

# Chemical composition, rumen degradability and crude protein fractionation of some commercial and improved cowpea (*Vigna unguiculata* L. Walp) haulm varieties

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## Abstract

Seasonal chemical composition, *in sacco* organic matter (OM) and crude protein (CP) degradabilities and CP fractions of haulms of three improved (ITA2, ITA6 and ITA8) and three commercial (Oloyin, Peu and Sokoto) cowpea varieties harvested in wet and dry seasons were evaluated in a 2 × 2 factorial arrangement of treatments. Effective degradation of OM and CP was estimated at assumed outflow rates of 2 and 4% h<sup>-1</sup>. Commercial haulms (all the other parts of the cowpea minus the grains) had greater ( $P < 0.001$ ) CP than improved varieties, whereas neutral detergent fibre and acid detergent fibre were greater ( $P < 0.001$ ) in improved vs. commercial haulms. Interactions between variety group (improved vs. commercial) and season were observed for CP ( $P = 0.002$ ), lignin ( $P = 0.003$ ) and hemicellulose ( $P = 0.030$ ) contents of the haulms. Similarly, a group × season interaction was observed for effective degradation of OM at an outflow rate of 2%. The proportion of substrate degraded in the samples harvested in the wet season was generally less ( $P < 0.001$ ) than in the dry season. Effective degradability values of OM at the assumed passage rates were greater ( $P < 0.001$ ) for improved vs. commercial cowpea haulms. Interactions between group and season were observed for all but one of the CP fractions.

Seasonal differences in the quality of haulms showed that attention must be given to handling of haulms to minimize the amount of leaves lost during the wet season.

**Keywords:** legume, protein value, ruminant production, season, substrate degradability, tropics

## Introduction

A limited feed supply is the greatest impediment to improved livestock production in the sub-Saharan African (SSA) countries (Agyemang, 2002). With a prolonged annual dry season, feed supplies vary as a result of shortages in the quantity and quality of forage from natural pastures that provide most of the feed for animals. The role of livestock in providing protein vital for human nutrition is increasingly being recognized. The low level of productivity in the livestock industry in SSA is exacerbated by a combination of poor husbandry and animal health issues. Of immediate concern, therefore, in any development programme for improved livestock productivity, is the need for improved management, especially nutrition.

Expanding crop production activities and decreases in fallow periods in most of the SSA countries are already leading to competition between crop and livestock production. Land fallowing, a traditional agricultural system that helps to restore land fertility, is likely to disappear in the next 50 years (Thornton *et al.*, 2002). As much of the arable lands in these countries are already under cultivation, increased livestock productivity will need to come from improving the productivity per unit area or by expansion of marginal lands that

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have traditionally supplied a grazing resource to livestock (Delgado *et al.*, 1999).

In contrast to the situation in the rainy season, when there are abundant forages with fairly high nutrient quality, researchers and farmers in SSA countries are faced with the challenge of providing adequate feed for ruminant animals during the dry season. As indicated by the FAO (1997) report, large increases in animal production could be achieved by alterations to the feed base. According to that report, animal production could be increased about five times above the present level by providing the critical nutrients that are deficient in animal diets and by balancing the available nutrients closer to requirements.

Animal productivity could be increased by the introduction of low-cost technologies that would improve the current systems of management. Acceptable and successful feeding systems are described as those that are simple, practical, consistently reproducible and within the limits of the farmer's resources (Douthwaite, 2002). This approach underscores the importance of dual-purpose crops that provide food (grain) and feed (residues) in meeting household needs under the current and foreseeable future scenarios (Delgado *et al.*, 1999).

Cowpea (*Vigna unguiculata* L. Walp) 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, 2009). Going beyond its importance for food and feed, cowpea can be regarded as a fulcrum of sustainable farming in regions characterized by farming systems that make limited use of purchased inputs (Anele *et al.*, 2010). Cowpea is grown extensively in sixteen African countries, with the continent producing more than three-quarters of the world total. Two countries – Nigeria and Niger – produce 2 916 000 and 1 569 300 tonnes annually, representing approximately 75% of the world crop (FAO, 2009). The bulk of this production comes from smallholder farmers in semiarid zones of the region.

The present study investigated the chemical composition, *in sacco* degradation of major nutrients and crude protein (CP) fractions of three improved and commercial varieties of cowpea haulms during wet and dry seasons.

