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Influence of *Cassia fistula* leaf powder on *in vitro* ruminal fermentation, gas production and degradability of diets for ruminants

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Abstract

Medicinal plants with high phytochemical values are critical for enhancing the productivity of livestock systems, while reducing their environmental impact. This study investigated the influence of graded levels of *Cassia fistula* leaf powder (CFLP) on *in vitro* ruminal fermentation parameters, gas production and degradability of diets for ruminants. Five concentrate diets were formulated to contain varying levels of CFLP (0, 15, 30, 45 and 60 mg/g dry matter (DM), denoted CF0, CF15, CF30, CF45 and CF60, respectively). The concentrate diets combined with guinea grass hay at the ratio of 4 : 6 (concentrate : forage) served as a substrate for the study. *In vitro* gas production test was performed by incubating 200 mg DM of the substrate for 48 h. At the end of the incubation period, the total gas and methane production, nutrient digestibility, *in vitro* fermentation and post-incubation parameters were evaluated. Results revealed a linear decrease ($P < 0.001$) in gas and methane production across the treatment groups with an increase in CFLP levels. The amount of methane produced varied from 9.3 ml/200 mg DM in the CF60 diet to 20 ml/200 mg DM in the control diet. Nutrient digestibility was highest in the control diet and lowest in the CF60 diet. There was a linear decrease in ammonia-nitrogen and total volatile fatty acid (TVFA) concentration with an increase in the CFLP level. Inclusion of CFLP up to 30 mg/g DM ruminant diet was found to reduce methane production and ammonia-nitrogen concentration with minimal effects on nutrient degradation and TVFA concentration.

Introduction

Worldwide, livestock production is one of the strong pillars of food industry and they contribute to global climate change by emitting greenhouse gases from enteric fermentation and manure management (FAO, 2006). Methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3) have been recognized as some of the atmospheric polluting gases largely from livestock production with a share contribution of 0.53, 0.21 and 0.75, respectively, to total agricultural emission in the European Union (UNFCCC, 2016). This has generated public concerns as methane and nitrous oxide are major greenhouse gases that contribute to climate change (EEA, 2015). Ruminants are the main contributors to the greenhouse gases with approximately 0.8 of the total livestock sector's emission (Opio *et al.*, 2013). In addition, methane and excess ammonia production in the rumen are major nutritional inefficiency manifestations in ruminant animals. For instance, only 0.1–0.2 of nitrogen consumed is retained in beef cattle (Cole and Todd, 2009) with the remaining being excreted in urine and faeces. Johnson and Johnson (1995) also reported 0.02–0.12 loss of ingested energy through methane emission. Therefore, modulating rumen fermentation to lower methane and ammonia-nitrogen production has both nutritional and environmental benefits.

The use of chemical additives such as antibiotics, ionophores, defaunating agents and methane inhibitors to improve rumen fermentation by lowering total amount of methane and ammonia-nitrogen produced has been reported in ruminants (Raymond *et al.*, 2006; Patra and Saxena, 2009). However, public concern over the use of antibiotics due to their residues in animal products such as meat and milk (Busquet *et al.*, 2006) has been on the increase and hence a ban on their usage by the European Union since 2006. Based on this, there is increasing considerable efforts by ruminant nutritionists in exploring natural alternative antibiotics which are considered safe for human consumption and will favourably help in modifying rumen metabolism to increase feed-use efficiency and animal productivity. This includes the use of medicinal plants and their extracts, organic acids, yeast, antibodies and probiotics (Elghandour *et al.*, 2014). Medicinal plants contain certain plant secondary metabolites (PSMs) such as tannin, saponin, alkaloids, flavonoids, etc. which are capable of manipulating rumen metabolism with improved production and reduced negative impact on the environment. A synergetic positive effect of secondary metabolites on ruminal microorganisms, nutrient digestion and microbial protein production has been reported (Salem *et al.*, 2011).

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Furthermore, secondary metabolites have been revealed to improve protein metabolism, suppress or stimulate microbial growth and reduce methane emission (Makkar *et al.*, 1998). Therefore, continuous investigation into natural resources such as fodder trees, shrubs and herbaceous plants with potential to improve feed utilization efficiency through methane and ammonia-nitrogen reduction with concomitant environmental friendly effects is critical in tropical livestock systems.

