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Nutrient composition, *in vitro* digestibility, and methane production of two tropical grasses: Effect of grazing frequency and plant spacing

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ABSTRACT

This study aimed to evaluate the dry matter yield, nutrient composition, *in vitro* and methane produced from *Megathyrsus maximus* (Ntchisi) and *Cenchrus purpureus* grazed at two grazing frequency (3 and 6-week, (GF)) with two plant spacing (0.5 m x 1 m and 1 m x 1 m). The design of this experiment was split-split plot design in 2 x 2 x 2 factorial arrangement. Milled samples were analyzed for chemical composition, *In vitro* dry matter digestibility (IVDMD), total gas production, methane (CH_4) production, total volatile fatty acid content, content of acetate, butyrate, propionate and acetate: propionate ratio. The *M. maximus* grazed at 6-week GF with 1 m x 1 m spacing had the highest dry matter yield. The crude protein (CP) content of the grasses ranged from 104.24 – 181.35 g/kg DM with *C. purpureus* grazed at 3-week with 1 m x 1 m spacing ranked highest in CP content and protein fraction A, B_1, and B_2. *Megathyrsus maximus* at 6-week GF with 1 m x 1 m spacing produced the highest content of acid detergent fibre. Metabolizable energy (ME), short chain fatty acid and volume of gas produced at 48 h of incubation was highest for *M. maximus* grazed at 3-week GF with 0.5 m x 1 m spacing. *Megathyrsus maximus* at 3 and 6-week GF with 0.5 m x 1 m spacing displayed highest CH_4 value. Acetate, propionate, and total volatile fatty acid were significantly ($P<0.05$) highest for *M. maximus* grazed at 6-week with 0.5 m x 1 m spacing.

Keywords: *Megathyrsus maximus*, gas production, nutritive quality, dry matter yield

INTRODUCTION

Forages are crucial feed resource in ruminant animal production and have been reported to be sustainable and inexpensive feed that are easily obtainable for consumption by ruminants (Kubkomawa et al., 2015). The persistent in variation in terms of quantity and quality, due to season, species, environmental and management factors have resulted in low intake rate and ineffective usage of the forage plants. The inefficiency of farmers to utilize the growth advantage and the dry matter accumulation that is prominent to tropical forage species contributed significantly to the reduction in their productivity (Hughes et al., 2013).

The quality of grass as feed for cattle largely depended on the digestibility rate, chemical composition and the quantity of dry matter ingested (Lounglawan et al., 2014). Several factors like frequency and stage of cut, varietal differences, soil and climate condition have been reported to affect the chemical composition and the utilization of forage grasses (Buxton and Fales, 1994).

However, *Megathyrsus maximus* and *Cenchrus purpureus* have shown their ability of producing huge quantity of herbage biomass, digestible dry matter and consequently, increase the live weight gain of grazing animals (Almeida et al., 2000, Kozloski et al., 2005). The high dry matter accumulation that is prominent in tropical grasses and reduction in quality as a result of season and growth stage before cut or grazed has been reported (Dele, 2012, Hughes et al., 2013). The reduction in quality as a result low grazing frequency due to stem elongation, lignification and senesces of leaves has greatly affected the productivity of animals negatively which has adverse effect on farmers' output. It was reported by Jimoh et al. (2018) that the accumulation of dry matter is majorly determine by plant spacing as sparsely planted pasture produced less herbage as compared with the densely planted pasture.

The need for careful selection of grazing frequency that encourage high quality forage and at the same time produces high dry matter accumulation as a result of plant spacing is an important tool in pasture management, hence the objective for this study.

MATERIALS AND METHODS

Experimental site history and vegetation types

The experiment was conducted at the Cattle Production Venture Unit of the Federal University of Agriculture, Abeokuta in Ogun State, Nigeria. Grazing experimental site lies within the savanna agro-ecological zone of Southwestern Nigeria with annual temperature of about 35.08 to 36.98 °C during the raining season, and average annual precipitation of 1037 mm. The relative humidity ranges between 78.63 – 90.49% and 59.72 – 76.76% in the rainy season usually April - October and in the dry season usually November - March respectively, with an annual average of 78.4% (Table 1). A total area of 2496 m^2 of an existing plot was used for this study. The grasses were planted in April 2019 with the use of crown splits and stem cuttings for *M. maximus* and *C. purpureus*, respectively as vegetative propagules. The experimental plot area was fenced to avoid uncontrolled grazing of straying animals and the two grasses *M. maximus* and *C. purpureus* were spaced at 0.5 m x 1 m and 1 m x 1 m spacing. Prior to the commencement of the study, in April 2020, soil samples were randomly collected from the experimental plots at the depth of 0 – 15 cm top soil 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. The analysis of soil samples had the following values: pH: 5.84; organic carbon: 0.93%; nitrogen: 0.04%; phosphorus: 9.87 ppm; potassium :20.60 ppm; magnesium :51.34 ppm; calcium: 191.55 ppm; manganese: 30.36 ppm; copper: 0.65 ppm; zinc: 1.10 ppm; iron: 117.85 ppm; sand: 72%; silt: 10% and clay: 18%; texture: sandy loam. In May 2020, the grasses were cut back at the commencement of this experiment to 15 cm above ground surface and NPK 20:10:10 fertilizer was applied 8 days after cut back at the rate of 120 kgN/ha single application to stimulate regrowth.

Experimental design and pre-grazing herbage mass determination

The study was 2 x 2 x 2 factorial arrangement laid out in a split-split plot design. Each sub and sub-sub plot measured 20 m x 10 m and 10 m x 5 m respectively with 2 m spacing between plots. 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 (1 m x 1 m and 0.5 m x 1 m) as the sub-sub plot with three (3) replicates. Prior to grazing, 1 m^2 quadrat was thrown three times on each sub-sub plot at every grazing session and forages that fell within quadrat were clipped to 15 cm above the ground level, weighed and dried and dry matter yield estimated as described by Dele (2012). Sub samples were taken from the clipped forages, weighed and oven dried at 65 °C until constant weight was attained, milled and stored for chemical analysis. Twelve white Fulani yearling calves were used for this study with three animals per plot at each grazing session. The study was for a period of eighteen weeks from May 2020 to September 2020 with six sessions of 3-week grazing frequency and three sessions of 6-week grazing frequency. After every grazing session which is a 2-hour grazing period per day, there were cutback of the stubble to 15 cm height with no post grazing yield determined.