## Materials and methods

### Experimental site

The field experiment was conducted at the Teaching and Research Farm, University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria. The *in situ* incubations and laboratory analyses were carried out at the

Institute of Animal Science University of Bonn, Germany. The experimental site lies within the savanna agro-ecological zone of south-western Nigeria (latitude: 7°N, longitude 3.5°E, average annual rainfall: 1037 mm). The soil is loamy sand and slightly acidic with pH ranging between 5.40 and 5.45. The soil is low in organic C and total N. Abeokuta has a bimodal rainfall pattern that typically peaks in July and September with a break of 2–3 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, and the lowest night temperature of around 24°C during the harmattan wind period between December and February. Relative humidity is high during the rainy season with values between 63 and 96% compared with dry season values of 55–84%. The temperature of the soil ranges from 24.5 to 31.0°C (Source: Agrometeorology Department, UNAAB).

### Forage establishment and management

The experimental area, measuring 2600 m<sup>2</sup>, was ploughed twice and harrowed. The area was divided into eight blocks, and each block was subdivided into 10 plots that each measured 5 × 4 m<sup>2</sup>. Three improved (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 ('Oloyin', 'Peu', 'Sokoto') dual-purpose cowpea varieties constituted the treatments. The dual-purpose cowpea varieties were semi-erect types and had days to pod maturity of 70–86 d. The improved varieties (obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria) were bred for greater agronomic (biomass and grain) yield.

In both seasons, treatments were allocated randomly to plots within each block. The inner six blocks and plots (thirty-six plots) were selected for sampling to avoid a border effect. Samples collected from two blocks were bulked together and constituted one field replicate. As a result, three field replicates were obtained from the six blocks. The cowpea was planted in rows 0.4 m wide with a 0.3 m plant spacing in May 2007 for the wet season, and the second planting was carried out in August 2007 for the dry season. Wet and dry season plants were harvested in August and November, respectively. The experimental area was maintained weed-free throughout the first month to decrease competition. The cowpea formed a tight canopy within a short period after planting, which smothered weeds. Grain was harvested approximately 3 months after planting. The haulms, comprising the vine, leaves and roots, were later uprooted, manually rolled and chopped into particles of 2- to 4-cm lengths and milled with a hammer mill (Model DFZH, Bühler AG, Uzwil, Switzerland) using a 3-mm sieve. Because of rainfall,

several leaves were lost in the process of drying haulms harvested during the wet season. There was no fertilizer application in either seasons. Details of the agronomic part of this study are described elsewhere (Anele *et al.*, 2011b).

### Animals and diet

Three 12-year-old German Red Pied steers, with a mean live weight of 1300 kg, were used in the experiment. Each steer was fitted with a 10-cm internal diameter ruminal cannula (Model 1C; Bar Diamond, Parma, ID, USA) and housed indoors in a temperature-controlled stall (18°C). The steers received a mixed diet consisting of 6 kg maize silage, 2 kg hay and 2 kg mixed concentrates. Animals were fed the diets to meet their maintenance energy requirements (Agricultural Research Council, 1980). The daily allotment of feed was offered in two equal meals at 07:00 and 19:00 h. The steers had continuous access to water during the experiment. Before the experiment began, a period of 2 weeks was allowed for dietary adaptation.

### In situ procedure

Samples of milled haulms (about 5 g) were weighed into 10- × 20-cm polyester bags (R510, Ankom Technology, Macedon, NY, US) with a pore size  $50 \pm 15 \mu\text{m}$ . Duplicate samples of each variety were incubated in the rumen of the three steers. The bags were tied to an 800 g cylindrical plastic weight with 20 cm cable binders. All bags were inserted into the ventral sac of the rumen at 07:00 h, immediately before the morning feeding. Incubation periods were 6, 12, 24, 48, 72, 96 and 336 h. At the end of each incubation period, bags were removed and immersed in ice water to stop further microbial activity and then washed for 30 min on a cold rinse cycle in a washing machine. Zero time disappearance values (0 h) were obtained by washing pre-soaked, non-incubated bags in a similar fashion.