Cassia fistula L. is a medicinal plant that belongs to the family Fabacae, and it is generally known as golden flower in English. It grows well on shallow, poor, rocky and stony soil almost everywhere. All parts of the plant are recognized for their medicinal purpose (Sakulpanich and Gritsanapan, 2008). Antioxidant, anti-inflammatory and hepatoprotective activities of this plant have been reported in rats (Manonmani *et al.*, 2005). Phytochemicals present in the plant parts include alkaloids, flavonoids, tannins, saponin, terpenoids and glycosides (Neelam *et al.*, 2011). Luximon-Ramma *et al.* (2002) found that the young and old leaves of *C. fistula* showed higher total phenolic, flavonoid and proanthocyanidin contents among the vegetative organs investigated. Among the major factors that influenced the effects of PSMs in ruminant nutrition are the chemical nature of PSMs and their concentration in diet (Wanapat *et al.*, 2008). Although, several studies have been carried out on the use of medicinal plants to modify rumen fermentation, there is no available information on the use of *C. fistula* plant for this purpose despite having abundant bioactive compounds. It was therefore hypothesized that supplementing diet of ruminant with varying levels of *Cassia fistula* leaf powder (CFLP) as an additive will affect the rumen fermentation *in vitro*, by reducing the methane and ammonia-nitrogen production. Hence, this study investigated the effects of varying levels of CFLP as an phytogenic feed additive on *in vitro* gas production, fermentation parameters and degradation of diets for ruminants.

Materials and methods

Study area and experimental treatments

The study was conducted at the Laboratory of Animal Nutrition Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (7°13′N, 3°26′E 145 m a.s.l.). *C. fistula* leaves were freshly harvested at the premises of the University. The leaves were air-dried under a shade for 7 days to a constant moisture content level after that it was milled and made to pass through a 1 mm mesh sieve to form CFLP. Five concentrate diets were formulated to contain CFLP at 0 (CF0), 15 (CF15), 30 (CF30), 45 (CF45) and 60 (CF60) mg/g dry matter (DM) as presented in Table 1. Concentrate diets and guinea grass hay were combined at the ratio 4 : 6 and served as the substrate for the *in vitro* study.

In vitro *gas production study*

The *in vitro* gas production study was carried out according to the procedure of Menke and Steingass (1988). Rumen fluid was collected from six West African dwarf goats through the use of a suction tube as described by Babayemi and Bamikole (2006) before morning feeding (ethics number COLANIM/ACURC/022/010). The animals were fed with 0.40 concentrate feed and 0.60 guinea grass the previous day. The fluid was mixed, strained through four layers of muslin under a continuous flushing with carbon dioxide (CO_2). This served as a source of inoculum. Substrates (200 mg/g DM) which consisted of concentrate diets (CF0, CF15, CF30, CF45 and CF60) and guinea grass hay in the ratio 4 : 6 were weighed into 100 ml capacity glass syringes fitted with silicon tubes. Thereafter, 30 ml of incubation medium (consisting of buffer solution and rumen liquid in the ratio 2 : 1) was added. The syringes were tapped and pushed upwards by a piston to eliminate air in the inoculum. Controls (blanks) containing 30 ml buffered rumen fluid only were included in triplicates for correction of gas produced from rumen fluid due to the presence of small particles in the fluid. Each treatment was replicated ten times in a completely randomized design and gas produced was measured at 3 h intervals for 48 h. Total gas values were corrected for blank incubation by deducting the mean gas volume produced in the blank syringes from the volume of gas produced in the samples. However, gas volumes at 24 and 48 h of incubation periods were reported and considered very important in the current study as organic matter digestibility, metabolizable energy and short-chain fatty acids (SCFAs) of substrate incubated were calculated using the volume of gas produced at 24 h of incubation period according to Menke and Steingass (1988) and Getachew *et al.* (2002). Methane produced was determined from the final gas volume which was obtained at 48 h of incubation period based on the design of the study.

