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Chemical characterization, *in vitro* dry matter and ruminal crude protein degradability and microbial protein synthesis of some cowpea (*Vigna unguiculata* L. Walp) haulm varieties

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ABSTRACT

A study was carried out to evaluate the chemical composition, *in vitro* apparently and truly degraded dry matter (DM), utilizable crude protein at the duodenum (uCP) (total CP at the duodenum minus endogenous CP), methane production, and short chain fatty acid production of haulms of six cowpea varieties. The study was arranged in a $2 \times 2 \times 2$ factorial design, with three replicates. Three improved (ITA2, ITA6 and ITA8) and three commercial (Oloyin, Peu and Sokoto) cowpea varieties harvested during wet and dry seasons were used for the study. After an initial gas test to evaluate 96 h gas production profiles of haulms with and without polyethylene glycol (PEG), the time to half maximal gas production was calculated and a second incubation conducted with fermentation stopped at substrate specific half time ($t_{1/2}$) and 24 h for each substrate. True DM degradability was measured from incubated residues and combined with gas volume to estimate the partitioning factor. Crude protein flow to the duodenum was estimated by combining gas volume with the measured ammonia nitrogen in the incubated fluid. Addition of PEG did not have any effect ($P>0.05$) on all the variables determined. Interaction between group (improved *vs.* commercial) and season was observed for CP ($P=0.002$), lignin ($P=0.003$) and hemicellulose ($P=0.030$) contents of the haulms. A group × season interaction was also observed for some of the variables at both substrate specific $t_{1/2}$ and 24 h. Commercial cowpea haulms had greater ($P=0.002$) microbial mass and produced less ($P<0.05$) methane than the improved cowpea haulms. The improved cowpea haulms were less ($P<0.001$) degraded in the rumen and as a result ensured greater ($P<0.001$) amount of uCP. The results validated that cowpea haulm is an important agro-based by-product that is adequate in protein and energy to sustain ruminant animal production in Nigeria and other Sub-Saharan African countries during the extended dry season.

Abbreviations: ADFom, acid detergent fibre expressed exclusive residual ash; Lignin(sa), lignin solubilized with sulphuric acid; CP, crude protein; DM, dry matter; EE, ether extract; ivADDM, *in vitro* apparently degraded dry matter; ivTDDM, *in vitro* truly degraded dry matter; MN, microbial nitrogen; NDFN, neutral detergent fibre bound nitrogen; NDFom, neutral detergent fibre expressed exclusive residual ash; NFC, non-fibre carbohydrates; PEG, polyethylene glycol; PF, partitioning factor; RDN, rumen degraded nitrogen; SCFA, short-chain fatty acids; uCP, utilizable crude protein at the duodenum; UDN, undegraded dietary nitrogen; UNAAB, University of Agriculture, Abeokuta.

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1. Introduction

Insufficient quantity and quality of traditional forage resources in many tropical regions, particularly those with acidic, low-fertility soils and prolonged annual dry season arising from fluctuations in environmental factors (which directly and indirectly limit the quantity of herbage available to animals either from natural grazing and/or crop residues) are major constraints for farmers to improve livestock productivity.

Agriculture in the Sub-Saharan African countries is intensifying in response to increasing populations of humans and livestock. Estimates have shown that ignoring crop residues as a feed resource will result in serious feed shortages. In these scenarios, crop farmers may own their own livestock for ready access to manure, while simultaneously selling some marginal land to livestock keepers who settle and begin crop farming using the manure from their animals (and possibly traction) as an input (Delgado et al., 1999). As a result, increased productivity demands are placed on integrated crop-livestock systems with more emphasis on legumes such as cowpea (*Vigna unguiculata* L. Walp). Cowpea has the potential to function as a key integrating factor in intensifying systems through supplying protein in human diets, and fodder for livestock, as well as bringing N into the farming system through fixation (FAO, 2000). Going beyond its importance for food and feed, cowpea can be regarded as a fulcrum of sustainable farming in regions characterized by systems for farming that make limited use of purchased inputs (Anele et al., 2010).

Cowpea is grown extensively in 16 African countries, with the continent producing two-thirds of the world total. Two countries–Nigeria and Niger–produce 850,000 and 271,000 tonnes annually or, together, 49% of the world crop (FAO, 2000). The bulk of this production comes from smallholder farmers in semiarid zones of the region.

The use of an *in vitro* gas technique in evaluating feedstuffs (through the measurement of variables like methane, microbial mass and short chain fatty acids (SCFA)) is a very effective and robust way of estimating energy loss from diets, microbial and feed nitrogen supply to ruminants.

