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Full Length Research Paper

Anti-nutrient components of guinea grass (*Panicum maximum*) under different nitrogen fertilizer application rates and cutting management

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This research was carried out in 2004 in the Department of Crop Science, University of Nigeria, Nsukka. The study investigated the anti-quality factors in *Panicum maximum* under different N-fertilizer application rates and cutting management. The experiment was a 4 × 4 factorial laid out in a randomized complete block design with three replications. The treatments comprised four levels of nitrogen of 0, 150, 300 and 450 kg N ha^{-1} and four harvesting frequencies of 3-6-9 and 12 weekly intervals, resulting in 16 treatment combinations per block. The grass analyzed in this study had low levels of anti-nutrient constituents such as tannin, phytate, hydrogen cyanide, saponin and alkaloid. The alkaloid, hydrogen cyanide and tannin contents were depressed significantly ($P < 0.05$) with infrequent cutting interval. Nitrogen fertilizer application significantly ($P < 0.05$) increased the alkaloid, hydrogen cyanide and phytate content when compared with the control. The 9-weeks cutting interval gave significantly ($P < 0.05$) lowest alkaloid and phytate contents when 150 kg N ha^{-1} was applied. The hydrogen cyanide and tannin contents were lower at the cutting interval of 12 weeks when 300 kg N ha^{-1} was applied. Application of 300 kg N ha^{-1} produced the lowest saponin content when cutting was done every 6 weeks.

Key words: *Panicum maximum*, cutting frequency, N-fertilizer, anti-nutrient factors, alkaloid, saponin, tannins, hydrogen cyanide and phytate.

INTRODUCTION

The feeding value of any forage is a function of a number of characteristics of the species, including its availability, accessibility, nutrient availability, chemical composition and presence or absence of anti-nutritional factors (Dynes and Schlink, 2002). Good quality forage is leafy, green and succulent under normal condition. Any forage material that does not provide nutrients adequate for growth or milk productions, however, in its looks, smells, tests, protein or fiber contents, it is not a good quality forage (Adesogan et al., 2006). A true measure of forage quality is animal performance (Pinkerton and Cross, 1992). Another factor determining the feeding value of forage crops is the presence of anti-quality components such as tannins, hydrogen cyanide, glycosides, etc. (Cheeke, 1995). These anti nutrient factors may limit forage intake and digestibility, result in mineral deficiencies, toxicities, nutritional imbalances and affect animal health adversely (Provenza, 1995; Allen and Segarra, 2001).

The anti-nutritional contents of a pasture could be manipulated by management to reduce the opportunity for toxicity (William and Jeremiah, 2004). No research work has been carried out on the effect of cutting frequency and fertilizer–N application on the anti-nutritional components of *Panicum maximum* grown in Nigeria.

Therefore, the present investigation was carried out to: (1) Evaluate the effect of cutting frequency on anti nutrient factors in *P. maximum*; (2) assess the effect of nitrogen fertilizer application on anti-nutrient factors in *P. maximum*; (3) find the best combination of nitrogen and cutting frequency for higher quality pasture.

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Table 1. Effect of cutting frequency and nitrogen application on the alkaloid content (%) of *P. maximum*.

Cutting frequency (week)	Nitrogen fertilizer (kg N ha^{-1})				Mean
	0	150	300	450	
3	1.50(1.34)	3.00(1.85)	2.33(1.65)	3.17(1.84)	2.50(1.67)
6	1.00(1.21)	2.50(1.73)	2.67(1.74)	4.00(2.11)	2.54(1.70)
9	0.67(1.07)	0.67(1.07)	1.33(1.35)	1.50(1.14)	1.04(1.23)
12	2.33(1.65)	1.67(1.46)	1.83(1.53)	3.83(2.06)	2.42(1.67)
Mean	1.38(1.32)	1.96(1.53)	2.04(1.57)	3.12(1.85)	2.12(1.57)

The comparison is based on transformed means in parenthesis because of zero data. s.e.d for 2 cutting frequency means (C) = 0.141; s.e.d for 2 nitrogen means (N) = 0.141; s.e.d for 2 C × N means = 0.283.

