In vitro digestibility and methane production of two tropical grasses: Plant spacing and grazing frequency

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Abstract
This study was carried out to investigate the influence of plant spacing and grazing frequency on the *in vitro* gas production, digestibility, and methane production of Guinea grass (*Megathyrsus maximus*) and Elephant grass (*Cenchrus purpureus*) in the humid southwest part of Nigeria. The experiment was laid in a 2 x 2 x 2 factorial arrangement in a split-split-plot design with three replicates, which consisted of two grazing frequency (3 and 6-week) as the main plot, two grass species: Guinea grass (*M. maximus*) and Elephant grass (*C. purpureus*) as the sub plot and two plant spacing (1m x 1m and 0.5m x 1m) as the sub-sub plot. A total area of 2496 m^2 of an existing plot established April 2019 was used in conducting this experiment. In May 2020, the grasses were cut back at the commencement of this experiment to 15cm above ground surface and NPK 20:10:10 fertilizer was applied 8 days after cut back at the rate of 120 kg N/ha. The result showed a significant ($P<0.05$) effect of grazing frequency x species x plant spacing on the crude protein (CP), *in vitro* gas production, digestibility (IVDMD) and methane gas production. The CP content of *C. purpureus* (18.14 %) grazed at 3-week GF with 1 m x 1 m spacing was significantly ($P<0.0001$) highest and the least CP was recorded for *M. maximus* that was grazed at 6-week GF with a narrower plant spacing. The highest gas volume (31.33 ml/200mg DM) was produced by *M. maximus* grazed at 3-week at the narrower spacing with the IVDMD been the highest (64.53% DM) and the least gas volume was produced by *C. purpureus* grazed at 3-week with a narrower spacing, whereas the least digested was *C. purpureus* grazed at 6-week with narrower spacing. The methane production of *C. purpureus* grazed at 3-week with wider spacing was the least (5.53 ml/200mg DM) while the highest methane was produced by *M. maximus* also grazed at 3-week with 0.5m x 1m spacing though not statistically ($P>0.05$) different from the methane of same species with same plant spacing at 6-week grazing frequency. It can be concluded that grazing at 3-week grazing frequency and narrower spacing will help reduce methane for *C. purpureus* and improve digestibility for *M. maximus.*

Introduction
Natural grasses are the most abundance feed for cattle especially in the tropics which could be supplemented with concentrate, agricultural waste and industrial by-products (Man and Wiktorsson, 2003). Acquiring high nutritive quality and forage production could be achieved by sowing improved variety of forage grasses. *Cenchrus purpureus* grows erectly upwards and the tillers form clumps; thick and sugarcane like stems with nodes and internodes. *Cenchrus purpureus* stems are tall and vary in height from 2 to 6 m. The leaves are long, spine-like, sharp, very hairy and narrow. Nyambati *et al.* (2010) reported the morphological characteristics of 12

cultivars *C.* purpureus with leaf length of 61.2cm, leaf width 2cm and tiller number 60. *Megathyrsus maximus* is a tufted perennial or sometimes annual plant with slim to vigorous and smooth stem (nodes and internodes) that vary in height from 25 to 450 cm. The leaves are long, narrow and hairless with length ranges from 6 – 100cm and wideness of about 4 – 35mm (Muir and Jank, 2004). Grazing or cutting management of *Cenchrus purpureus* and *Megathyrsus maximus* (Ntchisi) is a practical management in encouraging the productivity and persistent of these grasses. Delayed grazing or cutting has resulted in declined nutritive quality, affected the *in vitro* dry matter digestibility and also methane production of tropical grasses (Dele, 2012). Plant spacing effect on nutritive quality of these grasses have not been carefully dealt with and thus the reason for this study to investigate the effect of grazing frequency and plant spacing on the *in vitro* digestibility and methane production of *Cenchrus purpureus* and *Megathyrsus maximus* (Ntchisi).

