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CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION OF *Brachiaria decumbens* HARVESTED AT DIFFERENT STAGES OF GROWTH IN THE HOT HUMID REGION

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ABSTRACT: The study evaluated the effect of harvesting date on the chemical composition and *in vitro* gas production of the botanic fractions of *Brachiaria decumbens* grass. The botanic fractions (leaf, stem and whole plant) of the grass at two maturities (60 and 120 days) in a Completely Randomised Design with factorial arrangement. Samples of botanic fractions at the different harvest dates were chemically analyzed for dry matter (DM), crude protein (CP), fibre concentrations and *in vitro gas* production (IVGP) was measured at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h to estimate the volume and rate of gas production. Short-chain volatile fatty acids, microbial protein production, *in vitro* organic matter digestibility, and metabolizable energy were estimated from established models. Organic matter, crude fibre, NDF, ADF and ADL increased ($P<0.05$) with increasing maturity whereas the reverse was so for CP and ash contents ($P<0.05$). Significant interactions ($P<0.05$) between harvest date and plant fraction were present for both '*b*' and '*c*' attributable to treatment effects. Potential gas production '*b*' elicited a negative response for all plant fractions across the two harvest dates as the values decreased linearly. The rate at which the gases were produced '*c*' also induced a negative response for the leaf and whole fraction but a positive one for the stem fraction. The nutrient composition and gas production characteristics of grasses harvested at day 60 offer a better potential as high quality forage for improved intake and digestibility. The leaf fractions performed relatively better based on the afore-mentioned methods of quality assessment at both maturity periods.

Keywords: Botanic fraction, *Brachiaria decumbens,* Chemical composition, Feedstuff, *In vitro* gas production.

INTRODUCTION

The shortage of forage and its varying availability in quantity and quality throughout the year has been the bane of increased ruminant productivity in the developing world (Makkar, 2002). For improved animal productivity, feeds with high dry matter digestibility that ensure a higher supply of protein post-ruminally should be the choice ruminant feed (Gusha et al., 2015; Rehman et al., 2020). This therefore calls for sustainable pasture establishment practices with high-yielding species and the characterization of their nutrient composition as well as their nutritive value (Muschler, 2016; Leiber, 2022). Chemical composition and digestibility of forages in the tropics are influenced by plant species, variety, levels of maturity, the prevailing climatic conditions and soil fertility (Minson, 1990; Moore et al., 2020). Plant leaves are known to have higher levels of crude protein and digestible carbohydrates (cell contents) but lower structural carbohydrates than the stems. There is rapid accumulation of cell wall carbohydrate as grasses mature which leads to a reduction in crude protein content thereby affecting digestibility (Seyoum et al., 1997). Different plant botanic fractions (leaf, stem and whole plant) have different chemical constituents and digestibility with an increase in maturity among plant species and varieties.

Grasses, crop residues and browse plants are the traditional feed resources for ruminants in the West African sub Region. Most ruminant farmers in developing countries have been reported to harvest grasses daily or at most weekly for feeding their animals owing in part to limited or unavailable storage facilities (Ansah et al., 2013) or nutrient losses in storage (Antwi et. al., 2010). This harvest practice may lead to increased fibre deposition as the grass stays on the field for subsequent harvests which may in turn affect the leaf to stem ratio and the quality of the grass as well. For farmers to choose the most suitable harvesting period for the various plant fractions, the assessment of the nutrient composition and the nutritive value of grasses at different stages of growth cannot be overemphasised. Several studies have however reported the chemical components and *in vitro* gas production of some Brachiaria cultivars including *Brachiaria decumbens* (Ribeiro et al., 2014: Nguku et al., 2016), the gap in the literature on the evaluation of the various botanic fractions of *B. decumbens* in the hot humid regions of Ghana remains wide. The study therefore sought to assess the influence of different harvest dates (60 and 120 days on the chemical composition and *in vitro* gas production profiles of the leaf, stem and whole plant fractions of *B. decumbens* grass.

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MATERIALS AND METHODS

Ethical considerations

All the necessary standard operating procedures outlined by the Animal Research Ethics Committee (AREC, 2018) of the Quality Assurance and Planning Unit of the Kwame Nkrumah University of Science and Technology, Kumasi were followed.

Location and climate of the experimental site

The study was conducted at the Dairy/Beef Cattle Research Station of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi Ghana. The experimental site lies between Latitude 06˚43'N and Longitude 1˚36'W and is within the humid semi-deciduous forest belt of Ghana with a bimodal rainfall pattern. Annual rainfall for the site averages about 1194 mm.