Water-soluble material (WS) was estimated by washing duplicate samples through a folded filter paper (No. 595<sup>1/2</sup>, Schleicher and Schuell, Dassel, Germany). All washed bags and filter paper residues were freeze dried. Water-insoluble OM and CP escaping in small particles (SP) from the bags during washing were estimated by subtracting water-soluble OM and CP from 0-h values. The single values obtained for CP disappearance (DI<sub>i</sub>) were then corrected (C) for SP by the equation (Weisbjerg *et al.*, 1990):

$$\text{CDI}_i = \text{DI}_i - \text{SP} \\ \times (1 - ((\text{DI}_i - (\text{SP} + \text{WS})) / (1 - (\text{SP} + \text{WS}))))$$

Degradation of OM and CP (CDEG) was calculated using the equation of McDonald (1981):

$$\text{CDEG} = a + b(1 - e^{-c(t-L)}) \text{ for } t > L,$$

where CDEG = disappearance at time  $t$  corrected for SP,  $a$  = an intercept representing the proportion of OM and CP solubilized at initiation of incubation (time 0; soluble fraction),  $b$  = the fraction of OM and CP insoluble but degradable in the rumen,  $c$  = a rate constant of disappearance of fraction  $b$ ,  $t$  = time of incubation and  $L$  = lag phase. The non-linear parameters  $a$ ,  $b$ ,  $c$  and  $L$  were estimated by an iterative least squares procedure (SAS<sup>®</sup>, 2002). The effective degradability (ED) of OM and CP was calculated using the following equation (McDonald, 1981) with the modification of Wulf and Südekum (2005), which assumes no degradation occurs during the lag phase:

$$\text{ED} = a + (bc/(c+k)) \times e^{-kL},$$

where  $k$  is the estimated rate of outflow from the rumen, and  $a$ ,  $b$ ,  $c$  and  $L$  are the same parameters described previously. The ED of OM and CP was estimated as ED<sub>2</sub> and ED<sub>4</sub> assuming rumen solid outflow rates of 2 and 4% h<sup>-1</sup>, which is representative for low and medium/high passage rates typical for forages. The *in sacco* ruminally undegraded fraction was estimated by subtracting the ED from 100 (100 - ED).

Correction for microbial attachment ( $A$ ) (% residue N) to undegraded particles was carried out according to Krawielitzki *et al.* (2006) using the exponential equation:

$$A = A_{\text{max}}(1 - e^{-Ct}),$$

where  $A_{\text{max}}$  is the maximum extent of bacterial contamination at  $t \approx \infty$ ,  $C$  is the rate of contamination (% h<sup>-1</sup>), and  $t$  denotes the incubation time (h).  $A_{\text{max}}$  was estimated by treating a subsample of the residue with neutral detergent solution (NDS) ( $t > 16$  h) with the assumption that the residues only contained cell wall-bound protein and microbial matter was neutral detergent soluble. Duplicates of 0.5 g were boiled for 1 h in NDS, rinsed thoroughly with distilled water, reweighed and analysed for CP. The difference in CP between pre- and post-NDS-treated residues was taken as microbial CP. The mean from the 24, 48, 72, 96 and 336 h residues was used as the  $A_{\text{max}}$  parameter. The rate of microbial attachment ( $C$ ) was calculated as:

$$C(\% \text{ h}^{-1}) = 13 \cdot 3 + 0 \cdot 09 \text{ NDF} - 0 \cdot 35 \text{ CP} \text{ (Krawielitzki } et al., 2006).$$

### Chemical analyses

Feed samples were successively ground in mills with 3- and 1-mm sieves to determine the chemical composition. Before milling, samples were oven-dried at 60°C for 96 h (the longer time was allowed for the cowpea roots), whereas dry matter (DM) was determined by oven-drying at 100°C for 24 h. Total nitrogen (N) was

estimated by combustion assay (LECO Instrumente, Mönchengladbach, Germany), CP was calculated as  $N \times 6.25$ , and ash (ID 942.05) and ether extract (EE) (ID 963.15) were analysed according to the standard methods of AOAC (1990). Neutral detergent fibre (NDFom) was determined according to Van Soest *et al.* (1991) without use of  $\alpha$ -amylase or sodium sulphite and expressed without residual ash. Acid detergent fibre (ADFom) was analysed according to AOAC (1997; method 973.18) and expressed exclusive of residual ash. Lignin was determined by solubilization of cellulose with sulphuric acid in the ADF residue (Lignin(sa); Van Soest *et al.*, 1991). Non-fibre carbohydrates (NFC) were calculated as:  $NFC = 1000 - CP - Ash - EE - ND-Fom$ , hemicellulose as:  $ND-Fom - ADFom$  and cellulose as:  $ADFom - lignin(sa)$ , with all variables expressed as  $g\ kg^{-1}\ DM$ .

