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ORIGINAL ARTICLE

Effect of cashew nut shell liquid at varying inclusion levels on rumen fermentation and methane production *in vitro*

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Abstract Essential oils are possible natural antibiotic alternatives for manipulating ruminal fermentation in order to improve the utilization of nutrient in ruminants. This study evaluated the effect of cashew nut shell liquid (CNSL) at varying inclusion levels on *in vitro* gas production, nutrient degradation, and post-incubation parameters. Experimental diets consisted of *Panicum maximum* (Ntchisi) grass as basal diet and supplemental pellets treated with 0, 5, 10 and 15 ml/kg of cashew nut shell liquid. Proximate composition of experimental diets was analysed. *In vitro* experiment was carried out on experimental diets (P. maximum and concentrate pellets at ratio 70:30) for 24 hours using goat rumen liquor as inoculum. Results showed that CNSL inclusion had a significant effect ($P < 0.05$) on the ether extract and metabolizable energy of experimental pellets. Dietary treatment with 15 ml/kg of CNSL had the lowest ($P < 0.05$) *in vitro* gas production. Inclusion of CNSL significantly inhibited methane production at 5 ml/kg (18%), 10 ml/kg (34%) and 15 ml/kg (57%) CNSL inclusion levels ($P < 0.05$). Ammonia-N and TVFA decreased significantly with CNSL inclusion. Significant reduction ($P < 0.05$) in short-chain fatty acid, metabolizable energy, and organic matter degradability were recorded with increasing CNSL inclusion levels.

Keywords: dietary, eco-friendly feed additive, essential oils, methane inhibition, ruminants

Introduction

Fermentation of feed in the rumen often results to substantial nutritional loss in form of methane and ammonia. The reduction in nutritional component consequently results to wasteful utilization of feed nutrients by animals and can also lead to increased feed costs (Holmes et al 2006; Gebrehiwot 2014). Improving nutritional utilization through the inhibition of methane could result in improved nutrient utilization and on the other hand, limit environmental pollutions from enteric sources (Santra and Karim 2003).

Ruminants contribute to environmental pollution due to the production of greenhouse gases (CO_2, CH_4 and N_2O) which is the cause for climate change. The global contribution to GHG emissions by the animal sector are 9% for cabondioxide (CO_2), 35–40% for methane (CH_4) and 65% for nitrous oxide (N_2O) (Ooninc x et al, 2010). Ammonia from manure and urine is transformed via nitrification and denitrification into N_2O (Oonincx et al, 2010). Nitrous oxide (N_2O) is a greenhouse gas that is capable of undergoing stratospheric reaction with atomic oxygen to form nitric oxide (NO) which in turn destroys stratospheric ozone (Wrage et al., 2001). The process of producing N_2O from ammonia has also been identified to lead to soil nitrogen losses (Hong et al, 2017).

Therefore, the manipulation of rumen fermentation can be considered as a nutrient optimization process (Santra and Karim 2003) and mitigation strategy for GHG emissions. To improve efficiency of nutrient utilization in ruminants and mitigate methane production, antibiotics have been progressively embraced over the years (McDougall et al 2004; Gallardo et al 2005). However, there is rise in scientific and public concern about the use of antibiotics as feed additives in animal production. This concern is driven by the occurrence of antibiotic resistance in various human pathogenic bacteria (Manero et al 2006; Parveen et al 2006) die and increased risk of growth-promoting antibiotic residues in foods of animal products (Yang and Carlson 2004). In order to combat such

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residues, there has been a great need amid animal producers and consumers for alternative feed additives which are naturally safe in human food supply chain (Jouany and Morgavi 2007). Essential oils are possibly promising natural replacements to antibiotics and ionophores for manipulating ruminal fermentation. This is due to their capacity in modifying cell permeability in microbes and toxicity to some strains of rumen microorganism (McIntosh et al 2003; Calsamiglia et al 2007). Cashew nut shell liquid is a typical essential oil that is known for its antimicrobial activities (Kozubek et al 2001). However, limited studies have been conducted to determine the effect of dietary inclusion levels of cashew nut shell liquid in ruminant diet. This study therefore, aim at evaluating the effect of cashew nut shell liquid (CNSL) on *in vitro* gas production, nutrient degradation and post incubation parameters.

Materials and Methods

Experimental diets and procedure

A complete concentrate supplement was compounded (Table 1) to contain approximately 14% crude protein, which is adequate for growing goats (NRC 1981). Cashew nut shell liquid was added to the concentrate at varying levels of 0, 5, 10, and 15 ml/kg of complete diet, which made up to four dietary treatments. The concentrate was processed into pellets using cassava flour in the composition as binder, according to the different levels of CSNL inclusion.