Management practices and Experimental design

A 200 m^2 established *B. decumbens* pasture demarcated by lining and pegging. All grasses within the demarcated plot were cut to a stubble height of 5 cm. The plot was then divided into four sub-plots with 1m alley. Weeding was carried out once at 30 days after the commencement of the experiment by pulling out all unwanted plants. A 2×3 factorial arrangement in a Completely Randomised Design with four replications was used. The harvest dates (60 and 120 days) and botanic fractions (leaf, stem and whole plant) were the factors. The treatments imposed were leaf at 60 days (designated as L60), stem at 60 days (S60), whole plant at 60 days (W60), leaf at 120 days (L120), stem at 120 days (S120) and whole plant at 120 days (W120) for treatments 1–6 in that order. The four sub-plots served as replications.

Harvesting and sample fractionation

The grass samples were taken from the pasture at each harvest date (60 and 120 days), using a quadrant of 1m x 1m in area. Within quadrant grass samples were cut at a height of approximately 5 cm above ground on all sub-plots that served as replications. The harvested grass from each sub-plot was divided into 3 groups and separated into leaves, stems and whole plant fractions.

Chemical analyses

Samples of the botanic fractions of the grass at each harvest date were milled to pass through a sieve size of 2mm and analysed according to the procedures of AOAC (1990) for dry matter (DM), crude protein (CP), ash and ether extract (EE). Representative samples were also assessed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) with sulphite without amylase (Van Soest et al., 1991). Hemicellulose and cellulose were estimated from ADF, NDF and ADL.

Gas production

Rumen digesta or liquor was obtained from two bulls (*Bos indicus*) at the Kumasi Abattoir, Ghana receiving grass hay before slaughter. The digesta was collected early in the morning after the bulls had been starved for 12 hours prior to slaughter. The digesta was collected immediately during evisceration into a flask and conveyed to the laboratory. The *in vitro* gas production was determined from gas production measurements using the method of Menke and Steingass (1988) in which 200mg±2mg dried samples were incubated in triplicate with 20 ml of artificial saliva (bicarbonate buffer, macro- and micro-minerals, resazurin and a reducing solution) and 10 ml of rumen liquor. Gas volume was measured as the displacement of the syringe plunger at 0, 3, 6, 12, 24, 48, 72 and 96 h of incubation. Gas readings were fitted into the model described as $Y= b\ (1-e^{-ct})$ using the SigmaPlot for windows (version 14.0) software (Systat Software Inc, 2017).

$Y= b\ (1-e^{-ct})$;

where: Y = volume of gas produced at time t (ml); b = potential gas production (ml/200 mg DM); c = rate at which gas was produced from the insoluble fraction (ml/hr); t = incubation time.

In vitro organic matter digestibility (IVOMD, g/kg DM), metabolisable (ME MJ/kg DM), net energy (NE, MJ/kg DM), short chain fatty acids (SCFA) and microbial protein (MP) were predicted according to the following stoichiometric equations of Menke and Steingass (1988) and Close and Menke (1986):

IVOMD (g/kg DM) = [14.88+0.889 IVGP24 +0.45CP (%DM) +0.0651 Ash]

ME (MJ/kg DM) = 2.20+(0.136 IVGP24) +0.057 X CP

NE (MJ/kg DM) = 2.20+(0.0272 X IVGP 24) +(0.057 X CP) +(0.149 X EE)/14.64

SCFA = [−0.00425+0.0222× IVGP24 (ml/0.5g DM)] X 100

MP = 1.93×IVOMD /10

Where, IVGP24= *in vitro* gas production after 24 hours

Statistical analysis

The data obtained from the gas production technique were analyzed using the Minitab Statistical Package, version 19.1 (Minitab Inc., 2019). The means were separated by Bonferroni Pairwise Comparisons test. Probability level of significance was set at 5%.

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RESULTS AND DISCUSSION

Effects of harvest date on the chemical composition and estimated metabolizable energy of plant botanic fractions

The nutrient characteristics of the leaf, stem and whole plant fractions as influenced by harvest date is presented in Table 1. The results revealed significant (P<0.05) plant fractions x harvest date interactions for chemical composition and ME except for EE, cellulose and hemicellulose. Harvest dates resulted in different (P<0.05) nutrient compositions, ME and the fibre concentrations (NDF and ADF). In addition, estimates of the chemical composition as well as NDF, ADF were influenced (P<0.05) by the various plant fractions except for ME which recorded no differences. Ether extract was similar among the plant fractions (P=0.832) but tended to approach significance (P=0.073) at different harvest dates.