MATERIALS AND METHODS

The experiment was a 4 × 4 factorial laid out in a randomized complete block design and was replicated three times. Treatments comprised four levels of nitrogen at 0, 150, 300 and 450 kg N ha^{-1} and four harvesting intervals of 3-6-9 and 12 weeks. There were 16 treatment combinations. A portion of land measuring 21.2 × 11.2 m was ear marked for this experiment and was divided into three blocks of 19.2 × 2.4 m each. Each block was separated by 1 m path-way. The treatment combinations were allocated completely at random in each of the three blocks.

Harvesting

Cutting was done at uniform height of about 15 cm with shears. The harvest intervals of 3-6-9 and 12 weeks gave 6-3-2 and 1- samples respectively.

Chemical analysis

Phytochemical analysis of dried and ground grass was carried out in the laboratory of the Department of Crop Science, University of Nigeria, Nsukka. The following anti-nutrient compositions were measured: Alkaloid, saponin, tannins, hydrogen cyanide and phytate. The method used for the determination of alkaloid was as described by Harborne (1973). Determination of saponin was according to the method described by Obadiri and Ochuko (2001). The method used for the determination of hydrogen cyanide was described by Onwuka (2005). While the method used for the determination of tannin and phytate was as described by Pearson (1976).

Statistical analysis

All data collected were statistically analyzed using the procedure outlined by Steel and Torrie (1980) for factorial experiment in randomized complete block design (RCBD) using GENSTAT (2007) statistical package. Means were subsequently separated using the standard error of the difference between two means (s.e.d). Square root transformation of the form x + 0.5, where x is the observation, was employed whenever there is zero value.

RESULTS

The 9-weekly cutting interval produced significantly ($P < 0.05$) lowest alkaloid content when compared with the other cutting intervals which had similar effects (Table 1). Nitrogen fertilization significantly increased the alkaloid content when compared with the control treatment. The 450 kg N ha^{-1} produced significantly ($P < 0.05$) higher alkaloid content than the 150 kg N ha^{-1} but had similar effect with the 300 kg N ha^{-1}. There was no significant difference in the alkaloid content between the 150 and 300 kg N ha^{-1}. The 6-weeks cutting interval gave significantly highest alkaloid content when 450 kg N ha^{-1} was applied, while the application of 0 and 150 kg N ha^{-1} produced the least alkaloid content when cutting was done at 9-weeks.

The hydrogen cyanide content decreased non-significantly ($P > 0.05$) with increase in interval between cuts. There was no significant difference in hydrogen cyanide content between the 3- and 6-weeks cutting intervals (Table 2). Nitrogen fertilizer application increased significantly ($P < 0.05$) the hydrogen content as compared to when no nitrogen was applied. Fertilizer-N application at 300 kg N ha^{-1} gave significantly ($P < 0.05$) higher hydrogen cyanide content than the 450 kg N ha^{-1} but was similar to the 150 kg N ha^{-1}. The 6-weeks cutting interval gave significantly highest hydrogen cyanide content when 300 kg N ha^{-1} was applied.

The phytate content remained statistically the same for all the intervals between cuts (Table 3). Application of N-fertilizer increased significantly ($P < 0.05$) the phytate content when compared with when no nitrogen was applied. The application of 450 kg N ha^{-1} had significantly higher ($P < 0.05$) phytate content when compared with the 150 kg N ha^{-1} but was similar to the 300 kg N ha^{-1}. The 9-weeks cutting interval gave the highest phytate content when 450 kg N ha^{-1} was applied.

The saponin content was statistically similar for all the cutting intervals (Table 4). Fertilizer application did not affect significantly the saponin content. The 6-weeks cutting interval gave the least saponin content at 300 kg N ha^{-1}, while 0 kg N ha^{-1} produced the highest saponin content when cutting interval was at 6-weeks.

