

EFFECT OF CINNAMON (*Cinamonum cassia*) POWDER ON *IN VITRO* METHANE PRODUCTION AND FERMENTATION OF MAIZE STOVER-BASED SUBSTRATE

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ABSTRACT

Methane (CH₄) production and fermentation of a maize stover-based substrate in response to inclusions of cinnamon powder (CNP) were evaluated using the *in vitro* gas technique. Substrate (200 mg) consisting of maize stover and concentrate in ratio 6: 4 was incorporated with CNP at levels of 0, 15 and 30 mg/g dry matter (DM) to make three different treatments. These were incubated *in vitro* in 100 ml glass syringes at 39°C for 48 h with total gas and CH₄ production measured. Dry matter degradability (DMD), partitioning factor, microbial biomass and volatile fatty acids were also determined. Results showed that CNP reduced ($P < 0.05$) total gas volume, CH₄ production and DMDs with the least reduction at 30 mg/g DM inclusion. The partitioning factor increased ($P < 0.05$) with inclusion of CNP at both levels of inclusion. It was concluded that, inclusion of cinnamon powder at 15 and 30 mg/g DM has potentials to reduce CH₄ production in maize-stover based diet. The higher fermentation efficiency implied that cinnamon powder reduced the amount of gas produced per gram of substrate degraded which implied better rumen fermentation efficiency.

Keywords: Maize stover, *in vitro*, fermentation, methane, cinnamon.

INTRODUCTION

Maize stover is one of the abundant crop residues for ruminant feeding, particularly during the dry seasons (Methu *et al.*, 2001; Tang *et al.*, 2011). Limitations to feeding these fibrous carbohydrates to ruminants include low digestibility and CH₄-shifted digestion (Gemedu and Hassan, 2015). Ruminants fed crop residues produce more CH₄ than those fed high quality forages (Berhanu *et al.*, 2019). Rumen CH₄ aside from posing a threat to the environment via increase in greenhouse gas emissions, also reduces feed energy utilization by ruminants. It accounts for up to 12% loss of dietary gross energy intake (Chaudhry and Khan, 2012) or up to 15% depending on the diet (Patra *et al.*, 2010). With the value of crop residues as feed resource for ruminants, it is beneficial to optimize their utilization by reducing CH₄ emission attributed to their digestion within the rumen. Spices are recognized as potential agents to reduce enteric CH₄ emission due to the presence of plant secondary metabolites which exert antimicrobial activities (Patra *et al.*, 2006; Khan and Chaudhry, 2010; Chaudhry and Khan, 2012). According to Khan and Chaudhry (2010), they can be used to enhance the utilization of low quality forages and reduce nutrient wastages by ruminants. This study therefore, evaluated the effect of cinnamon powder on CH₄ production and rumen fermentation of maize stover-based diets for ruminants.

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°13'23.8" N and Longitude 3°26'25.4" E (Google map, 2018).

Preparation of cinnamon powder

Cinnamon sticks were sourced from a local market within Abeokuta and was ground to pass through 1 mm sieve. Ground samples were stored at room temperature in air-tight glass container for subsequent use for chemical analysis and for *in vitro* study.

Experimental diets used as substrate

The substrate degraded consisted of 60% Maize stover and 40% concentrate (DM basis). Cinnamon powder was included in the substrate as feed additive at varying levels of 0, 15 and 30 mg/g DM to make three different experimental treatments. The maize stover used was collected from a maize field after the harvesting of maize grains. Samples (n=3) of the maize stover were chopped, weighed and

oven-dried at 60°C until constant weight for DM determination and subsequently milled through 1 mm sieve. The compounded concentrate consisted of 44% wheat offal, 38% rice bran, 15% brewers' dried grain, 2% bone meal and 1% salt. Concentrate samples (n=3) of known weight were similarly oven-dried for DM determination.

***In vitro* total gas and CH₄ production measurement**

The *in vitro* total gas production was measured using the *in vitro* gas technique (Menke and Steingass, 1988). Approximately 200 mg of substrate was weighed into 100 ml glass syringes using ten syringes per treatment. Each of the syringes were filled with 30 ml inoculum. The inoculum was a mixture of a medium solution and rumen liquor at ratio 2: 1. The tip of the syringes were fitted and sealed with a rubber tube to prevent escape of gas. The syringes were incubated at 39°C for 48 h with gas readings recorded at three hours interval from 0 to 48 h. The incubation procedure was handled under continuous anaerobic conditions. Four blanks were included in the run to correct for gas production not arising from feed degradation. Methane was determined (n=4 per treatment) at 48 h incubation according to Fievez *et al.* (2005).

Determination of dry matter degraded and fermentation parameters

At 48 h incubation, contents of each syringe (n=6 per treatment) were filtered through Whatman filter paper (grade 1, 11µm pore size). The supernatants were collected into tubes and used for total volatile fatty acids determination following the titration method of Barnett and Reid (1957). The filtered residues were oven-dried at 105°C overnight to determine DM degraded. The DM degradability (mg/g DM) was then calculated as difference between dry matter content of substrate incubated and that of its residue. Partitioning factor and microbial biomass production were estimated according to Blummel *et al.* (1997).

