



In vitro digestibility and methane gas production of fodder from improved cowpea (*Vigna unguiculata* L.) and groundnut (*Arachis hypogaea* L.) varieties



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ABSTRACT

In vitro substrate degradability and methane gas production of fodder from cowpea and groundnut plants were evaluated in this study. Duplicate samples and three batch replicates ($n = 3$) of three groundnut varieties (Samnut 22, Chinese and Samnut 23) and two cowpea varieties (Padi Tuya and Songotra) were incubated in a buffered rumen fluid. The crude protein (CP) concentration of Songotra and Padi Tuya varieties was in the range of 112 to 154 g kg⁻¹ dry matter (DM), respectively. Both neutral detergent fiber (NDF) and acid detergent fiber (ADF) were found to be higher in Samnut 22 with the other varieties having values below 400 g kg⁻¹ DM. Significant differences were found among treatments for all the *in vitro* kinetic parameters. The highest ($P < 0.05$) DM and organic matter (OM) degradability were observed in cowpea variety Padi Tuya. Methane gas production expressed as ml g⁻¹ DM incubated and ml g⁻¹ DM degraded were both higher ($P < 0.05$) in cowpea varieties Padi Tuya, Songotra and groundnut variety Chinese. Total volatile fatty acid and the ratio of acetate: propionate did not differ among the treatments. Pearson correlation showed a significant positive association between CP and metabolizable energy (ME) and a negative association between CP and methane. The association between NDF, ADF and methane production, IVOMD and IVDMD was found to be negative. It can be concluded from the study that the cowpea varieties possessed superior and efficient degradability compared to the groundnut varieties.

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Introduction

Leguminous crops are very important in the farming system of most tropical countries. They provide highly nutritious grain, fix nitrogen to boost soil fertility and provide highly palatable fodder for livestock production [8,18,21].

[44] In order to meet the high demand of protein in human diets, especially from plant sources in Sub-Saharan Africa, plant breeders have focused their attention on identifying and selecting high yielding, drought and pest resistant varieties of

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Table 1
Agronomic characteristics, grain and fodder yield of legume varieties.

Specie	Variety	Growth form	Maturity (days)	Leaf:Stem	Potential grain yield (t/ ha)	Fodder yield (t/ha)
Groundnut	Chinese	Spreading	90	1.8	1.7	2.0
	¹ Samnut 22	Semi-erect	110	1.0	2.5	3.5
	¹ Samnut 23	Spreading	100	1.4	2.5	3.0
Cowpea	² Padi-Tuya	Semi-erect	75	-	1.8	2.5
	² Songotra	Semi-erect	65	-	2.0	3.5

¹ Ndjeunga et al. [29]

² IITA AgriSTAT [1]

leguminous crops [35,36,44]. The economic value of these legumes in recent times has been extended to cover the haulms. Plant breeders have been exploring high yielding and nutritious haulms alongside grain yield to satisfy both human and animal nutrition [33,41].

Fodder from groundnut and cowpea is among the common feeds traded in emerging livestock feed markets across Africa [9,24,41]. The cost of cowpea fodder has been found to be higher than that of groundnut in Africa, which may be linked to the fodder quality. It is however, unclear how newly released varieties will impact on the quality of the legume species, hence the need for this study.

Carbon dioxide and methane are greenhouse gases that are natural products of enteric fermentation. Methane, is 25 times more potent greenhouse gas than carbon dioxide [22] and therefore its production needs to be minimized. It has been estimated that nearly 96 million tonnes of methane gas from enteric fermentation and 18 million tonnes from livestock manure are released into the atmosphere globally [19,37]. Methane emission from enteric fermentation represents a significant loss of feed energy [23] and its production from tropical livestock production systems have been found to be high and inefficient [32], largely due to poor feed quality [14,39]. Investigating the enteric methane production of improved cowpea and groundnut varieties is therefore very relevant and justified considering the important role of fodder from these crops plays in ruminant nutrition.