The objective of this study was to estimate intervarietal differences of the nutritive value of some cowpea haulms. This was achieved by determination of *in vitro* apparent and true degraded dry matter (DM), utilizable crude protein at the duodenum (uCP; total CP at the duodenum minus endogenous CP), methane production, protozoa population, microbial mass and efficiency of three improved and three commercial varieties of cowpea haulms.

2. Materials and methods

2.1. Experimental site

The field experiment was conducted at the Teaching and Research Farm, University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria. The site lies within the savanna agro-ecological zone of south-western Nigeria (latitude: 7°N, longitude 3.5°E, average annual rainfall: 1037 mm). Abeokuta has a bimodal rainfall pattern that typically peaks in July and September with a break of two to three weeks in August. Temperatures are fairly uniform with daytime values of 28–30 °C during the rainy season and 30–34 °C during the dry season with the lowest night temperature of around 24 °C during the harmattan period between December and February. Relative humidity is high during the rainy season with values between 63 and 96% as compared to dry season values of 55–84%. The temperature of the soil ranges from 24.5 to 31.0 °C (Source: Agrometeorology Department, UNAAB).

2.2. Forage establishment and management

The experimental area, measuring 2600 m^2, was ploughed twice and harrowed. The area was divided into eight blocks and each block was sub-divided into 10 plots each measuring 5 m × 4 m. Three improved (*i.e.*, IITA 97k-1069-6, IITA 98k-311-8-2, IITA 98k-476-8; hereafter designated ITA-6, ITA-2 and ITA-8) and three commercial (*i.e.*, "Oloyin", "Peu", "Sokoto") dual-purpose cowpea varieties constituted the treatments. The dual-purpose cowpea varieties were semi-erect types and had 70–86 days to pod maturity. The improved varieties were modified for greater biomass and grain yields. Treatments were randomly allocated to plots within block. The inner six blocks (36 plots) were selected for sampling to avoid border effects. Samples collected from two blocks were bulked together to constitute one field replicate. As a result, three field replicates were obtained from the 6 blocks. The cowpea was planted in rows 0.4 m wide with a 0.3 m plant spacing in May, 2007. The experimental area was maintained weed-free throughout the first month to reduce competition. The cowpea formed a tight canopy within a short period after planting which smothered weeds. Grains were harvested approximately three months after planting to represent wet season. The second planting was carried out in August and harvested in November 2007 to represent dry season. The haulms, comprising of the vine, leaves and roots were later uprooted, manually rolled and chopped into particles of 2–4 cm lengths and milled with a hammer mill (Model DFZH-Bühler AG, Uzwil, Switzerland) using a 3 mm sieve.

2.3. Chemical analyses

Feed samples were successively ground in mills with 3 and 1 mm sieves. Prior to milling, samples were oven-dried at 60 °C for 96 h while DM was determined by oven-drying at 100 °C for 24 h. Total nitrogen (N) was estimated by combustion

assay (LECO Instrument, Monchengladbach, Germany), CP was expressed as N × 6.25, ash (ID 942.05) and ether extract (EE) (ID 963.15) were analyzed according to the standard methods of AOAC (1990). Neutral detergent fibre (NDFom) was determined according to Van Soest et al. (1991) without use of α-amylase or sodium sulphite and expressed without residual ash. Acid detergent fibre (ADF) was analyzed according to (AOAC, 1997; method 973.18) and expressed exclusive residual ash. Lignin(sa) was determined by solubilisation of cellulose with sulphuric acid in the ADF residue (Van Soest et al., 1991). Non-fibre carbohydrates (NFC) were calculated as:

NFC = 1000 − CP − ash − EE–NDFom, with all variables expressed as g/kg DM. Short chain fatty acids concentrations were determined using a splitless injector Perkin-Elmer auto system gas chromatograph (Perkin-Elmer, Inc., Shelton, CN, USA).

2.4. In vitro gas production measurement

In vitro gas production was determined according to Menke and Steingass (1988). Rumen fluids were collected prior to feeding from three fistulated Merino sheep (≈70 kg body weight) fed a standard diet (600 g grass hay/600 g pelleted concentrate). Feeds were offered in two equal meals at 07:00 and 19:00 h. Rumen fluid was strained through two layers of cheesecloth into a prewarmed, insulated flask. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Samples (200 ± 2 mg) with and without PEG (200 ± 2 mg) of the air-dry haulms were accurately weighed into 100 ml glass syringe, the syringe pistons were lubricated with vaseline and inserted into the syringes. The inclusion of PEG was necessary because we had no data on the content of tannins especially the improved varieties. *In vitro* incubation of the samples was conducted in triplicate. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution as described by Menke and Steingass (1988) except that the concentration of $NaHCO_3$ was reduced to 33 g/l and that of $(NH_4)HCO_3$ increased to 6 g/l. Three blanks containing 30 ml of medium only were included. The syringes were placed in a rotor inside the incubator (39 °C) with about one rotation per min.