### Fractionation of CP

Protein fractions of samples were partitioned into five fractions according to the Cornell Net Carbohydrate and Protein System (Sniffen *et al.*, 1992) modified according to Licitra *et al.* (1996). These are fraction A, non-protein nitrogen, estimated as the difference between total nitrogen and true CP nitrogen precipitated with sodium tungstate (0.30 M) and 0.5 M sulphuric acid; fraction B<sub>1</sub>, buffer soluble protein, calculated as the difference between true protein and buffer-insoluble protein, estimated with borate-phosphate buffer (pH 6.7–6.8) and freshly prepared 10% sodium azide solution. Fraction B<sub>2</sub>, neutral detergent soluble protein, was estimated as buffer-insoluble protein minus ND insoluble protein, whereas fraction B<sub>3</sub>, acid detergent soluble CP, was estimated as the difference between ND insoluble protein and acid detergent insoluble CP. Fraction C is assumed to be indigestible. All fractions, including CP, were analysed in triplicate and the N content determined by Kjeldahl (4.1.) according to the German Handbook of Agricultural Experimental and Analytical Methods (VDLUFA, 2007).

### Statistical analyses

Data were subjected to analysis of variance using the GLM procedure of SAS<sup>®</sup> (2002) in a  $2 \times 2$  factorial arrangement with three field replicates. The model used was:

$$Y_{ijklm} = \mu + G_i + H_j(G_i) + S_k + (GS)_{ik} + \epsilon_{ijklm}$$

Where:  $Y_{ijklm}$  = observation,  $\mu$  = population mean,  $G_i$  = group effect (improved vs. commercial) ( $i = 1-2$ ),  $H_j(G_i)$  = haulms within group effect,  $S_k$  = season effect ( $k = 1-2$ ),  $(GS)_{ik}$  = interaction between group and season and  $\epsilon_{ijklm}$  = residual error.

Means were then compared by applying the probability of difference 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  $< 0.001$  are expressed as  $P < 0.001$ . A stepwise regression analysis was used to establish regression models for predicting effective degradation of OM from chemical components at an assumed outflow rate of 2%.

## Results

### Chemical composition

Haulms of commercial varieties had greater ( $P < 0.001$ ) CP than those of improved varieties (Table 1). Likewise, the NDFom and ADFom contents were greater ( $P < 0.001$ ) in improved vs. commercial haulms. Despite their high fibre contents, haulms from both groups of cowpea varieties contained substantial amounts of NFC which were higher ( $P < 0.001$ ) in improved haulm varieties. Crude protein and NFC were greater ( $P < 0.001$ ) in the dry vs. wet season. Lower ( $P < 0.001$ ) fibre contents were observed in the dry season. Group  $\times$  season interactions were observed for CP, lignin and hemicellulose contents of the cowpea haulms.

### Non-linear parameter estimates and effective degradability values

Non-linear parameter estimates and effective degradability values of OM and CP of the cowpea haulms are shown in Tables 2 and 3, respectively. Significant group  $\times$  season interactions were observed ( $P < 0.05$ ) for the insoluble but degradable fraction 'b' and effective degradation at an outflow rate of 2% for the OM. The proportion of substrate degraded in the samples harvested in the wet season was generally less ( $P < 0.001$ ) than those harvested in the dry season. More ( $P < 0.05$ ) OM was degraded in improved cowpea haulms than in commercial varieties. The effective degradability values of the OM at the two assumed passage rates were greater ( $P < 0.001$ ) for improved than for commercial cowpea haulms. Significant group  $\times$  season interaction was only observed ( $P < 0.05$ ) for the soluble fraction 'a' for the CP of the haulms. As observed in OM degradation, greater ( $P < 0.001$ ) proportions were degraded in the samples harvested in the dry season, but more CP was degraded in commercial than in improved haulms. No lag time was observed in the degradation of CP. The effective degradability values of the CP at the two assumed passage rates were similar for the two haulm groups.