The objective of the study was to evaluate the *in vitro* substrate degradability, kinetics and methane production of fodder from improved cowpea and groundnut varieties.

Materials and methods

Collection and processing of samples

Fresh fodder, comprising leaves and twigs of two cowpea (*Vigna unguiculata* L.) varieties (Songotra and Padi Tuya) and three groundnut (*Arachis hypogaea* L.) varieties (Samnut 22, Samnut 23 and Chinese) were collected from the Savelugu and Kassena-Nankana Districts of Ghana. The agronomic characteristics, grain and fodder yield are shown in Table 1. The fodder was harvested from the crop fields of the International Institute of Tropical Agriculture (IITA), in the cropping seasons of 2016 and 2017. The harvested fodder was dried in a forced air oven (60 °C) and then milled with a hammer mill to 1 mm particle size. The milled samples were stored in airtight containers and brought to the Animal Nutrition Division of ICAR-Central Sheep and Wool Research Institute (CSWRI), India.

Experimental procedure

The *in vitro* gas production was carried out using 100 ml calibrated glass syringes (häberle LABORTECHNIK, Lonsee-Ettlenschieß, Germany) for each fodder in duplicates. The samples were incubated in three separate batches. Anaerobic media was prepared following the procedure of [26]. Rumen fluid was obtained from three male Malpura sheep (2–2.5 years of age) before feeding in the morning through stomach tube into a pre-warmed thermos flask and immediately brought to the laboratory. The rams were maintained on pasture dominated by *Cenchrus ciliaris* and supplemented with concentrate consisting of maize, barley, mustard oil cake, groundnut cake, mineral mixture and salt. The rumen fluid was filtered through a four-layered cheese cloth into a conical flask placed in a warm water bath with continuous flushing of carbon dioxide.

Approximately, 400 mg of fodder samples were weighed into the glass syringes and 40 ml of buffered rumen fluid was drawn in to the syringe and incubated in a water bath (39 °C) for studying *in vitro* rumen fermentation kinetics and true substrate degradability.

The gas reading for the kinetics study was recorded over a period of 72 h (2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 60 and 72 h). At each time of reading, the contents of the syringes were gently shaken and the gas vented when it exceeded 50 ml of the volume of the syringes. Blank was run simultaneously without any substrate and gas volumes recorded for calculating the net gas production.

The incubation for *in vitro* dry matter degradability (IVDMD) was terminated after 24 h and the content of the syringes transferred to a 200 ml beaker. It was refluxed with 50 ml neutral detergent solution [13], filtered through sintered silica crucibles and oven dried (60 °C). The *in vitro* organic matter degradability (IVOMD) and metabolizable energy (ME) were calculated using the prediction equation:

Table 2
Chemical composition (g kg⁻¹DM) of fodder from cowpea and groundnut varieties.

Species	Varieties	OM	CP	NDF	ADF	Ash
Groundnut	Samnut 22	803±0.2	132±0.3	552±9.2	518±7.3	195±0.1
	Chinese	847±0.5	120±0.1	356±4.7	312±6.3	227±0.1
	Samnut 23	835±0.4	134±0.3	360±1.8	325±3.7	168±0.1
Cowpea	Padi Tuya	858±0.2	154±0.1	365±0.7	344±0.7	143±0.1
	Songotra	853±0.2	112±0.3	361±2.6	336±2.8	144±0.2

DM, dry matter; OM, Organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

IVOMD (%) = 16.49 + 0.9042 × GP + 0.0492 × CP + 0.0387 × ash [26], ME (MJ/kg DM) = 2.20 + 0.136 × GP + 0.057 × CP [27] where: GP = gas production (ml/200 mg DM at 24 h) and CP = crude protein (g/kg DM).

A different set of incubation involving same fodder as substrate was carried out to measure production of methane gas, volatile fatty acid and ammonia nitrogen. The samples were incubated for 24 h, after which the total gas was recorded, sampled and transferred to the gas chromatograph for methane estimation. The content of the syringes was filtered through a four-layer cheese cloth and the filtrate stored at -20 °C for ammonia nitrogen and VFA analysis.