Cumulative gas volume measurements of samples were read manually from the three replicates each at 0, 4, 8, 12, 18, 24, 32, 48, 56, 72, 80 and 96 h of incubation. After subtraction of gas production from blank syringes, data were fitted to exponential model (Ørskov and McDonald, 1979) as:

$$y = B(1 - \exp -c \times [t - \text{lag}]),$$

where '*y*' is the cumulative volume of gas produced at time '*t*' (h), '*B*' the asymptotic gas volume, '*c*' the rate constant and 'lag' is the time (h) between inoculation and commencement of gas production.

Halftime of gas production ($t_{1/2}$) [*i.e.*, the time (h) when half of the asymptotic gas volume (*B*; ml) was produced] was calculated as:

$$t_{1/2} = \left(\ln \frac{2}{c}\right) + \text{lag}.$$

2.5. In vitro apparently (ivADDM) and truly (ivTDDM) degraded dry matter

After the initial 96 h gas run, substrate specific $t_{1/2}$ was calculated and a second incubation with the samples as substrates were conducted to obtain degradability measures at substrate-specific $t_{1/2}$ and 24 h for each substrate. Collection and handling of ruminal fluid was the same as that described for the 96 h incubations. Eight syringes were prepared for each substrate, providing four syringes for apparent and four for true degradability measurements (with and without PEG) in each incubation run. The incubations were terminated at $t_{1/2}$ and 24 h, and the volume of gas was recorded. The whole process was repeated to have four analytical replicates. True substrate degradability of diets (Van Soest et al., 1991) at $t_{1/2}$ and 24 h was measured by refluxing the incubation residue with ND solution (prepared without sodium sulphite) for 1 h with subsequent recovery of the truly undegraded substrate in Dacron fibre bags (4 cm × 12 cm, 40 μm pore size: Ankom, Macedon, NY, USA).

The ivTDDM coefficient was calculated as:

Feed (DM) incubated − residue (DM) recovered in the crucibles/Feed (DM) incubated.

In vitro apparent degraded DM was determined at $t_{1/2}$ and 24 h by high-speed centrifugation (20,000 × *g*) of incubation residues at 20 °C for 30 min (Blümmel and Lebzien, 2001) following placement in iced cubes (about −4 °C) to stop fermentation. Blanks were also centrifuged and residues weighed and used to correct for residues from the ruminal inoculum.

In vitro apparent degraded DM coefficient was calculated as:

Feed (DM) incubated − [pellet (DM) − blank pellet (DM)]/Feed (DM) incubated.

Partitioning factor (PF) was calculated as the ratio of mg of ivTDDM to ml of gas thereby produced.

2.6. In vitro microbial N analysis

Undegradable dietary N (UDN) *in vitro* was quantified by N determination in the truly undegraded $t_{1/2}$ and 24 h dry residues recovered in the fibre bags after ND solution treatment. In other words UDN was estimated as fibre bound N (NDFN) after incubation. Rumen degradable N (RDN) was calculated as: RDN = feed N − NDFN ($t_{1/2}$ or 24 h). *In vitro* microbial

N production (MN) was estimated directly by using the N content of the apparently degraded substrate after centrifugation (pellet N) and NDFN in diets at $t_{1/2}$ and 24 h, using the equation:

Microbial N production at $t_{1/2}$ (24 h) = pellet N at $t_{1/2}$ (24 h) − blank pellet N at 0 h incubation − NDFN at $t_{1/2}$ (24 h).

2.7. Estimation of the contents of utilizable crude protein at the duodenum (uCP)

For uCP determination (Lebzien and Voigt, 1999), a method was used which combined the Hohenheim feed test with the measurement of ammonia in the incubated fluid (Steingass et al., 2001). Samples were incubated in two runs at $t_{1/2}$ and 24 h. The gas production was recorded after $t_{1/2}$ and 24 h. Ammonia N was determined using steam distillation (Vapodest 50 s Carousel; Gerhardt, Königswinter, Germany). To alkalize the incubated fluid prior to distillation, 5 ml of 1 M sodium hydroxide solution was added. For estimating the uCP after $t_{1/2}$ and 24 h of incubation, the following formula was used:

$$\text{uCP(g/kgDM)} = \frac{[(\text{NB} + \text{NF} - \text{NI}) * 6.25]}{(\text{IW} * \text{DM})}$$

where NB (mmol/ml) = average ammonia N content of the blanks, NF (mg) = N content of the forage sample, NI (mmol/ml) = ammonia N content of the incubated fluid after $t_{1/2}$ or 24 h, IW (mg) = initial weight of the incubated sample and DM (g/kg).