**Table 1** Chemical composition (g kg<sup>-1</sup> dry matter (DM) unless stated) of the cowpea haulms.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H(Group)	Season	G × S
DM (g kg <sup>-1</sup> )	948	942	933	931	2.3	ns	ns	***	ns
CP	181 <sup>b</sup>	147 <sup>c</sup>	217 <sup>a</sup>	212 <sup>a</sup>	4.0	***	ns	***	**
EE	18.9	16.3	42.2	28.6	3.34	*	ns	***	ns
Ash	94.3	30.5	71.4	30.5	10.35	***	ns	ns	ns
NDFom	569	612	379	403	14.9	*	ns	***	ns
ADFom	399	419	208	233	15.1	ns	ns	***	ns
Lignin(sa)	206 <sup>a</sup>	162 <sup>b</sup>	107 <sup>c</sup>	113 <sup>c</sup>	8.1	*	*	***	**
Hemicellulose	169 <sup>b</sup>	193 <sup>a</sup>	171 <sup>b</sup>	170 <sup>b</sup>	5.4	*	ns	*	*
Cellulose	194	257	101	120	11.9	**	ns	***	ns
NFC	136	193	289	325	12.7	***	ns	***	ns

Means with different letters within rows differ ( $P < 0.05$ ).

H(Group), haulms within group; CP, crude protein; EE, ether extract; NDFom, neutral detergent fibre expressed exclusive residual ash; ADFom, acid detergent fibre expressed exclusive residual ash; Lignin(sa), lignin was determined by solubilization of cellulose with sulphuric acid in the ADF residue; NFC, non-fibre carbohydrates; ns, non-significant.

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Table 2** Non-linear estimates and effective degradability coefficients of organic matter of the cowpea haulms.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H(Group)	Season	G × S
<i>a</i>	0.02	0.04	0.13	0.17	0.007	***	ns	***	ns
<i>b</i>	0.30 <sup>c</sup>	0.37 <sup>b</sup>	0.56 <sup>a</sup>	0.56 <sup>a</sup>	0.018	*	ns	***	*
<i>c</i>	0.019	0.026	0.056	0.053	0.0029	ns	ns	***	ns
lag	2.25	1.78	0.01	0.62	0.536	ns	ns	**	ns
ED (2%) <sup>†</sup>	0.16 <sup>c</sup>	0.23 <sup>b</sup>	0.54 <sup>a</sup>	0.57 <sup>a</sup>	0.011	***	**	***	*
ED (4%)	0.11	0.17	0.46	0.48	0.009	***	**	***	ns
Undegraded	0.68	0.59	0.31	0.26	0.016	***	ns	***	ns

*a* = The portion of OM solubilized at initiation of incubation; *b* = the fraction of OM insoluble but degradable in the rumen; *c* = the constant rate (percent per hour) of disappearance of fraction *b*; Lag = lag phase (h) before the commencement of degradation of fraction *b*; Undegraded = 100 - (*a* + *b*).

Means with different letters within rows differ ( $P < 0.05$ ).

H(Group), haulms within group; ns, non-significant; ED, effective degradability; OM, organic matter.

<sup>†</sup>Effective degradability at two ruminal passage rates (2 and 4%).

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

The CP and NFC of the haulms were positively correlated with the degradation constants in both seasons, whereas NDF and ADL had negative relationships with the degradation constants (Table 4). Results also showed that effective degradation could be predicted from the chemical constituents of the haulms ( $r^2 = 0.64-0.73$ ; RMSE = 0.010 - 0.034) as shown in Table 5.

### Protein fractions of the cowpea haulms

The contents of CP fractions of the cowpea haulms are shown in Table 6. The *B* fraction, which is assumed to

represent true protein ( $B_1$ ,  $B_2$  and  $B_3$ ), accounted for more than half the CP in the haulms, with the exception of improved haulms during the dry season. Interactions between group and season were observed for all the fractions except  $B_1$ . Greatest ( $P < 0.05$ ) proportions of *A* and  $B_3$  fractions were observed in improved haulm varieties during the dry and wet seasons, respectively, whereas the commercial haulm varieties had the greatest ( $P < 0.001$ ) content of fractions  $B_2$  and *C* during the wet season. The proportion of potentially degradable protein fractions (*A*,  $B_1$ ,  $B_2$  and  $B_3$ ) ranged from 741 to 828 g kg<sup>-1</sup> CP, representing between 65 and 79% of total CP content of the haulms.