The tannin content decreased significantly ($P < 0.05$) as the interval of cutting increased (Table 5). The 3-weeks cutting interval had significantly ($P < 0.05$) higher tannin content when compared with the 9- or 12-weekly cutting interval but was statistically similar to the 6-weeks cutting interval. The tannin content remained statistically

Table 2. Effect of cutting frequency and nitrogen application on hydrogen cyanide (HCN) content (%) of *P. maximum*.

Cutting frequency (week)	Nitrogen fertilizer (kg ha^{-1})				Mean
	0	150	300	450	
3	0.06(0.75)	0.15(0.80)	0.20(0.83)	0.17(0.82)	0.15(0.80)
6	0.17(0.82)	0.11(0.78)	0.25(0.86)	0.11(0.78)	0.16(0.80)
9	0.14(0.80)	0.11(0.78)	0.11(0.78)	0.09(0.77)	0.12(0.70)
12	0.10(0.77)	0.11(0.78)	0.06(0.75)	0.09(0.77)	0.09(0.77)
Means	0.12(0.78)	0.12(0.79)	0.16(0.81)	0.12(0.78)	0.13(0.79)

The comparison is based on transformed means in parenthesis because of zero data. s.e.d for 2 cutting frequency means C) = 0.013; s.e.d for 2 nitrogen means (N) = 0.013; s.e.d for C × N means = 0.026.

Table 3. Effect of cutting frequency and nitrogen application on phytate content (%) of *P. maximum.*

Cutting frequent (week)	Nitrogen fertilizer (kg N ha^{-1})				Mean
	0	150	300	450	
3	0.17(0.80)	0.25(0.87)	0.33(0.91)	0.25(0.87)	0.25(0.86)
6	0.25(0.87)	0.25(0.87)	0.25(0.87)	0.25(0.87)	0.25(0.87)
9	0.33(0.89)	0.00(0.71)	0.25(0.87)	0.25(0.97)	0.25(0.86)
12	0.25(0.84)	0.25(0.87)	0.25(0.87)	0.42(0.87)	0.25(0.86)
Mean	0.25(0.84)	0.19(0.83)	0.27(0.88)	0.29(0.89)	0.25(0.87)

The comparison is based on transformed mean in parenthesis because of zero data. s.e.d for cutting frequent means (C) = 0.022; s.e.d for 2 nitrogen means (N) = 0.022; s.e.d for 2 C x N means = 0.045.

Table 4. Effect of cutting frequent and nitrogen application on saponin content (%) of *P. maximum.*

Cutting frequency (week)	Nitrogen fertilizer (kg N ha^{-1})				Mean
	0	150	300	450	
3	1.07(1.19)	1.02(1.21)	1.03(1.23)	1.03(1.24)	1.04(1.22)
6	1.77(1.49)	1.70(1.45)	0.05(1.00)	1.17(1.28)	1.28(1.31)
9	1.03(1.23)	2.20(1.63)	1.70(1.47)	0.87(1.17)	1.45(1.37)
12	0.09(1.18)	0.87(1.17)	1.23(1.31)	0.73(1.11)	0.93(1.19)
Mean	1.19(1.27)	1.45(1.36)	1.12(1.25)	0.95(1.20)	1.18(1.27)

The comparison is based on transformed means in parenthesis because of zero data. s.e.d for 2 cutting frequent means (C) = 0.092; s.e.d for Nitrogen means (N) = 0.092; s.e.d for 2 C × N means = 0.184.

the same for 0, 150, 300 and 450 kg N ha^{-1}. The 6-weeks cutting interval gave the highest tannin content when 300 kg N ha^{-1} was applied.