Methods and study site

This study was carried out on an existing plot which was established with two grasses *Megathyrsus maximus* and *Cenchrus purpureus* with 0.5 m x 1 m and 1 m x 1 m spacing each in separate plots in April 2019 with total land area of 2496 m^2. Soil samples were randomly collected from the experimental plots at the depth of 0 – 15 cm topsoil layer using soil auger. The soil samples were bulked per replicate, mixed thoroughly and sub samples were taken for analysis to determine the nutrient status of the soil. May 2020, the grasses were cut back at the commencement of this experiment to 15cm above ground surface and NPK 20:10:10 fertilizer was applied 8 days after cut back at the rate of 120 kg N/ha to stimulate regrowth (Onasanya *et al.*, 2009). The study was 2 x 2 x 2 factorial arrangement laid out in a split-split plot design. The treatments consisted of two grazing frequency (3 and 6 weeks) as the main plot, two tropical grasses (*M. maximus* and *C. purpureus*) as the sub-plot and two plant spacing (1m x 1m and 0.5m x 1m) as the sub-sub plot with three (3) replicates. Crude protein was determined according to A. O. A. C. (1995) and *In vitro* gas production was determined following the modified of Menke and Steingass. Methane production was determined after recording the final gas volume produced from the incubated contents, 4.0ml of NaOH (10M) was injected into each incubated syringe, which absorbs CO_2 content while the remaining gas volume in the syringe was considered to be methane (Demeyer *et al.*, 1988). After 48 hours digestion, the syringe contents were centrifuge at 15,000 rpm for 10 minutes and residues were decanted into pre weighed crucibles and were oven-dried at 105 ^{0}C for 24 hours. The dry residues were weighed, and the dry matter digestibility was carried out as described by Dele (2012)

Results and Discussion

The interaction effect of grazing frequency x grass species and plant spacing significantly ($P<0.0001$) affected the crude protein content, *In vitro* gas volume, methane produced and the *In vitro* dry matter digestibility of the two tropical grasses (Table 1). *Cenchrus purpureus* grazed at 3-week with 1 m x m spacing produced the highest crude protein content (18.14 %). The volume of gas produced at 24 and 48 h of incubation was highest for *M. maximus* at 3-week GF with 0.5 m x 1 m spacing (17.43 and 31.33 ml/200mg DM respectively) and the least gas volume was recorded for *C. purpureus* at all interaction levels. *Megathyrsus maximus* grazed at 3 and 6-week GF with narrower spacing generated more methane while the 3-week GF *C. purpureus* with 1 m x 1m spacing had the least methane production. *Megathyrsus maximus* grazed at 3-week with 0.5 m x 1 m spacing displayed the highest (64.53 %) *In vitro* dry matter digestibility value.

The quality of tropical grasses cannot be over emphasized in ruminant animal production. The decline in the crude protein content from the more frequently grazed (3-week grazing frequency) to the less frequently grazed grasses (6-week grazing frequency) could be due to the lignification and stem elongation that is common with grasses as the age increases. Ansah *et al.* (2010), Dele (2012) and Tilahun *et al.* (2017) affirmed the report of this findings of decline in CP content of desho grass with increase in grass age. Planting orientation in terms of spacing contributed to the differences in crude protein content of grasses examined in this study. Wider spaced grasses produced more crude protein content compared with narrower spaced grasses. This could be as a result of less competition with soil water, nutrient and sunlight in the wider spaced grasses as it enhanced sufficient utilization of the nutrients tapped and formation of leafy parts. *Cenchrus purpureus* displayed higher content of crude protein over *M. maximus* in this study, which is as a result of differences in plant morphology especially the leafy parts which contributed most to the variation in crude protein content of forages. *Cenchrus purpureus* exhibited higher number of leaves, wider width, longer leaf length and higher leaf to stem ratio, although growth parameters of these grasses were not disclosed in this study. Dele (2012) reported significantly higher CP for *C. purpureus.*

