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ORIGINAL RESEARCH ARTICLE

Effects of Herbal Mix Supplementation on In Vitro Gas Production Fermentation Parameters and Methane Mitigation Potential

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ABSTRACT

The study was conducted to evaluate the effects of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) mix supplementation on chemical composition, anti-nutritional factors, *in vitro* total gas characteristics and methane reduction potential. Six goats fed a mixed diet of *Pannicum maximum* (60% DM) and standard concentrates (40% DM) for 14 days were used as sources of inoculum. Treatments were T1 (Concentrate diet), T2 (concentrate diet + 1g/kg ginger), T3 (concentrate diet + 1g/kg turmeric powder), T4 (concentrate diet + 0.5g/kg ginger powder + 0.5g/kg turmeric powder). There were significant differences ($P < 0.05$) in all the nutrients evaluated except crude protein. Non-fibre carbohydrate (NFC) was highest (19.56% DM) in T3 (Concentrate diet + 1g/kg turmeric powder) and lowest in T1 (concentrate diet). Condensed tannins (CT) and saponins were higher in T4 (4.68 and 2.32 % DM) respectively, while the lowest CTs and saponnins were obtained for T1. The total gas production (GP24) steadily increased and was most pronounced (P<.0.05) in T3 (27.00 mL/200mg DM). Methane reduction potential (18%), organic matter digestibility (52.11%) and microbial protein supply (10.06%) were also highest (P<.0.05) in T3 relative to other diets. The findings revealed either ginger or turmeric and their combination in total meal ration supplementation maintained CT and saponins within acceptable limits, improved degradability (%DM) and methane reduction potential, qualifying them as suitable phytogenic feed additives for ruminants.

Keywords: Herbal plants, chemical composition, in vitro gas degradability, methane

INTRODUCTION

In the recent decade, an evolving trend in the livestock industry has been the restriction of the use of antibiotics and other synthetic feed additives. In ruminant nutrition, the use of antibiotic as a supplement has been widely applied to alter rumen fermentation and methane emission for better animal performance. However, the indiscriminate use of antibiotics is an increasing concern (Gunun *et al*., 2016), due to drug resistance, as well as residual effects of antibiotics in ruminant product (milk or meat) which could threaten human health (Khattab *et al*., 2020). This radical change resulted in an intensive development of research relating to finding effective natural compounds that could inhibit greenhouse gases (GHG) and modulate the rumen fermentation (Matloup *et al*., 2017). Researchers and livestock farmers have thus sought for alternatives to synthetic growth promoters and antibiotics in livestock production for improved growth and reproductive performances of ruminant animals. An increased interest in the utilization of growth promoters of natural origin (herbs) has been observed, with some investigators confirming an impact on productive performance and health (Khattab *et al*., 2020). Phytobiotics are phytogenic feed additives or plant-derived products supplemented to animal feeds in order to increase production and enhance performance. They find their origin in tubers, roots, fruits or leaves of herbs, spices and other plants (Alam *et al*., 2013, Ali *et al*, 2016). Phytogenic feed additives are used in ruminant nutrition due to their effects on rumen microbial populations, by improving ruminal fermentation efficiency and mitigating methane emissions (Khiaosa-ard and Zebeli, 2013). Methane is a metabolite released due to the inability of the animal to benefit from H_2 and CO_2 production during fermentation. Methanogenic bacteria can utilize hydrogen and carbon dioxide to produce methane (Beauchemin *et al*, 2020). It has been estimated that dairy farms contribute more than 3% of the total greenhouse gasses (Knapp *et al*., 2014), which is considered a loss in feed energy by up to 12% in the form of emitted methane. Many herbs have been reported to contain essential oils and/or active compounds which modify rumen fermentation and positively affect methane emission, volatile fatty acids (VFA's), protein, carbohydrates degradation and reduce ruminal bio-hydrogenation (Khattab *et al*., 2020). The objective of the present study was thus to evaluate the effect of using different levels of ginger (*Zingiber officinale*) andturmeric (*Curcuma longa*) mix (natural feed additive) as a feed additive on *in vitro* ruminal fermentation parameters, methane concentration and methane reduction potential.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Small Ruminant Unit of the Teaching and Research Farm of the Federal College of Wildlife Management, New Bussa, Nigeria located in the Guinea Savannah vegetation zone (9° 53 N, 4° 31 E), with a humid tropical climate, a mean annual rainfall of about 1040 mm and mean temperature of 34°C.