Chemical analysis

The milled samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash contents was determined according to AOAC (1995). Neutral

Table 1. Agro-climatological data for the year 2020

	Rainfall (mm)	Evaporation (mm)	Relative Humidity (%)	Mean Temp. °C
January	0	5.9	63.75	37.21
February	0	6.03	59.72	36.42
March	109	4.84	76.76	35.25
April	60.4	4.74	78.63	35.08
May	106.8	4.4	82.2	35.19
June	168.3	3.94	85.13	36.13
July	124.09	2.17	90.49	36.87
August	1	2.78	85.5	36.98
September	191.7	2.17	86.75	36.67
October	63.48	3.14	86.53	35.51
November	3.4	4.83	71.96	36.16
December	1.8	5.03	72.96	36.27

Source: Ogun- Oshun River Basin Development Authority, Abeokuta, 2020

detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) contents of the samples were determined according to Van Soest et al. (1991). Cellulose was calculated as the difference between ADF and ADL while hemicellulose was calculated as the differences between NDF and ADF. The crude protein fractions of the samples were estimated following procedure of Sniffen et al. (1992) modified by Licitra et al. (1996). A: crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1: true protein soluble in buffer solution and precipitated by the tungstic solution; B_2: true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3: true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; and C: true protein insoluble in the acid-detergent solution. Carbohydrate fractions of the samples were estimated following Cornell Net Carbohydrate and Protein (CNCP) system (Sniffen et al., 1992). CA: rapidly degradable CHO composed mainly of starch, CB_1: intermediately degradable CHO composed mainly of soluble fibers such as pectic polysaccharides, ß-glucans and fructans, CB_2: slowly degradable CHO composed of available NDF and CC: completely undegradable NDF

***In vitro* gas production and dry matter digestibility**

The *in vitro* gas production was determined following the modified of Menke and Steingass (1988) procedure. Milled samples of 200 ± 0.05 mg were weighed into 100 ml incubation syringes as the only substrate (*n* = 5). In addition, quintuplicate syringes without substrate were included as blanks to assist in estimating the net gas production. Rumen content was sourced from culled cattle at an abattoir located in Ojoo, Ibadan early in the morning and sieved with 4-layers cheese cloth and it was used as inoculum. Macro and micro elements, reduction and resazurin dye solutions were mixed together with distilled water, refluxed with CO_2 continuously until the pH was adjusted to 6.9 with buffer solution. This solution was mixed together in ratio 2:1 with the rumen fluid and the volume of solution-mixture used is 30 ml for each syringe. The syringes were placed in a water bath with temperature regulated at 39 °C. Gas produced in each syringe was read at 24 and 48 hours of incubation. Gas volume at each incubation time was expressed per unit of incubated dry matter (DM).

Methane production determination and post incubation estimations

After recording the final gas volume produced from the incubated contents, 4.0 ml of NaOH (10 M) was injected into each incubated syringe, which absorbs CO_2 content while the remaining gas volume in the syringe was considered to be methane (Fievez et al., 2005).

Organic matter digestibility (OMD) was estimated as:
OMD = 14.88 + 0.889GV+ 0.45 CP + 0.651 ash (Menke and Steingass, 1988).

Short-chain fatty acids (SCFA) was estimated as:
SCFA = 0.0239GV−0.0601 (Getachew et al., 2000).

Metabolizable energy (ME) was calculated as:
ME = 2.20 + 0.1357GV+ 0.0057 CP + 0.0002859 EE^2 (Menke and Steingass, 1988).

Total gas volume (GV) was expressed as ml/200mg DM, CP and ash as g/kg DM, ME as MJ/kg DM and SCFA as µmol/g DM.

In vitro dry matter digestibility (IVDMD)

After 48 hours' digestion, the contents of the syringes were centrifuge at 15,000 rpm for 10 minutes and residues were decanted into pre weighed crucibles and were oven-dried at 105 °C for 24 hours. The dry residues were weighed, and the digestibility was calculated using the formula below:
IVDMD (g/kg DM) = ((Initial DM input – DM Residue-Blank)/ Initial DM input) * 1000

In vitro ruminal volatile fatty acids

The decanted supernatant from the centrifuged after the 48-hour incubation were preserved with metaphosphoric acid (25%) and total volatile fatty acids (VFAs), proportions of acetate (A), propionate (P) and butyrate (B) were determined as described by Ikyume et al. (2020).

Statistical analysis

Data collected were analyzed using the General Linear Model (GLM) and the Tukey's HSD was used for separating the means (SAS, 1999).

RESULTS

The grazing frequency effect on the dry matter yield (DMY) differed (P<0.0001) significantly with the DMY of the grasses grazed at 6-GF greater than that of 3-GF by more than 144%. The species effect was also observed to significant (P<0.0001) with *M. maximus* more yield than the *C. purpureus* by over 67%. The effect of spacing on the DMY was not statistically different (P=0.07) though the narrowed spaced had yield over 16% than the wider spaced grasses (Figure 1).

The grazing frequency x species x spacing interaction significantly (P<0.0001) affected the DMY which ranged from 1.70 t/ha for *C. purpureus* with 1 m x 1 m spacing at 3-week grazing frequency to 9.41 t/ha for *M. maximus* with wider spacing and 6-week grazing frequency (Figure 2).

At 3-GF, the two grasses had higher accumulation of DMY when narrowed spaced but at 6-GF, the widely spaced *M. maximus* accumulated more DMY though statistically similar to the narrowed spaced.

