In vitro Evaluation of Siam Weed (*Chromolaena odorata*) Additive as a Potential Rumen Modifier in West African Dwarf Bucks

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Summary

Recently, bioactive components of plants and plant parts of most trees and browse species have been used as rumen modifiers to reduce methane gas production in ruminants, thereby reducing their contribution to the implicated greenhouse effect. This study, therefore, evaluated the probable use of *Chromolaena odorata* (L.) R.M.King & H.Rob. (Siam weed) leaves as rumen modifier in West African Dwarf Bucks. Fresh *C. odorata* leaves were harvested, air dried for 3 weeks, milled using a 2 mm sieve size and bagged for both proximate and phytochemical analysis. Concentrate diets were formulated with *C. odorata* leaf meal included in the diet at 0, 2, 4 and 6% of the whole diet. Rumen fluids were collected from West African Dwarf (WAD) bucks (averaged 25 kg) using suction tube and assigned to the 4 experimental diets in a completely randomized design (CRD). The incubation of inocula was performed for 96 hours with 12 replicates per treatment in a single run and data obtained were subjected to one-way analysis of variance and the mean values were compared with Tukey's Test. The results indicated that *C. odorata* had 969.0mg/kg dry matter, 17.51 % crude protein, 20.43% crude fibre, 52.16% nitrogen free extract, 1.99% saponin, 2.57% tannin, 1.08% flavonoid and 1.26% alkaloid. Addition of 2 and 4% *C. odorata* leaves to the diets resulted in increased ($P < 0.05$) *in vitro* gas production while *C. odorata* at 2% reduced ($P < 0.05$) the methane gas (%) estimate. *In vitro* organic and dry matter digestibility, total digestible substrates and short chain fatty acids were increased ($P < 0.05$) by the addition of *C. odorata* leaves to the diets. This study concluded that the use of *C. odorata* as an additive at 2 and 4% inclusion increased total gas output. However, 2% inclusion is beneficial as it reduces the net methane production while maintaining higher gas production and digestibility.

Key words

Chromolaena odorata, rumen modifier, methane, feeds and feeding

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Introduction

Global warming and its associated greenhouse effect are major issues for agriculturists, politicians, scientists and the society at large (FAO, 2009). The emanation of greenhouse gases (GHG) like carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) from agricultural practices (Weiske, 2005) contributes greatly to the greenhouse effect (global warming). Ruminant's livestock production accounts for 80% of the GHG emissions within the agricultural sector (FAO, 2008). According to Idris et al. (2011), a lot of research has been initiated varying from nutrient manipulation, breeding strategy to water treatment in order to improve the animal performance (milk production, meat and other products). Due to the long dry season leading to lignification of available forages, a higher volume of methane per kilogram of milk or meat has been recorded in developing countries compared to developed countries. It is therefore necessary to reduce the contributory ruminant methane production per kilogram of milk or meat produced in order to increase the animal's efficiency (Carlsson Kanyama, 1998; Garnet 2009). Feed supplement and nutraceutical plants from trees and browse species of high nutritive value for ruminants will be beneficial to boost the animal productivity as well as reducing fattening time and the contributory methane production (Goodland, 1997; Schils et al., 2007). Rumen fermentation represents a distinctive symbiotic relationship between the host and the rumen microflora, which lends the ruminant several benefits in digestive and metabolic processes over non ruminants (Nagaraja et al., 1997). However, products from ruminal fermentation such as ammonia and methane represent a loss of energy and nitrogen, respectively. Methane produced during rumen fermentation represents a loss of 2–15% of gross energy intake and thus decreases the potential conversion of digesta to metabolisable energy. The efficiency of energy and protein utilization in the rumen may be improved through the manipulation of microbial population and their activity (Casamiglia et al., 2007). This may be achieved using feed supplement and herbs from existing tree species. Emphatically, the use of nutriceutical plants (*Moringa oleifera* Lam., *Chromolaena odorata* (L.) R.M.King & H.Rob., *Leucena luecocephala* (Lam.) de Wit, etc.) in ruminant nutrition has been well documented as they are said to be highly nutritious with numerous phytochemicals that can improve performance and modify the rumen microbes (Yusuf et al., 2018, Kholif et al., 2018). In this case the study evaluates the nutritive, *in vitro* gas and methane gas production of *C. odorata*, which is a perennial flowering evergreen herb as alternative nutraceutical plant in ruminants.

