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INFLUENCE OF GROWTH STAGE OF *PANICUM MAXIMUM* ON THE NUTRIENT COMPOSITION, *IN VITRO* DIGESTIBILITY AND ENERGY VALUE FOR RUMINANTS

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ABSTRACT

Panicum maximum was harvested at 6, 8, 10 and 12 weeks of growth to determine its nutrient composition, *in vitro* nutrient digestibility and energy value (metabolizable energy, short chain fatty acids and methane production) using standard analytical methods and equations. The dry matter content of *Panicum maximum* increased with increased growth. Crude protein content reduced ($P < 0.05$) with maturity from 6 weeks (10.55%) to 12 weeks (7.39%). Panicum at 6, 8 and 10 weeks of growth had lower ($P < 0.05$) Neutral Detergent Fibre (42.87–46.93%), Acid Detergent Fibre (23.19–30.69%) and lignin (7.82–10.58%) contents compared with values at 12 weeks of growth. At 24 and 48 h post-incubation, the *in vitro* dry matter, crude protein and organic matter digestibility, short-chain fatty acids and metabolizable energy at 6 and 8 weeks were higher ($P < 0.05$) with less methane produced per gram of dry matter degraded. It can be concluded that, harvesting *Panicum maximum* for ruminant feeding at ages between 6 and 8 weeks will supply animals with high crude protein and adequate fibre which can be better digested in the rumen. The higher short chain fatty acids, higher metabolizable energy and lower methane production at these growth stages indicates an efficient supply of dietary energy in the rumen for utilization by ruminants.

Key words: *Panicum maximum*, growth stage, digestibility, energy value, ruminants

INTRODUCTION

Grasses constitute the bulk of ruminant diet and where they are high in nutritive value, they can make up the sole diet for ruminants. *Panicum maximum* (Guinea grass) is a major grass in the tropics serving as forage for ruminants in form of pasture, cut and carry, hay and silage (Aganga and Tshwenyane, 2004; Heuze and Tran, 2015). The combination of the physical, structural and chemical characteristics of a forage often determines the pasture quality and the availability of nutrients to ruminants (Newman *et al.*, 2006). The amount of nutrients in forage coupled with nutrient digestibility and energy value are significant indices of the nutritive quality of the forage, and these vary with plant growth. As plants mature, cells become lignified and this interferes with digestibility of nutrients as there is often a negative relationship between digestibility and maturity (Moore and Jung, 2001). According to Babayemi (2009), ruminants benefit little from over mature grass due to lignification. Asides from influencing digestive efficiency, the stage of maturity of the plant can also influence enteric methane emissions (Ribeiro *et al.*, 2014). Grasses at different stages of development have been reported to cause ruminants to produce different amounts of methane and the more fibrous the grass is, the greater the methane emission (Bassett, 2013). The emission of methane during

microbial degradation of forages in the rumen, both contribute to anthropogenic greenhouse gas emission and to loss of feed energy in ruminants (Hristov *et al.,* 2015). It is therefore believed that reducing the amount of methane production within the rumen might consequently increase the energy value of the forage. This study determined the influence of different growth stages of *Panicum maximum* on its nutritional value, digestibility and energy value for ruminants using the *in vitro* gas production technique in order to ascertain an appropriate growth stage which matches enhanced nutrient supply.

MATERIALS AND METHODS

Location of the Experiment

The experiment was conducted at the Research Farm of the College of Animal Science and Livestock Production (for pasture establishment), and the laboratory of the Department of Animal Nutrition (for *in vitro* studies), Federal University of Agriculture, Abeokuta, Nigeria. The region is located in the Derived Savannah vegetation zone of South-Western Nigeria and it falls within Latitude 7°13'23.8" N and Longitude 3°26'25.4" E (Google, 2018). The climatic condition is humid with a mean annual rainfall of 1037 mm. The mean annual temperature and humidity are 34.7°C and 82%, respectively (Meteorological Unit, FUNAAB).