As shown in Table 2, the grazing frequency and plant spacing of the grasses significantly (P<0.05) influenced the percentage of the measured proximate parameters except for dry matter content. The crude protein (CP) contents of the grasses were significantly (P<0.0001) influenced by the grazing frequency, species and spacing at the main and interaction effect levels. CP (181.35 g/kg DM) of *C. purpureus* grazed at 3-week GF with 1 m x 1 m spacing was significantly (P<0.05) highest. It was observed that the two grasses more frequently grazed (3-week GF) had higher CP content compared to the less frequently grazed (3-week GF), this might be as a result of decline in quality that have been associated with maturity as plants advanced in age are known to have low leaf to stem ratio and the wider spaced had better quality in terms of CP than the narrower spaced for both species as this could be attributed to competition of nutrients which is known to favour wider spaced plants. The CP of *C. purpureus* grazed at 3-week GF with 1 m x 1 m spacing which was the highest CP value in this study was 42.6% better than *M. maximus* grazed at 6-week GF with 0.5 m x

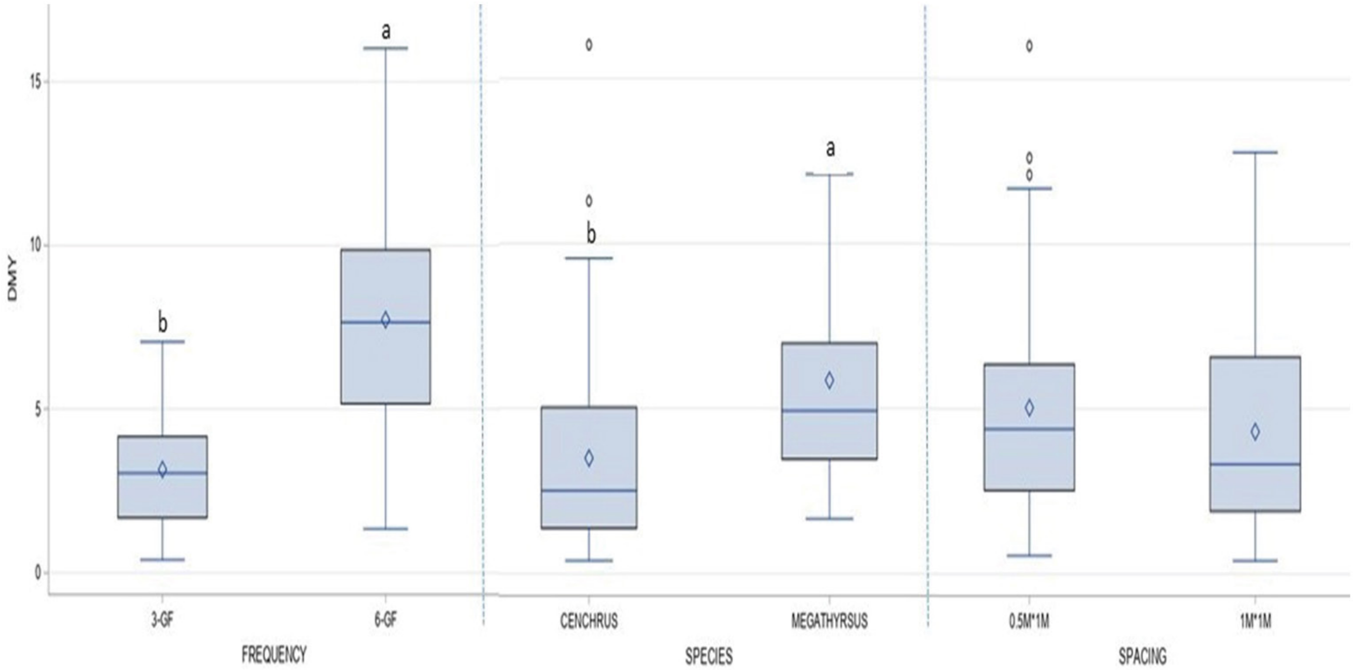

Figure 1. Main effect of grazing frequency and plant spacing on the dry matter yield (t/ha) of two tropical grasses

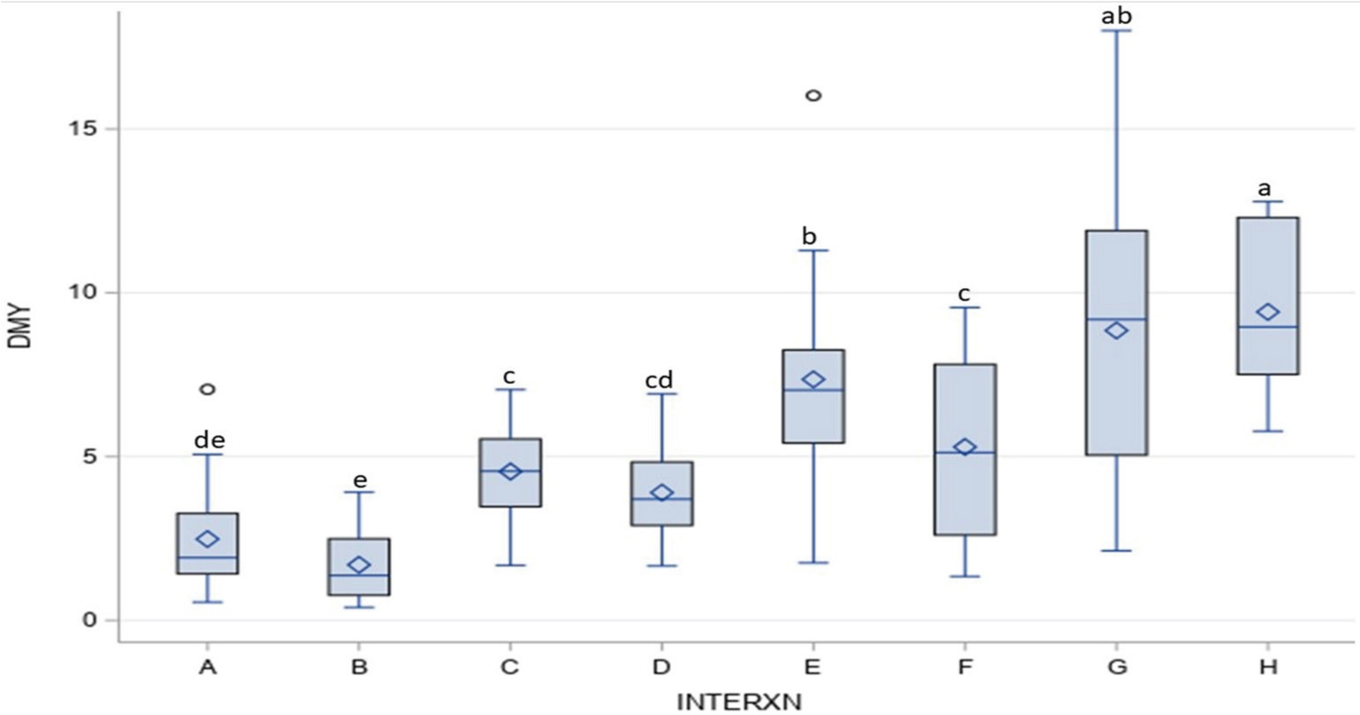

Figure 2. Interaction effects of grazing frequency and plant spacing on the dry matter yield (t/ha) of two tropical grasses