Chemical composition

Proximate composition of experimental diets samples was determined according to the procedure of AOAC (1990). Crude protein was obtained by total N determination using the micro Kjeldahl technique (nitrogen content (N) × 6.25). The fibre fractions of experimental diets and faecal samples were determined according to Van Soest et al (1991). Cellulose and hemicellulose were calculated as differences between ADF and ADL and NDF and ADF respectively.

$$\text{Hemicellulose} = \text{NDF} - \text{ADF}$$

$$\text{Cellulose} = \text{ADF} - \text{ADL}$$

Organic matter content was calculated as 100 – ash content in feed. Non-structural carbohydrate (NSC) was calculated as (100 – (CP + EE + Ash + NDF) (AOAC, 2000). Metabolizable energy content was estimated as 37×% crude protein+81×% ether extract+35.5×% nitrogen-free extract (Pauzenga 1985).

Table 1 Gross composition of experimental concentrates diets.

Ingredients	Composition (g/kg)			
Wheat offal	533.0	533.0	533.0	533.0
Brewers dried grain	100.0	100.0	100.0	100.0
Groundnut cake	170.0	170.0	170.0	170.0
Cassava flour	167.0	167.0	167.0	167.0
Bone meal	20.0	20.0	20.0	20.0
Salt	10.0	10.0	10.0	10.0
CSNL levels	-	+	++	+++
TOTAL (kg)	1000	1000	1000	1000

CNSL: Cashew nut shell liquid; - 0% inclusion level of cashew nut shell liquid; + 5% inclusion level of cashew nut shell liquid; ++ 10% inclusion level of cashew nut shell liquid; +++ 15% inclusion level of cashew nut shell liquid.

In vitro gas production of experimental diets

Rumen fluid used for the incubation was collected from three West African dwarf goats prior to morning feeding using suction tube as described by Babayemi and Bamikole (2006). The goats were previously fed with 40% concentrate (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soyabean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% *Panicum maximum* grass. Oven-dried and milled samples of the *Panicum maximum* leaves and concentrate pellet according to the different treatments was incubated at the ratio 70:30 respectively with the rumen fluid in 100 mls calibrated transparent syringes fitted with silicon tube following the procedure of Menke and Steingass (1988). Incubation was carried out at 39°C in an incubator and the volume of gas produced was measured at 24 hours. The methane gas produced from each sample was determined according to the procedures of Fievez et al (2005).

The post incubation parameters were determined by calculation as follows:

Metabolizable energy (ME, MJ/kg DM) = 2.20 + 0.136GV + 0.057CP + 0.0029CF (Menke and Steingass, 1988).

Organic Matter Digestibility (OMD, %) = 14.88 + 0.889GV+ 0.45CP + 0.651XA (Menke and Steingass, 1988).

Short chain fatty acids (SCFA, µmol/g DM) = 0.0239GV-0.0601(Getachew et al. 2001)

where GV, CP, CF and XA are total gas volume at 24 h, crude protein, crude fibre and ash values, respectively.

After 24 hours incubation period, the residues was decanted into pre-weighed crucibles and then oven-dried at 105^{o}C for 24 hrs. The dry residue was weighed and degradability calculated using the equation below:

$$\text{IVDMD (\%)} = \frac{\text{Initial dry matter incubated} - \text{dry matter of residues x 100}}{\text{Initial dry matter incubated}}$$

For determination of crude protein and organic matter degradability, samples of feed incubated and the residues was collected and analysed for crude protein (CP) and organic matter (OM) content. Crude protein degradability (CPD %) and organic matter degradability (OMD %) was calculated thus:

$$\text{CPD (\%)} = \frac{\text{CP content of incubated sample} - \text{CP content of residues}}{\text{CP content of incubated sample}} \text{ x 100}$$

$$\text{OMD (\%)} = \frac{\text{OM content of incubated sample} - \text{OM content of residues}}{\text{OM content of incubated sample}} \text{ x 100}$$

Statistical analysis

Data generated from *in vitro* study was subjected to analysis of variance (ANOVA) in a completely randomized design according to the procedure of SAS (2010). Significant means were separated using Duncan multiple range test ($P < 0.05$).