DISCUSSION

The decrease in the alkaloid and hydrogen cyanide (HCN) contents observed in this study with infrequent cutting interval was expected according to the previous work of McDonald et al. (1973) who reported that immature potato tubers were found to contain more alkaloid salanidine than mature tubers. These authors also reported that young shoots of potatoes were found to be rich in alkaloid solanidine and abortion may result when pregnant animals consume alkaloid-rich foliage. Pandey et al. (2011) working with sorghum fodder samples in Gujarat, India, indicated the presence of comparatively higher hydrogen cyanide (HCN) content in the immature plants. Significantly ($P < 0.05$) decreasing trend in HCN content was also observed by these authors with the advancement in the plant growth.In this study, nitrogen fertilization significantly ($P < 0.05$) increased the alkaloid content of the grass forage as was reported by Arechavaleta et al. (1992). As was expected, nitrogen fertilization enhanced the alkaloid formation, since alkaloids are aromatic nitrogenous compounds containing nitrogen on their carbon skeletons (Al-Humaid, 2003). In addition, fertilization provides the nutrient elements that contribute in the synthesis of various amino acids, the precursor of a variety of alkaloids (Salisbury and Ross, 1978). Alkaloid biosynthesis was shown to

Table 5. Effect of cutting frequency and nitrogen application on tannin content (%) of *P. maximum.*

Cutting frequency (week)	Nitrogen fertilizer (kg N ha^{-1})				Mean
	0	150	300	450	
3	0.05(0.75)	0.16(0.81)	0.21(0.74)	0.13(0.80)	0.14(0.80)
6	0.13(0.79)	0.12(0.84)	0.12(0.84)	0.06(0.75)	0.11(0.78)
9	0.13(0.79)	0.09(0.77)	0.08(0.76)	0.09(0.77)	0.10(0.77)
12	0.07(0.75)	0.08(0.76)	0.04(0.74)	0.05(0.74)	0.06(0.75)
mean	0.09(0.77)	0.11(0.78)	0.11(0.78)	0.08(0.76)	0.10(0.77)

The comparison is based on transformed means in parenthesis because of zero data. s.e.d for 2 cutting frequency mean (C) = 0.011; s.e.d for 2 nitrogen means (N) = 0.011; s.e.d for 2 C x N means = 0.021.

depend on enzyme systems that diminished in plants grown in nutrient-poor conditions (Johnson et al., 1989).

The significant ($P < 0.05$) increase in HCN content observed with increase in nitrogen application is in agreement with Pandey et al. (2011) who reported that higher levels of nitrogen application increased the hydrogen cyanide content in sorghum. Siegler et al. (1989) indicated that most commonly consumed grasses are cyanogenic.

However, the quantity of hydrogen cyanide (HCN) produced by *P. maximum* species is too low to pose major animal health problems (kumar and D'Mello, 1996). The levels of hydrogen cyanide reported in this study are unlikely to pose toxicity problem to the animals since they are much below the toxic levels reported by Enechi and Odonwodu (2003). Generally, only plants that produced more than 20 mg HCN/100g fresh weight are considered deleterious (Everist, 1981). The lethal dose of HCN for cattle and sheep was reported (Kumar, 2011) to be 2.0 to 4.0 mg per kg body weight. The levels of HCN reported in this study are below these toxic levels. Excess cyanide ion was reported (Kumar, 2011) to inhibit the cytochrome oxidase. This stops ATP formation, tissues suffer energy deprivation and death follows rapidly.

The phytate levels reported in this study are similar to those reported on grass plants by Onwuka (1996). The levels of phytate in the present work are unlikely to have any adverse consequence in ruminant nutrition according to the previous work of Okoli et al. (2003). Presence of phytate in forage could be of dietary importance to monogastric animals since they lack the phytase needed to break down the phytin to release phosphorus.

A threshold concentration of tannin of 5% has been reported beyond which there may be rejection of browse by goats and wild browsers (Cooper and Owen-smith, 1985).