A = 3-week grazing frequency *Cenchrus purpureus* with 0.5 m x 1 m spacing
B = 3-week grazing frequency *Cenchrus purpureus* with 1 m x 1 m spacing
C = 3-week grazing frequency *Megathyrsus maximus* with 0.5 m x 1 m spacing
D = 3-week grazing frequency *Megathyrsus maximus* with 1 m x 1 m spacing
E = 6-week grazing frequency *Cenchrus purpureus* with 0.5 m x 1 m spacing
F = 6-week grazing frequency *Cenchrus purpureus* with 1 m x 1 m spacing
G = 6-week grazing frequency *Megathyrsus maximus* with 0.5 m x 1 m spacing
H = 6-week grazing frequency *Megathyrsus maximus* with 1 m x 1 m spacing

1 m spacing and 6.5% better than *C. purpureus* grazed at 3-week GF with 0.5 m x 1 m spacing. There was decline in percentage crude protein contents of the two grass species with decreased grazing frequency and increased plant spacing. The ether extract (EE) was influenced by the grazing frequency as the main effect whereas the species (P=0.063) and spacing (P=0.1427) did not exert influence on the EE but it was significantly (P<0.0001) influenced at all the levels of interactions. Percentage EE content was highest at 3-week *C. purpureus* with 0.5 m x 1 m spacing. The ash content was affected at all levels of interaction and main effects except for the grazing frequency (P=0.1283). Ash content ranged from 129.78 g/kg DM to 166.74 g/kg DM for *M. maximus* grazed at 3-week with 0.5 m x 1 m spacing and *C. purpureus* grazed at 6-week with 1 m x 1m spacing respectively.

Grazing frequency and plant spacing had significant (P<0.05) effect on the fibre composition of the grasses (Table 3). Neutral detergent fibre (NDF), ADF, and cellulose percentage contents of the grasses had higher value at 6-week GF. There was significant effect on the fibre composition of the two grasses. *Megathyrsus maximus* had significant (P<0.05) higher NDF and hemicellulose contents. The NDF content of the grasses were significantly (P<0.05) higher at 6-week GF. The fibre composition percentage of the grasses differs (P<0.05) between the interactions of the factors except for cellulose that had greatest percentage at 6-week GF for the two grass species and plant spacing (Table 3). However, the percentage concentration of NDF was highest at 6-week GF *M. maximus* for the two-plant spacing while ADF percentage had the highest concentration at 0.5

Table 2. Effects of grazing frequency and plant spacing on the proximate compositions (g/kg DM) of two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	Factors			
			DM	CP	EE	Ash
3	*C. purpureus*	0.5 x 1	935.78	169.68^{b}	41.45^{a}	151.64^{b}
		1 x 1	933.43	181.35^{a}	37.70^{bc}	163.44^{a}
	M. maximus	0.5 x 1	925.98	144.18^{cd}	38.53^{b}	129.78^{c}
		1 x 1	925.92	152.95^{c}	38.68^{b}	136.75^{c}
6	*C. purpureus*	0.5 x 1	932.69	134.18^{de}	38.23^{b}	158.06^{ab}
		1 x 1	924.12	145.29^{c}	35.83^{cd}	166.74^{a}
	M. maximus	0.5 x 1	931.74	104.24^{f}	34.57^{d}	135.76^{c}
		1 x 1	935.00	124.56^{e}	37.37^{bc}	134.01^{c}
SEM			1.67	2.03	0.29	1.41
P-value						
Grazing frequency			0.8632	<.0001	<.0001	0.1283
Grass species			0.6037	<.0001	0.0630	<.0001
Plant spacing			0.5867	<.0001	0.1427	0.0028
Grazing frequency x grass species			0.1543	<.0001	<.0001	<.0001
Grazing frequency x plant spacing			0.9556	<.0001	<.0001	0.0266
Grass species x plant spacing			0.4882	<.0001	<.0001	<.0001
Grazing frequency x grass species x plant spacing			0.4687	<.0001	<.0001	<.0001

[a, b, c, d, e, f] Means in same column with different superscripts were significantly (P < 0.05) different
DM: Dry matter, CP: Crude protein, EE: Ether extract, SEM: Standard Error of Means

m x 1 m plant spacing only. Acid detergent lignin (ADL) concentration was highest at 3-week *C. purpureus* spaced at 0.5 m x 1 m. At 3 and 6-week GF of *M. maximus*, for the two-plant spacing were statistically the same and the highest for hemicellulose percentage concentration.

Grazing frequency significantly (P<0.05) influenced the protein fractions of the grasses with the 3-week GF having higher value across all fractions (Table 4). Grass species and plant spacing had no significant (P>0.05) effect on C fraction, however, *C. purpureus* had higher values across other fractions while 0.5 m x 1 m spaced grasses had lower protein fractions. Protein fractions percentage were significantly (P<0.05) affected by the interaction effect of grazing frequency, grass species and plant spacing (Table 3). The percentage protein fractions A, B_1, B_2 and B_3 of *C. purpureus* at 3-week GF spaced at 1 m x 1 m was more than the observed percentage for all other interactions. *Cenchrus purpureus* grazed at 3-week with the two-plant spacing resulted in a higher percentage protein fraction B_3. Protein fraction C percentage ranged from 11.22 g/kg DM – 13.93 g/kg DM when *C. purpureus* with 0.5 m x 1 m was grazed at 6-week and at 3-week respectively.

Grazing frequency and plant spacing effect on all the carbohydrate fractions (CA, CB_1, CB_2 and CC) of the grasses were significant (P<0.05). At 6-week GF all the carbohydrate fractions were significantly (P<0.05) higher. The carbohydrate fractions CA ranged from 90.33 g/kg DM to 100.44 g/kg DM significantly (P<0.05) with *M. maximus* grazed at 6-week at 0.5 m x 1 m spacing having