Results and Discussion

Table 2 shows the proximate composition of *Panicum maximum* (Ntchisi) fed as basal diets to West African Dwarf (WAD) goats. The *Panicum maxima* (Ntchisi) used in this study contained lower proportions of dry matter and this was similar to the values reported by Asaolu et al. (2014). Similarly, Aminah and Chen (1991) reported that most of the tropical pastures have crude protein contents ranging from 7 to 12% for grasses. The proximate analysis of supplemental diet is represented in table 3. The pelletised diets containing varying levels of CNSL fed in this study had a relatively high dry matter; this may be attributed to the dry feedstuffs used in compounding the feed and the effect of the pelleting process. Cashew nut shell liquid inclusion into pelletised diets resulted in ether extract increase and this may be due to the increase in fat content of diet as inclusion of oil increased. Metabolizable energy increased across the table indicating increase in the calorie content of supplemental pellets as CNSL increases, this agrees with the report of Geelen et al. (2001).

The effect of CNSL inclusion levels on gas production and fermentation parameters are represented in table 4. Addition of CNSL decreased gas production of supplemental diets from 5ml/kg to 15ml/kg inclusion levels ($P < 0.05$). The decrease in cumulative gas production recorded in this study is in line with the report of Nezhad et al (2011) who observed decrease in gas production of *in vitro* analysis of experimental diet treated with varying levels of spearmint essential oil. Inclusion of CNSL significantly inhibited methane production from 5ml/kg (18%), 10ml/kg (34%) and 15ml/kg (57%) CNSL inclusion levels ($P < 0.05$). This shows a reduction in loss of energy via methane production in ruminants.

Table 2 Nutrient composition (%DM) of Panicum maximum fed as basal diet.

Parameters	Composition	SEM
Dry matter (% as fed)	40.70	1.59
Crude protein	9.00	0.38
Ether extract	3.25	1.01
Ash	7.12	0.11
Non fibre carbohydrate	32.06	0.19
Organic matter	92.88	0.58
Neutral Detergent Fibre	48.56	2.04
Acid Detergent Fibre	30.92	2.32
Acid Detergent Lignin	10.05	0.53
Hemicellulose	17.64	0.52
Cellulose	20.87	1.85

NB: Average of four replicates.

Similar results have been observed in several studies. Wang et al (2009) reported in his study that inclusion of 0.25 g/day of essential oil mixture from oregano plants in the diet of sheep for 15 days decreased methane production. Evans and Martin (2000) observed that thymol (0.4 g L^{-1}), a main component of essential oils derived from Thymus and Origanum plants, was a strong inhibitor of methane in *in vitro* study**.** This reduction indicates that CNSL has the potential to manipulate ruminal fermentation process favorably and maximize nutrient utilization. This study proves that CNSL inclusion in ruminant feed will reduce the contribution of methane to the global GHG emissions, as it had been reported that enteric methane is the most significant GHG gas emitted (50% to 60%) in ruminant production systems (Ogino et al 2007).

Ammonia N production was decreased by CNSL with significantly lower values ($P < 0.05$) at 10mls CNSL inclusion level. This result is in accordance with the study of Castillejos et al (2006) which found a significant reduction in ammonia-N concentrations of rumen cultures incubated with thymol at 500-1000 mg/L. The reduction of ammonia in this study shows that the inclusion of CNSL in ruminant diet promises to make ruminant production more environmental-friendly as the nitrification and acidification of soils reduces and also the production of N_20.

Total volatile fatty acid increased significantly with the inclusion of CNSL compared to 0ml inclusion level. However, TVFA decreased in a CNSL inclusion level-dependent manner with the least value at 15mls when compare to 5ml/kg and 10 ml/kg CNSL inclusion level. This result supports the study of Busquet et al (2006) who reported a decrease in VFA concentration when anise oil (ANO) was added to rumen batch cultures at 3000 mg/L but no effects on VFA concentration when ANO was added at up to 300 mg/L. Higher doses of cinnamon oil and cinnamaldehyde also decreased total VFA and ammonia-N concentrations (Busquet et al 2006). This result indicates that CNSL is able to modify VFA production positively, however when added at higher doses, it may result in lower efficiency of nutrient utilization in the rumen.

Table 3 Chemical composition of supplemental diets with varying levels of cashew nut shell liquid.

