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ORIGINAL RESEARCH ARTICLE

In vitro gas production assessment of concentrate diet containing ginger rhizome meal at varying levels

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ABSTRACT

Ginger rhizome is a spice and may be beneficial in ruminant nutrition due to its inherent anti-microbial factors. Being an emerging additive, in vitro assessment may be necessary to evaluate its nutritive value. Thus, varying levels of ginger rhizome as dietary inclusion was examined. Dried ginger rhizome was ground into powder and added to a concentrate diet at 0, 50, 100, 150 and 200g/kg. The diet samples were incubated using in vitro gas production technique. Gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h post incubation to estimate total gas volume, methane (CH_4), metabolisable energy (ME; MJ/kg DM), organic matter digestibility (OMD; %) and short chain fatty acids (SCFA; µmol/200 mg DM)) were estimated using 4mL of 10M NaOH. Dynamics of gas production characteristics over time were described by equation $y = a + b\ (1\text{-}e^{-ct})$. DM was similar across all the treatments. The CP ranged between 14.98% for 150g of ginger/Kg and 18.13% for 200g/Kg ginger supplementation. The total volume of gas produced by the diets consistently increased from the control diet to 150g/Kg ginger inclusion and thereafter declined significantly ($p<0.05$). The values ranged from 5.20 to 6.42 for ME, 39.19 to 46.08 for OMD and 0.43 to 0.65 for SCFA. However, the CH_4 production varied from 8.00 mL to 10.00 mL with no significant difference. The highest level of methane production was obtained in the 50g/Kg ginger inclusion level. The result showed that the ginger inclusion in the diet enhanced the availability of nutrients resulting in higher digestible and metabolisable energy with reduced methane production.

Keywords: feedstuff, additive inclusion, gas production, ginger rhizome

INTRODUCTION

Plants or plant extracts containing essential oils, tannins, saponins, flavonoids and many other plant secondary metabolites have been shown to improve metabolism in the rumen, such as protein degradation in the rumen, increased microbial protein production and protein flow to the duodenum targeting specific rumen microbial populations (Patra and Saxena, 2009; Waghorn, 2008; Kamra *et al.*, 2008; and Wallace, 2004). In line with these, there has been a shift of interest to using plants and plant extracts as growth promoters, improve feed efficiency and animal productivity. Lots of plants contain secondary metabolites in the form of essential oil to serve this purpose (Calsamiglia *et al.*, 2006, 2007, Sallam *et al.*, 2009). One of such plant is the Ginger (*Zingiber officinale* Roscoe) with its rhizome (ginger root) being widely used as an herbal remedy for some common ailments. It contains zingiberol, gingerol and shagoals which are active constituents with antipyretic, antiemetic, analgesic, anti-inflammatory (Evans and Trease, 1979), antioxidant and anti-stress activities (Lakshmi and Sudhakar, 2010).

In vitro gas production is quick and less expensive means of determining the nutritive value of feeds for ruminants (Babayemi *et al.*, 2004; Babayemi and Bamikole, 2006). Total gas production can predict methane (CH_4), Volatile Fatty Acids (VFA) and the individual molar VFA (Fievez *et al.*, 2005) and prediction of feed intake (Khazaal *et al.*, 1995). The objective of this experiment was to estimate the effect of ginger supplementation on dry matter digestibility (DMD), organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA) of concentrate diet using *in vitro* gas production technique.

MATERIALS AND METHODS

Ginger rhizome samples: Dried ginger roots were purchased from a local market in Ibadan, Oyo state. All samples were ground in a laboratory mill to pass through a 1 mm screen.

Chemical analysis

Dry matter (DM) was determined by drying the samples at 105°C for 24 h and ash was determined by igniting the samples in muffle furnace at 525°C for 8 h and nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N × 6.25. Ether extract (EE) was determined by extracting the sample with ether according to the procedure outlined by AOAC (1995).

Table I: Non–supplemented feed composition

Ingredient	%
Dry Cassava peel	35
Brewers Dry Grain	40
Palm kernel cake	12
Rice Husk	8
Salt	3
Limestone	2

Treatments and experimental design

Four inclusion levels of dried ginger powder were added to the diet at 0, 50, 100, 150 and 200g/kg of concentrate respectively.

***In vitro* gas production**

Fermentation of ginger supplemented diets was carried out with rumen fluid which was obtained from five mature West African Dwarf goats via stomach tube. The goats were fed a diet of *Panicum maximum* (60%) and concentrate (40%) twice daily for 7days. The samples were incubated in the rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Gas production was measured as the volume of gas in the calibrated syringes and recorded before incubation at 3, 6, 9, 12, 15, 18, 21 and 24 h after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for concentrate meal samples were determined by subtracting the volume of gas produced in the blanks. Gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h post incubation to estimate total gas volume, methane (CH_4), metabolizable energy (ME; MJ/kg DM), organic matter digestibility (OMD; %) and short chain fatty acids (SCFA; µmol/200 mg DM)) were estimated using 4mL of 10M NaOH. Dynamics of gas production characteristics over time were described by equation $y = a + b\ (1\text{-}e^{-ct})$.