Estimation of methane gas from substrate incubated

At the end of incubation period (48 h), 4 ml of 10 M NaOH was introduced into three of the incubated syringes per treatment using a 5 ml syringe *via* the silicon tube just above the metal clip for methane production estimation. A heard of pop sound immediately the NaOH was introduced indicated the absorption of CO_2 and the remaining gas in the syringes was read as methane level (Fievez *et al.*, 2005).

Chemical analysis

Proximate composition of *C. fistula* leaf, guinea grass hay, concentrate diet and incubated residues were analysed according to the procedure of AOAC (2000). Briefly, DM was determined by drying samples to a constant weight at 65°C for 48 h followed by equilibration in a desiccator. Nitrogen content of the samples was measured using the Kjeldahl procedure and the crude protein content was calculated by multiplying the nitrogen content by 6.25. Ash was determined by combustion using a muffle furnace at 550°C for 5 h until all carbon were removed. Ether extract was analysed using the soxhlet extraction method. Neutral and acid detergent fibres were determined according to Van Soest *et al.* (1991).

The total tannin content in *C. fistula* leaf was determined using the Folin–Ciocalteu method as reported by Makkar (2003). Briefly, 0.25 ml Folin–Ciocalteu reagent (2 N) and 1.25 ml sodium carbonate solution (200 g Na_2CO_3/l) was added to an aliquot of the supernatant of the plant extract. Absorbance was read at 760 nm and a calibration curve was drawn for the tannic acid standard. Then, the concentration of total tannin was expressed as tannic acid equivalent (eq) in milligram per gram (mg/g) of dry plant materials weight.

The total phenol content was determined using the Folin–Ciocalteu colorimetric method according to the previously reported protocol (Do *et al.*, 2014). The total phenolic content was expressed as gallic acid equivalent in milligram per gram (mg/g) of dry sample.

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Flavonoid content was analysed following a modified aluminium chloride method as reported by Nasseri *et al.* (2019). The flavonoid content was expressed as gallic acid equivalent (GAE) in mg/g of dry sample.

A method reported by Mir *et al.* (2016) was used for the saponin content determination. In this, 20 ml of diethyl ether was added to the extracted sample in a 250 ml separating funnel and was shaken vigorously. Then, 60 ml of *n*-butanol was added to the aqueous layer and was washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was allowed to evaporate by heating in a water bath and later oven dried. The saponin content was calculated in percentage.

Alkaloid content was determined by the gravimetric method as outlined by Adeniyi *et al.* (2009). Here, few drops of concentrated ammonium hydroxide solution were added to the sample extract to precipitate the alkaloids. Then, the precipitate was recovered through a weighed filter paper and was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was oven dried at 60°C to a constant weight. Thereafter, the percentage of alkaloids was calculated.

In vitro *DM and crude protein degradation of substrate*

The fermentation residue was oven dried at 105°C overnight and digestibility coefficient of DM and crude protein is calculated thus:

$$\text{Digestibility coefficient of dry matter } (DM_d) = \frac{[DM_s - (DM_r - DM_b)]}{DM_s}$$

where DM_s = dry matter content of the substrate, DM_r = dry matter content of the residue and DM_b = dry matter content of the blank:

$$\text{Digestibiity coefficient of crude protein } (CP_d) = \frac{[CP_s - (CP_r - CP_b)]}{CP_s}$$

where CP_s = crude protein content of the substrate, CP_r = crude protein content of the residue and CP_b = crude protein content of the blank.

Metabolizable energy, organic matter digestibility and SCFAs of the substrate incubated

Volume of gas produced at 24 h of incubation period was used as an index to calculate the metabolizable energy, organic matter digestibility and SCFAs of the substrate as follows:

$$ME\ (MJ/kg) = 2.20 + 0.136GV + 0.057CP + 0.0029(CF)^2$$
(Menke and Steingass, 1988)

$$OMD\ (\%) = 14.88 + 0.889GV + 0.45CP + 0.651A$$
(Menke and Steingass, 1988)

$$SCFA\ (\mu mol/g\ DM) = 0.0239GV - 0.0601$$
(Getachew *et al.*, 2002)

where GV = gas volume at 24 h of incubation period; CP = crude protein of the substrate (g/kg DM), CF = crude fibre (g/kg DM) and A = Ash content of the substrate (g/kg DM).

