Tropical Animal Health and Production (2021) 53:312
https://doi.org/10.1007/s11250-021-02751-x

REGULAR ARTICLES

Extracts of herbs and spices as feed additives mitigate ruminal methane production and improve fermentation characteristics in West African Dwarf sheep

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Received: 5 November 2020 / Accepted: 26 April 2021

Abstract
The study aims to evaluate the effect of aqueous and methanolic extracts of three herbs, namely, neem (*Azadirachta indica*), drumstick (*Moringa oleifera*) and scent (*Ocimum gratissimum*), and three spices garlic (*Allium sativum*), ginger (*Zingiber officinale*) and onion leaflets (*Allium cepa*) on ruminal methane production and fermentation characteristics. The feed samples (200 mg substrates plus extracts of the selected herbs and spices) were incubated with rumen liquor taken from three mature West African dwarf ewes at 3, 6, 9, 12, 15, 18, 21 and 24 h. The results show that extracts of the selected herbs and spices increased the gas produced, from the insoluble fraction, degradability rate, volume of gas produced at time and time of most rapid change in gas produced. The organic matter digestibility (OMD), metabolizable energy (ME) and short-chain fatty acid (SCFA) contents of aqueous extracts of the selected herbs and spices (1 ml/200 mg substrates samples) were between 32.82 and 71.34 g/100 g OM, 4.10 and 10.25 MJ/kg DM and 0.28 and 1.31 μmol, respectively. Furthermore, the OMD, ME and SCFA contents of methanolic extracts of the selected herbs and spices (1 ml/200 mg substrates samples) were between 32.82 and 99.50 g/100 g OM, 4.10 and 14.37 MJ/kg DM and 0.28 and 2.07 μmol, respectively. Methane produced from the methanolic extracts of the herbs and spices highly differed ($P < 0.05$). This study suggests that extracts of the selected herbs and spices have the potential to affect rumen fermentation and also to reduce the methane production in sheep.

Keywords Digestibility · Herbs extract · Methane production · Spices extracts · Sheep · Gas production

Introduction

In livestock production, antibiotics are commonly fed or used in animal production to prevent diseases and metabolic disorders, to manipulate the rumen environment for proper feed utilization/efficiency and also as growth promoters. Quite a number of synthetic feed additives (mostly chemical substances) have been used in ruminant nutrition to manipulate, modify or improve rumen efficiency and reduce energy and nitrogen losses (Faniyi et al. 2016a, b; Adegbeye et al. 2018). Recently, there is increasing interest in the use of plant parts and their extracts as an alternative to antibiotics growth promoter (Faniyi et al. 2016a, b; Adegbeye et al. 2018). This is because of the ban on the use of antibiotics in livestock rations as growth promoter (WHO 2017) as a result of residual effect of antibiotics in animal products which may be detrimental for human consumption (i.e. report on the additives leaving residue in the milk of most dairy cow). Several studies have been carried out to ascertain the efficacy of phytochemicals as natural feed additives especially those of phytogenic origin. Some phytogenic additives have been found to enhance rumen fermentation efficiency, reduce methane gas production and improve rumen ecology (Salamat Azar et al. 2012; Faniyi et al. 2016a, b, 2019; Adegbeye et al. 2018).

In vitro gas production technique is effective for the prediction of the nutritive value of feeds, feed intake, digestibility,

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Published online: 09 May 2021