Materials and Methods

Sample Collection

The *C. odorata* plant was harvested and identified at the Forestry Department, College of Environmental Science, Federal University of Agriculture, Abeokuta, Nigeria. Fresh *C. odorata* leaves were sourced before inflorescence to harvest more leafs. The leaves were air dried for 3 weeks, ground until they passed through a 2 mm sieve, bulked and stored for subsequent analysis.

Animal Donors and Collection of Rumen Fluid

Inoculum donors comprise of four West African dwarf (WAD) bucks averaged 25 kg. They previously fed with 500 g kg^{-1} DM of Maize stover and 500 $g.kg^{-1}$ DM of concentrated diet. The concentrate consisted of (as fed basis, g kg^{-1}) 160 corn, 520 wheat offal, 240 palm kernel cake, 60 soybean meal, 10 common salt and 10 bone meal. Equal proportions of rumen fluid were collected from the donor bucks into thermo flasks, the rumen fluid was further strained through a four-layered cheesecloth with the temperature maintained at 39 °C. Handling of the rumen fluid was done under a continuous flow of CO_2. The rumen liquor and the buffer solution were mixed in the ratio 1:2 (v/v), as pronounced by Menke and Steingass (1988).

Chemical Analysis of Test Ingredients and Experimental Diets

The nitrogen (N) content was carried out using the Kjeldahl method (AOAC, 2000; ID 973.18). The N content was multiplied by 6.25 to calculate the CP content of the sample, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as enunciated by Van Soest et al. (1991). The neutral detergent fibre was determined without amylase and sodium sulphite. Lignin was determined by solubilization of cellulose with H2SO4 on the ADF residue (Van Soest et al. (1991).

In vitro Procedure

The samples were incubated at 39°C while gas volume was measured after 3, 6, 9, 18 24, 48, 72, and 96 h (Menke and Steingass, 1988). Three blanks were included in the run as the controls. The gas volume produced by the blanks was subtracted from the gas volume produced per sample. The resultant gas volumes recorded at different incubation hours were fitted to the non-linear equation model of France et al. (2002):

$$A = b\ (1-e-c(t\text{-}L))$$

where:
A is the volume of gas produced at time *t, b* is the potential/asymptotic gas production (ml g^{-1} DM) from the fermentable fraction of forage, *c* is the fractional rate of gas production (/h) from the slowly fermentable fraction and *L* is the discrete lag time prior to gas production.

In vitro organic matter digestibility (IVOMD) and metabolisable energy (ME) of forage were computed as described by Menke et al. (1979).

Formula:

$$OMD\ \% = 14.88 + 0.889GP + 0.45CP + 0.651A$$

$$ME\ (MJ/Kg\ DM) = 2.20 + 0.136GP + 0.0574CP + 0.029CP^2$$

Short chain fatty acid SCFA (µmole g^{-1} DM) = 0.0239GPT – 0.0601 (Getachew et al., 1999)

where GP = 24 hours net gas production (mL 200mg^{-1} DM)
CP = crude protein content of substrate
A = ash content of substrate.

In vitro Dry Matter Degradation

At 96 h, the *in vitro* dry matter degradation was determined by agitating the incubation residues at 20, 000 x g for 30 min after placing iced cubed (-4°C) to end fermentation process. The residues obtained were strained and oven-dried to determine the dry weight. The blanks were centrifuged and the filtrate was weighed and used as correction factor for residues from the rumen inoculum. *In vitro* dry matter degradation was then calculated as:

(Substrate dry matter incubated – (residue dry matter – blank dry matter))/(Substrate dry matter incubated)

Determination of Methane Gas Estimate

Approximately 4 ml of sodium hydroxide (NaOH, 10M) was introduced to estimate the methane production at post incubation (Fievez et al., 2005). The average of the volume of gas and methane produced from the blanks was deducted from the total volume of gas and methane produced per sample to determine net gas and methane produced, respectively.