**Table 3** Non-linear estimates and effective degradability coefficients of crude protein of the cowpea haulms.

	Wet season		Dry season		SEM	<i>P</i>			
	Commercial	Improved	Commercial	Improved		Group	H(Group)	Season	G × S
<i>a</i>	0.05 <sup>c</sup>	0.04 <sup>c</sup>	0.15 <sup>b</sup>	0.20 <sup>a</sup>	0.010	ns	ns	***	*
<i>b</i>	0.40	0.34	0.50	0.42	0.014	***	ns	***	ns
<i>c</i>	0.015	0.019	0.046	0.041	0.0036	ns	ns	***	ns
lag	0	0	0	0	0	–	–	–	–
ED (2%) <sup>†</sup>	0.21	0.21	0.50	0.47	0.011	ns	ns	***	ns
ED (4%)	0.15	0.16	0.42	0.40	0.009	ns	ns	***	ns
Undegraded	0.55	0.61	0.35	0.38	0.013	**	**	***	ns

Means with different letters within rows differ ( $P < 0.05$ ).

H(Group), haulms within group; ns, non-significant; ED, effective degradability.

*a* = The portion of CP solubilized at initiation of incubation; *b* = the fraction of CP insoluble but degradable in the rumen; *c* = the constant rate (percent per hour) of disappearance of fraction *b*; Lag = lag phase (h) before the commencement of degradation of fraction *b*; Undegraded = 100 – (*a* + *b*).

<sup>†</sup>Effective degradability at two ruminal passage rates (2 and 4%).

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Table 4** Relationships between organic matter (OM) degradation characteristics and chemical constituents of the cowpea haulms.

Season	Variety	CP	NDFom	Lignin(sa)	NFC	
Wet	Commercial	<i>a</i>	0.19 <sup>ns</sup>	–0.08 <sup>ns</sup>	–0.01 <sup>ns</sup>	0.36 <sup>ns</sup>
		<i>b</i>	0.66 <sup>ns</sup>	–0.33 <sup>ns</sup>	–0.29 <sup>ns</sup>	0.01 <sup>ns</sup>
		<i>c</i>	0.14 <sup>ns</sup>	–0.42 <sup>ns</sup>	–0.60 <sup>ns</sup>	0.48 <sup>ns</sup>
		ED	0.69*	–0.33 <sup>ns</sup>	–0.51 <sup>ns</sup>	0.24 <sup>ns</sup>
	Improved	<i>a</i>	0.21 <sup>ns</sup>	–0.10 <sup>ns</sup>	–0.25 <sup>ns</sup>	0.16 <sup>ns</sup>
		<i>b</i>	0.33 <sup>ns</sup>	–0.59 <sup>ns</sup>	–0.63 <sup>ns</sup>	0.69*
		<i>c</i>	0.09 <sup>ns</sup>	0.04 <sup>ns</sup>	–0.67*	0.10 <sup>ns</sup>
		ED	0.26 <sup>ns</sup>	–0.43 <sup>ns</sup>	–0.83**	0.62 <sup>ns</sup>
Dry	Commercial	<i>a</i>	0.36 <sup>ns</sup>	–0.23 <sup>ns</sup>	–0.43 <sup>ns</sup>	0.10 <sup>ns</sup>
		<i>b</i>	0.34 <sup>ns</sup>	–0.38 <sup>ns</sup>	–0.61 <sup>ns</sup>	0.48 <sup>ns</sup>
		<i>c</i>	0.11 <sup>ns</sup>	–0.50 <sup>ns</sup>	–0.45 <sup>ns</sup>	0.52 <sup>ns</sup>
		ED	0.25 <sup>ns</sup>	–0.59 <sup>ns</sup>	–0.86**	0.47 <sup>ns</sup>
	Improved	<i>a</i>	0.73*	–0.29 <sup>ns</sup>	–0.46 <sup>ns</sup>	0.75*
		<i>b</i>	0.59 <sup>ns</sup>	–0.11 <sup>ns</sup>	–0.15 <sup>ns</sup>	0.56 <sup>ns</sup>
		<i>c</i>	0.72*	0.02 <sup>ns</sup>	0.31 <sup>ns</sup>	0.59 <sup>ns</sup>
		ED	0.66*	–0.01 <sup>ns</sup>	–0.08 <sup>ns</sup>	0.52 <sup>ns</sup>

CP, crude protein; NDF, neutral detergent fibre; ADL, acid detergent fibre; NFC, non-fibre carbohydrates; NDFom, neutral detergent fibre expressed exclusive residual ash; Lignin(sa), lignin was determined by solubilization of cellulose with sulphuric acid in the ADF residue; ED, effective degradability at ruminal passage of 2%; ns, non-significant.