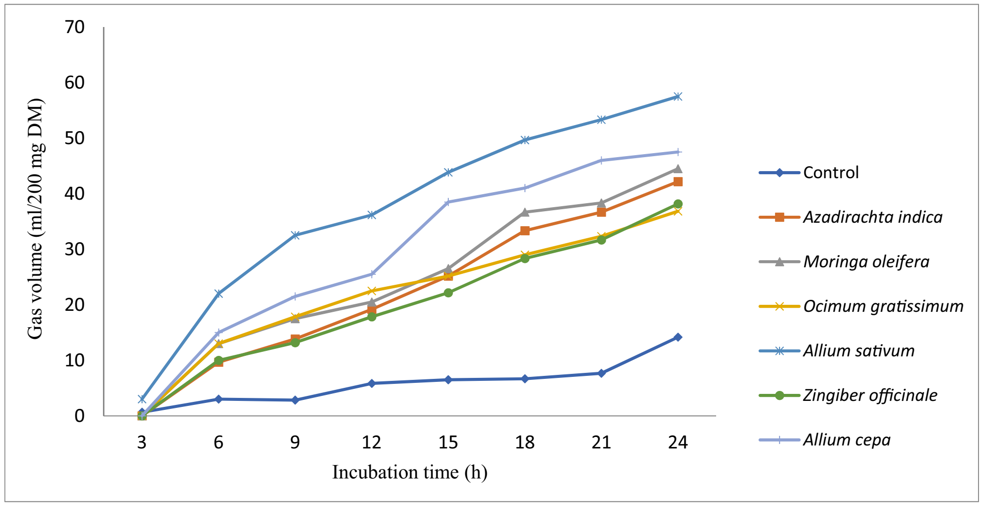

Fig. 1 In vitro gas production of aqueous extracts of some herbs and spices

microbial nitrogen supply and animal performance (Fievez et al. 2005; Babayemi and Bamikole 2006; Daodu and Babayemi 2009). For some years now, in vitro technique has been used to determine some parameters such as the amount of short-chain fatty acids (SCFA), organic matter degradability (OMD), carbon dioxide (CO_2) and metabolizable energy (ME) of feed for all ruminants (Blummel and Becker 1997; Getachew et al. 1999; Binuomote and Babayemi 2012). Thus, using such technique to simulate digestion can help to screen potential herbs and spices before use in vivo.

The objective of this study is to evaluate the effect of some selected herbs and spices extracts on methane production and fermentation characteristics in rumen of the West African Dwarf sheep using in vitro gas production technique.

Materials and methods

Collection and preparation of samples

All materials were procured/gathered in their unprocessed state. Neatly plucked herbs (neem, drumstick and scent leaves) were harvested within and around the University of Ibadan campus, Ibadan. Spices (ginger, garlic and onion leaflets) were obtained from Bodija Market in Ibadan. The outer skin of the washed rhizomes of ginger was peeled off using a blunt knife and light scaly leaves on the garlic cloves were removed and the naked cloves were chopped into smaller particles. All herbs and spices were air dried at 60 °C and afterwards milled. The finely ground samples were screened through mesh 300 μm and stored in an airtight cellophane bag and kept in a refrigerator (4 °C) till further analyses.

Extract preparation of herbs and spices

Plant extracts were prepared in aqueous methanol according to (water and methanol 50/50 ml) (Santra et al. 2012; Sirohi et al. 2012a, b). Twenty-five grams powder of herbs and spices each were deposited in a 500-ml conical flask and mixed with 250 ml of water and 250 ml (50%) aqueous methanol. The flask was tightly sealed and kept in a shaker at 25 °C and

Table 1 In vitro fermentation parameters of some herbs and spices aqueous extracts