Statistical Analysis

Data collected during the experimental period were subjected to one-way analysis of variance (ANOVA) in a completely randomized design using SAS (1999) significantly differing means were separated using Tukey's Test as contained in the same software at 5% level of significance. The model for the study is given below:

$$Y_{ij} = \mu + T_j + \varepsilon_{ij}$$

where, Y_{ijk} is individual observation, μ is population mean, T_j is the effect of *Chromolaena odorata*, ε_{ij} is random residual error.

Results

Proximate composition and fibre fractions of *C. odorata*, experimental concentrate diet and maize stover

The proximate composition and fibre fractions of the experimental diets are presented in Table 1. The nutrient composition (on dry matter basis) of the plant are dry matter (DM) 969.0 g kg^{-1}, crude protein (CP) 175.1 g kg^{-1}, crude fibre (CF) 204.3 g kg^{-1}, ether extract (EE) 13.9 g kg^{-1}, ash 85.2g kg^{-1}, nitrogen free extract (NFE) 521.6g kg^{-1}, organic matter (OM) 914.8g kg^{-1}, neutral detergent fibre (NDF) 626.5 g kg^{-1}, acid detergent fibre (ADF) 377.7 g kg^{-1}, acid detergent lignin (ADL) 107.2 g kg^{-1}, hemicellulose 248.8 g kg^{-1}, cellulose 270.5 g kg^{-1}. For the phytochemicals investigated, the values are saponin 1.99%, tannin 2.57%, flavonoid 1.08% and alkaloid 1.26%.

The four experimental diets significantly ($P < 0.05$) differed in nutritive value and fibre fractions. Diet containing 0% inclusion of *C. odorata* had the highest ($P < 0.05$) DM content (93.32%) while the lowest (92.05%) was obtained in the diet with 6% *C. odorata* inclusion. The highest ($P < 0.05$) CP value (14.97%) was observed in the diet with 6% *C. odorata* inclusion while the lowest (13.35%) was recorded in the diet with 0% *C. odorata* inclusion. The highest ($P < 0.05$) values of 5.00% and 9.44% for ash and ether extract (EE) were observed in the diets with 4% *C. odorata* and 6% *C. odorata* inclusion, respectively, while the least values 3.85% and 8.13% for EE and ash in diets with 2% and 0% *C. odorata* inclusion for ash and ether extract, respectively. For crude fibre, values ranged from 6.21%- 6.52% with increase in *C. odorata* inclusion. The values for NFE and OM decreased significantly progressively ($P < 0.05$) from 61.00% to 56.73% and 91.87% to 90.56% from 0% to 6% *C. odorata* inclusion respectively.

Table 1. Nutritional composition of experimental concentrate diet and maize stover fed to West African Dwarf Bucks

Parameters (g kg^{-1})	MS	*C. odorata*	T_1	T_2	T_3	T_4	SEM	P-value
Dry matter	929.5	969.0	933.2^{a}	927.2^{b}	926.9^{b}	920.5^{c}	0.10	0.01
Crude protein	42.8	175.1	133.5^{d}	139.4^{c}	141.3^{b}	149.7^{a}	0.13	0.01
Crude fibre	308.0	204.3	62.1^{b}	63.0^{b}	64.5^{a}	65.2^{a}	0.03	0.05
Ether extract	7.0	13.9	46.3^{b}	38.5^{d}	50.0^{a}	43.9^{c}	0.10	0.01
Ash	100.2	85.2	81.3^{d}	91.3^{c}	93.0^{b}	94.4^{a}	0.12	0.01
Nitrogen free extract	542.1	521.6	610.0^{a}	595.0^{b}	578.1^{c}	567.3^{d}	0.33	0.01
Organic matter	899.9	914.8	918.7^{a}	908.7^{b}	907.0^{b}	905.6^{c}	0.12	0.01
Neutral detergent fibre	766.1	626.5	661.4^{a}	624.8^{b}	591.9^{c}	581.4^{d}	0.72	0.01
Acid detergent fibre	199.5	377.7	327.0^{a}	356.3^{b}	388.1^{c}	396.1^{d}	0.62	0.01
Acid detergent lignin	55.3	107.2	43.6^{a}	36.6^{b}	35.8^{c}	43.8^{a}	0.86	0.01
Hemicelluloses	596.6	248.8	333.8^{a}	268.5^{b}	203.8^{c}	185.3^{d}	1.34	0.01
Cellulose	144.2	270.5	284.0^{c}	319.7^{b}	35.23^{a}	352.3^{a}	0.65	0.01
*ME (MJ kg^{-1} DM)	141.4	ND	14.10^{a}	13.66^{c}	13.91^{b}	13.66^{c}	0.04	0.01