*a* = The portion of OM solubilized at initiation of incubation; *b* = the fraction of OM insoluble but degradable in the rumen; *c* = the constant rate (percent per hour) of disappearance of fraction *b*; Lag = lag phase (h) before the commencement of degradation of fraction *b*; Undegraded = 100 – (*a* + *b*).

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

**Table 5** Linear regression analysis to predict effective degradation of organic matter from chemical constituents.

Season	Variety	Model	RMSE	r <sup>2</sup>	P
Wet	Commercial	ED = 0.45253 - 0.00138 CP - 0.00022177 Lignin(sa)	0.025	0.65	*
	Improved	ED = 0.39851 - 0.00098473 Lignin(sa)	0.103	0.70	**
Dry	Commercial	ED = 0.77334 - 0.00215 Lignin(sa)	0.146	0.73	**
	Improved	ED = 0.14682 + 0.00233 CP - 0.00057763 Lignin(sa)	0.012	0.64	**

RMSE, root mean square error; Lignin(sa), lignin was determined by solubilization of cellulose with sulphuric acid in the ADF residue; ns, non-significant; ED, effective degradability.

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

**Table 6** Crude protein (CP) fractions ( $\text{g kg}^{-1}$  CP) of the cowpea haulm varieties.

	Wet season		Dry season		SEM	P			
	Commercial	Improved	Commercial	Improved		Group	H(Group)	Season	G × S
A	100 <sup>d</sup>	185 <sup>c</sup>	285 <sup>b</sup>	342 <sup>a</sup>	9.5	***	*	***	ns
B <sub>1</sub>	106	140	114	109	11.3	ns	ns	ns	ns
B <sub>2</sub>	267 <sup>a</sup>	145 <sup>b</sup>	182 <sup>b</sup>	159 <sup>b</sup>	13.6	***	ns	*	***
B <sub>3</sub>	268 <sup>b</sup>	310 <sup>a</sup>	223 <sup>c</sup>	218 <sup>c</sup>	8.9	*	ns	***	*
C	259 <sup>a</sup>	220 <sup>b</sup>	196 <sup>bc</sup>	172 <sup>c</sup>	7.8	***	*	***	ns

Means with different letters within rows differ ( $P < 0.05$ ).

H(Group), haulms within group; A, non-protein nitrogen; B<sub>1</sub>, buffer soluble protein; B<sub>2</sub>, neutral detergent soluble protein; B<sub>3</sub>, acid detergent soluble protein; C, indigestible protein; ns, non-significant.

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

## Discussion

### Chemical composition

Although detailed agronomic results are described elsewhere (Anele *et al.*, 2011b), it is relevant to note here that there was better performance from the haulms of the improved varieties than haulms of commercial varieties. Chemical composition of the cowpea haulms showed substantial variation across group and season. Dry matter and CP contents of the commercial cowpea haulms were greater than those of the improved haulms. The CP concentrations in the current study are above the range of 110–130  $\text{g kg}^{-1}$  DM, which is adequate for maintenance and growth of small ruminants (NRC, 2007). This implies that cowpea haulms, especially the haulms of commercial varieties, can be used as a CP supplement to poor-quality grasses during the dry season. Without details of the relative proportions of the individual minerals, one can only speculate on the importance of the 2–3 fold greater ash content for commercial haulms compared with improved haulms. Further analyses of specific mineral concentrations will be required to draw conclusions relative to this difference.

Fibre concentrations were greater in haulms of improved varieties than commercial cowpea haulms.

Despite the greater fibre concentration in improved haulms, a significant proportion of DM was in the form of NFC, which is easily degraded, in contrast to the commercial haulms, which had greater lignin concentration.