Table 1. Ingredient composition (g/kg DM) of concentrate diet

	Levels of inclusion of CFLP (mg/g DM)				
Ingredients	CF0	CF15	CF30	CF45	CF60
Wheat offal	490	490	490	490	490
Maize bran	280	280	280	280	280
Palm kernel cake	200	200	200	200	200
Bone meal	15	15	15	15	15
Premix	5	5	5	5.00	5
Salt	10	10	10	10.00	10
CFLP	–	+	++	+++	++++
Total	1000	1000	1000	1000	100

CF0, 0 mg/g DM CFLP (–); CF15, 15 mg/g DM CFLP (+); CF30, 30 mg/g DM CFLP (++); CF45, 45 mg/g DM.

Determination of pH, ammonia-nitrogen and total volatile fatty acid (TVFA) concentrations of the incubated fluid

At the end of the incubation period, the pH of the incubation fluid was immediately determined using a pH meter (PH-200 model). Ammonia-nitrogen (NH_3-N) concentration of the fluid was determined using the steam distillation procedure (Ogubai and Sereke, 1997). A 10 ml conc. NaOH was added to 10 ml rumen fluid under a Kjeldahl distillation system. The released ammonia was collected in boric acid solution and the distillate was titrated against a standard acid (0.1 N HCl). Concentration of ammonia-nitrogen in the fluid was then calculated. TVFA concentration was determined using Markham apparatus as described by Barnett and Reid (1956). The procedure involved addition of 2 ml rumen fluid to 1 ml of 10% potassium oxalate buffer and 1 ml oxalic acid injected into the Markham apparatus and a 100 ml distillate was collected which was subsequently titrated against a standard 0.01 N NaOH with two drops of phenolphthalein as an indicator:

$$NH_3\text{-}N\ (mg/dl) = \frac{\text{HCl normality} \times \text{HCl volume} \times 100}{\text{rumen fluid volume}}$$

$$TVFA\ \ (mM) = \frac{[\text{NaOH volume} \times \text{NaOH normality}]}{\text{rumen inoculum volume}} \times 1000$$

Statistical analysis and model

All data obtained were subjected to one-way analysis of variance in a completely randomized design using the generalized linear model of SAS (2014). Orthogonal polynomial contrasts were used to examine the linear and quadratic effects of the increasing level of CFLP with the same statistical procedure. The significance level was set at $P < 0.05$. The mathematical model for the study is as follows:

$$Y_{ij} = \mu + T_i + \Sigma_{ij}$$

where Y_{ij} = observed values of dependent variables, μ = population mean, T_i = effect of different levels of CFLP and Σ_{ij} = random residual error, $P < 0.05$.

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Results

Chemical composition of concentrate diet, C. fistula *leaf and guinea grass hay used for the study*

The chemical composition of feed ingredients used in this study is presented in Table 2. Concentrate diet contained 913 g/kg, 135 g/kg DM, 284 g/kg DM and 210 g/kg DM for dry matter, crude protein, neutral detergent fibre and acid detergent fibre contents, respectively. The values obtained for DM, crude protein, neutral detergent fibre and acid detergent fibre contents in *C. fistula* leaf were 948 g/kg, 163 g/kg DM, 677 g/kg DM and 39 g/kg DM, respectively, while that of guinea grass hay were 824 g/kg, 68 g/kg DM, 690 g/kg DM and 405 g/kg DM, respectively. The determined phytochemical contents in *C. fistula* leaf were alkaloids (34 g/kg DM), saponin (102 g/kg DM), tannins (386.486 mg/100 g), phenol (286.481 mg/100 g) and flavonoids (392.2 mg/100 g).

In vitro *gas production and post-incubation parameters of substrate*

The additive effects of CFLP on *in vitro* gas volume and post-incubation parameters are presented in Table 3. The volume of gas produced at 24 and 48 h of incubation periods decreased linearly with an increase in the level of CFLP ($P < 0.001$). Increasing the level of CFLP reduced methane gas volume linearly at the end of the incubation period. Organic matter digestibility of the substrate decreased across varying levels of CFLP (linear: $P < 0.001$). SCFAs were higher in CF0 and CF15 compared to other levels of inclusion (linear: $P < 0.001$) while metabolizable energy decreased linearly as CFLP inclusion increased.