Note: Means on the same row having different superscripts are significantly different according to the Tukey HSD test ($P < 0.05$); * Calculated using MAFF 1984 equation; M.S; Maize stover. T_1: 0% *C. odorata*, T_2: 2% *C. odorata*, T_3: 4% *C. odorata*, T_4: 6% *C. odorata*

NDF and hemicellulose values ranged from 58.14% - 66.14% for NDF and 18.57% - 33.38% for hemicellulose, the values decreased from 0% to 6% *C. odorata* inclusion. ADF and cellulose increased significantly ($P>0.05$) with increasing inclusion of *C. odorata* with values ranged from 32.23% - 31.69% for ADF and 28.40% - 35.23% for cellulose. The highest ($P < 0.05$) value (4.38%) for ADL was recorded in diet with 6% *C. odorata* inclusion while the lowest value (3.58%) was recorded in the diet with 4% *C. odorata* inclusion. The proximate composition of maize stover determined in this study was: DM 92.95%, CP 4.38%, CF 30.80%, EE 0.70%, ash 10.02%, NFE 54.21%, OM 89.99%, NDF 79.61%, ADF 19.95%, ADL 5.53%, hemicellulose 59.66% and cellulose 14.42% (Table 3).

In vitro Gas Production and Fermentation Kinetics of West African Dwarf Bucks Rumen Fluid with *C. odorata* as Additive

The result of the effect of the experimental diets on *in vitro* gas production (ml 200 mg^{-1}) is presented in Table 2. The result showed a significant difference ($P < 0.05$) in the volumes of gas produced by the various experimental diets at 3, 6, 9, 12, 18, 24, 30, 42, 48, 60, 72, 84 and 96 hours of incubation. The treatment with 4% *C. odorata* inclusion recorded the highest ($P < 0.05$) gas volumes while treatment with 0% *C. odorata* inclusion recorded the lowest ($P < 0.05$) values. Generally, gas volumes increased from 0% *C. odorata* inclusion and peaked at 4% *C. odorata* inclusion. Potential gas production (b), fractional rate of gas production (c) and lag time (L) were significantly ($P < 0.05$) different.

Post Incubation Parameters of West African Dwarf Bucks Rumen Fluid with *C. odorata* as Additive

Table 3 shows the result of the effect of *C. odorata* additive on post incubation parameters of WAD bucks. Parameters investigated such as total gas volume (TGV), net gas volume (NGV), net methane proportion (NMP), *in vitro* organic matter digestibility (IVOMD), *in vitro* dry matter digestibility (IVDMD), short chain fatty acid (SCFA) and metabolizable energy (ME) were significantly influenced ($P < 0.05$) by the experimental diets.

The highest value for TGV, NGV, SCFA, and IVOMD were observed in the 4% *C. odorata* inclusion while the lowest were obtained in the 0% *C. odorata* inclusion. The highest values were: 30.67ml, 30.67ml, 0.15μmol g^{-1} DM, 31.99% while the lowest values were 21.42 ml, 21.12 ml, 0.09 μmol g^{-1} DM, and 29.30% for TGV, NGV, SCFA and IVOMD respectively.