Aqueous extracts	a (ml)	b (ml)	a + b (ml)	c (ml/h)	t (hrs)	Y (ml)
Control (no herbs and spices)	0.67 ± 1.15	14.17 ± 1.15^{c}	14.83 ± 1.15^{c}	0.06 ± 0.02	9.00 ± 0.00^{b}	6.33 ± 0.58^{d}
Azadirachta indica	0.00 ± 0.00	42.17 ± 7.37^{b}	42.17 ± 7.37^{b}	0.10 ± 0.04	18.00 ± 3.00^{a}	33.83 ± 8.25^{abc}
Moringa oleifera	0.00 ± 0.00	44.50 ± 2.65^{ab}	44.50 ± 2.65^{b}	0.10 ± 0.03	18.00 ± 0.00^{a}	36.67 ± 4.93^{ab}
Ocimum gratissimum	0.00 ± 0.00	36.83 ± 12.90^{b}	36.83 ± 12.90^{b}	0.12 ± 0.08	17.00 ± 3.46^{a}	29.00 ± 9.01^{bc}
Allium sativum	3.00 ± 5.20	57.50 ± 5.29^{a}	60.50 ± 4.36^{a}	0.09 ± 0.02	14.00 ± 4.58^{ab}	43.00 ± 6.06^{a}
Zingiber officinale	0.00 ± 0.00	38.17 ± 1.15^{b}	38.17 ± 1.15^{b}	0.08 ± 0.01	16.00 ± 3.46^{a}	27.17 ± 5.00^{bc}
Allium cepa	0.00 ± 0.00	38.83 ± 14.15^{b}	38.83 ± 14.15^{b}	0.08 ± 0.04	13.00 ± 1.73^{ab}	23.17 ± 4.93^{c}
SEM	1.16	4.63	4.67	0.02	1.65	3.52

[abcd] means within the same column with different superscript, differ significantly ($P < 0.05$); a = gas produced from soluble fraction, b = gas produced from insoluble fraction, c = gas production rate for the insoluble fraction (b), Y = volume of gas produced at time t, t = time of most rapid change in gas produced

120 rpm for 24 h. After shaking, the contents in the flask were squeezed through muslin cloth and further filtered through Whatman No. 1 paper, while the aqueous methanol extracts were filtered directly using Whatman No. 1 filter paper. The residues were re-extracted with 125 ml solvent and filtered through Whatman No. 1 filter paper. Filtrates were combined and stored at 4 °C for further use.

In vitro gas production test

The rumen fluid was collected from three ewes prior to morning feeding. The fluid was collected through the suction method (by means of the suction/stomach tube or hose) from three ewes under the same feeding regime. The animals were fed according to their body weights; they were fed with 60% cassava peel, 20% wheat offal, 12% palm kernel cake, 2% soya bean meal, 2% dicalcium phosphate, 1% limestone, 1% common salt, 1% premix and 1% urea. The collected rumen fluid was filtered through a four-layered cheese cloth into a flask flushed with carbon dioxide (CO_2) gas and stirred using a stirrer. Incubation was carried out using the method of Menke and Steingass (1988). The buffer solution prepared was the McDougall's buffer containing $NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ + urea in a ratio 1:2 v/v. Thirty millilitres of the inoculums were introduced into each of the pre-warmed syringes through the silicon tubes. Air bubbles trapped in the syringes were removed by shaking the syringe and then pushing the piston upwards after which the steel (Hoffman's) clips on the tubes were screwed tightly. Blanks were also prepared with 30 ml of inoculums, without the feed sample. The incubation period lasted for 24 h, and the gas production was observed and recorded at an interval of 3 h, including the blanks. Gas production was terminated at the end of 24 h. After termination, 10 molar solution of NaOH was introduced through the silicon tube. After opening the steel clip, the mixture was thoroughly shaken; NaOH absorbed the CO_2 gas present in the syringe, leaving only the methane gas. The volume of methane gas was recorded, and the volume of the CO_2 was determined by subtracting the volume of methane from total gas produced. In vitro gas production data were analysed with $Y = a + b\ (1 - e^{-ct})$ equation described by Ørskov and McDonald (1979)

Table 3 Methane produced by aqueous extracts of some herbs and spices from the in vitro gas production

Treatment	Methane (ml/200mgDM)	%/gas volume
Control (no herbs and spices)	10.0^{b}	67.4
Azadirachta indica	24.3^{a}	57.7
Moringa oleifera	22.7^{a}	50.9
Ocimum gratissimum	19.7^{a}	53.4
Allium sativum	24.0^{a}	39.7
Zingiber officinale	19.7^{a}	51.5
Allium cepa	24.0^{a}	61.8
SEM	2.16	3.88