Generally, estimates from the plant cell wall fractions (hemicellulose and cellulose) were not significantly different among the plant fractions (P>0.05) and the harvest dates (P>0.05) with the exception of ADL which tended to approach significance at the various harvest dates (P=0.063). The influence of harvest dates on plant fraction reported in this study supports the study by Crowder and Chheda (1982) who stated that as grasses advance in age, there is generally increase in dry matter which increases the cell wall contents with a concomitant decrease in the cell contents. The increase in DM content with maturity observed in this study is consistent with those of Ansah et al. (2010) and Osman et al. (2019). The linear increase (P<0.05) in DM of the various plant fractions from the study may be due to photosynthetic activity leading to the accumulation of dry matter with advancing age (Crowder and Chheda, 1982). On the contrary, McDonald et al., (1995) reported increases in fibre concentration as plants advance in age.

Similarly, the organic matter (OM) content significantly increased (P<0.05) among the botanic fractions from day 60 of harvest to day 120. This pattern of increase was reported by Tilahun et al. (2017) in a study that evaluated harvesting date on the chemical composition of *Pennisetum pedicellatum*. However, the OM contents reported during the 60-day harvest for all botanic fractions were lower than the 83.65 – 84.71% reported by Umami et al. (2017).

Conversely, CP and ash contents decreased (P<0.05) consistently for botanic fractions with advancement in maturity. Seyoum et al., (1997) reported a dilution effect of protein by swift build-up of cell wall carbohydrates towards the end of the grass growth phase. The decline in CP contents observed in this study was consistent with the results of Peiretti (2009), Ansah et al. (2010), Tilahun et al. (2017) and Osman et al. (2019).

It is however noteworthy that the CP contents recorded for all botanic fractions across the two harvest dates were higher than the 7% minimum CP level needed to sustain rumen microbes according to Lazzarini et al. (2009). The CP concentrations of the leaves at the harvesting dates were 1-1.32 times higher than the CP content reported by Faria et al., (2018) when bromatological characteristics of *B. decumbens* were evaluated. These differences in the chemical composition according to Low (2015) may partly be attributable to the season of grass establishment, application of fertilizer, combination with leguminous species, growing conditions and sampling procedures (i.e. plot harvest or picked selection). With regards to ash content, the decreasing trend observed with an increase in harvest date in the current study was in consonance with the findings of Tilahun et al. (2017).

Fibre content represents the amount of indigestible components in the feed and supplies the bulk required for the needed peristalsis in the rumen (McDonald et al., 2010, Bhardwaj et al., 2018). Fibers also support the structure of growing plants and their accumulation with age is inversely proportional to CP content (McDonald, 2011). The contents of NDF, ADF, cellulose and ADL reported in the study significantly increased (P<0.05) with increasing days of growth and was consistent with the findings of Anele et al. (2008) and McDonald et al. (2011). The trends observed with NDF, ADF and ADL in the current study are consistent with earlier findings (Zinash et al., 1995; Seyoum et al., 1997; Tilahun et al., 2017; Wassie et al., 2018).

NDF, according to Nguku et al. (2016), gives an indication of forage dry matter intake. Therefore, forages high in NDF content will consequently have a lower intake. ADF refers to the cell wall fractions composed of cellulose and lignin. Values for ADF are essential as they relate to the animal's ability to digest forage, that is, grasses that are high in ADF have low energy values and are less digestible. ADF content of all botanic fractions over the 3 harvest dates were all within the 200 and 450 g/kg DM reported for tropical forages by Asaolu et al. (2011), McDonald et al. (2011) and Martens et al. (2012). According to Nussio et al. (1998), forages with 40% or more of ADF are indicative of poor intake and subsequent digestibility. With the exception of treatment S120 (stem at 120 days), all other treatments registered ADF contents of less than 40% and could therefore promote intake and digestibility.

The increasing levels of ADL with increasing harvest dates observed in this study confirm the reports of Kidunda et al. (1990), Tesema et al. (2002) and McDonald et al. (2002). Lignin, according to Xu et al. (2003), is a complex, phenolic polymer found in plant cell walls indispensable for providing mechanical support and transporting water and minerals. That is, the stem continues to become lignified as plants grow to provide the needed support to the plant, hence the highest levels of lignin found in the stem fractions over the differing harvest dates. Digestibility of cellulose decreases with high lignin contents (Nguku et al., 2016). Plants produce fibrous tissues as they age according to McDonald et al. (1995) which lead to an increase in structural carbohydrates and lignin. Plant maturity also has been reported by Rambau et al. (2016) as the primary factor that affects the nutritive value of forages.