2.8. Protozoa count and methane estimation

For methane (CH_4) estimation, 150 ± 2 mg (with and without PEG) of the samples were used. The rationale behind the reduced quantity of sample is to limit the volume of gas below 60 ml. After recording the final gas volume at the end of incubation ($t_{1/2}$ and 24 h), the lower end of the syringe was connected to the lower end of a pipette containing 4.0 ml of NaOH (10 M). The NaOH (10 M) was then introduced from the latter into the incubated contents, thereby avoiding gas escape. Mixing of the contents with the NaOH solution allowed for the absorption of CO_2, with the gas volume remaining in the syringe considered to be CH_4 (Demeyer et al., 1988). For protozoan enumeration, 1 ml of sample was added to 9 ml of 4% formaldehyde solution. Total protozoan count was made in 30 microscopic fields at a magnification of 100× using Fuchs-Rosenthal counting chamber (depth 0.2 mm, 0.0125 μl per grid).

2.9. Statistical analysis

Data were subjected to analysis of variance using the GLM procedure of SAS (2002) in a 2 × 2 × 2 factorial arrangement with 3 field replicates. The model used was:

$$Y_{ijklm} = \mu + G_i + H_j(\text{Gi}) + S_k + P_l + (\text{GS})_{ik} + (\text{GP})_{il} + (\text{SP})_{kl} + (\text{GSP})_{ikl} + \varepsilon_{ijklm}$$

where Y_{ijklm} = observation, μ = population mean, G_i = group effect (improved *versus* commercial) (i = 1–2), H_j(Gi) = haulms within group effect, S_k = season effect (k = 1–2), P_l = PEG effect (l = 1–2), $(\text{GS})_{ik}$ = interaction between group and season, $(\text{GP})_{il}$ = interaction between group and PEG, $(\text{SP})_{kl}$ = interaction between season and PEG, $(\text{GSP})_{ikl}$ = interaction between group, season and PEG; and ε_{ijklm} = residual error. After the initial analysis, PEG did not have any effect on the measured variables, so a reduced model was subsequently used for the statistical analyses.

$$Y_{ijk} = \mu + G_i + H_j(\text{Gi}) + S_k + (\text{GS})_{ik} + \varepsilon_{ijk}$$

All variables are already explained above. Means were then compared by applying the probability of difference (PDIFF) option of the least squares means statement in the GLM procedure. Differences among means with P<0.05 were accepted as representing statistically significant differences. Probability values less than 0.001 are expressed as 'P<0.001' rather than the actual value.

3. Results

3.1. Chemical composition

Group × season interactions were observed for CP, lignin(sa) and hemicellulose contents of the cowpea haulms. Crude protein content of the cowpea haulms was greater (P<0.001) in the commercial cowpea haulms and during the dry season (Table 1). The NDFom content though not significant (group × season) was greater (P<0.05) in the improved than commercial haulms with values of 612 *vs.* 569 and 403 *vs.* 379 g/kg DM during the wet and dry seasons, respectively. Despite their high fibre contents, haulms from both cowpea varieties contained significant amounts of NFC.

Table 1
Chemical composition (g/kg DM unless stated) of the cowpea haulms.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
DM[b](g/kg)	948	942	933	931	2.3	0.114	0.241	<0.001	0.409
CP[c]	181b	147c	217a	212a	4.0	<0.001	0.633	<0.001	0.002
EE[d]	18.9	16.3	42.2	28.6	3.34	0.023	0.577	<0.001	0.116
Ash	94.3	30.5	71.4	30.5	10.35	<0.001	0.233	0.281	0.279
NDFom[e]	569	612	379	403	14.9	0.033	0.244	<0.001	0.546
ADFom[f]	399	419	208	233	15.1	0.155	0.192	<0.001	0.835
Lignin(sa)	206a	162b	107c	113c	8.1	0.028	0.011	<0.001	0.003
Hemicellulose	169b	193a	171b	170b	5.4	0.041	0.604	0.045	0.030
Cellulose	194	257	101	120	11.9	0.002	0.330	<0.001	0.072
NFC[g]	136	193	289	325	12.7	<0.001	0.088	<0.001	0.405

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.
[b] DM, dry matter.
[c] CP, crude protein.
[d] EE, ether extract.
[e] NDFom, neutral detergent fibre expressed exclusive residual ash.
[f] ADFom, acid detergent fibre expressed exclusive residual ash.
[g] NFC, non-fibre cabohydrates.