Statistical analysis

Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model of SAS (2000).

RESULTS AND DISCUSSIONS

In vitro gas production characteristics of concentrate supplemented with ginger powder is presented in Table 3. The *In vitro* gas production characteristics varied significantly ($P < 0.05$) among the ginger supplemented concentrates. The intercept value (a) for all the treatments ranged from 4.00 to 6.67 at 24 h. The extent of gas production 'b' values were similar across the treatments except for the control which was significantly ($P < 0.05$) different from other treatments such that, it has the lowest gas production value among all the treatments. Potential gas production (a+ b) was not significantly ($P > 0.05$) different across treatments. There were significant ($P < 0.05$) differences in gas production rate ('c') and 'y' of the incubated samples. Incubation time ('t') did not follow similar trend. The rate of gas production 'c' ranged from 0.12 to 0.06 ml h^{-1} for all the treatments while the volume of gas 'y' produced at time ('t') ranged from 8.67 to 14.00 for all the treatments. Time of rapid gas production't' ranged from 12.00 h in the control and 9.00 h in diets supplemented with ginger at 50g/kg, 100g/kg and 150g/kg but was least in 200g/kg supplemented diet at 6.00 h. Hillman et al. (1993) reported that gas production is positively related to microbial protein synthesis. Although gas production is a nutritional wasteful product (Mauricco et al., 1990) but it provides a useful basis from which metabolizable energy, organic matter digestibility and short chain fatty acids may be predicted.

More importantly, gas production helps to measure digestion rate of soluble and insoluble fractions of feedstuff (Menke and Steingass, 1988, Pell and Schofield, 1993). The gas produced is directly proportional to the rate at which substrate are degraded (Doano *et al.*, 1997). Also, gas volumes have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993). Metabolisable energy (ME), short chain fatty acid (SCFA) and dry matter degradability (DMD) production all differed significantly ($P<0.05$) for the five treatments. The value for the ME, SCFA and DMD ranged from 5.20 to 6.42, 0.43 to 0.65 and 66.33 to 73.33 respectively.

Table II. Proximate analysis (g/100 g DM) of concentrate diet supplemented with *Zingiber officinale* root at varying quantity

	0g/kg	50g/kg	100g/kg	150g/kg	200g/kg	SEM
Dry matter	92.18	91.65	91.76	91.30	91.07	
Crude Protein	15. 80^{b}	18.09^{a}	17.29^{a}	14.97^{b}	18.13^{a}	0.266
Crude Fibre	4. 80^{b}	3.20^{c}	2.17^{d}	5.33^{a}	5.40^{a}	0.056
Ether extract	16.30^{c}	17.60^{ab}	17.80	17.40^{b}	17.80	0.07
ASH	5.48^{b}	5.96^{a}	5.55^{b}	3. 89^{d}	5.14^{c}	0.06
Nitrogen Free Extract	49.80	46.80	48.95	49.71	44.60	

abc: Means on the same column with similar superscript are not significantly ($P > 0.05$) different

A correlation between ME values measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feed was reported by Menke and Steingass (1988). When feedstuffs are incubated with buffered rumen fluid (inoculums) *in vitro*, gas production is basically the result of microbial degradation of carbohydrates under anaerobic condition to acetic, propionic and butyric acids (Steingass and Menke 1988, Getachew *et al.* 2002; Khazaal *et al.* 1995 and France and Siddon 1993). Gas production from protein fermentation is relatively small compared to carbohydrate fermentation. The contribution of fat to gas production is negligible. (Beuvink and Spoelstra (1992) further stated that gas is produced mainly when feedstuff carbohydrates are fermented to acetate and butyrate with fermentation to propionate yielding gas only from buffering of the acid, therefore forage which produce high amount of propionate should produce low gas volume. Acetate and butyrate are lipogenic which leads to synthesis of butter fat in milk while propionate is glucogenic which leads to production of lean meat. Gas production was directly proportional to SCFA (Beuvink and Spoelstra, 1992), the higher the gas produced, the higher the short chain fatty acids. Short chain fatty level indicates that the energy is available to the animal and it contributes up to 80% of animal daily energy requirement (Fellner, 2004). Short chain fatty acid (SCFA) is directly proportional to metabolisable energy (ME) (Menke *et al.*,1979).

Furthermore, methane (CH_4) range of value was 8.00 – 10.00%, although there were no significant differences. Organic Matter Digestibility (OMD %) of the ginger supplemented diets ranged from 39.19 to 46.08% without any significant differences, however, the un-supplemented diet has the lowest OMD% while it was highest at the 150g/kg of ginger inclusion in the diet. The metabolisable energy (ME) followed the same trend as OMD%. The Metabolisable Energy (ME) ranged between 5.20 and 6.42 MJ/kg DM. The short chain fatty acid (SCFA) had similar values across the treatments, although it followed the same trend as observed in OMD and ME. The mean values of SCFA ranged from 0.43 to 0.65µml. The dry matter degradability (DMD %) had no statistical difference ($P < 0.05$). DMD values ranged from 53.33 to 72.33% with the highest value (73.33%) observed in 50g/kg ginger supplemented diet with the least value (53.33%) recorded for 200g/kg ginger supplemented diet shown in Table 4.