Chemical analysis

Dried samples of maize stover, concentrate, and CNP were analyzed for proximate and fibre composition following the procedure of AOAC (2000) and Van Soest *et al.* (1991), respectively. Metabolizable energy was estimated according to De Boever *et al.* (1997). Samples of cinnamon powder were further analyzed for phytochemical constituents such as tannin, saponin, flavonoids, oxalate, alkaloids and total phenol following standard procedures.

Experimental design and statistical analysis

The experiment was laid out as a completely randomized design. All data were subjected to one-way analysis of variance (SAS, 2002). Significant differences were accepted at $P \leq 0.05$. Significant means were separated using Duncan multiple range test (SAS, 2002).

RESULTS AND DISCUSSION

Chemical composition of diets used as substrate is presented in Table 1. Maize stover had a high neutral detergent fibre, acid detergent fibre and low crude protein which is typical of maize stover as previously reported (Fon *et al.*, 2012). The concentrate with a higher crude protein content served as an additional source of protein. Phytochemicals in CNP is shown in Table 2. Cinnamon powder contained a high flavonoids content followed by saponin and tannin. These phytochemicals are plant secondary metabolites (PSM) described as naturally occurring biochemical compounds in plants and considered to serve as defense mechanism for plants survival (Bodas *et al.*, 2012).

Table 1. Chemical composition (%) of experimental maize stover and concentrate

Parameters	Maize stover	Concentrate
Dry matter ¹	38.12	95.43
Crude protein	5.27	15.04
Organic matter	92.20	93.64
Neutral detergent fibre	68.75	51.32
Acid detergent fibre	37.28	23.70
Acid detergent lignin	11.53	8.86
Metabolizable energy (MJ/kg DM) ²	12.32	12.68

¹On as-received basis; ²Estimated according to De Boever *et al.* (1997)

Table 2. Phytochemical composition (g/kg) of cinnamon powder

Parameters	Concentration
Tannin	56.36
Flavonoids	72.08
Total phenolic acid	44.10
Saponin	60.21
Oxalate	1.45
Alkaloids	32.32

Total gas volume, CH₄ production and DM degradability reduced ($P < 0.05$) with CNP inclusion in maize stover-based substrate at both 15 and 30 mg/g DM inclusion levels while total volatile fatty acids and microbial biomass were unaltered ($P > 0.05$) compared to the control (Table 3). The reduction effect suggested inhibition of microbial activity to ferment substrate since gas production in the rumen is a direct product of substrate fermentation by rumen microbes. Similar reduction effect of cinnamon on CH₄ and DM degradability from wheat-based substrate was reported by Chaudhry and Khan (2010). The observed reduction effect of CNP was attributed to the antimicrobial properties of phytochemicals in CNP. Condensed tannins have been reported to reduce methanogenesis through direct toxic effect on methanogenic archaea (Bodas *et al.*, 2012). Saponin reduction effect on methanogenesis was linked to inhibition of protozoa activity (Bodas *et al.*, 2012). Antimicrobial effect of flavonoids on dry matter degradation and methane production *in vitro* has similarly been reported (Oskoueian, 2013). A higher ($P < 0.05$) partitioning factor was observed with increasing levels of cinnamon powder. Partitioning factor is a measure of fermentation efficiency (Jimenez-Peralta *et al.*, 2011). This implied that less gas was produced per unit of substrate degraded suggesting better utilization of organic matter in substrate.

Table 3. *In vitro* methane production and fermentation of maize stover-based substrate containing varying levels of cinnamon powder

Parameters	Levels of CNP in substrate degraded			SEM	P-value
	0	15	30		
<i>In vitro</i> dry matter degraded, mg/g	345.0 ^a	249.2 ^b	236.7 ^b	18.30	0.019
<i>In vitro</i> total gas production, ml/g DM	162.5 ^a	136.7 ^b	124.2 ^b	5.52	0.006
CH ₄ production, ml/g DM	57.5 ^a	29.6 ^b	28.1 ^b	3.63	<0.001
Partitioning factor	3.3 ^b	4.1 ^a	4.5 ^a	0.16	0.034
Microbial biomass production (mg/g DM)	218	184	175	13.42	0.399
Total volatile fatty acids (mM/100 ml)	12.19	11.41	10.95	0.37	0.394

^{a,b}Means along the same row with different superscripts are significantly different ($P < 0.05$); CNP: cinnamon powder

CONCLUSION AND RECOMMENDATION

From this study, 15 and 30 mg of cinnamon powder per gram of maize stover-based substrate reduced CH₄ production and dry matter degradation similarly, with no change in total volatile fatty acids and microbial biomass *in vitro*. Inclusion level of 15 mg/g DM can therefore be recommended for mitigating methane production in ruminant diets based on maize stover. However, studies *in vivo* are required to confirm the effects of these reductions on animal performance.

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