The highest value for IVDMD was 77.08% (2% *C. odorata* inclusion) while the lowest was 66.25% (0% *C. odorata* inclusion). For ME, 6% *C. odorata* inclusion had the highest value (8.06 MJ kg^{-1} DM) while the lowest was recorded in the 0% *C. odorata* inclusion (7.48 MJ kg^{-1} DM). The means for methane gas output was not significantly different ($P>0.05$). Net methane proportion

Table 2. Effect of *Chromolaena odorata* additive on *in vitro* gas production (ml 200 mg^{-1}) and fermentation kinetics of West African Dwarf Bucks

Incubation hours	T_1	T_2	T_3	T_4	SEM	P-value
3	0.75^b	1.42^a	1.50^a	1.17^a	0.009	0.12
6	2.08^b	3.00^a	3.25^a	2.25^b	0.001	0.12
9	3.17^b	3.92^{ab}	4.42^a	3.25^b	0.001	0.15
12	4.08^b	5.00^a	5.58^a	4.00^b	0.001	0.17
18	5.25^b	6.83^a	7.17^a	5.08^b	0.001	0.22
24	6.17^b	8.25^a	8.75^a	6.42^b	0.001	0.27
30	7.17^b	10.50^a	10.75^a	8.08^b	0.001	0.34
36	8.42^b	13.25^a	14.17^a	10.92^b	0.001	0.47
42	11.08^c	16.33^a	17.50^a	14.00^b	0.001	0.52
48	13.92^c	18.83^a	20.17^a	16.58^b	0.001	0.51
60	18.17^c	25.42^a	27.75^a	21.92^b	0.001	0.76
72	21.42^c	29.17^a	30.67^{ab}	26.58^b	0.001	0.75
84	21.42^c	29.17^a	30.67^{ab}	26.58^b	0.001	0.75
96	21.42^c	29.17^a	30.67^{ab}	26.58^b	0.001	0.75
B	28.56^c	37.51^b	43.59^a	35.82^b	0.001	1.152
C	0.11^b	0.13^{ab}	0.16^a	0.11^b	0.008	0.017
L	1.13^a	1.07^b	1.05^b	1.11^a	0.004	0.008

Note: Means on the same row having different superscripts are significantly different according to the Tukey HSD test ($P < 0.05$); b: potential/ asymptotic gas production (ml g^{-1} DM), c: fractional rate of gas production (/h), *L*: lag time; T_1: 0% *C. odorata*, T_2: 2% *C. odorata*, T_3: 4% *C. odorata*, T_4: 6% *C. odorata*

Table 3. Effect of *Chromolaena odorata* additive on post incubation parameters of West African Dwarf Bucks

Parameters	T_1	T_2	T_3	T_4	SEM	P-value
TGV (ml)	21.42^{c}	29.17^{ab}	30.67^{a}	26.58^{b}	0.75	0.01
NGV (ml)	20.42^{c}	28.87^{ab}	30.67^{a}	26.58^{b}	0.75	0.01
Methane (ml 200 mg^{-1})	4.17	4.00	3.92	4.42	0.22	0.87
NMP	0.20^{a}	0.14^{b}	0.13^{b}	0.16^{ab}	0.01	0.03
NMP %	19.78^{a}	14.20^{b}	16.35^{ab}	16.35^{ab}	0.90	0.03
IVOMD %	29.30^{b}	31.60^{a}	31.99^{a}	30.11^{b}	0.25	0.01
IVDMD %	66.25^{b}	77.08^{a}	76.25^{a}	74.17^{a}	1.51	0.03
TDS (g)	132.50^{b}	154.17^{a}	152.50^{a}	148.33^{b}	2.93	0.03
SCFA (μmol g^{-1} DM)	0.09^{b}	0.14^{a}	0.15^{a}	0.09^{b}	0.01	0.01
ME (MJ kg^{-1} DM)	7.48^{b}	7.94^{a}	7.99^{a}	8.06^{a}	0.04	0.01

Note: Means on the same row having different superscripts are significantly different according to the Tukey HSD test ($P < 0.05$); TGV- Total gas volume, NGV- Net gas volume. NMP- Net methane proportion, IVOMD- *in vitro* organic matter digestibility, IVDMD- *in vitro* dry matter digestibility, SCFA- Short chain fatty acid, ME- Metabolizable energy, TDS: Total digestible substrate, T_1: 0% *C. odorata*, T_2: 2% *C. odorata*, T_3: 4% *C. odorata*, T_4: 6% *C. odorata*

declined significantly progressively ($P < 0.05$) from 0.20 in 0% *C. odorata* inclusion to 0.13 in 4% *C. odorata* inclusion.