Gas production is an indication of microbial degradability of samples (Babayemi *et al.*, 2004, Fievez *et al.*, 2005). All parameters observed in this study indicated no significant effect on the nutritive value of diets supplemented with or without ginger powder; however, the values obtained for the non-supplemented diets are lower for all the parameters measured except for the dry matter digestibility. Meanwhile, the total gas volume (TGV) of non – supplemented diet was lowest and the highest value was recorded in the 150g/kg supplemented diet. In most cases, feedstuff that showed high capacity for gas production were also observed to be synonymous for high methane production. Methane is a dietary energy loss and is an important greenhouse gas contributing to global warming (Johnson and Johnson, 1995) by trapping outgoing terrestrial impaired radiation 20 tons more effectively than CO_2. The IPCC (2001) reported domestic livestock as one of the largest single sources of methane with 80 to 115 million tonnes per year equivalent to 0.15-0.20 of total anthropogenic methane. Therefore, reduction of methane production leads to greater efficiency in feed utilisation. Depending on the level of feed, composition of the diet and digestibility, 2.15% of the gross energy in the feed is lost through methane production (Johnson et al., 1991; Holter and Young, 1992). The intercept value (a) for all the treatments including the unfortified diet ranged from 2.00 to 6.67 at 24 h. The extent of gas production 'b' values were similar across the treatments except non –. supplemented diet which was significantly ($P < 0.05$) different from other diets such that, it was the lowest among the diets. Potential gas production (a+ b) was significantly ($P < 0.05$) different with the 100g/Kg diet being significantly higher than other diets but the non –supplemented and 50g/Kg diets were significantly similar ass well as the 150 and 200g/Kg supplemented diet.

Table III. *In vitro* gas production characteristics of concentrate fortified with ginger root meal

	0g/kg	50g/kg	100g/kg	150g/kg	200g/kg	SEM	P-Value
A	4.00^{a}	4.67^{a}	6.67^{bc}	2.00^{c}	4.67^{a}	0.30	0.01
B	11.33^{b}	12.67^{ab}	18.00^{ab}	22.67^{ab}	12.67^{ab}	1. 89	0.15
C	0.11^{ab}	0.08^{ab}	0.12^{a}	0.06^{b}	0.06^{b}	0.01	0.07
a+b	15.33	17.33	20.67	24.67	17.33	1.730	0.27
T	12.00	9.00	9.00	9.00	6.00	1.34	0.53
Y	11.33^{ab}	11.33^{ab}	14.00^{a}	8.67^{b}	9.33^{b}	0.69	0.07

abc= Means on the same column with similar superscript are not significantly (P > 0.05) different.

å = intercept (gas produced from the soluble fraction), b = Potential gas production (ml/g DM) from the insoluble fraction, c = gas production rate constant (h^{-1}) for the insoluble fraction (b), t = incubation time, y = volume of gas produced at time't'.

Table IV: In vitro fermentation parameters of the test diets

	0g/kg	50g/kg	100g/kg	150g/kg	200g/kg	SEM	P-Value
CH_4 (%)	8.00	10.00	8.67	9.33	8.67	0.58	
SCFA(µml)	0.43	0.47	0.55	0.65	0.47	0.04	0.27
ME(MJ/Kg DM)	5.20	5.60	6.00	6.42	5.61	0.23	0.32
OMD (%)	39.19	42.31	44.65	46.08	41.79	1.53	0.44
TGV(ml)	15.33	17.33	20.67	24.67	17.33	1.73	
DMD (%)	72.33	73.33	66.33	62.00	53.33		

[a,b c] Means along the same row with different superscripts are significantly ($P < 0.05$) different

TVG = Total Volume Gas, CH_4 = Methane, ME = Metabolisable Energy, OMD = Ogamic Matter Digestibility, SCFA = Short Fatty Acid, DMD = Dry Matter Degradability

There was no significant ($P > 0.05$) difference in gas production rate ('c') but significant difference was observed in the volume of gas produced at time 't' 'y' of the incubated samples. Also, incubation time ('t') had no significant differences. The rate of gas production 'c' had no significant difference. Time of most rapid increase in gas produced 't' ranged from between 6.00 to 12.00 h. The potential gas production from the insoluble fraction, extent and rate of gas production, volume produced and time of production at 24 h incubation period are presented in Table 3. The *in vitro* gas production characteristics of the substrate in the liquors from the animals showed that there were significant differences in the 'a' and 'b' but non-significant with 'a + b' values.

Getachew *et al.*, (2002) stated that it is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate). Also, Menke and Steingass (1988) reported that fermentable carbohydrate , increase gas production while degradable nitrogen compound decrease gas production to some extent due to binding of carbohydrate with ammonia.

CONCLUSION

The result showed that the addition of ginger rhizome increased the dry matter, enhanced metabolisable energy, organic matter digestibility and short chain fatty acids. The total gas was high with increasing level of ginger but methane production was substantially reduced with increasing addition of ginger. Thus, the ginger inclusion in the diets of ruminants may be beneficial.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest concerning the submission of this manuscript for publication.

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