3.2. *In vitro* gas production characteristics

Gas production profiles of the cowpea haulms at wet and dry seasons are presented in Fig. 1. There were differences in the asymptotic (b) gas production of the cowpea haulms with greater values recorded for the commercial cowpea haulms harvested during the dry season. Substrate specific $t_{1/2}$ was on the average shorter during the dry season, being 8.19 and 8.26 h and for wet season, 10.3 and 13.3 h for commercial and improved haulms, respectively.

3.3. *In vitro* dry matter digestibility, partitioning factor and protozoa population

In vitro apparently degraded DM, ivTDDM, microbial mass, protozoa population, methane and partitioning factor of the haulms at substrate specific $t_{1/2}$ are presented in Table 2. Interactions between group and season were observed for microbial mass, methane and PF of the commercial and improved cowpea haulms. Commercial cowpea haulms had greater (P=0.003) microbial mass while the improved cowpea haulms produced more (P=0.002) methane. Both ivADDM and ivTDDM coefficients were greater (P>0.05) in the improved than commercial haulms. Greater ivADDM and ivTDDM coefficients were recorded during the dry season. The PF values of the haulms at substrate specific $t_{1/2}$ ranged from 2.49 to 4.36 with greater (P<0.001) values for commercial cowpea haulms. After 24 h incubation, group × season interactions were also observed for ivADDM, microbial mass, methane and protozoa population of the haulms (Table 3). Microbial mass, methane and PF at 24 h followed similar trend observed at substrate specific $t_{1/2}$. Improved cowpea haulms had greater ivADDM coefficient and protozoa population.

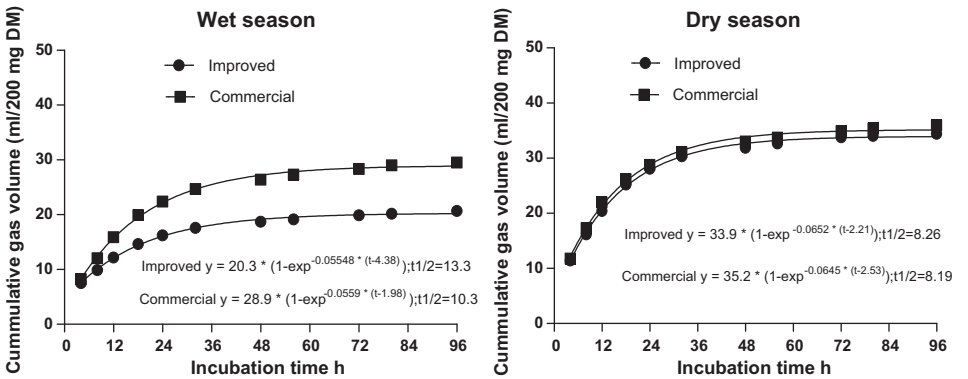

Fig. 1. *In vitro* gas production profiles of the cowpea haulms at wet and dry seasons. Gas production profiles have been fitted to curves using the equation ($y = B^*(1 - \exp - c^*[t - \text{lag}])$) with time to half maximal gas production ($t_{1/2}$) expressed in hours. Standard errors for maximal gas production and rate of gas production for wet season were 0.18, 0.0031; 0.29, 0.0032; for dry season, 0.23, 0.0029; 0.31, 0.0036 for improved and commercial haulms, respectively.

Table 2
Half time ($t_{1/2}$) *in vitro* apparent and true dry matter degradability coefficients, microbial mass (g/kg DM), efficiency of microbial production (partition factor) (mg/ml), methane (g/kg dry matter (DM)) and protozoa population (10^6/ml) of the cowpea haulms.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
IVADDM[b]	0.177	0.254	0.323	0.359	0.0126	<0.001	0.034	<0.001	0.106
IVTDDM[c]	0.469	0.492	0.652	0.679	0.0137	0.076	0.374	<0.001	0.833
Microbial mass	316a	226b	310a	302a	13.6	<0.001	0.111	0.016	0.003
Protozoa population	1.39	1.39	1.29	1.62	0.085	0.052	0.324	0.457	0.061
Methane	12.73b	16.40a	16.13a	15.53a	0.097	0.022	<0.001	0.059	0.002
PF[d]	3.98ab	3.09c	4.36a	3.39bc	0.320	<0.001	<0.001	0.002	<0.001

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.
[b] IVADDM, *in vitro* apparently degraded dry matter.
[c] IVTDDM, *in vitro* truly degraded dry matter.
[d] PF, partioning factor.