Establishment of *Panicum maximum* Pasture

Panicum maximum pasture was establishment on an area of land of about 10, 000 m^2 within the University farm. The area of land was cleared and ploughed. Thereafter, the land was allowed to rest for a period of two weeks before harrowing. *Panicum maximum* (Ntchisi) was planted from crown splits. The crown splits of *Panicum maximum* (Ntchisi) was sourced from established plots within Abeokuta. The grass was planted at a spacing of 0.5 m x 0.5 m. After establishment, weeding was done at an interval of three weeks.

Harvesting and Preparation of the Forage

Panicum maximum grass was harvested at two weeks intervals (from six (6) to twelve (12) weeks of growth) for the determination of its nutritive value, *in vitro* nutrient degradation and energy value using *in vitro* technique. Aliquot samples of *Panicum maximum* harvested were collected in triplicates, which were weighed and oven-dried at 65°C until constant weight was attained for dry matter determination. Oven-dried samples were used for proximate and fibre analyses and for *in vitro* studies.

Chemical Analyses

Oven-dried samples of *Panicum maximum* were taken to the laboratory for determination of proximate and fibre composition. Nitrogen (N), ether extract and ash contents were determined according to the standard methods of AOAC (2000). Neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined by the methods of Van Soest *et al*. (1991). Crude protein was estimated as N x 6.25. Organic matter content was calculated as 100 – ash content. Non-structural carbohydrate was calculated as 100% ‾ % crude protein ‾ % neutral detergent fibre ‾ % ether extract ‾ % ash (NRC, 2001).

In vitro Digestibility Studies

In vitro gas production was determined according to the method of Menke and Steingass (1988) with slight modification. The rumen fluid used for incubation was collected prior to feeding from three West African dwarf goats which had been previously fed on grass and concentrate diet in the ratio 60: 40 of total dry matter offered. The concentrate diet was composed of 40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% brewers dried grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Rumen fluid was collected using suction tube into thermo flask pre-warmed to a temperature of 39°C. Five-hundred milligrams (500 mg) of oven-dried and milled samples of *Panicum maximum* harvested at 6, 8, 10 and 12 weeks of age were weighed into dacron bags of known weights and incubated *in vitro* in 100 ml calibrated transparent glass

syringes fitted with silicon tube in eight replicates. Each of the syringes was then filled with 30 ml inoculum containing rumen liquor strained through a four-layered cheese-cloth and buffer. The rumen liquor and buffer solution were mixed in a ratio of 1:2 (v/v). The mixture was handled under continuous anaerobic condition and immediately dispensed using 50ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston to eliminate air completely in the inoculums. The silicon tube in the syringe was tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 39^{o}C and the volume of gas produced was measured at three hours interval from 0 to 48 hrs. Three blanks containing 30 ml of medium only were included in the run. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample to correct for gas production not arising from substrate fermentation.

Estimation of Energy Value of *Panicum maximum*

Energy value of *Panicum maximum* harvested at 6, 8, 10 and 12 weeks of growth was estimated by determining post-incubation parameters (metabolizable energy, short chain fatty acids and methane production) from the different samples. Metabolizable energy (ME, MJ/kg DM) was calculated as 2.20 + 0.136GV + 0.057CP + 0.0029CF according to Menke and Steingass (1988). Short chain fatty acids (SCFA, µmol/g DM) was determined using the formula 0.0239GV – 0.0601 according to Getachew *et al.* (2000). The gas volume (GV) in the equations is the total gas volume at 24 hrs of incubation, while CP and CF are values for the crude protein and crude fibre, respectively. The volume of methane gas produced from each sample incubated was determined by dispensing 10 M sodium hydroxide into the incubated syringes at the end of 24 and 48 hrs of incubation using two of the replicated samples.

The sodium hydroxide was introduced into the incubated syringes via the silicon tube just above the metal clip through the tip of the needle of a 5 ml syringe. The clip was carefully unscrewed and the reagent introduced. Sodium hydroxide was added to absorb carbon-dioxide produced during the process of fermentation and the remaining gas in the syringe was recorded as methane according to Fievez *et al.* (2005). A pop sound which gave rise to the jacking upward movement of the piston indicated the absorption of carbon-dioxide by the sodium hydroxide. The syringe was then turned upside down for the reading of methane level.