^{abcd} means within the same column with different superscript, differ significantly ($P < 0.05$); *OMD* = organic matter digestibility

Calculations

The gas volume produced from the blank was subtracted from the volume of gas produced from each feed sample. This gave the net gas produced for each sample. The values obtained were fitted into the exponential equation, $Y = a + b\ (1 - e^{ct})$ to estimate gas production characteristics as described by Ørskov and McDonald (1979):

Where

Y volume of gas produced,
t time of incubation,
a intercept (gas produced from the soluble fraction),
b gas produced from insoluble fraction,
c gas production rate for the insoluble fraction (b).

Table 2 Estimated metabolizable energy (ME), organic matter digestibility (OMD) and short-chain fatty acid (SCFA) of aqueous extracts of some herbs and spices

Aqueous extract	ME (MJ/kg DM)	OMD (g/100 g OM)	SCFA (mmol)
Control (no herbs and spices)	4.10 ± 0.15^{c}	32.82 ± 1.03^{c}	0.28 ± 0.03^{c}
Azadirachta indica	8.26 ± 0.96^{b}	57.71 ± 6.55^{b}	0.95 ± 0.18^{b}
Moringa oleifera	8.56 ± 0.34^{ab}	59.79 ± 2.35^{ab}	1.00 ± 0.06^{ab}
Ocimum gratissimum	7.56 ± 1.67^{b}	52.97 ± 11.46^{b}	0.82 ± 0.31^{b}
Allium sativum	10.25 ± 0.56^{a}	71.34 ± 3.88^{a}	1.31 ± 0.10^{a}
Zingiber officinale	7.74 ± 0.15^{b}	54.16 ± 1.03^{b}	0.85 ± 0.43^{b}
Allium cepa	7.82 ± 1.84^{b}	54.75 ± 12.58^{b}	0.87 ± 0.34^{b}
SEM	0.60	4.11	0.11

^{abc} means within the same column with different superscript, differ significantly ($P < 0.05$); *ME* = metabolizable energy, *OMD* = % organic matter digestibility, *SCFA* = short-chain fatty acids

Table 4 In vitro fermentation parameters of some herbs and spices methanolic extracts

Treatment	a (ml)	b (ml)	a + b (ml)	c (ml/h)	t (hrs)	Y (ml)
Control (no herbs and spices)	0.67 ± 1.15^{b}	14.171.15^{d}	14.83 ± 1.15^{d}	0.06 ± 0.02^{ab}	9.00 ± 0.00^{e}	6.33 ± 0.58^{e}
Azadirachta indica	0.00 ± 0.00^{b}	55.50 ± 11.14^{bc}	55.50 ± 11.14^{c}	0.06 ± 0.02^{ab}	10.67 ± 1.53^{de}	38.83 ± 11.37^{cd}
Moringa oleifera	6.67 ± 5.86^{a}	70.17 ± 14.74^{b}	76.83 ± 10.79^{ab}	0.05 ± 0.02^{b}	17.00 ± 1.73^{a}	63.83 ± 9.25^{b}
Ocimum gratissimum	0.00 ± 0.00^{b}	57.83 ± 5.77^{bc}	57.83 ± 5.77^{c}	0.03 ± 0.01^{b}	16.00 ± 3.46^{ab}	46.83 ± 13.36^{cd}
Allium sativum	0.00 ± 0.00^{b}	89.17 ± 6.93^{a}	89.17 ± 6.93^{a}	0.12 ± 0.03^{ab}	12.00 ± 0.00^{cde}	81.50 ± 6.00^{a}
Zingiber officinale	0.00 ± 0.00^{b}	52.17 ± 9.61^{c}	52.17 ± 9.61^{c}	0.12 ± 0.02^{ab}	13.00 ± 1.73^{bcd}	34.50 ± 3.61^{d}
Allium cepa	0.00 ± 0.00^{b}	67.50 ± 8.19^{b}	67.50 ± 8.19^{bc}	0.16 ± 0.14^{a}	15.00 ± 0.00^{abc}	51.83 ± 6.51^{bc}
SEM	1.30	4.55	5.05	0.03	0.98	4.81