Table 3
Twenty-four hour (24 h) *in vitro* apparent and true dry matter degradability coefficients, microbial mass (g/kg DM), efficiency of microbial production (PF) (mg/ml), methane (g/kg dry matter (DM)) and protozoa population (10^6/ml) of the cowpea haulms.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
IVADDM[b]	0.209c	0.347b	0.431a	0.446a	0.0143	<0.001	<0.001	<0.001	<0.001
IVTDDM[c]	0.502	0.569	0.750	0.767	0.0167	0.013	0.062	<0.001	0.132
Microbial mass	275a	209b	300a	303a	9.7	0.002	0.006	<0.001	<0.001
Protozoa population	1.07ab	1.26a	1.13ab	0.95b	0.069	0.952	0.004	0.073	0.010
Methane	18.46c	27.20b	31.90a	31.80a	0.126	<0.001	<0.001	<0.001	<0.001
PF[d]	2.98	2.79	3.97	3.16	0.145	0.229	0.521	<0.001	0.217

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.
[b] IVADDM, *in vitro* apparently degraded dry matter.
[c] IVTDDM, *in vitro* truly degraded dry matter.
[d] PF, partioning factor.

3.4. *In vitro feed N partitioning, microbial N and utilizable CP at the duodenum*

In vitro N partitioning of the cowpea haulms, microbial N and uCP at substrate specific $t_{1/2}$ and 24 h are shown in Tables 4 and 5, respectively. There were strong interactions (P<0.001) between group and season for the N content, NDFN and RDN contents of the haulms at both time periods. At both substrate specific $t_{1/2}$ and 24 h, the N compounds of the commercial haulms were degraded more (P<0.001) in the rumen than the improved varieties. The improved cowpea haulms supplied greater (P<0.001) uCP at both times. The amount of uCP was consistently greater during the dry season and it decreased with increasing incubation time as more time was available for CP to be degraded in ruminal fluid.

3.5. *Short chain fatty acid production*

Interactions between group and season were observed for the iso- and n-valerate contents of the haulms at substrate specific $t_{1/2}$ (Table 6) and for acetate and total SCFA contents of the haulms at 24 h (Table 7). On the average, improved

Table 4
Half time ($t_{1/2}$) microbial nitrogen, utilizable crude protein at the duodenum (uCP), ammonia N (NH_3-N), neutral detergent fibre-bound N (NDFN) and rumen degraded N (RDN).

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
Sample N (mg)	2.89b	2.35c	3.47a	3.39a	0.036	<0.001	0.101	<0.001	<0.001
Microbial N (mg)	1.88a	1.21b	1.79a	1.76a	0.130	0.007	0.337	0.076	0.015
uCP (g/kg DM)	83.51	94.61	102.18	110.16	1.202	<0.001	<0.001	<0.001	0.196
NH_3-N (mmol/ml)	0.36	0.33	0.35	0.31	0.003	<0.001	<0.001	<0.001	0.718
NDFN (mg)	0.21a	0.15c	0.19b	0.16c	0.004	<0.001	<0.001	0.135	<0.001
RDN (mg)	2.66b	2.21c	3.22a	3.21a	0.025	<0.001	0.002	<0.001	<0.001

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.

Table 5
Twenty-four hour (24 h) microbial nitrogen, utilizable crude protein at the duodenum (uCP), ammonia N (NH_3-N), neutral detergent fibre-bound N (NDFN) and rumen degraded N (RDN).

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
Sample N (mg)	2.89b	2.35c	3.47a	3.39a	0.036	<0.001	0.101	<0.001	<0.001
Microbial N (mg)	1.62	1.64	2.12	2.02	0.098	0.675	0.481	<0.001	0.529
uCP (g/kg DM)	71.81	88.33	78.03	94.02	1.628	<0.001	<0.001	<0.001	0.871
NH_3-N (mmol/ml)	0.42	0.38	0.44	0.39	0.006	<0.001	0.038	<0.001	0.393
NDFN (mg)	0.19a	0.12b	0.12b	0.11b	0.005	<0.001	0.005	<0.001	<0.001
RDN (mg)	2.69b	2.25c	3.29a	3.26a	0.03	<0.001	<0.001	<0.001	<0.001

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.