***In vitro* Dry Matter, Crude Protein and Organic Matter Degradability**

After 24 and 48 hrs of incubation, the dacron bags were removed and rinsed properly under tap water until water runoff was clear. The bags were then oven-dried at 105^{o}C overnight for 6 h. The weight of dry residues was determined by removing the weight of dacron bag from the total weight of the bag and residue. The dry matter degradability (IVDMD) was then calculated using the equation below:

$$\text{IVDMD (\%)} = \frac{\text{Initial dry matter incubated – dry matter of residues}}{\text{Initial dry matter incubated}} \times 100$$

For determination of crude protein (CP) and organic matter (OM) degradability, residues were collected and analysed for CP and OM content as was done for the incubated samples. Crude protein degradability (CPD %) and organic matter degradability (OMD %) were calculated using the respective formula below:

$$\text{CPD (\%)} = \frac{\text{CP content of incubated sample – CP content of residue}}{\text{CP content of incubated sample}} \times 100$$

$$\text{OMD (\%)} = \frac{\text{OM content of incubated sample – OM content of residues}}{\text{OM content of incubated sample}} \times 100$$

Statistical Analysis, Experimental Design and Model

Data collected were subjected to one-way Analysis of Variance procedure of SAS (1999). Significant differences between means were compared at 5% probability level using Duncan Multiple Range Test (SAS, 1999). The statistical model used was:

$\Upsilon ij = \mu + T_i + \Sigma_{ij}$

Where:

Υ_{ij} = the observation;

μ = population mean;

T_i = effect of the i^{th} growth stages of *Panicum maximum* (i = 6, 8, 10, 12);

Σ_{ij} = residual error.

RESULTS AND DISCUSSION

The nutrient composition of *Panicum maximum* at different growth stages is presented in Table 1. At two weeks interval (from 6 weeks to 12 weeks of growth), the dry matter content of *P. maximum* increased ($P < 0.05$) from 33.55 to 47.03%. This reflects a higher biomass yield with advancement in age and could be an indication that ruminant animals will consume more dry matter from it at an advanced stage of growth relative to consumption from the grass at an earlier growth stage. According to Katoch *et al.* (2013), the dry matter content of forage determines how much of the forage an animal gets and the determination of dry matter in forage is important to ensure that animals receive proper amount of nutrients through their diet. While dry matter increased with age, the crude protein content of the plant reduced ($P < 0.05$) with advancement in age of the plant from 10.55% at 6 weeks to 7.39% at 12 weeks of growth. In line with our result, it has been reported that as plant matures, dry matter yield increases while crude protein content declines (Rusdy, 2014). Crude protein content is positively correlated with forage quality (Katoch *et al.*, 2013). Therefore, the lower crude protein content observed with increase in the growth of *P.maximum* signifies a reduction in its quality. Although ruminants can cope with low protein diets due to extra nitrogen supplied by microbial proteins in the rumen, a minimum requirement of 7 or 8% CP has been reported for normal rumen function and to supply rumen microbes with minimum ammonia levels for adequate rumen activity (Norton, 1998; Stan *et al.*, 2011).

Table 1: Chemical Composition (%) of *Panicum maximum* at Different Stages of Growth