Preparation of herbal mixture

Fresh rhizomes of ginger and turmeric were sourced from a local market in New Bussa, Niger State, Nigeria. The rhizomes were sorted, washed with potable water, followed by draining and spreading in a cool dry place on clean trays, and allowed to air-dry for about 24 hours. The air-dried rhizomes were chopped into smaller pieces and oven dried at 60ºC to a constant weight. The dried rhizomes were milled into fine powder using an electric blender (Panasonic, Malaysia) before its inclusion in the experimental diets and necessary analyses carried out.

Experimental diets

T1: Concentrate diet
T2: Concentrate diet + 1g/kg Ginger
T3: Concentrate diet + 1g/kg Turmeric powder
T4: Concentrate diet + 0.5g/kg Ginger +0.5g/kg Turmeric powder
The composition of basal concentrate diet is presented in table 1.

Chemical Analysis and calculation

Dry matter of diets tested was determined by drying at 105ºC for 48h (AOAC, 1990; method 930.15). Samples were analyzed for CP, ether extract, and ash according to AOAC (2000). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined using the (Van Soest, *et al*, 1994) procedure. Phytochemical screenings were carried out for all the extracts (saponin, and condensed tannins) as per the standard methods as described by (Solomon *et al*., 2013).

Source of rumen liquor, collection and processing

Six goats fed a mixed diet of *Pannicum maximum* (60% DM) and standard concentrates (40% DM) for 14 days were used as sources of inoculum. The animals had free access to water *ad libitum*. Rumen fluid was collected from the goats with the use of suction tube into thermos-flask pre-warmed to 39°C prior to morning feeding. The rumen liquor was flushed with CO_2 after straining with cheese cloth and taken to the laboratory for the preparation of incubation inoculums. Temperature and pH of the rumen liquor were determined immediately using a pH meter. Samples (200 mg) of the milled oven-dried dietary feed rations were accurately weighed and dispensed into 60 ml calibrated plastic syringes filled with 30 ml of medium consisting of cheese-cloth strained rumen liquor and buffer solution (pH 7) containing 292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)2SO_4$, 480 mg NaCl, 100 mg $MgSO_4.7H_2O$, 64 mg $CaCl_2.2H_2O$, 4 mg $Na2CO_3$ and 600 mg cysteine hydrochloride in 1litre of double distilled water (ddH_2O) and dispensed anaerobically in the 1:2 (v/v) ratio. The syringes were subsequently incubated at 39°C for 24 hours. The mixture was handled under continuous flushing with CO_2 and immediately dispensed using 60 ml plastic calibrated syringes. The syringes were tapped and pushed upward by the piston to eliminate air completely in the inoculum. The silicon tube in the syringe will then tightened by a metal clip so as to prevent escape of gas.

Table 1: Gross ingredient composition of experimental diet

	Herbal mix level of inclusion			
Ingredient	T1	T2	T3	T4
Maize offal	40.00	40.00	40.00	40.00
Wheat offal	25.00	25.00	25.00	25.00
Cowpea husk	20.00	20.00	20.00	20.00
Groundnut cake	10.00	10.00	10.00	10.00
Ginger	0.00	1.00	0.00	0.50
Turmeric	0.00	0.00	1.00	0.50
Bone meal	3.00	3.00	3.00	3.00
Salt	1.50	1.50	1.50	1.50
Premix	0.50	0.50	0.50	0.50
Total	100.00	101.00	101.00	101.00
Calculated analysis				
Crude protein	16.56	16.71	16.69	16.58
NDF	34.04	34.15	34.08	34.14
ADF	20.75	20.92	20.72	20.74
ME (MJ/kg)	3.35	3.36	3.35	3.34

T1, concentrate diet; T2, Concentrate diet + 1g/kg Ginger; T3, concentrate diet + 1g/kg turmeric powder; T4, concentrate diet + 0.5g/kg ginger +0.5g/kg turmeric powder.