Analytical procedures for nutrient, methane gas, vfa and ammonia nitrogen

The concentrations of DM, OM, CP (N × 6.25) and ash of the fodder sample were determined following the AOAC [6] procedures. The NDF and ADF were analyzed according to the methods described by Van Soest et al. [46]. Both NDF and ADF contents were determined inclusive of residual ash, and NDF was determined without α-amylase and sodium sulfite.

Methane production was determined by collecting 50 μl of gas after 24 h fermentation [34]. The sampled gas was injected into a gas chromatograph (GC-1000, Dani, Milan, Italy) fitted with a flame ionization detector and packed column (Chromatopak, length-3 m, Diameter- 1/8 in, liquid-15%). The concentration of methane in the standard was 999.98 ml⁻¹ L (Sigma-Aldrich; Missouri, United States). The temperature of injector oven, column oven and detector were 120, 50 and 120 °C, respectively. Concentration of ammonia nitrogen in the fermentation solution was determined according to the method of Chaney and Marbach [16]. For analyzing VFA in the fermented incubation media approximately 0.9 ml of filtrate from the incubated samples was mixed with 0.1 ml of metaphosphoric acid (25%; w/v) and centrifuged at 8000 rpm for five min and the supernatant injected into the gas chromatograph (GC-1000, Dani, Milan, Italy) using flame ionization detector fitted with the appropriate column (10% SP-1000, 6 ft × 1/8 × 2.1 mm SS).

Statistical analysis

Net gas production in the kinetics study was fitted to the non-linear equation $Y = b(1 - e^{-c(t-L)})$ using Sigma plot 10th edition, where 'Y' is the cumulative gas production at time 't' (h), 'b' the asymptotic gas production and 'c' the rate constant of gas production, 'L' discrete lag time. The half time of asymptote gas production ($t_{1/2}$ (h)); was calculated as $\ln 2/c$. All data were analyzed using one-way ANOVA in GenStat 11th edition [38]. The model used was $Y_{ij} = \mu + V_i + e_{ij}$. Where Y_{ij} is an observation, μ is experimental mean, V_i is treatment effect, and e_{ij} is random error. Means were separated at 5% significant level using the Tukeys mean separation method. Pearson correlation was used to explore if there was any association between the nutrient composition and *in vitro* gas production parameters. Means and standard deviations were computed for the chemical analysis data.

Results

Chemical composition

The OM concentration of the fodder varieties ranged between 800 and 850 g kg⁻¹ DM, respectively (Table 2). The concentration of CP ranged from 112 to 154 g kg⁻¹ DM for Songotra and Padi Tuya, respectively. Both NDF and ADF were found to be higher in Samnut 22 with the other varieties having values below 400 g kg⁻¹ DM.