	Stages of Growth of *P. maximum* (Weeks)				
Parameters (%)	**6**	**8**	**10**	**12**	**SEM**
Dry Matter (as fed basis)	33.65^{d}	38.02^{c}	44.11^{b}	47.03^{a}	1.59
Organic Matter	92.07^{b}	92.90^{a}	93.47^{a}	93.06^{a}	0.19
Crude Protein	10.55^{a}	9.44^{b}	8.68^{b}	7.39^{c}	0.38
Ether Extract	3.66^{a}	3.28^{ab}	3.10^{ab}	2.95^{b}	0.11
Ash	7.93^{a}	7.10^{b}	6.94^{b}	6.53^{b}	0.19
Non-Structural Carbohydrate	34.98^{a}	34.41^{a}	34.76^{a}	24.07^{b}	1.72
Neutral Detergent Fibre	42.87^{b}	45.76^{b}	46.93^{b}	58.68^{a}	2.04
Acid Detergent Fibre	23.19^{c}	27.21^{bc}	30.69^{b}	42.59^{a}	2.32
Acid Detergent Lignin	7.82^{d}	9.32^{c}	10.58^{b}	12.50^{a}	0.53

$^{a.b.c.d}$ Means along the same row having different superscripts are significantly different ($P < 0.05$). SEM = Standard Error of Mean.

When crude protein is below 8%, rumen bacteria responsible for digesting forage cannot maintain adequate growth rates and then forage intake and digestibility will decrease (Parish, 2008). From our results, *P. maximum* at 6, 8 and 10 weeks of age was considered adequate in crude protein to supply rumen bacteria with minimum ammonia levels required for proper rumen functioning.

The values for neutral detergent fibre in *P. maximum* at 6, 8 and 10 weeks of age, were similar ($P > 0.05$) but lower ($P < 0.05$) than the value obtained at 12 weeks of age. While the value for acid detergent fibre significantly ($P < 0.05$) increased at 12 weeks of age relative to earlier weeks of growth. The increase in neutral detergent fibre and acid detergent fibre fractionswith increase maturity of *P. maximum* could have implication on its intake and digestibility by ruminants. According to Blezinger (2013), neutral detergent fiber has traditionally been used as a predictor of forage intake, while acid detergent fibre has been used as a predictor of forage digestibility. The acid detergent lignin of *P. maximum* increased ($P < 0.05$) with maturity signifying a greater lignification of the cell wall of the plant. Lignin is a component of fibre that is essentially indigestible, accumulates mostly at maturity and acts as a barrier to fibre degradation by rumen microbes (Newman *et al.,* 2006). For grasses, when the neutral detergent fiber values fall below 50 percent, the grass is of a high quality. Above 60 percent however, it is considered of low quality (Van Saun, 2016). Although ruminants require some level of fibre in their diet for proper rumen function, our study found that *P. maximum* harvested at 6 to 10 weeks of growth had neutral detergent fibre values below 50 percent which is an indication of high nutritive quality above which quality begins to decline. However, according to Andualem *et al.* (2016), nutrient composition of feed which is commonly determined by its chemical composition does not provide sufficient information to determine the nutritive value of feeds.

Table 2 shows the *in vitro* gas production from degradation of *P. maximum* at different stages of growth. At the first 21 hours of incubation, *P. maximum* at 6 weeks had higher ($P < 0.05$) gas volumes relative to gas volumes at later growth stages. This suggests that *P. maximum* at this stage contained higher amounts of fermentable nutrients. According to Getachew *et al.* (2004), gas production reflects all nutrients fermented, soluble as well as insoluble; and fractions that are not fermentable do not contribute to gas production. From 24 to 48 hrs of incubation, a constant trend was observed in gas production with Panicum growth. Gas production of *P. maximum* at every two weeks interval from 6 to 8 weeks, 8 to 10 weeks and 10 to 12 weeks were similar ($P > 0.05$), while gas production at every four weeks interval from 6 to 10 weeks and 8 to 12 weeks reduced significantly ($P < 0.05$). This suggests that within a shorter process of growth, the amount of fermentable nutrients in the grass will remain unaltered but as growth exceeds two weeks, fermentable nutrients will decline. The higher gas produced from degradation of *P. maximum* at earlier growth stages was attributed to the higher crude protein content and lower fibre fractions at these stages. A positive correlation has been reported between crude protein and gas volumes (Andualem *et al.,* 2016) while a negative correlation is reported between neutral detergent fibre and gas volumes in grasses (Kulivand and Kafalzadeh, 2015).