In vitro *fermentation parameters and digestibility coefficient of substrate*

The effects of varying levels of CFLP on *in vitro* fermentation parameters and digestibility coefficient of the substrate are shown in Table 4. No significant differences were observed in the pH of the incubation fluid as the CFLP level increased. Ammonia-nitrogen concentration decreased linearly with an increase in the CFLP level ($P = 0.001$). There was a linear decrease of TVFA concentration ($P < 0.001$) across the varying levels of CFLP. *In vitro* DM digestibility was affected by CFLP levels in linear and quadratic trends ($P < 0.001$; $P = 0.018$). With the increasing CFLP level, *in vitro* crude protein digestibility decreased linearly ($P < 0.001$).

Table 2. Chemical composition (g/kg DM) of experimental feed materials

Parameters	Concentrate	*C. fistula* leaf	Guinea grass hay
Organic matter	943	921	887
DM	913	948	824
Crude protein	135	163	68
Ether extract	79	71	16
Crude fibre	12	286	301
Neutral detergent fibre	284	677	690
Acid detergent fibre	210	385	405
Alkaloids	–	34	–
Saponin	–	102	–
Tannin (mg/100 g)	–	386	–
Phenol (mg/100 g)	–	286	–
Flavonoids (mg/100 g)	–	392	–

Discussion

The phytochemical composition of CFLP revealed the presence of some secondary plant metabolites (tannins, saponin, flavonoids, alkaloids and phenol) which play critical roles in plants, to ensure normal growth development, defence against infection and injury. Similar detections had been made in previous studies with *C. fistula* plants (Salem *et al.*, 2007; Panda *et al.*, 2011). Generally, secondary metabolites such as tannins and total phenolic component concentrations in plant materials have an impact on rumen microorganisms and fermentation pattern in ruminants (Khejornsart *et al.*, 2021) which subsequently affect the fermentation products such as gas production, volatile fatty acid concentration and methane gas production.

In vitro gas production method has been identified as a more efficient method of assessing PSM effects and can measure substrate degradation indirectly (Elberg *et al.*, 2018). Reduction in gas volume with an increase in the level of CFLP inclusion may be linked with the presence of the PSMs in the leaf powder whose effects depend on their concentration in the diets (Wanapat *et al.*, 2008). The presence of the PSMs such as tannin in the *Cassia* leaf powder might have limited ruminal carbohydrate fermentation by interfering with the digestive enzymes which might indirectly affect carbohydrate fermentation, hence, the resulted low gas output. Aletor (2005) stated that high tannin contents in the diet may depress enzyme activities including cellulose and intestinal digestion. Although, tannin effect is said to be evident mainly on protein, its effect has also been reported on carbohydrates particularly on hemicelluloses, cellulose, starch and pectin (Leinmüller *et al.*, 1991). This may be justified in this study as TVFA concentration decreased as the level of CFLP increased in the diet. Addition of medicinal plant materials to ruminant feed has been observed to modify rumen fermentation and influence gas emission in several *in vitro* and *in vivo* studies (Cieslak *et al.*, 2016). Reduction in methane production with the addition of CFLP confirmed the findings by Zeleke *et al.* (2006) and Kamra *et al.* (2006) where secondary compounds in medicinal plants have been reported to reduce methane production *in vitro*. In this study, tannins, saponins, flavonoids, phenol and alkaloids were all present at appreciable levels in *Cassia* leaf powder but identification of the specific fraction responsible for methane reduction is difficult. It can therefore be stated that all these phytochemicals might have been involved in the process in one way or the other through different mechanisms. Tannin has been reported to reduce methane in sheep (Rira *et al.*, 2014) probably through a direct effect on methanogens and indirect effect on fibre digestion. In addition, supplementation of *Antidesma thwaitesianum* Muell. Arg. seed meal (MOSM) containing condensed tannins decreased ruminal methane when goats were fed with MOSM at 0.024 of total DM intake (Gunun *et al.*, 2016). Saponin reduced methanogenesis through an effect linked to anti-protozoal activity (Newbold and Rode, 2006). Similarly, Supapong *et al.* (2017) discovered 42.4% methane reduction *in vitro*, when diet was supplemented with *Delonix regia* seed meal (12% saponin) at 16.7 mg. Flavonoids are