Incubation was carried out at 39 ° C and the volume of gas produced measured at three-hour interval from 0 to 24 hours. Three blanks containing 30 ml of medium only were included in the run. The average of the volume of gas that was produced from the blanks was deducted from the volume of gas produced per samples. The methane gas produced from three replicates per treatment was also determined by dispensing 4 ml of 10M sodium hydroxide (NaOH) into the calibrated syringes after 24 hours of incubation using 5 ml plastic calibrated syringe. The metal clip was carefully unscrewed and NaOH was introduced to absorb CO_2 produced during fermentation and the remaining gas will be recorded as methane according to (Fievez *et al.*, 2005). The average methane gas produced from the blanks was deducted from total methane gas produced per sample to determine net methane production. Fermentation in the remaining three replicates per treatment was terminated after 24 hours by removing the calibrated plastic syringes from the incubator and placing them on ice. The post-incubation inoculum in these syringes was stored at -20 ° C until analyzed for ammonia, nitrogen and volatile fatty acid.

Determination of *in vitro* post incubation parameters

Metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFAs) and microbial protein were estimated according to Menke and Steingass (1988) and (Close and Menke, 1986) respectively.

Equation 1: ME (MJ/kg DM) = 2.20 + (0.136*Gv) + (0.057*CP) + (0.0029*EE);

Where = GV (Gas volume at 24 hr), EE (ether extract), CP (Crude protein)

Equation 2: OMD (%) = 14.88 + 0.889GV+ 0.45 CP + 0.651 ash (Menke and Steingass, 1988).

Equation 3: SCFA (µmol/g DM) = 0.0222 GP (at 24 hr) - 0.00425

Where, Gv, CP, EE and XA are corrected 24 h gas volume (ml/200 mg DM), crude protein, ether extract and ash (g/kg DM) of the incubated samples, respectively. Microbial protein (MP) were predicted as MP = 1.93×IVOMD /10

Methane Reduction Potential Determination

Methane concentration (MC) was determined according to Jayanegara *et al.* (2009): Methane concentration (MC %) = Net methane production/ Net gas production × 100. Methane production reduction potential (MRP) was calculated by taking the highest % net methane values as 100 %: MRP = %Net methane in control − %Net methane in the test / %Net methane × 100.

Statistical analysis

Data was statistically analyzed using GLM procedure of Statistical Analysis System (SAS, 2009), version 9.2. Significant differences between means of treatments were carried out by Duncan's Multiple Range Test, and the significance threshold was set at $p < 0.05$.

RESULTS AND DISCUSION

Chemical composition and fibre fraction

There were differences ($P < 0.05$) in the chemical composition of the dietary treatments except for crude protein and metabolizable energy (Table 2). Non-fibre carbohydrate (NFC) values were highest ($P < 0.05$) in T3 (19.86% DM) and lowest in T1 (8.28 % DM). The NFC contents of the browse species should be adequate to stimulate NH_3–N utilization in the rumen (Tylutki *et al*., 2008). There were variations ($P < 0.05$) in the NDF (55.39-56.34% DM) and ADF (25.39-36.34% DM) which were moderate for ruminants. Low to moderate fibre fractions can effectively improve dry matter degradability (Arigbede and Tarawali, 1997). The low to moderate fibre contents of any ruminant diet suggests their high nutritive value since fibre plays a significant role in voluntary intake and digestibility both *in vitro* and *in vivo* (Okunade *et al*., 2014).