Metabolisable energy refers to the energy left after losses from fecal and urinary sources. It represents the energy used for growth or reproduction and for supporting metabolic processes. Forages with ME values beyond 2000.5 kcal/kg (8.37 MJ/kg DM), according to National Council of Science and Technology (1975), are regarded to be of high quality. The study recorded ME values ranging from 1781kcal/kg in S120 to 2066.58kcal/kg in L60.

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Table 1 - Effects of harvest date and plant botanic fraction on the chemical composition of *Brachiaria decumbens.*

	Treatments						Significance			
Harvest date	60			120			P values			
Plant fraction	Leaf (L60)	Stem (S60)	Whole (W60)	Leaf (L120)	Stem (S120)	Whole (W120)	SEM	HD	PF	HD*PF
Component										
DM (g/kg)	353.30^{e}	381.63^{cd}	370.40^{de}	403.18^{bc}	442.80^{a}	420.15^{ab}	6.590	0.0001	0.0001	0.042
OM (g/kg DM)	783.90^{d}	801.53^{bc}	792.50^{cd}	804.83^{b}	824.53^{a}	807.18^{b}	2.810	0.0001	0.0001	0.012
CP (g/kg DM)	129.03^{a}	98.88^{c}	113.83^{b}	100.08^{c}	78.58^{d}	88.88^{cd}	3.560	0.0001	0.0001	0.029
Ash (g/kg DM)	94.63^{a}	73.48^{bc}	82.40^{b}	71.83^{c}	51.90^{d}	69.78^{c}	2.820	0.0001	0.0001	0.028
EE (g/kg DM)	31.65	32.00	32.65	34.33	33.00	33.33	0.368	0.073	0.832	0.523
NDF (g/kg DM)	606.03^{b}	648.60^{a}	645.93^{a}	652.88^{a}	658.58^{a}	656.73^{a}	4.760	0.003	0.014	0.045
ADF (g/kg DM)	320.70^{b}	370.18^{ab}	368.73^{ab}	365.28^{ab}	405.98^{a}	391.43^{a}	7.610	0.007	0.011	0.025
Cellulose (g/kg DM)	257.10	299.30	299.20	295.08	314.98	317.95	7.260	0.083	0.103	0.754
Hemicellulose (g/kg DM)	285.33	278.43	277.20	287.60	252.60	265.30	6.630	0.439	0.509	0.747
ADL (g/kg DM)	63.60^{b}	70.88^{ab}	69.53^{ab}	70.20^{ab}	91.00^{a}	73.48^{ab}	2.610	0.039	0.063	0.032
ME (kcal/kg)	2066.58^{a}	2053.65^{ab}	1926.87^{abc}	1895.28^{bc}	1781.86^{c}	1912.96^{abc}	23.700	0.0001	0.129	0.005

^{a,b,c,} Mean values with different superscripts on the same row differ significantly (P<0.05).

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A declining pattern in ME with advancing age was observed in the study and it is in consonance with the observation by Umami et al. (2017) who reported a constant decline in ME when the nutritive value of *Brachiaria sp* was accessed.

It can also be seen from Table 1 that in respect of the plant botanic fractions, the leaf fraction registered the highest CP and ash contents for both harvest dates followed by the whole fraction with the stem fraction recording the least levels of CP and ash. The high content of CP in the leaf fraction is advantageous to microbes in the rumen that utilize dietary sources of nitrogen for the synthesis of their body proteins. Ansah et al. (2010) also found that the leaf fraction of Napier grass had more CP than the stem fractions. Levels of NDF, ADF and ADL were highest in the stem followed by the whole fraction with the leaf fraction registering the lowest values. The least content of lignin in the leaves is required as it suggests minimum binding of cellulose and hemicellulose thereby making them available to be utilized efficiently by rumen microbes. CP content of the leaf and whole fractions at 60 days met the recommended 110g/kgDM to 130g/kgDM required for maintenance and growth for ruminant livestock (Freer et al., 2007; Asaolu et al., 2011).

Effects of harvest date on gas production profile of plant botanic fractions

The cumulative *in vitro* gas production of the various botanic fractions over the 3 harvest dates as a function of incubation time is shown in figures 1 and 2. *In vitro* fermentation is generally a reflection of the extent of the fermentation and digestibility of feed (Getachew et al., 1998). Gas production generally increased cumulatively with increase in incubation time from 3 hours to 96 hours for all plant fractions across the two harvest dates.