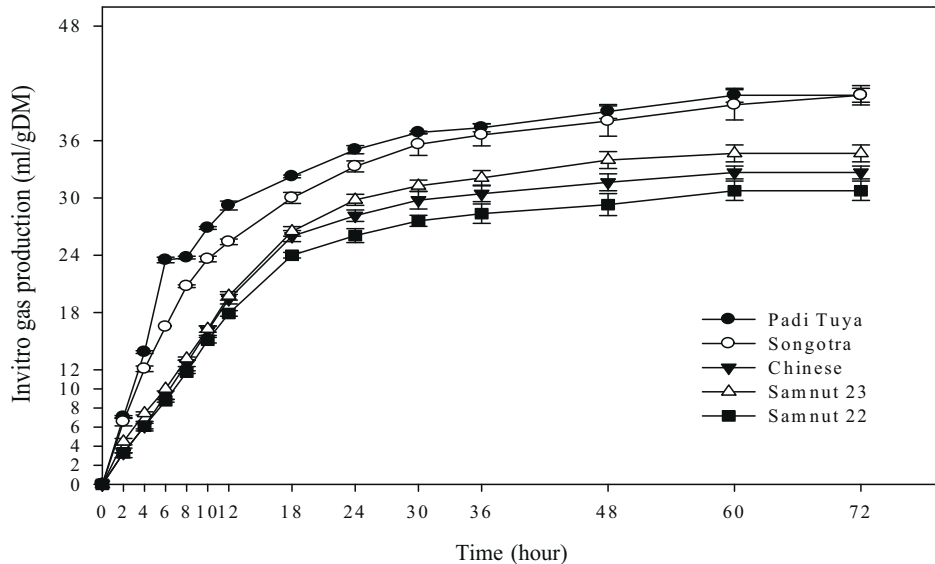
In vitro gas production kinetics

The results of *in vitro* gas production kinetics are shown in Table 3. Asymptotic gas production (b) was found to be higher ($P < 0.05$) for varieties Padi Tuya and Songotra relative to the groundnut varieties Samnut and Chinese. The rate constant (c) differed ($P < 0.05$) between Padi Tuya and all the other varieties. Generally, the groundnut varieties had a slower rate of fermentation compared to cowpea. The cowpea varieties had a relatively shorter lag time than the groundnut. The $t_{1/2}$ (h) of fodder from groundnut varieties were significantly greater than fodder from the cowpea varieties. The cumulative gas production is shown in Fig. 1 with the fodder from the cowpea varieties maintaining a relatively higher gas production than their groundnut counterparts throughout the fermentation period of 72 h.

Table 3*In vitro* fermentation kinetics of fodder from different cowpea and groundnut varieties.

Species	Varieties	b (ml g ⁻¹ DM)	c	L(h)	t _{1/2} (h)
Groundnut	Samnut 22	31.14 ^a	0.07 ^{ab}	1.8 ^a	9.7 ^c
	Chinese	33.26 ^a	0.07 ^{ab}	1.6 ^a	9.5 ^{bc}
	Samnut 23	35.53 ^{ab}	0.06 ^a	0.9 ^b	10.1 ^c
Cowpea	Padi Tuya	39.02 ^b	0.11 ^c	0.2 ^c	5.9 ^a
	Songotra	39.25 ^b	0.08 ^b	0.7 ^{bc}	8.1 ^b
	SEM	1.05	0.003	0.17	0.29
	<i>P</i> value	0.001	0.001	0.001	0.001

b, asymptote gas production; c, rate constant; L, discrete lag time; t_{1/2} (h), half-time of asymptotic gas production; SEM, standard error of mean ^{ab} Means within the same column with different superscripts differ significantly (*P*<0.05).

**Fig. 1.** Mean (\pm SE) cumulative *in vitro* gas production of fodder from different cowpea and groundnut varieties.**Table 4***In vitro* digestibility and methane production parameters of fodder from different cowpea and groundnut varieties.

Species	Varieties	IVDMD (%)	IVOMD (%)	ME (MJ kg ⁻¹ DM)	Total Gas (ml/g ⁻¹ DM)	Methane (ml g ⁻¹ DM)	Methane (ml g ⁻¹ DMD)	Methane (ml g ⁻¹ OMD)
Groundnut	Samnut 22	47.10 ^a	53.52 ^a	13.19 ^b	127.1 ^a	17.55 ^a	37.3 ^a	46.4 ^a
	Chinese	53.71 ^b	54.93 ^a	12.38 ^a	122.1 ^a	35.75 ^b	63.1 ^c	74.5 ^c
	Samnut 23	52.92 ^b	53.93 ^a	13.51 ^b	134.6 ^a	18.55 ^a	35.1 ^a	42.0 ^a
Cowpea	Padi Tuya	65.90 ^c	63.25 ^b	16.02 ^c	186.2 ^b	39.40 ^b	59.8 ^{bc}	69.7 ^{bc}
	Songotra	61.22 ^c	58.68 ^b	13.24 ^b	172.1 ^b	34.80 ^b	56.8 ^b	66.6 ^b
	SEM	0.83	0.79	0.16	6.00	1.06	2.04	2.50
	<i>P</i> Value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

SEM, standard error of mean ^{ab}. Significant difference at *P* <0.05.