^{abcd} means within the same column with different superscript, differ significantly ($P < 0.05$). a = gas produced from soluble fraction, b = gas produced from insoluble fraction, c = gas production rate for the insoluble fraction (b), Y = volume of gas produced at time t, t = time of most rapid change in gas produced

The following parameters were estimated:

$$\text{Metabolizable energy (ME) (Menke and Steingass 1988)} = 2.20 + 0.13{*}GV + 0.057{*}CP + 0.0029{*}CF; \text{Organic matter digestibility (Menke and Steingass 1988)} = 14.88 + 0.889{*}GV + 0.45{*}CP + 0.651XA; \text{Short-chain fatty acid (Getachew et al. 1999)} = 0.0239{*}GV - 0.0601$$

where GV, CP, CF and XA are total gas production (ml/200 mg DM), crude protein, crude fibre and ash of the incubated samples, respectively.

Statistical analysis

Completely randomized design was used and data obtained were analysed using analysis of variance (ANOVA) at $P < 0.05$.

Results

Aqueous extract

Gas production characteristics of the aqueous extract of selected herbs and spices are presented in Fig. 1. The control produced the lowest gas, while *Allium sativum* extract produced the highest gas from insoluble fraction ($P < 0.05$). The control had the lowest rate of gas production from insoluble fraction, while *Ocimum gratissimum* had the highest. The least volume of gas produced was observed in the control, while *Allium sativum* had the highest (Table 1). There were significant differences ($P < 0.05$) for all the OMD values of the selected herbs and spices aqueous extracts including the control. The OMD and SCFA values for the control were the lowest, and *Allium sativum* extract values were the highest (Tables 2 and 3). However, the methane production in all herbs were similar, but it is quite surprising that all herbal and spices aqueous extract produced more methane than the control (Table 3). Nevertheless, the percentage of methane produced per

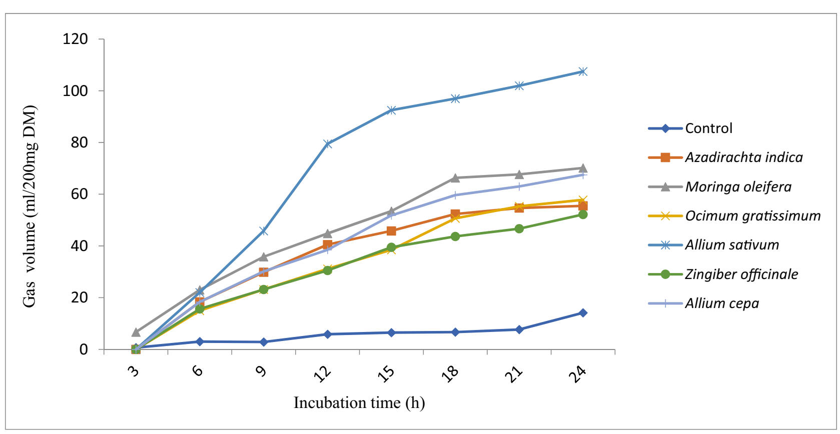

Fig. 2 In vitro gas production of methanolic extracts of some herbs and spices

Table 5 Estimated metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of methanolic extracts of some herbs and spices