It can be seen from Figures 1 and 2 that the maximum amount of gas was typically produced within the first 3 hours of incubation, which is consistent with Sarkwa et al. (2020)'s report on various browse plants. Gas produced by the various botanic fractions decreased consistently with an increase in harvest date throughout the incubation period. The decline in gas production associated with maturity is in harmony with the report of Kamalak et al. (2005) to the effect that maturity affects gas production which decreases with advancement in age of the plant. Since cumulative *in vitro* gas production is proportional to substrate degradation, it can be said that maturity decreases the proportion of easily degradable portions of the plant fractions.

It is also worth noting that at both harvest dates, leaf fractions produced more gas, followed by the whole plant fraction with the stem producing less gas throughout the incubation period as illustrated in figures 1 and 2. This observation agrees with the report of Tang et al. (2008) who recounted that cumulative gas production was constantly higher for the leaf sheath portion relative to the stem in maize.

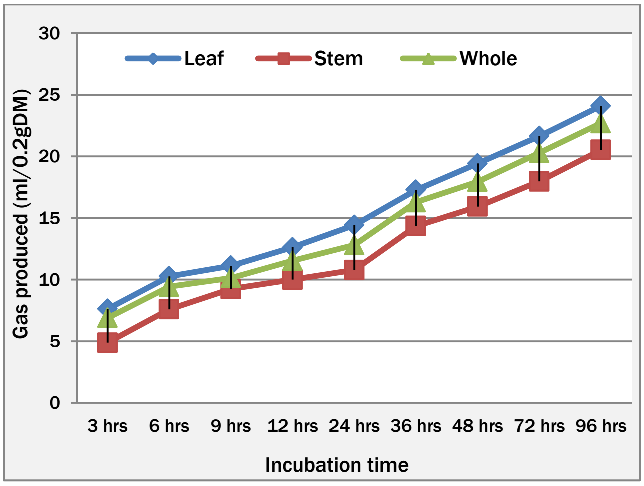

Figure 1 - *In vitro* gas production of leaf, stem and whole fractions at 60 days.

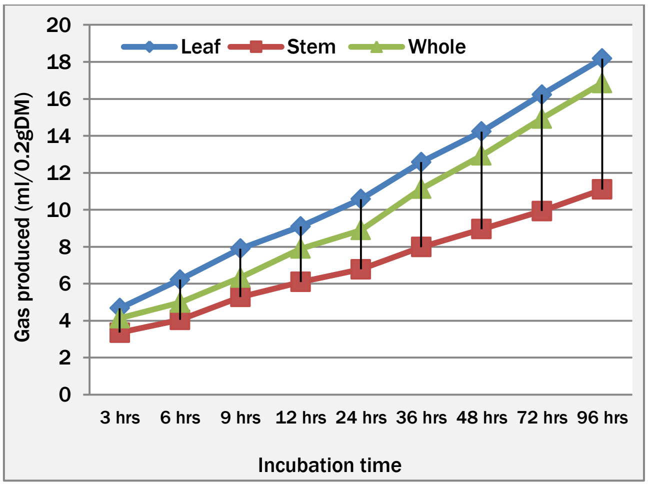

Figure 2 - *In vitro* gas production of leaf, stem and whole fractions at 120 days.

The highest gas production rate in the leaves relative to the stem and whole fractions suggests that the microbes in the rumen were able to degrade the leaf fractions better possibly because there was a higher level of fermentable nutrients present. Gasmi-Boubaker et al. (2005) found a positive correlation between crude protein and gas production, so the higher gas volume for the leaf fractions corresponded to the higher CP and lower cell wall contents. In addition, the leaf fractions recorded lower contents of NDF and ADF (Table 1), which are negatively correlated with gas production (De Boever et al., 2005) and fermentation parameters (Heidary and Kafilzadhe, 2012). Thus, the low fibre content according to van Soest (1994) can enhance feed utilization by rumen microbes which could lead to higher fermentation rates. This trend however conflicts the observation of Cone and Gelder (1999) that feed items rich in CP normally produce small amounts of gas during fermentation as the fermentation of protein results in ammonia production, which affects the carbonate buffer equilibrium by neutralizing H^+ ions from VFA without release of carbon dioxide.

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Again, the least gas accumulation from the stem fractions was due to large amounts of cell wall constituents, that is, crude fibre and lignin. Jung and Deetz (1993) have reported lignin content to be negatively correlated with gas production. Lignification of cell walls, according to the authors, limits the activities of rumen microbes responsible for the fermentation or enzymatic breakdown of forage polysaccharides.