IVDMD, *in vitro* dry matter degradability; IVOMD, *in vitro* organic matter degradability; ME, metabolizable energy; DMD, Dry matter digested; OMD, organic matter digested.

Substrate degradability and methane production

Significant differences were found between the treatments for all the *in vitro* degradability parameters (Table 4). IVDMD was higher (*P* <0.05) in the cowpea varieties. The lowest IVDMD was observed in Samnut 22. A similar trend was observed in IVOMD of cowpea varieties, which was superior to groundnut. The fodder cowpea had higher ME content than the groundnut, but the variety Padi Tuya had the highest (*P*<0.05) ME, while it was lowest in Chinese groundnut variety (Table 4).

Total gas production expressed as ml g⁻¹ DM were both higher in the cowpea varieties than the groundnut varieties. The least (*P* <0.05) amount of methane gas was produced in groundnut varieties Samnut 23 and Samnut 22, while the Chinese variety produced similar methane as that of cowpea varieties. The methane production per g of DMD or OMD

Table 5

Volatile fatty acids and ammonia nitrogen concentration of fodder from different cowpea and groundnut varieties.

Species	Varieties	Acetic	Propionic	Butyric	Iso-butyric	Valeric	Iso-valeric	TVFA	AP-ratio	NH ₃ -N
Groundnut	Samnut 22	10.2	2.0	0.3	1.4	0.4	0.30	14.7	5.1	102.3 ^c
	Chinese	10.4	2.9	0.3	1.8	0.5	0.22	16.1	3.5	86.2 ^{bc}
	Samnut 23	11.6	2.6	0.4	1.5	0.4	0.23	16.7	4.5	98.0 ^c
Cowpea	Padi Tuya	13.4	3.2	0.3	1.8	0.4	0.37	19.4	4.3	77.3 ^b
	Songotra	12.9	2.8	0.3	1.7	0.4	0.24	18.5	4.6	56.9 ^a
	SEM	1.18	0.31	0.03	0.16	0.03	0.04	1.63	0.34	4.17
<i>P Value</i>		0.359	0.347	0.356	0.501	0.359	0.089	0.425	0.109	0.001

TVFA (mM/L), total volatile fatty acid; AP-ratio, acetate propionate ratio; NH₃-N (mg/dl), ammonia nitrogen; SEM, standard error of mean ^{ab}, Significant difference at $P < 0.05$.

Table 6Pearson correlation (r) between chemical composition and rumen fermentation parameters of fodder from different cowpea and groundnut varieties.

Parameters	CP	NDF	ADF	Ash
IVOMD	0.425	-0.409	-0.326	-0.664**
IVDMD	0.265	-0.653**	-0.577*	-0.701**
Methane (ml g ⁻¹ DMD)	-0.027	-0.439	-0.406	-0.149
ME (MJ kg ⁻¹ DM)	0.829**	-0.157	-0.073	-0.686**
Total volatile fatty acid	0.155	-0.391	-0.346	-0.453
B	0.103	-0.621*	-0.549*	-0.770**
C	0.573*	-0.289	-0.205	-0.617*

IVOMD, *in vitro* organic matter degradability; IVDMD, *in vitro* dry matter degradability; ME, metabolizable energy; b, asymptote gas production; c, rate constant; *, $P < 0.05$; **, $P < 0.01$.

revealed a similar trend showing the highest value in groundnut Chinese variety followed by cowpea varieties and the lowest in Samnut 22 and 23.