Table 2: Chemical composition (% DM) of dietary treatments

	Herbal mix level of inclusion					
Ingredient	T1	T2	T3	T4	S.E.M	P- value
Dry matter	97.43^{a}	96.82^{b}	96.72^{b}	95.59^{c}	0.07	0.001
Organic matter	93.03^{a}	90.37^{b}	91.93^{a}	88.97^{c}	0.40	0.001
Crude protein	17.63	17.60	17.68	17.89	0.08	0.304
Non fibre carbohydrate	8.28^{c}	16.87^{b}	19.86^{a}	9.83^{c}	0.07	0.001
NDF	56.34^{a}	58.75^{c}	55.39^{d}	54.43^{b}	0.10	0.001
ADF	36.34^{a}	28.75^{c}	25.39^{d}	34.45^{b}	0.09	0.001
ME (MJ/kg)	5.54^{c}	6.00^{b}	6.65^{a}	6.05^{b}	0.85	0.001
Tannins	1.35^{d}	4.01^{b}	3.79^{c}	4.68^{a}	0.06	0.001
Saponins	2.30	2.11	2.07	2.32	0.15	0.156

Means in the same column with different superscripts are significantly different (P < 0.05)

T1, concentrate; T2, concentrate + 1g/kg ginger; T3, concentrate + 1g/kg turmeric; T4, concentrate + 0.5g/kg ginger +0.5g/kg turmeric.

The major phytochemical components (tannins and saponins) in the experimental diets were different ($P < 0.05$). Tannins content was lowest in T1 (1.35% DM) and highest (4.68% DM) for T4. The levels of CTs recorded in this study are much below the range of 6% DM to 10% DM, considered to depress feed intake and growth (Mbomi *et al*., 2011). Therefore, the experimental diet contained CTs at levels beneficial to ruminants because CTs at low level produce mild or low protein binding effect (Olafadehan, 2013). Saponin levels in all the samples were comparable to the tolerable level of 15-20 g/kg DM reported for goats (Onwuka, 1983), which suggests the levels reported herein are not likely to affect nutritional potentials of the browses to ruminants.

In vitro gas production study

In vitro total gas, methane (CH_4) and CO_2 of the different experimental diets are presented in Table 3. The final net gas volumes at 24 h after incubation and CH_4 production were significantly different ($P < 0.05$) among the diets. Ratio of methane/total gas volume (v:v) produced, which indicates the methanogenic property of the diets, was least ($P < 0.05$) for T3 (56.67 v:v). Many factors such as the nature and level of fibre, the presence of secondary metabolites and potency of the rumen liquor for incubation have been reported (Babayemi *et al*., 2004) to determine the amount of gas to be produced during fermentation. In the current study, it appears the secondary metabolites and NFC content more than fibre content influence *in vitro* gas production and hence degradability. Higher gas production and the extent of *in vitro* fermentation of T3 suggest that these substrates are of higher nutritional value than the other browse species, in agreement with (Isah *et al*., 2012). The low gas production of T1 could be attributed to its high amount of tannins and saponnins.

In vitro fermentation parameters

It has been reported that when ranking plants according to their methanogenic property, the proportion of methane-to-total gas ratios is more relevant than absolute methane formation: a low value for this proportion indicates a low methanogenic potential of the digestible part of the feed, i.e. fewer methane production per unit net gas volume production (Moss *et al*., 2000). In the light of this, T3 (18.00%) had the highest ($P<0.05$) MRP, (52.11%) OMD and (10.6%) estimated microbial protein supply (Table 4).

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Short chain fatty acids are major source of metabolic fuel while microbial protein serves as a major portion of protein absorbed from in gut in ruminants (Osafo *et al*., 2023). The relatively high *in vitro* OMD in T2 and T3 is an indication of the high nutritive value ginger and turmeric added to dietary T2 and T3 respectively when used in ruminant feeding. Lower fibre fractions in T2 and T3 relative to other diets may have resulted in the higher values for *in vitro* OMD and SCFA (Van Soest, 1994).