High lignin contents also limit access of rumen bacteria to easily fermentable cell contents (van Soest 1994). The least gas production from the stem fractions could also be linked to the low degradability of CP in the rumen. This ultimately affects the extent of ammonium nitrogen available for microbial cell synthesis thus resulting in low microbial fermentation and gas production.

Since the production of gas is positively correlated with feed fermentation, the stem fraction can be thought of as possessing a relatively low feeding value because of the low fermentative gas production. The differences observed in volume of gas produced in this study could be ascribed to constituents and method of preparation of culture used to incubate the test sample, size and variability of microbial inoculum, reading intervals used to record gas pressure and volume from the fermentation bottles and atmospheric pressure (Zinash et al., 1998).

Gas production kinetics of plant botanic fractions at different harvest dates

Results of gas production kinetics of the various plant botanic fractions over the two harvest dates are shown in Table 2. Kinetics of gas production deal with the quantities of gas produced by a material, '*b*' and the rate at which these gases are produced, '*c*'. These are indicative of how digestible a feeding material is and for that matter how long it will remain in the animal's gut (Getachew et al., 2005). Significant interactions ($P<0.05$) between harvest date and plant fraction were present for both '*b*' and '*c*' attributable to treatment effects. Potential gas production '*b*' elicited a negative response for all plant fractions across the two harvest dates as the values decreased linearly. The rate at which the gases were produced '*c*' also induced a negative response for the leaf and whole fraction but a positive one for the stem fraction.

The potential gas production '*b*' of the leaf fraction was consistently the highest followed by that of the whole plant fraction with the stem fraction registering the least at both harvest dates. Potential gas production ranged from 9.640 ml/0.2g DM in S120 (stem fraction at 120 days) to 20.759 ml/0.2g DM in L60 (leaf fraction at 60 days). The leaf fractions had the lowest levels of fibre contents which were indicative of higher levels of soluble carbohydrates which eventually translated to the uppermost potential gas production. A higher potential gas production is desirable as it can contribute significantly to energy supply through the synthesis of short chain fatty acid (Remesy et al., 1995). The gas production rate '*c*' was also constantly highest for the leaf fraction followed by the whole plant fraction with the stem fraction having the least. However, at 120 days, the rate for the stem fraction was higher than what was recorded for the whole fraction (Table 2). The whole plant fraction at 120 days (W120) was digested at the lowest rate 0.0481/hour while the leaf fractions at 60 days were digested at the highest rate of 0.0811/hour. The highest rate of fermentation which was observed in the leaf fractions was a result of the highest CP and lowest fibre (NDF, ADF and ADL) levels (Osuga et al., 2006) as well as carbohydrate portions that were easily available to microbes (Afshar et al., 2011).

The greater values obtained for the '*b*' and '*c*' for the leaf fractions across all harvest dates may be indicative of the fact that nutrients were much more easily available for the rumen microbes. The rates of fermentation for different chemical components reflect the growth of rumen microbes and access to feed by microbial enzymes (Getachew et al., 2004). The differences in the rate at which gases were produced are indicative of the fact that the plant botanic fractions used different times to degrade all potentially digestible materials and for that matter different rate of passage in the digestive system. The disparities in gas production kinetics across the various treatments were attributable to the treatments imposed as stages of growth and portions of plants used as feed among other factors have been reported by Akinfemi et al. (2009) to influence gas production.

Effects of harvest date on some estimated parameters of the plant botanic fractions

Effects of the different harvest dates on short chain fatty acids (SCFA), estimated energy values (ME and NE), *in vitro* organic matter digestibility (IVOMD) and microbial protein (MP) of the plant botanic fractions are given in Table 3. Harvest date by plant fraction interaction was present ($P<0.05$) for all parameters estimated. For SCFA, ME, IVOMD and MP, the interactions observed were due to the differing harvest dates. The said estimated parameters were significantly not affected ($P>0.05$) by plant fractions within the same harvest date (Table 3). In the case of NE, the interaction observed was due to the synergy between harvest date and plant fraction. Generally, all estimated parameters decreased with advancement in maturity.