Table 3. Effects of grazing frequency and plant spacing on the fibre composition (g/kg DM) of two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	Factors				
			NDF	ADF	ADL	Hemicellulose	Cellulose
3	*C. purpureus*	0.5 x 1	613.14^{b}	415.67^{cd}	39.99^{a}	197.47^{abc}	375.69^{b}
		1 x 1	591.49^{cd}	406.91^{de}	34.82^{abc}	184.58^{c}	372.09^{bc}
	M. maximus	0.5 x 1	611.77^{bc}	398.07^{e}	33.07^{abc}	213.70^{a}	365.00^{bc}
		1 x 1	604.78^{bcd}	394.15^{e}	32.44^{bc}	210.63^{a}	361.71^{c}
6	*C. purpureus*	0.5 x 1	615.63^{b}	424.09^{bc}	30.53^{c}	191.34^{bc}	393.56^{a}
		1 x 1	590.40^{d}	424.08^{bc}	30.15^{c}	166.31^{d}	393.93^{a}
	M. maximus	0.5 x 1	656.46^{a}	442.04^{a}	36.21^{abc}	214.41^{a}	405.84^{a}
		1 x 1	638.91^{ab}	432.73^{ab}	38.11^{ab}	206.18^{ab}	394.62^{a}
SEM			2.68	2.08	0.8	2.11	1.77
P-value							
Grazing frequency			<.0001	<.0001	0.4227	0.0764	<.0001
Grass species			<.0001	0.8043	0.5126	<.0001	0.5078
Plant spacing			0.0004	0.1478	0.5196	0.0019	0.1478
Grazing frequency x grass species			<.0001	0.0002	0.0007	<.0001	0.0059
Grazing frequency x plant spacing			<.0001	<.0001	0.4154	0.0170	<.0001
Grass species x plant spacing			<.0001	0.2853	0.4323	<.0001	0.3255
Grazing frequency x grass species x plant spacing			<.0001	<.0001	0.0219	<.0001	<.0001

[a, b, c, d, e] Means in same column with different superscripts were significantly (P < 0.05) different

NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Lignin, SEM = Standard Error of Means

Table 4. Effects of grazing frequency and plant spacing on the protein fractions (g/kg DM) of two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	Factors				
			A	B_1	B_2	B_3	C
3	*C. purpureus*	0.5 x 1	69.94^{b}	4.74^{b}	30.88^{b}	47.74^{a}	13.93^{a}
		1 x 1	76.80^{a}	4.98^{a}	34.17^{a}	49.68^{a}	12.90^{ab}
	M. maximus	0.5 x 1	59.16^{d}	4.23^{cd}	28.51^{bc}	40.36^{b}	11.94^{b}
		1 x 1	63.29^{cd}	4.41^{c}	30.22^{b}	42.56^{b}	11.99^{b}
6	*C. purpureus*	0.5 x 1	60.14^{d}	4.03^{ed}	22.31^{d}	31.97^{c}	11.22^{b}
		1 x 1	65.39^{c}	4.26^{c}	26.29c	34.83^{c}	11.35^{b}
	M. maximus	0.5 x 1	45.19^{f}	3.44^{f}	14.43e	24.72^{d}	11.60^{b}
		1 x 1	51.81^{e}	3.84^{e}	20.86^{d}	32.98^{c}	12.57^{ab}
SEM			0.83	0.04	0.56	0.77	0.19
P-value							
Grazing frequency			<.0001	<.0001	<.0001	<.0001	0.0104
Grass species			<.0001	<.0001	<.0001	<.0001	0.3968
Plant spacing			<.0001	<.0001	<.0001	0.0008	0.9340
Grazing frequency x grass species			<.0001	<.0001	<.0001	<.0001	0.0004
Grazing frequency x plant spacing			<.0001	<.0001	<.0001	<.0001	0.0454
Grass species x plant spacing			<.0001	<.0001	<.0001	<.0001	0.1528
Grazing frequency x grass species x plant spacing			<.0001	<.0001	<.0001	<.0001	0.0029

$^{a, b, c, d, e, f}$ Means in same column with different superscripts were significantly ($P < 0.05$) different

A = Non Protein Nitrogen, B_1 = Soluble true protein, B_2 = Soluble protein in neutral detergent, B_3 = Insoluble protein in neutral detergent but soluble in acid detergent, C = Unavailable protein/ True protein, SEM = Standard Error of Means

the highest value. The carbohydrate fractions CB_1, CB_2 and CC followed similar pattern with *M. maximus* grazed at 6-week with narrowed spacing having the values 46.67 g/kg DM, 503.37 g/kg DM and 82.45 g/kg DM, respectively (Table 5).

In vitro dry matter and organic matter digestibility were influenced by grazing frequency such that the frequently grazed grasses (3-week) were higher in IVDMD and OMD values (Table 6). The gas volume produced at 24 and 48 h of incubation, SCFA, OMD and methane were significantly higher for *M. maximus*. *In vitro* dry matter digestibility, gas volume and methane were significantly (P<0.05) affected by the interaction of grazing frequency x plant species x plant spacing (Table 5). At 24 and 48 h of incubation, *M. maximus* at 3-week GF with 0.5 m x 1 m spacing produced highest gas volume (17.43 ml/200mg DM and 31.33 ml/200mg DM respectively) and the least gas volume was recorded at 3-week GF *C. purpureus* with 1 m x 1 m spacing. *Megathyrsus maximus* grazed at 3 and 6-week with narrower spacing are statistically same and highest in methane (8.08 and 7.89 ml/200mg DM respectively). The percentage of *in vitro* dry matter digestibility (DMD) ranged from 529.67 g/kg DM for *C. purpureus* spaced by 0.5 m x 1 m and grazed at 6-week GF to 645.33 g/kg DM for *M. maximus* spaced by 0.5 m x 1 m and grazed at 3-week GF.

Acetate, propionate and TVFA were significantly affected by the effect of grazing frequency, grass species and plant spacing. The total volatile fatty acid and acetate, butyrate and propionate of the two grass species were significantly (P<0.05) affected by the interaction of grazing frequency and plant spacing. Acetate, propionate and TVFA concentration were significantly highest (P<0.05) for *M. maximus* grazed at 6-week with 0.5 m x 1 m spacing while the highest butyrate and acetate: propionate concentration was recorded for *M. maximus* at 3-week GF with 0.5 m x 1 m spacing as well (Table 7).