Discussion

The percentage of dry matter recorded for *C. odorata* leaf meal in this present research was slightly higher than that reported by Aro et al. (2009), Ekeyem et al. (2010) and Kawed (2016) who recorded values of 87.40%, 91.44% and 90.49%, respectively. This disparity in dry matter percentages may be due to the growth stages of the leaf and seasonal variations in the study areas. Flowering and matured plants tend to have less moisture and more fibre compared to emerging plants, and dry matter percentage is always higher during the dry season than in the rainy season (Irwin et al., 2014). The crude protein values of *C. odorata* leaf meal in the present study competes well with those reported by Igboh et al. (2009) which was 16.17%, Aro et al. (2009), 18.67% and Ekeyem et al. (2010), 16.67%. This places Siam weed as essential source of protein in ruminants' diets as it is far above those recommended by NRC (2001) for maintenance production.

The observed value of saponins and tannins in *C. odorata* was lower than that reported by Agaba and Fawole (2016) which were 3.48% and 4.10%, respectively. However, the value of tannin was higher while saponins were similar to the report of Igboh et al. (2009) which were 0.37% and 1.98%, respectively. The percentage of flavonoid and alkaloid was higher than that reported by Agaba and Fawole (2010) and the values of phytate were also higher than that reported by Igboh et al. (2009) which were 0.77%, 1.55% and 0.54% for flavonoid, alkaloid and phytate, respectively. These slight variations can be due to factors such as stage of maturity of leaf, soil type and climatic variability. The recorded dry matter percentage of maize stover is similar to the reports of Biwi (1986), Tolera and Sundstol (1999) and Fabian (2011) who had 93.40%, 92.50% and 93.50% DM, respectively. However, Fabian (2011) reported a higher crude protein (CP) percentage of 5.60% compared to that reported in this study. Tolera and Sundstol (1999) had a similar CP percentage of 4.8% to the one reported in this study.

The crude protein content (CP) of the experimental diets was above 80 g kg^{-1} DM reported as adequate for rumen microbes digestive process (Orskov, 1982). The relative high CP content recorded could be used by rumen microbes to build up their body proteins for subsequent digestion in the abomasum. The increase in crude protein and ash contents of diets with *C. odorata* leaf additive justifies the possible feeding value of the leaf as protein and minerals supplement to feeds with lower level of protein and minerals.

A higher gas volume with an increase in *C. odorata* corresponded to a higher CP. Higher crude protein content in the diet has been reported to increase *in vitro* gas production (Ndlovu and Nherera, 1997; Gasmi-Boubaker et al., 2005; Aderinboye et al., 2016). Additionally, a higher crude protein diet encourages more microbial fermentation, the higher the CP in the diets, the higher the gas produced (Popova et al., 2012; Igbal and Hashim, 2014). A similar report has noted that different doses of *L. leucocephala* and *Salix babylonica* L. extracts (0.60, 1.20, 1.80 mL extract per g of DM) increased gas volume (Jiménez-Peralta et al., 2011).

The variation in the nutritive value (i.e., CP and CF) of the substrates could be responsible for that. However, the result of this work is contrary to the reports of several researchers (Kalita et al., 1996; Wang et al., 1997; Liu et al., 2003; Hess et al., 2003a; Hess et al., 2003b; Hu et al., 2005a; Hu et al., 2005b; Guo et al., 2008; Silivong, 2012). They all reported reduction in *in vitro* gas production by adding saponin extract or leaf meals rich in saponin such as *Yucca schidigera* Roezl ex Ortgies, *Sapindus saponaria* L. and *Camellia sinensis* (L.) Kuntze. The disparity in the results of this current research from those of the researchers above could be a result of species differences and the corresponding levels of saponin in the test leaf meals.