Accordingly, SCFA, ME, NE, IVOMD and MP contents decreased linearly for all plant fractions from 60 to 120 days. SCFA is a major source of metabolic fuel while MP serves as a major portion of protein absorbed from in gut in ruminants. ME values recorded for the various botanic fractions at 60 days were all lower than the 5.3MJ/kg DM reported by Umami et al. (2017) at 60 days. The ME values recorded in the present work were higher compared to those (314.6–1406.9kJ/g DM) reported by Sarkwa et al. (2020) for some browse forages. The current ME values for all botanic fractions (3.79 – 4.79MJ/kg DM) were lower relative to the 9.35MJ/Kg DM recounted by Aung et al. (2019) for Mulato II grass. The differences in ME could be attributable to growing conditions (soil and climatic) and sampling methods (Low, 2015).

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Table 2 - Fermentation kinetics of experimental feedstuffs (plant botanic fractions at different harvest dates

	Treatments						Significance			
Harvest date	**60**			**120**			**P values**			
Plant fraction	**Leaf (L60)**	**Stem (S60)**	**Whole (W60)**	**Leaf (L120)**	**Stem (S120)**	**Whole (W120)**	**SEM**	**HD**	**PF**	**HD*PF**
GP parameters										
b	20.759[a]	18.022[b]	19.607[a]	16.259[c]	9.640[d]	15.421[c]	0.754	0.0001	0.0001	0.0001
c (h^{-1})	0.0811[a]	0.0622[ab]	0.0750[ab]	0.0613[ab]	0.0788[a]	0.0481[b]	0.003	0.066	0.250	0.007

[a,b,c] Mean values with different superscripts on the same row differ significantly ($p<0.05$). '*b*' quantities of gas produced by a material; '*c*' the rate at which the gases were produced.

Table 3 - Effects of harvest day and plant part/fraction on some estimated parameters of *Brachiaria decumbens*.

	Treatments						Significance			
Harvest date	**60**			**120**			**P values**			
Plant fraction	**Leaf (L60)**	**Stem (S60)**	**Whole (W60)**	**Leaf (L120)**	**Stem (S120)**	**Whole (W120)**	**SEM**	**HD**	**PF**	**HD*PF**
GP parameters										
SCFA	27.94[ab]	32.71[a]	26.83[b]	19.33[c]	18.28[c]	23.05[bc]	1.100	0.0001	0.209	0.0001
ME (MJ/kgDM)	4.67[a]	4.79[a]	4.52[a]	3.98[bc]	3.79[c]	4.14[b]	0.080	0.0001	0.817	0.001
NE (MJ/kgDM)	3.32[a]	3.20[b]	3.22[b]	3.05[c]	2.91[d]	3.03[c]	0.029	0.0001	0.000	0.045
IVOMD (%)	32.66[a]	33.08[a]	31.45[a]	27.76[bc]	26.24[c]	28.73[b]	0.552	0.0001	0.410	0.001
MP	63.03[a]	63.84[a]	60.70[a]	53.58[bc]	50.65[c]	55.46[b]	1.070	0.0001	0.410	0.001

[a,b,c] Mean values with different superscripts on the same row differ significantly ($p<0.05$). SCFA: Short chain fatty acids, ME: Metabolisable energy, NE: Net energy, IVOMD: In vitro organic matter digestibility, MP: Microbial protein.

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Correlations between chemical composition and *in vitro* gas production

The correlation between chemical composition and *in vitro* gas production after 24 hours is presented in Table 4. The largest positive correlation was seen between 24 hr GP and '*c*' while the largest negative correlation was observed between CP and ADF. CP content correlated significantly and negatively with both NDF (r= -0.705; P=0.000) and ADF (r= -0.689; P=0.000). The content of CP was also significantly and positively correlated with ME (r=0.689; P=0.000) and GP at 24 hours (r=0.869; P=0.000). The observed relationship between CP and GP at 24 hours confirms the reports of Nsahlai et al. (1994), Gasmi-Boubaker et al. (2005), Basha et al. (2012) and Olfaz et al. (2018) who all found CP content to be positively correlated with gas production. Karabulut et al. (2007) however observed a negative correlation between CP and gas production at 24 hours. ME also correlated positively with CP which is one of the critical requirements for the growth of microbes (Larbi et al., 1998). A similar positive correlation between CP and *in vitro* gas production was found by Sarkwa et al. (2020) and Kulivand and Kafilzadeh (2015) for browse plants and pasture grasses respectively. A significant positive correlation (r= 0.861; P=0.000) between CP and potential gas production *'b'* was also observed. However, the correlation between CP and the rate of gas production *'c'* was not significant (r=0.289; P=0.171).