Table 5. Effects of grazing frequency and plant spacing on the carbohydrate fractions (g/kg DM) of two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	Factors			
			CA	CB_1	CB_2	CC
3	*C. purpureus*	0.5 x 1	93.81^c	43.59^b	470.16^b	77.01^c
		1 x 1	90.50^d	42.06^c	453.55^e	74.29^d
	M. maximus	0.5 x 1	93.60^c	43.50^b	469.10^c	76.84^{cd}
		1 x 1	92.53^{cd}	43.00^{bc}	463.75^d	75.96^{cd}
6	*C. purpureus*	0.5 x 1	94.19^c	43.77^b	472.07^b	77.32^c
		1 x 1	90.33^d	41.98^c	452.71^e	74.15^d
	M. maximus	0.5 x 1	100.44^a	46.67^a	503.37^a	82.45^a
		1 x 1	97.75^b	45.43^{ab}	489.92^{ab}	80.25^b
SEM			0.41	0.19	2.05	0.34
P-value						
Grazing frequency			<.0001	<.0001	<.0001	<.0001
Grass species			<.0001	<.0001	<.0001	<.0001
Plant spacing			0.0004	0.0004	0.0003	0.0004
Grazing frequency x grass species			<.0001	<.0001	<.0001	<.0001
Grazing frequency x plant spacing			<.0001	<.0001	<.0001	<.0001
Grass species x plant spacing			<.0001	<.0001	<.0001	<.0001
Grazing frequency x grass species x plant spacing			<.0001	<.0001	<.0001	<.0001

$^{a, b, c, d, e}$ Means in same column with different superscripts were significantly (P < 0.05) different

CA = Rapidly degradable carbohydrate, CB_1 = Intermediately degradable carbohydrate, CB_2 = Slowly degradable carbohydrate, CC = Completely undegradable carbohydrate

Table 6. Effects of grazing frequency and plant spacing on the *in vitro* gas production (ml/200mg DM), methane and post incubation calculations of the two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	ml/200mg DM			SCFA (µmol)	OMD (g/kg DM)	ME (MJ/kg)	CH_4/GV_{48h} (%)	DMD (g/kg DM)
			24 (hr)	48 (hr)	$CH_{4\text{-}48h}$					
3	*C. purpureus*	0.5 x 1	10.93[c]	21.53[de]	5.53[c]	0.20[c]	421.61[b]	3.79[cd]	25.40[c]	542.92[ab]
		1 x 1	9.67[c]	20.43[e]	5.85[bc]	0.17[c]	422.70[b]	3.62[d]	29.21[abc]	626.60[ab]
	M. maximus	0.5 x 1	17.43[a]	31.33[a]	8.08[a]	0.36[a]	453.23[a]	4.65[a]	26.13[bc]	645.33[a]
		1 x 1	14.90[ab]	27.47[abc]	7.13[abc]	0.29[ab]	439.14[ab]	4.31[ab]	26.30[abc]	638.19[ab]
6	*C. purpureus*	0.5 x 1	10.60[c]	22.73[cde]	6.93[abc]	0.19[c]	406.30[b]	3.72[d]	32.04[ab]	529.67[b]
		1 x 1	10.40[c]	22.80[cde]	7.48[ab]	0.19[c]	415.23[b]	3.69[d]	32.37[a]	574.61[ab]
	M. maximus	0.5 x 1	14.53[ab]	28.13[ab]	7.89[a]	0.29[ab]	413.29[b]	4.24[abc]	28.25[abc]	538.20[ab]
		1 x 1	12.88[bc]	25.47[bcd]	6.39[abc]	0.25[bc]	406.51[b]	4.02[bcd]	25.91[bc]	554.29[ab]
SEM			0.43	0.59	0.20	0.01	0.36	0.06	0.67	1.17
P-value										
Grazing frequency			0.1642	0.7170	0.2060	0.1642	0.0012	0.1146	0.0396	0.0086
Grass species			<.0001	<.0001	0.0260	<.0001	0.1131	<.0001	0.0267	0.2902
Plant spacing			0.0825	0.0944	0.3376	0.0825	0.7102	0.0941	0.7244	0.1556
Grazing frequency x grass species			<.0001	<.0001	0.0007	<.0001	0.0004	<.0001	0.0218	0.0124
Grazing frequency x plant spacing			0.1551	0.3339	0.5069	0.1551	0.0132	0.1356	0.1199	0.0271
Grass species x plant spacing			<.0001	<.0001	0.0024	<.0001	0.0940	<.0001	0.1429	0.0712
Grazing frequency x grass species x plant spacing			<.0001	<.0001	0.0027	<.0001	0.0057	<.0001	0.0745	0.0248

[a, b, c, d, e] Means in same column with different superscripts were significantly ($P < 0.05$) different

SCFA = Short Chain Fatty-Acid, OMD = Organic Matter Digestibility, ME = Metabolizable Energy, CH_4 = Methane, CH_4/GV = Methane/ Gas volume, DMD = Dry Matter Digestibility, SEM = Standard Error of Means

Table 7. Effects of grazing frequency and plant spacing on the acetate, propionate, butyrate, and total VFA production (mmol/g DM) of the two tropical grasses

Grazing frequency (week)	Grass species	Plant spacing (m^2)	Factors				
			Acetate	Butyrate	Propionate	TVFA	Acetate: Propionate
3	*C. purpureus*	0.5 x 1	1.04^{e}	0.39^{d}	1.02^{f}	2.54^{f}	1.06^{bc}
		1 x 1	1.03^{e}	0.39^{d}	1.01^{g}	2.52^{f}	1.02^{c}
	M. maximus	0.5 x 1	1.17^{b}	0.42^{a}	1.07^{c}	2.75^{b}	1.09^{a}
		1 x 1	1.14^{c}	0.41^{b}	1.06^{d}	2.70^{c}	1.08^{ab}
6	*C. purpureus*	0.5 x 1	1.12^{d}	0.39^{d}	1.06^{d}	2.67^{d}	1.06^{bc}
		1 x 1	1.11^{d}	0.40^{c}	1.04^{e}	2.64^{e}	1.07^{b}
	M. maximus	0.5 x 1	1.19^{a}	0.41^{b}	1.11^{a}	2.80^{a}	1.07^{b}
		1 x 1	1.16^{bc}	0.41^{b}	1.08^{b}	2.74^{b}	1.07^{b}
SEM			0.01	0.00	0.00	0.01	0.00
P-value							
Grazing frequency			<.0001	0.4690	<.0001	<.0001	0.1002
Grass species			<.0001	<.0001	<.0001	0.0011	<.0001
Plant spacing			0.0080	0.6548	<.0001	<.0001	0.8761
Grazing frequency x grass species			<.0001	<.0001	<.0001	<.0001	<.0001
Grazing frequency x plant spacing			<.0001	0.7352	<.0001	<.0001	0.3498
Grass species x plant spacing			<.0001	<.0001	<.0001	<.0001	<.0001
Grazing frequency x grass species x plant spacing			<.0001	<.0001	<.0001	<.0001	<.0001