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Table 3. Effects of CFLP on *in vitro* gas volume and post-incubation parameters of substrate

	Levels of CFLP (mg/g DM)[a]							Contrasts *P*-values[b]	
	CF0	CF15	CF30	CF45	CF60	S.E.M.	*P*-value	L	Q
In vitro gas volume									
Gas volume at 24 h (ml)	41	38	35	33	28	1.3	0.002	<0.001	0.657
Gas volume at 48 h (ml)	59.6	56.8	55.6	53.7	51.3	0.80	<0.001	<0.001	0.981
Methane (ml/200 mg/g DM)	20	16	13	10	9	1.1	<0.001	<0.001	0.136
Methane (%)	33	28	24	19	18	1.7	<0.001	<0.001	0.266
% methane reduction	0	14	28	43	46	5.4	0.005	<0.001	0.448
Post-incubation parameters									
OMD (%)c	71	68	66	50	50	2.5	<0.001	<0.001	0.073
SCFA (µmol/g DM)d	0.9	0.9	0.8	0.7	0.6	0.03	0.002	<0.001	0.608
ME (MJ/kg DM)[c]	9.8	9.4	8.9	8.7	8.1	0.17	0.002	<0.001	0.651

S.E.M., standard error of mean; OMD, organic matter digestibility; SCFA, short-chain fatty acids; ME, Metabolizable energy; CFLP, *Cassia fistula* leaf powder.
[a]CF0, 0 mg/g CFLP; CF15, 15 mg/g CFLP; CF30, 30 mg/g CFLP; CF45, 45 mg/g CFLP; CF60, 60 mg/g CFLP.
[b]Significant at $P < 0.05$; L, linear; Q, quadratic.
[c]Calculated according to Menke and Steingass (1988).
[d]Calculated according to Getachew *et al.* (2002).

Table 4. *In vitro* fermentation parameters and digestibility coefficient of diets containing graded levels of CFLP

	Levels of CFLP (mg/g DM)[a]							Contrasts *P*-values[b]	
	CF0	CF15	CF30	CF45	CF60	S.E.M.	*P*-value	L	Q
In vitro fermentation parameters									
pH	6.8	6.7	6.8	6.7	6.9	0.05	0.730	0.508	0.383
Ammonia-nitrogen (mg/dl)	36	31	26	23	19	1.9	0.010	0.001	0.624
TVFA (mM)	84.3	83.7	82.4	78.9	75.3	0.96	0.002	<0.001	0.097
In vitro digestibility coefficient on DM basis									
IVDMD	0.62	0.61	0.59	0.46	0.44	0.022	<0.001	<0.001	0.018
IVCPD	0.61	0.53	0.53	0.49	0.49	0.013	<0.001	<0.001	0.059

TVFA, total volatile fatty acids; IVDMD, *in vitro* dry matter degradation; IVCPD, *in vitro* crude protein degradation; S.E.M., standard error of mean; CFLP, *Cassia fistula* leaf powder.
[a]CF0, 0 mg/g CFLP; CF15, 15 mg/g CFLP; CF30, 30 mg/g CFLP; CF45, 45 mg/g CFLP; CF60, 60 mg/g CFLP.
[b]Significant at $P < 0.05$; L, linear, Q, quadratic.

known to positively or negatively interact with rumen microorganisms and its degradation products such as 3,4-dihydroxyphenylacetic acid might also alter microbial metabolism in the rumen (Broudiscou and Lassalas, 2000) which may in turn affect fibre degradation and methane gas production. Decreased organic matter and DM digestibility obtained could also be responsible for reduced methane production and SCFA recorded. Reduction in methane gas production with associated decreased feed digestibility has been reported by several authors (Cobellis *et al.*, 2016; Aderinboye *et al.*, 2020). Low SCFA concentration with medicinal plant treatment is an indicator of simultaneous methane reduction in the rumen tract (Busquet *et al.*, 2006) which was evident in this study. However, Cieslak *et al.* (2012) observed decreased methane gas production when *Vaccinium vitis idaea* extract was utilized without interfering with feed digestibility. In addition, SCFA concentration was lowered at 45 and 60 mg/g DM CFLP inclusion levels compared with other treatments. This is in agreement with the findings of Evans and Martin (2000) who observed reduction in SCFA concentration at high level of thymol (*Thymus vulgaris*) inclusion. Meanwhile, Castillejos *et al.* (2008) noted increased SCFA concentrations at all levels of thymol inclusion. Variations in observation could be as a result of different medicinal plant used with different levels of inclusion and phytochemical composition.