Table 1: Effects of grazing frequency and plant spacing on the crude protein, *in vitro* gas production (ml/200mg DM), methane and *in vitro* dry matter digestibility of the two tropical grasses

Factors			CP	24 (hr)	48 (hr)	CH_4	DMD (%)
			(%)	ml/200mg DM			
Grazing frequency (week)	**Grass species**	**Plant spacing (m^2)**					
3	*C. purpureus*	0.5 x 1	16.97^{b}	10.93^{c}	21.53^{de}	5.53^{c}	54.29^{ab}
		1 x 1	18.14^{a}	9.67^{c}	20.43^{e}	5.85^{bc}	62.66^{ab}
	M. maximus	0.5 x 1	14.42^{cd}	17.43^{a}	31.33^{a}	8.08^{a}	64.53^{a}
		1 x 1	15.30^{c}	14.90^{ab}	27.47^{abc}	7.13^{abc}	63.82^{ab}
6	*C. purpureus*	0.5 x 1	13.42^{de}	10.60^{c}	22.73^{cde}	6.93^{abc}	52.97^{b}
		1 x 1	14.53^{c}	10.40^{c}	22.80^{cde}	7.48^{ab}	57.46^{ab}
	M. maximus	0.5 x 1	10.42^{f}	14.53^{ab}	28.13^{ab}	7.89^{a}	53.82^{ab}
		1 x 1	12.46^{e}	12.88^{bc}	25.47^{bcd}	6.39^{abc}	55.43^{ab}
SEM			0.20	0.43	0.59	0.20	1.17
P-value							
Grazing frequency			<.0001	0.1642	0.7170	0.2060	0.0086
Grass species			<.0001	<.0001	<.0001	0.0260	0.2902
Plant spacing			<.0001	0.0825	0.0944	0.3376	0.1556
Grazing frequency x grass species x plant spacing			<.0001	<.0001	<.0001	0.0027	0.0248

$^{a, b, c, d, e, f}$: Means in same column with different superscripts were significantly (p0.005) different.
CP = Crude protein, CH_4 = Methane, DMD = Dry Matter Digestibility, SEM = Standard Error of Means

The volume of gas generated by forage materials has been considered by Blummel and Becker (1997) to deflect the fermentation end product of its substrate to volatile fatty acids (VFAs), microbes population and neutralization of VFA. *Megathyrsus maximus* produced higher gas volume than *C. purpureus* in this study. This contradicts the report of Dele *et al.* (2012). This could be as a result of high methane in *M. maximus* thus the gas volume observed. Gas volume produced by the grasses decreased with increasing plant spacing. Jimoh (2017) reported otherwise when the gas volume produced from *M. maximus* as affected by spatial distribution (dense and sparse) was studied. The variation in these studies can be attributed to the findings

that the ability of any forage material to produce a potential gas depends on the ability of the rumen liquor donor to meet its required nutrients (Babayemi, 2007). *Megathyrsus maximus* produced higher methane than observe for *C. purpureus*, this could be due to the level of lignification that is associated with plant age and also the differences in morphology characteristics of the two grasses. This corroborates the findings of Melesse *et al* (2017) of higher methane production for *M. maximus* against *C. purpureus.* This is because different plant species react differently to climate change which affect the quality of forage plants due to rise in temperature and dry conditions as a result of changes in water soluble-carbohydrate and nitrogen (Rojas-Downing *et al.,* 2017). Dry matter digestibility (DMD) of the grasses decreased with plant advancement in age. This was affirmed by Dele (2012) and Muhammad (2014) that DMD decline with maturity. It could also be attributed to increase in ADF contents with plant age.

Conclusion

Early grazing of *C. purpureus* with narrower spacing encouraged the decline in methane production this could help in better utilization of energy and conversion to animal products and also reduced green-house gas emission which promotes healthier environment. Feeding *M. maximus* improved digestibility.

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