$^{a, b, c, d, e, f, g}$ Means in same column with different superscripts were significantly ($P < 0.05$) different
TVFA= Total Volatile Fatty Acid, SEM = Standard Error of Means

DISCUSSION

Grazing frequency had influence on dry matter yield of the grasses. The results obtained from this study showed an increase in dry matter yield (DMY) with less frequently grazed grasses. This result was expected as there was a build-up of cell wall component and increased growth rate of less frequently grazed grasses. This corroborated the report of Tilahun et al. (2017) where latter harvested *P. pedicellatum* had higher yield than early harvested grass. Man and Wiktorsson (2003) had similar results with the observation of this study for elephant grass and two species of guinea grass harvested at different time intervals. On the other hand, this contradicts the report of Muhammad (2014) where sole panicum grass produced less dry matter yield with longer cutting intervals. Variation in these results could probably be due to the longer cutting interval used (up to 90 days) while this study's longest interval was 6 weeks and soil nutrients deficiency. The dry matter yield of *M. maximus* was substantially higher than recorded for *C. purpureus*. This agreed with the findings of Man and Wiktorsson (2003) where the dry matter yield same two species were influenced by cutting frequency. However, this result was contrary to the findings of Dele (2012) and Munyasi et al. (2015) where they recorded higher dry matter yield in *C. purpureus*. The difference in these results could be due to the fact that grass species respond differently when grazed compared to cutting.

In the current study, the crude protein of the two grasses differ as there were interplay with the plant spacing and grazing frequency. The higher CP observed at 3-week grazing frequency for *C. purpureus* at a wider plant spacing could be related to the interaction between genotype and management practices in terms of the grazing frequency and plant spacing. Previous studies have reported higher CP for *C. purpureus* to *M. maximus* (Man and Wiltorson, 2003; Dele, 2012). Grazing frequency (Ayala Torales et al., 2000) and maturity or stage of growth (Dele, 2012) have also been reported as factors that affect the quality of forages as it was observed in the decline of the CP where 3-week grazed grasses had higher CP than 6-week grazed grasses. This was expected as the less grazed tend to get fibrous than the frequently grazed (Ayala Torales et al., 2000). The wider spaced grasses in this study were observed to have higher CP than the narrowed spaced as the competition in terms of nutrients and sunlight within the wider spaced is expected to be lesser than those of the narrowed spaced. The interaction effect of grazing frequency and plant spacing resulted in variant nutrient contents of the grasses. The differences in the morphological characteristics of the grasses were considered as reason for the depletion in crude protein content of *Megathyrsus maximus* in this study.

Although, the growth parameters for these grasses were not reported, the result showed that *C. purpureus* with wider plant spacing produced more leaves because the competition for soil nutrient, water and light was reduced and studies affirmed that plant components is one of the major factors that affect crude protein content and that is the ratio of leaf to stem (Halk et al., 2015). Reduction in grazing frequency resulted in the production of lignified grasses which affected the nutrient composition and the digestibility of the grasses. Lounglawan et al. (2014) and Dele (2012) reported decrease in CP content with advancement in grass age due to stem elongation. Frequently grazed forages have higher CP because of the removal of old stocks and giving opportunity for regrowth which have higher leaf to stem ratio. It was generally observed that the CP contents reported in this study were higher than 7% and considered adequate protein level in the diet of ruminant for optimal growth and ruminal microorganisms' activities, therefore yielding desirable amount of microbial crude protein and optimizing ruminal fermentation (NRC 2000). The grasses grazed at 3-week GF had higher CP enough to replace creep feeds for nursing young ruminants which could help in gut development of young animals (Terler et al., 2022). The ash content recorded for the grasses in this study fell within the range (10-18%) reported to be common for the forages contaminated with soil (Hoffman, 2005) and this could be responsible for the non-significance of the grazing frequency which is in contrast to the reports of Dele (2012) and Weiss (2019) that reported decrease in the ash content of forages as plant mature. The EE content recorded showed that the narrowed spaced *C. purpureus* grazed at 3-weeks was the highest and was in line with the report of Jimoh (2017) that reported higher EE content for densely populated pasture than sparsely populated pasture. This also could infer that this grass could supply energy for optimum growth and maintenance of the calves.

The mean NDF of the grasses increased with the less frequently grazed (6-week). The increase in the NDF with the less frequently grazed grasses could be attributed to age, this is consistent with the findings of Costa et al. (2007) and Dele (2012), which reported that ageing of grasses lead to lignification. The recorded NDF in this study for most of the treatment combinations were less than 65% which was reported as critical limit above which ruminants cannot efficiently utilize the forages (Van Soest 1982; Muia, 2000). The moderate NDF recorded in this study can promote the activities of fibre degrading rumen microbiota, which can help improve higher rate of fermentation could promote digestibility, intake and better animal productivity. The wider spaced grasses were recorded to be relatively less fibrous than the closely spaced ones. This is consistent with the findings of Tadesse et al. (2021) that stated that closely spacing have high limitation with nutrient resource which is said to be and make them to have higher structural components to cope with the stress full environmental condition.

Fractionalization of crude protein in nitrogen component for accurate formulation of ruminant diet was recommended by Sniffen et al. (1992) because it reduced nitrogen loss by the animal and improved the performance of rumen microbial flora. Maturity of grass is a determinant factor for digestibility. However, the rate of rumen degradation for the frequently grazed grasses in this study was high which was as a result of their less fibrous nature. Protein fraction A and B_1 has high degradability in the rumen and as such *C. purpureus* was highly digestible when grazed early. Johnson et al. (2001) reported that different forage species at varying harvest date produced varying protein fraction results. This corroborates the findings of this study. The highest protein fraction A recorded for *C. purpureus* with a wider space grazed at 3-week frequency had the highest CP, it had a better protein fraction A than same species with same spacing grazed at 6-week frequency by 14.9% and 41.2% higher in protein fraction A than *M. maximus* with narrower spacing grazed at 6-week frequency with the least protein fraction A value (45.19%). The protein fraction A of *C. purpureus* with wider spacing grazed at 3-week been the highest could be due to the high amount of crude protein which is connected to the age which it was grazed as well as the spacing that gave favoured less competition for nutrient, hence improving the degradation rate. This higher CP and protein fraction A could lead to better animal productivity (Brandstetter et al., 2018). The higher protein fraction A as observed in this study have been reported to be good for ruminal functionality as ammonia has been a nitrogen source is used by microbes that ferment structural carbohydrate (Russel et al., 1992). The protein degradation in the rumen is known to be affected by age of the forage (Aufrere et al., 2002).