The pH values reported in this study fell within the acceptable range (6.2–7.2) for optimal microbial activities (Van Soest, 1994) and adequate fermentation process. Reduction of ammonia-nitrogen concentration observed revealed the potential of *Cassia* leaf powder in inhibiting deamination. Tannin may form complex with protein, thus, preventing its deamination in the rumen (Wanapat *et al.*, 2008; Khejornsart *et al.*, 2021). Min *et al.* (2002) established that population of ruminal proteolytic bacteria

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as well as ammonia-nitrogen concentration decreased when sheep was fed with diet containing condensed tannin from *Lotus corniculatus*. Furthermore, a large number of phenolic groups present in medicinal plants might have formed strong hydrogen bonds with protein at multiple sites thereby hindering its total degradation in the rumen and thus affecting ammonia-nitrogen concentration (Jayanegara and Palupi, 2010). Reduction in amino acid degradation in the rumen could also be related to inhibiting bacterial attachment to feed particle by various phytochemicals present in the medicinal plants (Wallace *et al.*, 2002). In the same vein, Cobellis *et al.* (2016) found that essential oil in medicinal plants reduced ammonia-nitrogen concentration in the rumen. Saponins in most medicinal plants have been shown to influence rumen microorganism population and hence improve microbial protein synthesis efficiency (Wang *et al.*, 2000). Similarly, reduction in ammonia-nitrogen concentration by supplementation of saponin-rich plant (Yucca schidigera) extract has been discovered in a continuous incubation system by Pen *et al.* (2006). Ammonia-nitrogen concentration has been revealed to be a crude predictor of efficient dietary nitrogen conversion into microbial protein in the rumen (Khejornsart *et al.*, 2021). However, when the rate of ammonia production exceeded the utilization capacity by the microorganisms, the excess is excreted leading to protein wastage and environmental pollution.

In this study, the DM, organic matter and crude protein degradation was above 0.50 in diets containing up to 30 mg/g DM CFLP. This is an indication of efficient fermentation and it shows that adding up to 30 mg/g DM CFLP to the diets of ruminants might not adversely affect the fermentation process. Organic matter digestibility and total gas production were lowest at the highest inclusion level (60 mg/g DM) which recorded lowest TVFA concentration. This might not be good for the animals because VFAs represent the main supply of metabolizable energy in ruminants. A positive correlation has been reported to exist between gas and volatile fatty acid production (Getachew *et al.*, 2002). This may explain the lower TVFA concentration at the highest level of CFLP inclusion (60 mg/g DM) which also recorded the lowest volume of gas production. The foregoing is in agreement with the work of Cieslak *et al.* (2016) who reported reduction in nutrient digestibility by PSMs only at higher levels of medicinal plant (*Sanguisorba officinalis*) supplementation. Gas production can be used to quantify VFA production as the digested substrate is partitioned into gas, VFA and microbial mass (Getachew *et al.*, 2002).

Conclusion

Supplementing diets with CFLP altered rumen fermentation, reduced methane production and ammonia-nitrogen concentration *in vitro*. Total gas production and nutrient digestibility coefficient were also reduced with dietary inclusion of CFLP. Therefore, diets of ruminant can be supplemented with up to 30 mg/g DM CFLP to reduce methane gas production and ruminal ammonia-nitrogen concentration with minimal effects on nutrient degradation and TVFA concentration. The finding is of importance to efforts aimed at controlling climate change as its adoption can reduce the emission of methane from the livestock industry which contributes to global warming. It also has the potential to assist livestock farmers in combating the problem of nutrient utilization inefficiency as a result of methane and excess ammonia-nitrogen production in the rumen of ruminant animals. *In vivo* study involving the use of CFLP up to 30 mg/g DM is encouraged to ascertain its effects on growth and animal health.

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