Table 4 - Correlations between chemical composition and *in vitro gas* production

	CP	NDF	ADF	ME	24hrsGP	b
CP						
NDF	-0.705***					
ADF	-0.689***	0.505*				
ME	0.689***	-0.526**	-0.615**			
24 hr GP	0.869***	-0.605**	-0.583**	0.612**		
b	0.861***	-0.533**	-0.616**	0.734***	0.912***	
c	0.289^{ns}	-0.299^{ns}	-0.072^{ns}	-0.032^{ns}	0.308^{ns}	-0.019^{ns}

CP: Crude protein (g/kg DM) NDF: Neural detergent fibre (g/kg DM) ADF: Aacid detergent fibre (g/kg DM) ME: Metabolisable energy (kcal/kg), *b*= potential gas production (ml/0.2 g DM) *c*= rate of gas production (/h). Level of significance: (*) P<0.05, (**) P<0.001, (***) P<0.0001, ns=not significant

There was also a positive and significant correlation (r=0.505; P=0.012) between the levels of NDF and ADF. Thus both NDF and ADF increased together with an increase in harvest dates for all botanic fractions. However, correlations between NDF and ME were negative and significant (r= -0.526; P=0.008). Correlation between NDF and 24hrGP followed a similar pattern (r= -0.605; P=0.002). NDF also correlated significantly and negatively with *'b'* (r= -0.533; P=0.007) and negatively but not significantly correlated with *'c'* (r= -0.299; P=0.156). Thus increased contents of NDF and ADF led to a decline in both potential gas production and the rate of fermentation. This trend agrees with the findings of Kamalak (2006) and Kulivand and Kafilzadeh (2015). The negative correlations observed between potential gas production *'b'* and both NDF and ADF could be a result of reduced growth of microbes and poor access to the feed by microbial enzymes as reported by Getachew et al. (2004). This trend contradicts the report of Abdulrazak et al. (2000) who reported positive correlations between NDF and ADF with gas production characteristics. However, the negative correlation between NDF and ADF with rate of gas production was in harmony with the results of the same authors. Nsahlai et al. (1994) and Basha et al. (2012) have both reported earlier that gas production of feeds negatively correlates with NDF content.

The concentration of ADF correlated significantly and negatively (r= -0.615; P=0.001) with ME as well as with GP (r= -0.583; P=0.003). Table 4 also shows that a very strong negative correlation was observed for ADF and *'b'* (r= -0.616; P=0.001). ADF correlated negatively but not significantly with *'c'* (r= -0.072; P=0.739). However, a significant positive correlation (r=0.583; P=0.001) was observed between ME and GP as well as between ME and *'b'* (r=0.73; P=0.0001). The correlation between ME and 'c' however, was not significant (r=0.032; P=0.882). Finally, 24hrGP correlated significantly and positively (r= 0.912; P=0.0001) with *b*'.

CONCLUSIONS

Harvest date influenced the chemical compositions of the leaf, stem and whole plant fractions of *B. decumbens* regrowth. While the CP and ash contents decreased with an increase in harvest date, OM, CF, ADF, NDF and ADL contents increased linearly with maturity. The leaf fraction that registered the highest CP and ash contents also had the lowest CF, ADF, NDF, cellulose and ADL contents across both harvest dates. The whole plant fraction consistently recorded values that were in between those of the leaf and stem fractions. The CP contents recorded for all botanic fractions across the two harvest dates met the least CP level of 7% required to sustain rumen microbes. In view of this when biomass yield is of interest, the whole plant fraction can be considered as the stem fraction also has some feeding value and could give bulk to the grass. At both harvest dates, the leaf fraction produced the most gas, followed by the whole plant fraction with the stem fraction producing the least amount of gas throughout the incubation period. Botanic fractions that were high in CP and low cell wall constituents (ADF, NDF, cellulose and ADL) showed better potential for gas production. *B. decumbens* regrowth should be harvested when it is 60 days in view of its comparatively superior chemical composition and highest *in vitro* gas production at that age.

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DECLARATIONS

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Consent

Not applicable

Author's contribution

E.L.K. OSAFO contributed to the design of the experiment, statistical analysis and interpretation, coordination of the study and the write up of the manuscript, A. OSMAN made substantial contribution to the conceptualisation and experimental design, data collection, statistical analysis and interpretation, laboratory analysis and write up of the manuscript, V. ATTOH-KOTOKU participated in the interpretation of results and contributed to the drafting of the manuscript and its revision, C. ANTWI was involved with interpretation of results and contributed to the drafting of the manuscript and its revision; Y. ABDUL AZIZ participated in the data collection, statistical analysis and drafting of the manuscript, F. IDAN contributed to data analysis and drafting of the manuscript and its revision. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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