Table 6
Half time ($t_{1/2}$) concentrations of short-chain fatty acids (SCFA, mmol/l) of cowpea haulms during incubation.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
Acetate	26.57	28.11	30.30	30.79	0.429	0.021	<0.001	<0.001	0.228
Propionate	6.50	6.66	7.37	7.63	0.138	0.139	<0.001	<0.001	0.722
Iso-butyrate	0.46	0.45	0.49	0.44	0.022	0.186	0.002	0.576	0.403
n-Butyrate	5.21	5.06	3.94	3.63	0.189	0.229	<0.001	<0.001	0.671
Iso-valerate	0.97a	0.79b	0.75bc	0.67c	0.024	<0.001	<0.001	<0.001	0.036
n-Valerate	0.48a	0.39c	0.44b	0.43b	0.011	<0.001	<0.001	0.774	<0.001
Total SCFA	40.21	41.46	43.3	43.6	0.539	0.153	0.023	<0.001	0.381
Acetate: propionate (mol/mol)	4.12	4.22	4.13	4.08	0.050	0.606	<0.001	0.183	0.164

Means with different letters within rows differ (*P*<0.05).
[a] H(Group), haulms within group.

cowpea haulms had greater SCFA concentration than commercial haulms. The SCFA concentration increased with increasing incubation time with greater values at 24 h than at substrate specific $t_{1/2}$. Acetate to propionate ratios ranged from 4.08 to 4.22 at substrate specific $t_{1/2}$ and from 3.79 to 4.03 after 24 h of incubation. Although not significant (P>0.05), acetate to propionate ratios were numerically greater for improved cowpea haulms at both time intervals.

4. Discussion

4.1. Chemical composition

The chemical composition of the cowpea haulms showed substantial variations across group and season. Dry matter and CP contents of the commercial cowpea haulms were greater compared to improved haulms. The CP levels in the current study are above the range of 110–130 g/kg DM which is adequate for maintenance and growth of small ruminants (NRC, 1985). This implied that the cowpea haulms especially the commercial haulms can be used as CP supplement to poor quality grasses during the dry season.

Fibre concentrations were greater in improved than commercial cowpea haulms. Despite the greater fibre concentration in improved haulms, significant proportion was in form of NFC and cellulose which are easily degraded unlike the commercial haulms which had greater lignin concentration.

Table 7
Twenty-four hour (24 h) concentrations of short-chain fatty acids (SCFA, mmol/l) of cowpea haulms during incubation.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H (Group)[a]	Season	$G \times S$
Acetate	29.71c	34.19b	37.36a	38.27a	0.442	<0.001	<0.001	<0.001	<0.001
Propionate	7.57	8.53	9.73	10.20	0.170	<0.001	<0.001	<0.001	0.164
Iso-butyrate	0.58	0.57	0.82	0.73	0.028	0.126	0.002	<0.001	0.149
n-Butyrate	6.18	6.45	5.11	4.85	0.268	0.989	<0.001	<0.001	0.336
Iso-valerate	1.32	1.23	1.43	1.23	0.043	<0.001	<0.001	0.227	0.157
n-Valerate	0.61	0.59	0.75	0.70	0.019	0.103	0.045	<0.001	0.461
Total	45.99c	51.57b	55.19a	55.99a	0.618	<0.001	<0.001	<0.001	<0.001
Acetate: propionate (mol/mol)	3.95	4.03	3.85	3.79	0.051	0.821	<0.001	<0.001	0.150

Means with different letters within rows differ (P<0.05).
[a] H(Group), haulms within group.

Higher NFC contents of improved cowpea haulms indicate that they should stimulate ammonia-N utilization in the rumen better than the commercial varieties (Tylutki et al., 2008). As N utilization by rumen microorganisms is related to the amount of available fermentable energy, the NFC in the cowpea haulms could improve the efficiency of microbial protein synthesis by promoting better utilization of rumen ammonia released from feeds with high content of rumen degradable CP (Cabrita et al., 2006).

4.2. In vitro DM digestibility, microbial efficiency and protozoa population

The observed differences in the ivADDM and ivTDDM of the cowpea haulms may be due to the differences in their content of potentially digestible materials. On the average, DM of the improved varieties of the cowpea haulms was degraded more *in vitro* at both time intervals than the commercial haulms. Greater lignin(sa) concentration observed in commercial haulms may have interfered with the DM digestibility by limiting the surface area for microbial attachment. The values observed for both apparently and truly degraded DM were about 50% greater in the dry than wet season. Expectedly, more DM was degraded at 24 h than at substrate specific $t_{1/2}$.

Greater DM degradability of the improved cowpea haulms did not translate to greater microbial mass as commercial cowpea haulms were able to partitioning more energy to microbial mass. This was also supported by greater PF values for commercial than improved haulms. Microbial biomass production estimated at substrate specific $t_{1/2}$ was on the average greater than when estimated at 24 h. The trend observed in ivADDM and ivTDDM was also noticed in microbial biomass production as values from haulms harvested during the dry season were greater than those harvested during the wet season. The achievement of maximum ruminal feed conversion into microbial biomass is a widely accepted concept of ruminant nutrition because high microbial efficiency improves microbial protein supply to the small intestine and, proportionally, reduces fermentative gaseous carbon losses (Beever, 1993).