Table 3 shows the amount of dry matter, organic matter and crude protein degraded at 24 and 48 hours of incubation from *P. maximum* at different stages of growth.

Table 2: In Vitro Gas Production (ml/g DM) from *Panicum maximum* at Different Growth Stages

	Stage of Growth of *P. maximum* (Weeks)				
Incubation Periods (hours)	**6**	**8**	**10**	**12**	**SEM**
3	5.33	2.76	2.67	2.00	0.58
6	8.00[a]	5.33[b]	4.00[c]	3.33[c]	0.58
9	14.67[a]	10.00[b]	6.00[c]	4.00[c]	1.38
12	22.00[a]	14.00[b]	10.67[c]	7.33[c]	1.81
15	30.00[a]	20.00[b]	16.00[c]	12.67[c]	2.16
18	32.00[a]	24.00[b]	18.00[c]	16.67[c]	2.04
21	34.67[a]	26.00[b]	20.00[c]	18.00[c]	2.11
24	36.67[a]	32.00[ab]	30.00[c]	24.00[c]	1.58
27	42.67[a]	37.33[ab]	32.67[c]	27.33[c]	1.93
30	44.00[a]	40.00[ab]	36.67[c]	32.00[c]	1.58
33	46.00[a]	43.33[ab]	38.00[c]	35.33[c]	1.50
36	48.00[a]	44.00[ab]	38.67[c]	36.67[c]	1.59
39	49.33[a]	43.33[ab]	36.00[c]	31.33[c]	2.35
42	50.67[a]	45.33[ab]	40.67[c]	36.00[c]	1.88
45	51.33[a]	47.33[ab]	42.00[c]	38.00[c]	1.83
48	52.00[a]	49.33[ab]	46.00[c]	42.67[c]	1.16

[a.b.c.] Means along the same row having different superscript are significantly different ($P < 0.05$). SEM: Standard error of mean

Table 3: Dry Matter, Organic Matter and Crude Protein Degradation (%) of *P. maximum* at Different Stages of Growth

	Growth Stages of *P. Maximum* (Weeks)				
Nutrient Degradation (%)	**6**	**8**	**10**	**12**	**SEM**
24 hours post incubation					
Dry Matter	61.31[a]	57.08[ab]	54.52[bc]	51.22[c]	1.49
Organic Matter	63.25[a]	61.56[ab]	58.71[bc]	56.17[c]	1.09
Crude Protein	58.01[a]	56.92[a]	53.48[ab]	50.37[b]	1.23
48 hours post incubation					
Dry Matter	70.31[a]	67.28[ab]	62.53[bc]	57.11[c]	2.02
Organic Matter	69.02[a]	65.11[ab]	60.10[bc]	55.89[c]	2.00
Crude Protein	65.37[a]	60.07[ab]	54.68[bc]	51.71[c]	2.05

[a.b.c.] Means along the same row having different superscript are significantly different ($P<0.05$). SEM: Standard Error of Mean

The degradation of dry matter, organic matter and crude protein at 24 and 48 hours were higher at 6 and 8 weeks relative to 12 weeks of age and this was related to the higher gas production at the early stages of *P. maximum* growth. Fievez *et al.* (2005) has reported digestibility to be synonymous to *in vitro* gas production. The higher organic matter digestibility of *P. maximum* at 6 and 8 weeks of growth compared with 12 weeks is a reflection of higher fermentation of nutrients by rumen microbes from less mature *P. maximum*. The organic matter in feed represents the crude protein, fat, soluble and structural carbohydrate fractions in the feed which are the nutrients supplying energy. A reduction in organic matter digestibility therefore, will imply a reduction in the supply of feed nutrients to ruminants with particular implication on dietary nitrogen supply for microbial protein synthesis.

The trend observed in the degradation of nutrients *P. maximum* at 24 to 48 hrs for the different growth stages was similar to the trend observed in the gas production. At two weeks interval, degradation of organic matter and crude protein proceeded similarly ($P > 0.05$) but within four weeks interval, significant reduction ($P < 0.05$) occurred. This implies that as growth proceeds over a longer period beyond two weeks, the forage nutrients become unaccessible to rumen microbes for breakdown and could lead to higher amounts of rumen by-pass nutrients.