Parameters (%)	Supplemental pellets with varying levels of CNSL				SEM
	0 ml	5 ml	10ml	15ml	
Dry matter	95.80	96.52	96.70	96.34	0.40
Crude protein	14.02	14.11	14.00	14.21	0.22
Crude fibre	10.64	10.70	10.78	10.66	0.18
Ether extract	8.38^{b}	10.62^{a}	11.15^{a}	11.52^{a}	0.42
Ash	10.70	10.55	10.60	10.50	0.15
Neutral detergent fibre	47.10	46.88	46.53	46.40	0.18
Acid detergent fibre	20.30	19.82	19.73	19.06	0.24
Acid detergent lignin	9.10	9.24	9.13	9.28	0.15
Organic matter	89.30	89.45	89.40	89.50	0.15
NSC	19.80^{a}	17.84^{b}	17.72^{b}	17.36^{b}	0.35
ME kcal/kg	3194.75^{b}	3296.69^{a}	3319.22^{a}	3344.30^{a}	19.19

NSC:Non-structural carbohydrate. ^{abc}Means on the same row having different superscripts are significantly different ($P < 0.05$).

Tabel 4 Effect of various levels of CNSL inclusion on in vitro gas production (ml/g DM) and fermentation parameters.

Fermentation parameter	Varying levels of CNSL				SEM
	0ml	5mls	10mls	15mls	
Gas production	54.50^{a}	51.50^{ab}	51.50^{ab}	47.50^{b}	0.88
CH_4	11.13^{a}	9.00^{ab}	7.25^{bc}	4.75^{C}	0.32
NH_3-N(mg/dL)	115.67^{a}	103.77^{b}	96.96^{d}	100.36^{C}	0.56
TVFA	9.55^{d}	15.50^{a}	13.50^{b}	11.50^{C}	0.40

^{abc} Means on the same row having different superscripts are significantly different ($P < 0.05$).
0ml - control diets, 5ml – contains 5ml of CNSL/kg DM pelletised diet, 10ml – contains 10ml of CNSL/kg DM pelletised diet, 15ml – contains 15ml of CNSL/kg DM pelletised diet

In the present study, short chain fatty acids recorded the lowest value when CNSL was included at 15 ml/kg in experimental diet. This agrees with the studies of Shinkai et al (2012), that feeding CNSL caused a decrease in acetate, methane emission and total short-chain fatty acid levels. *In vitro* dry matter degradability, crude protein degradability and organic matter degradability were affected significantly ($P < 0.05$) by CNSL inclusion. The decrease observed in this study is in line with the study of Sirohi et al (2012). Sirohi et al (2012) reported that the dry matter degradability of high fibre diet incubated with garlic oil decreased by 25.92%. The authors also reported that Eucalyptus oil decreased *in vitro* dry matter degradability by 27.13% and 23.28% in the medium fibre diet and low fibre diet respectively.

Table 5 Effect of varying levels of CNSL inclusion on *in vitro* post incubation parameter and nutrient degradability.

Post incubation parameters	Varying levels of CNSL				SEM
	0ml	5ml	10ml	15ml	
SCFA (µmol/g)	0.61^a	0.50^b	0.49^b	0.43^b	0.01
ME (MJ/kg)	6.66^a	6.00^b	5.93^b	5.53^c	0.09
Organic Matter digestibility (%)	45.49^a	41.10^b	40.54^b	37.92^c	0.57
In vitro dry matter degradability (%)	62.12^a	57.70^b	56.07^b	54.28^b	0.70
In vitro crude protein degradability (%)	64.67^a	60.61^b	59.89^b	58.80^b	0.71
In vitro organic matter degradability (%)	66.55^a	62.08^b	60.98^b	59.44^b	0.63

^{abc} Means on the same row having different superscripts are significantly different ($P < 0.05$)
0ml - control diets, 5ml – contains 5ml of CNSL/kg DM pelletised diet, 10ml – contains 10ml of CNSL/kg DM pelletised diet, 15ml – contains 15ml of CNSL/kg DM pelletised diet. SCFA– Short chain fatty acid. ME–Metabolizable energy.

Conclusions

The finding from this study can be concluded that cashew nut shell liquid inclusion at varying levels had a significant effect on *in vitro* gas, methane gas, ammonia-N and TVFA production. Although the addition of CNSL increased TVFA formation, it however decreased TVFA formation at higher inclusion levels. This result gives a good indication for improved utilization of dietary energy without the use of antibiotics thereby promising safer animal products for human consumption. It also supports the hypothesis that CNSL inclusion in ruminant diet can reduce methane and ammonia production thereby decreasing GHG (CH_4 and N_2O) emissions into the atmosphere. Thus, this paper adds to the evidence that CNSL, which is an essential oil, serve as an eco-friendly feed additive for nutrient optimization and for combating GHG emissions in ruminant production.

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Conflict of Interest

The author declare no conflict of interest.

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