This study confirmed the finding that *C. purpureus* grazed at 3-week with wider spacing to have higher protein fraction B_1 and B_2. This result can also be related to the fact that age has a correlation with forage quality in terms of protein fractions. The protein fraction B_1 in this study for *C. purpureus* is higher than that reported by Salazar-Cubillas and Dickhoefer (2021) for same species but fell within the range (0.3-9.2%) reported by same authors for tropical grasses. The higher crude protein of the grasses in the current study as it relates to age may have constituted to the high solubility of protein fractions A, B_1 and B_2 of the *C. purpureus*. The higher proportion of protein fraction C may be considered a limitation to protein utilization because of the protein linkage with lignin (Licitra et al., 1996). The protein fraction C in this study fell within the range (5.4-27%) reported in literature for tropical grasses (Salazar-Cubillas and Dickhoefer, 2021). The moderate protein fraction C in this study reiterate the fact that relatively younger forages have higher potential for better ruminant livestock productivity.

Carbohydrates are known to be majorly the source of energy that determine animal performance (Van Soest, 1994). *Megathyrsus maximus* grazed at 6-week with narrowed spacing had higher rapidly degradable fraction of soluble sugar CA which was slightly higher when compared with the wider spaced, this could mean that spacing has little effect on fraction CA of the grasses. Higher fraction CA value could mean that the *M. maximus* grazed at 6-week will be a higher source of quick energy because of the higher value of the rapidly degradable nature of the carbohydrate for ruminal microbes. Following the higher NDF value of the forages the higher fraction CA was not expected but could be related to the short frequencies (3 and 6-week) employed in this study, which might not amount to a huge difference in the maturity as reported by Yu et al. (2003), also, leaf soluble fibre has been reported to have tendency to increase with age (Hall et al., 1997). The higher CB_2, which is referred to as the slowly degradable carbohydrate of *M. maximus* spaced at 0.5m x 1m grazed at 6-week was expected because of maturity, which agreed with Van Soest (1994) that structural carbohydrate tend to increase with a corresponding decrease of non-structural carbohydrate with maturity. The higher carbohydrate fraction CC recorded for the narrowed spaced *M. maximus* grazed at 6-week was also expected for the reason of maturity which correlates with higher NDF, it is the fraction of the cell wall that is highly undegradable and unavailable for utilization by ruminal microorganisms. The higher level of fraction CB_2 and CC could mean that there might be the need to supplement energy source that could help make energy readily available for the ruminal microorganisms (Epifanio et al., 2014; Ferreira et al., 2018; Teixeira et al., 2022)

The *Megathyrsus maximus* produced relatively high gas volume in this study but had the least CP content. This follows similar trend with the report of Dele (2012), where grass with the least CP content produced the highest gas volume. This could be as a result of high content of ADL found in *C. purpureus* and Pazla et al. (2021) stated that low gas production could be due to high lignin content. The decrease in the total gas production with less frequently grazed (6-week) was expected as the *in vitro* dry matter digestibility also decreased which might be associated with the age and relatively higher NDF values, this is in line with the finding of Ribeiro et al. (2014). The methane production was scarcely affected by spacing but there was species differential effect as well as age effect. The relative higher methane recorded for *M. maximus* against *C. purpureus* in this current study agreed with the findings of Melesse et al. (2017) where *M. maximus* recorded relatively higher methane values than *C. purpureus*, this means that feeding *C. purpureus* to animals could amount to better energy utilization and conversion to animal products and as well promote a better and healthier environment. The higher methane associated with *M. maximus* in this study could be due to the higher NDF (Ellis et al., 2007) which is linked to maturity as matured forages are known to be more fibrous than relatively younger forages, hence the higher methane with 6-week grazed grasses. As methane production amounts to energy loss in ruminant production, this current study have its findings that grazing *C. purpureus* at 3-week will amount to much lesser energy loss, which in turn will amount to better animal productivity and healthier environment. High SCFA and ME derived from *M. maximus* is suggested to be due to the high gas volume it produced and lower ADL content. This was affirmed by Sisay et al. (2018) that high gas volume contributes tremendously to energy supply through short chain fatty acid production.

The total volatile fatty acid (TVFA) in this study showed that *M. maximus* had higher values as against those of *C. purpureus* and that the two grasses grazed less frequently (6-week) were higher than the more frequently grazed. The TVFA have been reported to be the major source of energy for ruminants (Van Soest, 1982), so the higher TVFA recorded for narrowed spaced *M. maximus* grazed at 6-week corresponded with a higher carbohydrate fraction CA, which is considered rapidly degradable soluble carbohydrate which also is noted as a source of quick energy for the use of ruminants. The higher TVFA for the less frequently grazed grasses is consistent with the finding of Vargas et al. (2018). The higher acetate of the narrowed spaced *M. maximus* grazed at 6-week correspond with the higher methane produced as recorded in our result as acetate have been reported to promote the release of hydrogen gas (H_2) which is a precursor to methane formation (Hegarty and Gerdes, 1998; McAllister and Newbold, 2008). The highest propionate recorded for the narrowed spaced *M. maximus* at 6-week could also be responsible for the higher TVFA which is considered the energy source for the ruminal microbial activities.

CONCLUSION

Cenchrus purpureus grazed at 3-week grazing frequency with 1 m x 1 m spacing are superior in nutritive quality in terms of crude protein, protein fractions and phosphorus and are less fibrous and highly digestible but with lowest dry matter yield, therefore cutting or grazing *Cenchrus purpureus* 3-week can improve and meet the nutrient requirement of animal and improve their productivity. However, all the interactions level examined in the study are capable of meeting the nutrient requirement of grazing animals. Grazing or cutting *Megathyrsus maximus* at 6-week with 1 m x 1 m spacing encouraged more dry matter yield which enhanced grazing time and fill. The increased gas volume that is associated with the frequently grazed (3-week) *M. maximus* spaced at 0.5 m x 1 m in this study promotes the rate of digestibility and nutrient utilization. *Cenchrus purpureus* grazed at 3-week with 0.5 m x 1 m spacing generated least methane this helps in reducing energy loss by the animal, improve feed conversion and reduce the rate of greenhouse gas emission.

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