In sheep and cattle, dietary tannin level of 2 and 5%, respectively have also been reported to have adverse effect on digestibility (Mcleod, 1974). The levels of tannin recorded in this work are below the toxic level. Tannin levels in excess of 50 g/kg dry matter was shown to reduce digestibility, lower feed intake, reduce palatability, inhibit digestive enzymes and was toxic to rumen micro-organisms (Kumar and Vaithiyanathan, 1990). The availability of sulphur and iron was shown (Dynes and Schlink, 2002) to be limiting to animals consuming tannin-rich foliage. The decrease observed in this study in the tannin content with increase in cutting interval is supported by Baloyi et al. (2001) who reported that proportion of condensed tannin in selected forage legumes grown in Zimbabwe, was greater in younger than in mature materials. Beck and Reed (2001) reported that most forage herbivores selectively consume plants of relatively low tannin concentration. These authors indicated that goats browsing blackbrush preferred previous year's growth to current year's growth apparently because it had lower tannin content.The saponin levels reported in this study are lower than those reported by Lu and Jorgensen (1987), who showed that alfalfa saponins inhibited microbial fermentation and synthesis in the rumen and altered the sites of nutrient digestion in sheep.

These authors indicated that total protozoa count in the rumen was reduced by 34 and 66% by saponins at levels of 2 and 4% dietary dry matter, respectively.

The levels of saponin in the present work are unlikely to have any adverse consequence in ruminant animal nutrition.

Conclusion

The grass analyzed in the present study had low levels of toxic constituents such as tannin, phytate, hydrogen cyanide, saponin and alkaloid. Feeding trails using ruminants and monogastric animals are recommended in order to fully ascertain the nutritional values of guinea grass.

Nitrogen fertilizer application had greater effects on alkaloid, hydrogen cyanide and phytate, while saponin and tannin were not affected by nitrogen application. The 9-weeks cutting interval gave the least alkaloid and phytate contents when 150 kg N ha^{-1} was applied. The hydrogen cyanide and tannin contents were lower at the infrequent cutting interval of 12 weeks when 300 kg N ha^{-1} was applied. Application of 300 kg N ha^{-1} produced the least saponin content when cutting was done every 6 weeks.

REFERENCES

Adesogan AT, Sollenberger LE, Moore JE (2006) Feeding value and anti-nutritive factors of forage. Tree Legumes, pp. 174-179.

Al-Humaid AI (2003) Effects of compound fertilization on growth and alkaloids of Datura (*Datura innoxia* Mill.) plant. J. Agricul. Rural Develop. Tropics Subtropics, 104 (2): 151-165.

Allen VG, Segarra E (2001) Anti quality components in forage: Overview, significance and economic impart. J. Range Manage. 54: 49-412.

Arechavaleta M, Bacon CW, Plattner RD, Hoveland CS, Radchliffe DE (1992) Accumulation of ergopeptide alkaloids in symbiotic Tall fescue grown under deficits of soil water and nitrogen fertilizer. Environ. Microbiol. 58(3): 857-861.

Baloyi JJ, Ngongoni NT, Topps JH, Acamovic T, Hamudikuwanda H (2001). Condensed tannin and saponin content of *Vigna unguiculata* (L.) Walp,*Desmodium uncinatum*, *Stylosanthes guianensis* and *Stylosanthes scabra* grown In Zimbabwe. Trop. Anim. Health Prod. 33: 57-66.

Beck JL, Reed JD (2001). Tannins: Anti-quality effects on forage protein and fibre digestion. In: Reed JD (2001) Effects of proanthocyanidins on the digestion and analysis of fibre in forages. J. Range Manage. 54: 466-473.

Cheeke PR (1995). Forage quality, Department of Animal science, Oregon state university, Corvallis 97331-6702, USA. J. Anim. Sci. 73(39): 09-918.

Cooper SM, Owen-Smith N (1985). Condensed tannins deter feeding by browsing ruminants in a South African savannah. Ecologia Berlin 67: 142-146.

Dynes RA, Schlink AC (2002). Livestock potential of Australian species of Acacia. Conservation Ssience W. Aust. 4(3): 117-124.

Everist SL (1981). Poisonous plants of Australia.Revised Edition. Angus and Robertson, Sydney.

Enechi OC, Odonwodu I (2003). An assessment of the phytochemical and nutrient composition of the pulverized root of *Cissus quadrangularius*. Bio-Research, 1: 63-68.