Table 3: In vitro gas production (ml/200 mg DM) of dietary treatments

Substrate	3hr	6hr	9hr	12hr	15hr	18hr	21hr	24hr	CO_2	CH_4
T1	3^{b}	4^{c}	9.66^{d}	13.33^{d}	15.00^{c}	19.66^{d}	22.66^{c}	23.66^{c}	9.32^{b}	14.38^{d}
T2	3^{b}	5^{b}	11.00^{b}	14.66^{c}	15.33^{b}	21.66^{b}	23.00^{b}	27.00^{a}	9.33^{b}	17.66^{a}
T3	4^{a}	6^{a}	11.66^{a}	15.66^{a}	16.33^{a}	23.00^{a}	25.00^{a}	27.00^{a}	11.66^{a}	15.33^{c}
T4	4^{a}	5^{b}	10.00^{c}	15.00^{b}	15.33^{b}	20.66^{c}	23.00^{b}	25.33^{b}	8.00^{c}	17.54^{b}
S.E.M	0.07	0.10	0.07	0.08	0.06	0.06	0.06	0.07	0..04	0.04
P. value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Means in the same column with different superscripts are significantly different ($P < 0.05$)

T1, concentrate; T2, concentrate + 1g/kg ginger; T3, concentrate + 1g/kg turmeric;
T4, concentrate + 0.5g/kg ginger +0.5g/kg turmeric

Table 4: *In vitro* gas fermentation parameters of dietary treatments

Dietary treatment	CH_4/Total gas Volume	MRP	ME	OMD	SCFA	Microbial protein
T1	0.61	12.70^b	6.44^c	46.69^b	0.52	9.01c
T2	0.65	5.56^c	6.93^a	51.52^a	0.59	9.94^a
T3	0.56	18.00^a	7.00^a	52.11^a	0.58	10.06^a
T4	0.69	0.00^d	6.66^b	48.12^b	0.55	9.30^b
S.E.M	0.08	0.08	0.09	0.76	0.01	0.13
P-value	0.154	0.001	0.001	0.001	0.831	0.001

Means in the same column with different superscripts are significantly different ($P < 0.05$)
CH_4, ml/200g DM; MRP, methane reduction potential; ME, metabolizable energy MJ/kg DM; OM, organic matter (%); SCFA, short chain fatty acids (µmol); MP, microbial protein (%)
T1, concentrate diet; T2, Concentrate diet + 1g/kg Ginger; T3, concentrate diet + 1g/kg turmeric powder; T4, concentrate diet + 0.5g/kg ginger +0.5g/kg turmeric powder

CONCLUSION AND RECOMMENDATION

The generally moderate *in vitro* degradability and estimated dry matter digestibility suggest their nutritive potential as alternative low cost sources of feed additives for ruminant feeding. Herbal supplementation generated moderate changes in diets *in vitro* degradability, gas production, methane reduction potential, *in vitro* organic matter digestibility, short chain fatty acids and VFAs production. However, it is noteworthy that the concentrate supplemented with ginger alone or combination of ginger and turmeric had lower CH_4 reduction potential and higher degradability than the rest of the diets. Supplementation of ginger or turmeric or their combination is recommended for future evaluations as a possible option in mitigating methane emissions and increasing the nutritional quality of animal diets. The CH_4 production and fermentation parameters depend on many factors, some of which were not evaluated in this study, such as type of carbohydrates and ruminal microorganisms; therefore, it is suggested to extend the analysis to these variables. In vitro gas technique is a valuable tool for evaluating the kinetic fermentation parameters and enteric methane reduction potential of ruminant feeds**,** further research should, however, be conducted to establish their phytobiotic property in *in vivo* trials**.**

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest

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