At both time intervals, improved cowpea haulms had greater protozoa population than commercial haulms. Expectedly, improved cowpea haulms produced more methane at both time intervals. Greater fibre content of improved cowpea haulms may have contributed to its higher methane volume. Greater methane values obtained at 24 h than at substrate specific $t_{1/2}$ was expected because of the extended period of time which will increase the amount of substrate degraded. Methane production was greater during the dry season at both time intervals. Methane is an estimate of the relative energy loss during fermentation.

The main component affecting methane production is the type of carbohydrate and relative rate of fermentation. Johnson and Johnson (1995) showed that there was decreased methane production with increased energy intake, when expressed relative to gross energy. Van Soest (1994) indicated that a high grain diet and/or the little addition of soluble carbohydrate with resulting shift in the fermentation pattern in the rumen are associated with hostile environment for methanogens in which passage rates are increased, ruminal pH is lowered and certain population of protozoa, ruminal ciliates and methanogens may be eliminated or inhibited. This was not the case with the cowpea haulms especially the improved haulms which had greater fibre concentration.

The PF of the cowpea haulms (3.09–4.36) at substrate specific $t_{1/2}$ and (2.79–3.97) after 24 h were within the theoretical range of 2.75–4.41 for feedstuffs, reflecting adenosine triphosphate (Y_{ATP}) of 10–32 mg, and Y_{ATP} of 32 mg is considered to be maximum microbial efficiency (Blümmel et al., 1997a). This implied that the haulms produced enough ATP for microbial growth. Greater PF values for commercial haulms were reflected in their greater microbial mass compared with improved haulms. The concept of the PF value is based on the stoichiometrical relationship between SCFA and gas volumes and on the fact that well-defined amounts of substrate in terms of carbon (C), hydrogen (H) and oxygen (O) are needed for the formation of SCFA and fermentative CO_2, CH_4 and H_2O (Blümmel et al., 1997b).

4.3. In vitro feed N partitioning, microbial N and utilizable CP in the duodenum

The N compounds of commercial cowpea haulms were more degradable in the rumen than that of the improved cowpea haulms which resulted in greater ammonia N concentrations observed in the former at both time intervals. Maximizing the utilization of rumen degraded N and its conversion into microbial protein is a key objective of protein feeding strategies. The RDN at substrate specific $t_{1/2}$ and 24 h were similar. While the rumen presents advantages, particularly when animals are offered low quality feeds, it can be a major cause of inefficiency of N utilization in ruminants. Determination of microbial CP degradability is thus important in formulating a sound supplementation strategy for efficient utilization of basal as well as supplementary diet components (Singh et al., 2005).

The microbial N content observed with the cowpea haulms followed the same trend observed in the ammonia N. On the average, commercial haulms had greater microbial N values than the improved varieties. Over half of the amino acids absorbed by ruminants, and often two-third to three-quarters, are derived from microbial protein (Agricultural and Food Research Council, 1992). The greater N content of commercial cowpea haulms may have contributed to greater ammonia N and microbial N concentrations for these haulms. The improved variety of the cowpea haulms had greater uCP values than their commercial counterpart. The greater uCP values for improved cowpea haulms may be due to the fact that they were less degraded in the rumen and thereby were able to deliver moderate amounts of ruminally undegraded CP to the small intestine.

4.4. Short chain fatty acids production

The amount of SCFA was about 10 units lower at substrate specific $t_{1/2}$ compared with 24 h but the relative proportions of individual SCFA were similar at both time intervals. The fact that improved cowpea haulms had greater SCFA concentration than the commercial haulms confirmed previous report that microbial mass and SCFA are inversely related (Hungate, 1966). The cowpea haulms tended to produce greater proportion of acetate with the ratio of acetate to propionate ranging from 3.79 to 4.22. The acetate to propionate ratio was marginally lower in the dry season.

5. Conclusions

Although the cowpea haulms are fibrous materials, the results of the *in vitro* degradability study showed they were effectively utilized by the rumen microbes which will result in the supply of energy and amino acids to the host. On the basis of CP content, microbial mass and PF, the commercial cowpea haulms varieties performed better while on the basis of SCFA production and uCP, improved varieties of the cowpea haulms were better. In conclusion, both improved and commercial haulms can be effectively utilized as supplements to low quality forages or as a basal diet for lactating dairy cattle during the dry season.

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