GENSTAT (2007). Genstat Release 7.2DE, Discovery Third Edition, Lawes Agricultural Trust Rothamsted Experimental station, U.K. England.

Harborne JB (1973). Phytochemical method. A guide to modern Techniques of plants analysis. Chapman and Hall limited. pp. 110-113.

Johnson ND, Rigney LP, Benttey BL (1989). Short-term induction of alkaloid production in jupines: differences between N_2-fixing and nitrogen-limited plants. J. Chem. Ecol. 15: 2425-2434, In: Gerson EA, Kelsey RG (1999) Piperidine alkaloids in nitrogen fertilized *Pinus ponderosa*. J. Chem. Ecol. 25(9): 2027-2039.

Kumar R, Vaithiyanathan S (1990). Occurrence, nutritional significance and effect of animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30: 21-38.

Kumar R, D'Mello JPF (1996). Anti-nutritional factors in forage legumes. In: tropical legumes in animal nutrition. D'Mello JPF and Devendra D (eds) CAB international Wallingford UK.

Kumar R (2011). Anti-nutritional factors: the potential risks of toxicity and methods to alleviate them. http://www.fao.org/ag/aga/agap/frg/ahpp102/102-145.pdf.

Lu CD, Jorgensen NA (1987). Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117: 919-927.

McDonald P, Edwards RA, Greenhalgh JFE (1973). Animal nutrition. Oliver and boyd, Edinburgh, p. 479

Mcleod MN (1974) Plant tannins- their role in forage quality. Nutrition Abstract, 44: 803-815.

Obadiri BO, Ochuko PO (2001). Phytochemical studies and comparative efficancy of the crude extracts of some homeostatic plants in Edo and delta states of Nigeria, Global J. Pure Appl. Sci. 8: 203-208.

Okoli IC, Anunobi MO, Obua BE, Enemuo V (2003). Studies on selection browse of south eastern Nigeria with particular reference to their proximate and some endogenous anti-nutritional constituents. Livestock Res. Rural Dev. 15(9): 1-7.

Onwuka GI (1996). Plant phytate, oxalates, and their effects on nutrient utilization by goat, Nigerian J. Anim. Prod. 23(1) 53-60.

Onwuka GI (2005). Food analysis and instrumentation theory and practice. Naphthali prints. Lagos Nigeria, pp. 142-143.

Pandey RK, Kumar D, Jadhav KM (2011). Assessment of determinants for reducing HCN content in sorghum used for ruminant in Gujarat, India. http://www.lrrd.org/lrrd23/3/pand2306.htm

Pearson DA (1976). The chemical Analysis of foods (7th ed) Churchill and livingstone, New York. pp. 145-160.

Pinkerton BW, Cross DL (1992). Forage quality. Forage Leaflet 16: 1 - 3. http://www.clemson.edu/Fairfield/local/news/quality.

Provenza FD (1995). Postingestive feedback as an elementary determinant of food preference to phytotoxicosis in herbivores. J. Range Manage. 45: 36-45.

Salisbury FB, Ross CW (1978). Lipids and aromatic compounds. Plant Physiology 2nd

ed.; Wadsworth Publishing Company, USA, In: Gerson EA, Kelsey RG (1999) Piperidine alkaloids in nitrogen fertilized *Pinus ponderosa*. J. Chem. Ecol. 25(9): 2027-2039.

Seigler DS, Maslin BR, Conn EE (1989) Cyanogensis in the leguminosis. In stirton HH and Zarchi JL (eds), Advances in legume Biology, Monograph systematic Botany. Missouri Botanical Garden, 29: 645-672.

Steel GD, Torrie JH (1980) Principles and procedures of statistics: A Biometrical Approach, 2nd Ed. Mc Graw-Hill Book company, Inc. New York, 633: p. 21.

William DP, Jeremiah F (2004). Cultivated pasture and rangeland forages. http:/www.arsgrm gov/eg.bin/npgs.