Volatile fatty acid composition and ammonia nitrogen concentration

The total VFA, composition of VFA and the ratio of acetate: propionate in the incubation media did not differ among the treatments (Table 5). The percentage of acetic acid in the total VFA was above 60 for all the treatments. Ammonia nitrogen concentration was higher ($P < 0.05$) in groundnut variety Samnut 22 with the least recorded in cowpea variety Songotra. The groundnut varieties generally had more ammonia nitrogen concentration than the cowpea varieties.

Correlation between chemical composition and *in vitro* fermentation parameters

Methane gas production (ml g⁻¹ DMD) was negatively correlated ($P > 0.05$) with CP, NDF, ADF and ash with a higher coefficient value in ADF and NDF (Table 6). The correlation between ME and CP was positive ($P < 0.01$; $r = 0.83$). However, NDF, ADF and ash all showed a negative correlation with ME. Whilst the correlation between IVOMD, IVDMD, TVFA, asymptote gas production (b), rate constant and CP were positive, that of IVOMD, IVDMD, TVFA, asymptote gas production (b), rate constant and NDF, ADF and ash were all negative.

Discussion

The mean CP recorded for all the treatments was similar to what has previously been reported by Anele et al. [2], Ansah et al. [3] and Ansah et al. [4] for fodder from other improved groundnut and cowpea varieties. The CP concentration in all the varieties was above the range of CP required for maintenance and growth of small ruminants [31,45]. The NDF and ADF fractions of forages harbor the bulk of digestible cell wall carbohydrate; cellulose and hemicellulose. The relatively higher NDF and ADF recorded in fodder from groundnut variety Samnut 22 is attributable to its longer days to maturity (110 days) compared to other varieties which were in the range of 62–100 days [10,17,33]. Plants with relatively longer days to maturity often accumulate more stem mass than leaves and this could result in the fodder from such plants having more cell wall carbohydrates than those of shorter days to maturity [47].

The differences recorded in observations on *in vitro* kinetics and substrate degradability parameters among the treatments is a reflection of the intrinsic chemical composition and anatomical structure of the fodder varieties. The higher asymptotic gas production found in Padi Tuya and Songotra compared to Samnut 22 and Chinese varieties suggests a relatively better degradation of cell wall solubles and other degradable carbohydrates. Similarly, Padi Tuya variety had a shorter half time ($t_{1/2}$; h) and lag compared to other forages indicating that a shorter time was required for half of the asymptotic gas to be produced and also, earlier access of the substrates for microbial degradation. Lag time has been reported to be

influenced by the microbial capacity, such as rate of hydration of feedstuff, microbial attachment to feed particles and nutrient limitations (Nolan and Dobos, 2005). It may further suggest that there was less limitation to microbial access and attachment, degradation and fermentation of fodder from the cowpea varieties.

The IVDMD and IVOMD was found to be superior in Padi Tuya than the other varieties. Access to DM and OM by rumen microbes is essential for degradability. It is apparent from relatively high IVDMD and IVOMD values that signifies enhanced microbial access and degradation and fermentation in Padi Tuya than the other varieties. All the forage varieties had degradability above the minimum 45% suggested as the requirement to support the maintenance of cattle in the tropics [28]. The correlation analysis showed a significant negative effect of NDF, ADF and ash on IVDMD. This corroborates the relatively low degradability recorded for fodder from groundnut varieties in this study. The cow pea variety Padi Tuya predicted the highest ME, which correlates well with its CP content. Moreover, its degradability and gas production were also higher that grouped Padi Tuya to have more ME (16.02 MJ kg⁻¹ DM) compared to other fodder varieties. The Chinese groundnut (12.38 MJ kg⁻¹ DM) had the lowest ME because of lower CP and least gas production, while an intermediate ME value was predicted for cowpea (Songotra) that had least CP content but high substrate degradability and gas production.

