves. The anti-methanogenic effect of saponin has been documented in literature and are related to its potentials as a defaunating agent (Jayanegara *et al*., 2012; Kholif, 2023). According to Guyader *et al*. (2017), a strong positive correlation has been established between protozoa and methanogenesis.

Table 2: Phytochemical composition of *Rauvolfia vomitoria* leaves

Phytochemicals	Composition (%)
Tannins	0.68
Total phenolic acid	0.76
Oxalates	0.11
Phytates	0.01
Saponins	10.34
Flavonoids	0.86

Table 3: *In vitro total* gas and methane production (mL/200mg DM) from fermentation of substrate containing varying levels of *Rauvolfia vomitoria* leaves

	Inclusion levels (%) of *Rauvolfia vomitoria*					
Incubation Hours	**0**	**1**	**2**	**3**	**SEM**	**P-value**
3	2.33	2.17	2.17	2.08	0.088	0.794
6	4.17	4.00	4.00	4.00	0.126	0.958
12	9.33	9.00	9.00	8.58	0.189	0.592
24	18.58	18.33	18.17	18.00	0.346	0.946
Methane production, mL/200 mg DM	6.67^{a}	6.33^{a}	4.67^{b}	3.33^{b}	0.509	0.039
Methane production, % of total gas	34.41^{a}	33.94^{a}	24.71^{b}	20.83^{b}	2.554	0.047

[a,b] Means on the same row with different superscript are significantly different ($P < 0.05$); SEM: Standard error of means

CONCLUSION

Inclusion of *Rauvolfia vomitoria* leaves as phytogenic additive up to 3% in substrate dry mater, had no significant effect on *in vitro* cumulative gas production. However, the percentage of methane in total gas production reduced at 2 - 3% inclusion of *R. vomitoria* leaves. *Rauvolfia vomitoria* leaves can be used as anti-methanogenic additive at 2% inclusion level for rumen methane mitigation.

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