Treatment	ME (MJ/kg DM)	OMD (g/100 g OM)	SCFA (mmol)
Control (no herbs and spices)	4.10 ± 0.15^{d}	32.82 ± 1.03^{d}	0.28 ± 0.03^{d}
Azadirachta indica	9.99 ± 1.45^{bc}	69.57 ± 9.90^{bc}	1.27 ± 0.27^{bc}
Moringa oleifera	11.90 ± 1.40^{b}	82.60 ± 9.59^{b}	1.62 ± 0.26^{b}
Ocimum gratissimum	10.29 ± 0.75^{bc}	71.64 ± 5.13^{bc}	1.32 ± 0.14^{bc}
Allium sativum	14.37 ± 0.90^{a}	99.50 ± 6.15^{a}	2.07 ± 0.17^{a}
Zingiber officinale	9.56 ± 1.25^{c}	66.60 ± 8.54^{c}	1.19 ± 0.23^{c}
Allium cepa	11.55 ± 1.06^{b}	80.23 ± 7.28^{b}	1.55 ± 0.20^{b}
SEM	0.59	4.04	0.11

[abc] means within the same column with different superscript, differ significantly ($P < 0.05$); *ME* = metabolizable energy, *OMD* = % organic matter digestibility, *SCFA* = short-chain fatty acids

volume of gas indicate that the control produced the highest while *Allium sativum* produced the least.

Methanolic extract

Gas production from insoluble fractions in *Allium sativum* methanolic extract had the highest, and the control (no herbs and spices) had the least value. Time of most rapid increase in gas produced "t" showed that the control (no herbs and spices extract) had the least value, while *Moringa oleifera* methanolic extract had the highest value (Table 4; Fig. 2). There were significant ($P < 0.05$) differences for all the OMD values of the selected herbs and spices methanolic extracts including the control. The OMD and SCFA for the control were the least, and the OMD for the methanolic *Allium sativum* extract was the highest (Tables 5). The volume of methane (CH_4) produced from these selected herbs and spices methanolic extracts were significantly different from the control (Table 6). The control (no herbs and spices) had the least CH_4 value, while *Allium sativum* had the highest. However, the percentage of methane produced per volume of gas indicate that the control produced the highest CH_4 while *Azadirachta indica* produced the least.

Discussion

Inclusion of all extracts increased gas production, metabolizable energy (ME) and organic matter digestibility (OMD). Gas production depicts microbial degradability of samples (Babayemi et al. 2004; Fievez et al. 2005), and in many cases, feedstuff showing high capacity for gas production also correlate with high methane production—which in itself is a product of an energy wasteful process that results in biogenic greenhouse emission. Gas produced from the anaerobic fermentation depends on the function of degradable carbohydrate (Blummel and Becker 1997; Getachew et al. 1998). However, OMD value is a measure of energy and evidence of fermentable substrates that is available to ruminants and could be used to measure microbial degradation of substrates in the presence of sufficient ammonia nitrogen (Taghizadeh et al. 2006; Paya et al. 2007; Akinfemi et al. 2009; Salamat Azar et al. 2012). The present study showed that all extract improved the OMD than the control. Janz et al. (2007) reported that phytogenic feed additives are capable of increasing digestive stimulant. Dey et al. (2021) study showed that inclusion of garlic oil during in vitro rumen digestion is capable of modifying fibrolytic enzymes such as CMCase, xylanase, β-glucosidase and acetyl esterase. The improvement in OMD

Table 6 Methane produced by methanol extracts of some herbs and spices from the in vitro gas production

Treatment	Methane (ml/200mg DM)	%/gas volume
Control (no herbs and spices)	10.0^{d}	67.4
Azadirachta indica	19.7^{c}	35.4
Moringa oleifera	33.0^{ab}	43.0
Ocimum gratissimum	25.7^{bc}	44.4
Allium sativum	37.7^{a}	42.3
Zingiber officinale	21.3^{c}	40.9
Allium cepa	31.3^{ab}	46.4
SEM	3.01	2.89