Ammonia nitrogen concentration in is reported to be a balance between dietary protein degradation and the uptake of ammonia for the synthesis of microbial protein [25]. However, in the absence of fermentable carbohydrates, particularly water-soluble carbohydrates, uptake of ammonia nitrogen by microbes is reduced leading to accumulation of in the rumen or the incubation media [30,40,43]. The high concentration of ammonia nitrogen in the groundnut varieties may suggest a deficit in the supply of fermentable carbohydrates and this may have also been influenced by the longer days to maturity required by groundnuts compared to cowpea. The days to maturity required by the groundnuts used in this study ranged from 90 - 110 vs 65 -75 days in cowpea. The concentration of fermentable carbohydrates reduces as plant matures and this is normally influenced by the increase in stem relative to leaf fraction as days to maturity prolong [47]. Generally, the cowpea varieties evaluated were superior to groundnuts in terms of CP, NDF, ADF and digestibility and this agrees with the findings of Ayantunde et al. [9] and Sapna et al. [42] who conducted similar studies in Mali and Niger respectively. It however, deviated from the findings of [41] et al., (2017) who conducted a similar study in Nigeria.

Methane production was however, found to be higher in cowpea varieties Padi Tuya and Songotra than the groundnut varieties Samnut 22 and 23, but similar to Chinese. Amongst these forages, the Chinese variety of groundnut exhibited higher concentration of methane (27.8%) in the total gas fermented. The less volume of methane produced per g DM digested in groundnut agrees with the findings of Bhatt et al. [12]. A report by Heuzé et al., [20] showed a generally higher condensed tannin concentration in groundnuts haulms than in cowpea haulms (16.8 g kg⁻¹DM vs 1.8 g kg⁻¹DM). Condensed tannins have been cited for their suppressive effect on methanogenic and other fermentative bacteria in the rumen [5,15]. It can therefore be suggested that the less volume of methane gas produced from the groundnut varieties after 24 h incubation was due the higher concentration of condensed tannins present in the groundnut haulms. However, it is worth noting that the higher volume of methane gas produced in the cowpea varieties within the 24 h corresponded to a higher IVDMD and IVOMD. The observation was not the same in the groundnut variety Chinese, which had a similar volume of methane gas production as the cowpea but did not match up with the cowpea in terms of IVDMD and IVOMD. This suggests that the Chinese variety will potentially contribute significantly to GHG emissions whilst contributing less to DM and OM digestibility when it is fed to ruminants as a sole diet. Since condensed tannins also affect fermentative bacteria in the rumen, it could be one of the reasons for the poor IVOMD and IVDMD in the groundnut varieties. The lack of significant difference in the total VFA production compared to significant difference in methane production among the varieties confirms the fact that feeding ruminants with the groundnut varieties in this study could not yield associative rumen fermentation metabolites although there was reduced methanogenesis in Samnut 22 and 23

The higher molar proportions of acetate and butyrate compared to propionate among the treatment is a reflection of the fibrous nature of the fodder varieties [25]. The percentage of acetate and propionate produced falls within the range of 45 to 70% and 15–40% respectively reported for most forage-based diets [7,11].

The negative correlation between NDF/ADF and methane production, asymptotic gas production, IVOMD and IVDMD agrees with the findings of Pal et al. [34]. The significant association between NDF/ADF and IVDMD, asymptotic gas production and rate constant confirms relatively slow nature of cell wall digestibility compared to cell contents. The lack of significant difference in the association between NDF/ADF and methane production in this study supports the reason that methane production was also influenced by other dietary factors such as condensed tannin concentration.

Conclusion

Both cowpea and groundnut varieties had over 40% degradability of dry matter and organic matter. The cowpea varieties evaluated in this study had a higher degradability and metabolizable energy than the groundnut varieties, and Padi Tuya was superior amongst all the varieties. Less methane and higher ammonia N were produced from the fodder of groundnut varieties compared to cowpea. Feeding the groundnut varieties as sole diet may thus lead to the accumulation of ammonia nitrogen in the rumen and subsequently excreted in urine as waste which can be collected as liquid manure. The Chinese variety in addition to the accumulation of high ammonia nitrogen in the rumen could also result in emission of high inefficient methane gas when fed as a sole diet.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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