Table 4 shows the energy value of *P. maximum* at different stages of growth estimated as short chain fatty acid, metabolizable energy and methane production. Short chain fatty acid and metabolizable energy were higher ($P < 0.05$) at 6 and 8 weeks compared with 12 weeks of age. Short chain fatty acid reduced from 0.787 µmol/g DM at 6 weeks of age to 0.489 µmol/g DM at 12 weeks.

Table 4: Energy Value of *Panicum maximum* at Different Stages of Growth.

	Growth Stages of *P. Maximum* (Weeks)				
Parameters	**6**	**8**	**10**	**12**	**SEM**
Organic Matter Digestibility (%)	51.66^{a}	46.48^{ab}	42.44^{bc}	39.05^{c}	1.02
Short Chain Fatty Acid (µmol/g DM)	0.787^{a}	0.663^{ab}	0.564^{bc}	0.489^{c}	0.03
Metabolizable Energy (MJ/kg DM)	7.624^{a}	6.852^{ab}	6.248^{bc}	5.742^{c}	0.16
Methane Production (ml/g DM degraded):					
At 24 hrs	4.50^{b}	5.00^{b}	10.50^{a}	11.00^{a}	2.15
At 48 hrs	8.00^{b}	9.50^{b}	11.00^{ab}	13.00^{a}	1.98

^{a,b,c} Means along the same row with different superscript are significantly ($P < 0.05$) different. SEM: Standard error of mean. DM: dry matter

Short-chain fatty acids are the end products of fermentation of dietary fibre by anaerobic intestinal microbiota which have been shown to exert multiple beneficial effects on mammalian energy metabolism (Besten *et al.,* 2013). The decrease in short chain fatty acid might be attributed to the decrease in fibre degradation. Similarly, metabolizable energy value of *P. maximum* decreased with age. Metabolizable energy gives an estimate of the energy available to an animal from feed. The reduction in feed metabolizable energy with increasing age of *P. maximum* suggests that as the forage matures,

less energy would be available for utilization by ruminants.

At 24 and 48 hrs post-incubation, methane production of *P. maximum* per gram of dry matter degraded increased ($P < 0.05$) from 6 to 12 weeks of growth. This suggests a higher loss of feed energy. Plant maturity has been shown to have some impact on methane production in the rumen with about 11% of gross energy intake being reported to be lost on low quality forage (Jenkins, 2014a). According to Johnson and Johnson (1995), about 2 – 15% of feed energy is lost as methane. The increased methane production as *P. maximum* advanced inage could be attributed to increased fibre fraction. As forage mature, soluble carbohydrates are converted to structural carbohydrate (Jenkins, 2014b) and neutral detergent fibre as well as acid detergent fibre positively correlates with methane concentration (Kulivand and Kafilzadeh, 2015). Similar to our results, certain researchers at Wageningen University and Research Centre conducted an experiment on cattle and reported an increased methane production per kg of milk as grass maturity at harvest increased (Wageningen, 2015). It has been suggested that when it is economically feasible, producers should manage pastures to reduce plant maturity in order to maximize ruminant performance and reduce methane production (Jenkins, 2014a).

CONCLUSION

Panicum maximum at 6 and 8 weeks of growth had higher crude protein, lower fibre, higher organic matter digestibility, higher metabolizable energy and short chain fatty acids and lower methane production per gram of dry matter degraded compared with its growth at 12 weeks indicating a better nutrient content, digestibility and energy value at a younger stage of growth. The plant can therefore, be harvested for ruminant feeding either at 6 or 8 weeks of growth at a time when its nutritive value is adequate for optimum nutrient utilization. When harvested at advanced stage of growth (up to 12 weeks), the crude protein content may be low and supplementation with higher sources of nitrogen may be required to improve its nutritive value.

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