[abcd] means within the same column with different superscript, differ significantly ($P < 0.05$); *OMD* = organic matter digestibility

suggests that garlic may be associated with increased enzymatic breakdown during in vitro. The short-chain fatty acid (SCFA) values for all the selected herbs and spices in the (aqueous and methanolic) extracts were significantly higher than the control. Gas production is directly proportional to SCFA, and the higher the gas produced, the higher the SCFA (Maheri-Sis et al. 2008; Salamat Azar et al. 2012). Thus, the increased SCFA and ME values are associated with high gas production and digestibility and this is evident in all the methanolic extract of the herbs and spices especially *Allium sativum* methanolic extract. The SCFA level indicates energy availability and can contribute up to 80% of animal daily energy requirement (Fellner 2004) and is directly proportional to ME and OMD (Menke et al. 1979). Moreover, SCFA can be used to relate feed composition to production parameters as well as the net energy value of the herbs and spices. The high gas production in this study relates with the study of Elghandour et al. (2015a, b) who used phytogenic additives; thus, the predominance of SCFA in the selected herbs and spices of (aqueous and methanolic) extracts could probably ascribe to increased proportion of volatile fatty acid present. Moreover, all the selected herbs and spices in the (aqueous and methanolic) extracts showed that they contained more fermentable substrates which are important for ruminal microbial growth (Van Soest 1994). It was observed that in both extracts, *Allium sativum* had the best fermentative performance and highest ME, OMD and SCFA. The increased digestibility and fermentation in *Allium sativum* may be attributed to the phytochemicals in the garlic such as high level of organosulphur compounds such as allicin, ajoene, s-ally cysteine, di-ally disulphide and methyl cysteine sulfoxide (Chi et al. 1982). This activity is similar to the activity of garlic in the gastro-intestinal tract where they help to maintain microbial ecosystem or improve microbes such as bacteria and protozoa (Ikyume et al. 2018). The increased fermentation in garlic group may be due to increased bacteria and fungi growth, which aided the degradation of organic material and subsequently increased SCFA and OMD and decreased CH_4. The increased fermentation in *Allium sativum* group is similar to the report of Adewumi (2008) where garlic improved rumen fermentation and energy utilization in vitro. The lowest methane output from garlic group may be attributed to decreased rumen protozoa. This is because Ikyume et al. (2018) reported that garlic powder decreased protozoa. Thus, due to the hydrogen exchange relationship between methanogens and protozoa, the decrease in methane may be attributed to this. Apart from garlic, the other extract that improved gas production, ME, OMD and SCFA was *Moringa oleifera*. The activity of *Moringa* in improving digestion in vitro has been reported by Elghandour et al. (2018) and Pedraza-Hernandez et al. (2019). This is due to the nutrient in it that can boost rumen microbial growth, digestible saccharides in phyto-constituents which results in good feed degradability, fermentation and reduced methane production.

Conclusions

Inclusion of the selected herbs and spices (aqueous and methanolic extract) increased gas production, digestibility, ME, OMD and SCFA with concomitant reduction in methane gas production in vitro. The increased gas production implies that more energy would be made available to animals when the selected herbs and spices are included in the diets. Garlic inclusion gave the highest value of ME, OMD and SCFA. Furthermore, extracts of *Allium sativum, Moringa oleifera, Allium cepa, Azadirachta indica, Ocimum gratissimum* and *Zingiber officinale* have the potentials to serve as natural alternatives for enhancing digestibility, efficient fermentative digestion, rumen microbial activities and therefore improve livestock productivity. The gas production translated to increased ME, OMD and SCFA that will be produced. The low methane production is important for promoting ruminant growth production efficiency. This is because methane production represents a significant loss of energy to ruminant, causing loss or reduction in animal products to the farmers, as well as cause environmental pollution and global warming.

Authors' contributions TOF and MKA conceived and designed the experiment; TOF, MKA and AAJ conducted the experiment; MKA supervised the experiment; TOF, MJA, MMMYE, AZM and ABP prepared the manuscript. All authors approved of the manuscript.

Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Ethics approval Animal studies have been approved by ethical committee. The research was performed in accordance with the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments.

Consent to participate All authors agree to participate in the current work.

Consent to for publication All authors agree to publish the findings of current research.

Conflict of interest The authors declare no conflict of interest.

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