The non-significantly differed *C. odorata* on methane gas estimate corroborate with the report of Jiménez-Peralta et al. (2011) and Sungchhang et al. (2016) who observed similar methane output for rumen liquor of goats and growing lambs with *Flemingia macrophylla* (Willd.) Merr., 1910 and *L. leucocephala* leaf meal supplemented diets, respectively. Gunun et al. (2011) also observed that methane output was not affected in goats supplemented with Mao (*Antidesma thwaitesianum* Müll. Arg.) seed meal. This result is contrary to that of Guo et al. (2008), Sliwinski et al. (2010), Hartanto et al. (2017) and Li et al. (2018) who observed reduced methane output when tea saponin, plants rich in tannins and saponins, monensin, monensin and vegetable oils, respectively, were included in the diets of goats. Saponin level, type of saponins in *C. odorata* and level of inclusion could be associated to the the non-significant effect of *C. odorata* on methane gas output. However, net methane proportion (NMP) reduced significantly with increase in the inclusion of *C. odorata* leaf meal, and this is an indication that *C. odorata* has methane reduction potentials.

A higher rate of gas production as well as an increased rate of digestion of experimental diets indicated that rumen microbes were able to degrade the diets faster owing to a higher content of digestible nutrients. Higher gas production can increase the carbohydrate supply through short chain fatty acid production (Remesy et al., 1995). This result is in line with the report of Olagoke (2015) who observed that variation of fibre fractions and fermentation kinetics with cashew nut liquid inclusion in the diet of WAD goats. Sirohi et al. (2012) observed a potential increased in gas production in high fibre, medium fibre and low fibre diets with the inclusion of various oils. For all the treatments in this study, values of gas production from soluble fraction (*a*) were positive and significantly different which can be associated with the significant increase in gas production rate constant and decrease in lag time.

There is a positive correlation among *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short chain fatty acid (SCFA) and gas production. This is a good predictor for volatile fatty acid production, which is directly related to microbial mass production (Menke and Steingass 1988, Liu et al., 2002). This validates the results of this study where IVOMD and SCFA values increased with an increase in *C. odorata* inclusion from 0% to 4% inclusion. This correlates with the work of Jiménez-Peralta et al. (2011) on growing lambs fed with *L. leucocephala* supplemented diet. Yan et al. (2007) found that garlic oil and juniper berry oil increased IVOMD, ME and SCFA of goats. However, this result is contrary to that of Olagoke (2015) who observed a decrease in IVOMD, ME and SCFA in WAD goats' diet with cashew nut liquid supplementation. A higher CP value of feeds and higher gas output could be the reason for the increased values in this study.

In vitro dry matter digestibility (IVDMD) can give an idea of the microbial population and activity during substrate fermentation (Kongman et al., 2010). This suggested that incremental level of *C. odorata* leaf meal encourages the growth of beneficial microbes through increased CP content. Kawed (2016) also observed increase in IVDMD percentage with increase in the inclusion of *C. odorata* leaf meal in the diet of SEA goats. This is contrary to the observation of Hartanto et al. (2017) who reported that monensin supplementation had no effect on IVDMD of female Boer goats. Higher *in vitro* dry matter digestibility of *C. odorata* supplemented diets was possibly due to higher level of CP of the diets and stage of plant maturity. The provision of protein may enhance the activity of the rumen microorganisms and improve digestibility of feedstuffs (McDonald et al., 2010).

Conclusion

C. odorata can serve as a suitable alternative for protein supplement in ruminants' diets due to a more increased content of crude protein than in common grasses. Also, the observed increase in *in-vitro* cumulative gas, *in vitro* organic matter digestibility, i*n vitro* dry matter digestibility, short chain fatty acid and metabolisable energy clearly indicate that addition of *C. odorata* encourages the growth of beneficial microbes. The reduced net methane gas production from the diets supplemented with *C. odorata* is an indication that *C. odorata* supplementation in the ruminants' diet may reduce their contribution to the greenhouse effect.

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