Greater NFC contents of improved cowpea haulms indicate that they might stimulate ammonia-N utilization in the rumen to a greater extent than the commercial varieties (Tylutki *et al.*, 2008). As N utilization by rumen micro-organisms is related to the supply of fermentable energy, the NFC in the cowpea haulms could improve the efficiency of microbial CP synthesis by promoting better utilization of rumen ammonia released from feeds with high content of rumen-degradable CP (Cabrita *et al.*, 2006).

### Non-linear parameter estimates and effective degradability values

Less than 50% of the samples were degraded in samples harvested in the wet season. Lower degradation observed in the wet season samples could be traced to the proportion (>80%) of vines in the samples. As a result of rainfall, significant amount of leaves were lost during drying (of the haulms), presumably leading to a lower leaf-to-stem ratio. Although the haulms were not partitioned into morphological fractions to prove this

(i.e. a lower leaf:stem), statistically but elevated fibre contents (especially lignin) in wet season compared with dry season samples agrees with this suggestion. This leaf loss did not affect haulms harvested during the dry season. All the haulms recorded more than 75% OM and CP degradabilities at 48 h. The fact that the CP contents were not extensively degraded ruminally is potentially positive for efficient utilization of CP. Extensive ruminal degradation of CP results in ammonia production above a concentration that can be utilized for microbial protein synthesis. If not recycled as urea to the rumen, the excess is excreted in urine. This constitutes a loss to the nitrogen economy of the animal and consequently limits animal production (Cabrita *et al.*, 2006).

The trend observed in OM and CP degradabilities agreed with an earlier *in vitro* study (Anele *et al.*, 2011a) in which more OM was degraded in the improved haulms and more CP was degraded in the commercial vs. improved haulms.

Correlation analysis showed that increment in NDFom and lignin(sa) contents will decrease OM degradation of the haulms. In contrast, the increase in CP and NFC contents should result in greater OM degradation values for the haulm varieties. Effective degradability of the cowpea haulms could be predicted from the chemical constituents in both seasons. The prediction of effective degradability of the haulms was improved when only lignin was used in the model (0.73 and 0.70, compared to 0.65 and 0.64 when CP was included with root mean square of 0.146, 0.103, 0.025 and 0.012, respectively).

### Protein fractions

There has not been any report on the CP fractions of cowpea haulms; hence, direct comparisons with previous findings cannot be made. The  $B_3$  fraction, which contains a high percentage of rumen undegradable CP with degradation rate of  $<1.5\% \text{ h}^{-1}$  (Sniffen *et al.*, 1992), was greater in the improved haulms. This finding was in agreement with earlier *in vitro* study (Anele *et al.*, 2011a) in which improved haulm varieties supplied greater utilizable CP at the duodenum (total CP at the duodenum minus endogenous CP). A greater proportion of the A fraction was also observed in the improved haulms (harvested in the dry season) making them suitable for feedstuffs with rapidly degradable carbohydrate to ensure synchronous release of nitrogen and carbohydrates ruminally. The importance of synchronous release of energy and nitrogen in the rumen is currently under intensive discussion. Dewhurst *et al.* (2000), Givens and Rulquin (2004) and Cabrita *et al.* (2006) described the relationship between ruminal CP and carbohydrate availability and its effect on microbial

CP synthesis in the rumen and protein supply to the small intestine. Fraction C contains proteins that are associated with lignin, tannin-protein complexes and Maillard products that are not degradable in the rumen and are indigestible in the intestine (Krishnamoorthy *et al.*, 1982). A greater proportion of fraction C in the wet season can be linked to the extended drying period because of rainfall which led to greater stem/leaf ratio compared with dry season. The resulting haulms (wet season) had elevated lignin content which is strongly associated with fraction C.

### Conclusions

The trend observed in organic matter (OM) and CP degradabilities agreed with the results of an earlier *in vitro* study (Anele *et al.*, 2011a) in which more OM was degraded in the improved haulms and more CP was degraded in the commercial haulms. Cowpea haulms harvested in the absence of rainfall and lower humidity were of greater quality than those harvested during the wet season. Results indicated that adequate attention must be given to handling of the haulms to minimize the amount of leaves lost during the wet season. This study and an earlier feeding study (Anele *et al.*, 2010) in which sheep gained weight when fed the haulms as supplements to a basal diet of poor-quality grass validated that cowpea haulm is an important agro-based by-product that is adequate in protein and energy to sustain ruminant production in SSA countries during the extended dry season.

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