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Mineral and anti-nutrient composition of *Pennisetum pedicellatum* Trin. grass

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ABSTRACT: The grass *Pennisetum pedicellatum* is an ornamental-like grass that is widely used in northern Nigeria as a source of animal feed especially by local animal farmers. The objective of this study was to determine the nutritional content of the grass and, hence, quantify their contribution to the animal feed so as to curb the unavailability of animal feed during the dry season. Both macronutrients and micronutrients were determined using flame photometry and atomic absorption spectrometric methods of analysis. The research investigates the mineral and anti-nutrients contents of *Pennisetum pedicellatum* grass. The anti-nutrients were determined using standard methods of food analyses. The mineral content of the sample showed a composition (mg/100gDW) of 11167 ± 3.82 sodium, 10850 ± 229.13 potassium, 108.3 ± 0.03 calcium, 28.3 ± 0.03 magnesium, 315.7 ± 0.03 phosphorus, 0.58 iron, 5.81 chromium and 4.07 nickel. However, copper, zinc and manganese were not detected in the sample. The anti-nutrient composition (mg/100 g DW) for oxalate, phytate, saponins, cyanide and tannins were 1.00 ± 0.0008, 3474 ± 0.0223, 56.00 ± 0.0035, 11.00 ± 0.0014 and 10.00 ± 0.0025, respectively. The anti-nutritional analysis reveals that the grass contains high amount of phytate and saponins and low oxalate content. The results revealed that the oxalic acid content of the grass is below the critical level while the phytate of the grass is above the critical level.

Keywords: Anti-nutrients, minerals, *Pennisetum pedicellatum* Trin.

INTRODUCTION

One of the major factors limiting the productivity of small ruminants in developing countries is over dependence on low digestibility feeds which during the dry season cannot meet even the maintenance requirements of these animals (Schoenian, 2003). The poor condition of livestock in the tropics is more likely as a result of inefficient digestion in the rumen and inefficient utilization of the nutrients absorbed from low quality feeds. Also, the poor utilization of available grasses in northern Nigeria is as a result of poor utilization of available dried grasses. Several attempts have been made to improve the nutritive quality of this class of livestock feeds which include physical, chemical and biological treatments, use of feeds additives as well as supplementation with non-protein nitrogen sources such as urea and molasses (Agumuo et al., 2016). There is an increase shortage of food or rather lack of balanced diet (malnutrition) in the animal feeds.

The term minerals refer to elements in their organic and inorganic forms. They are found in the body in ionic forms in which almost all the metabolic processes depend on (i.e. electrolyte) which include maintenance of pH, regulation of proper function of heart, muscles and digestion. Minerals are chemical substances that emanated from geological activities from which life on our planet is built upon. They get into the body through food. They are present in large percentage in the body tissue and are classified according to their metabolic roles as essential and non-essential (Umar, 2005). Essential minerals are further classified into macro and micro elements. The former comprises of calcium, phosphorus, potassium, chloride, sulphur and magnesium. Whereas the latter include copper, iodine, iron, manganese, etc; depending on the concentration

needed in the body. The deficiency of these nutrients is associated with diseases and disability (Umar, 2005). Anti-nutritional factors are natural or synthetic substances found in the human diet or animal feed that can adversely affect health and growth by preventing the absorption of the nutrients from food (D'Mello, 2000). They can also be generated in natural feedstuffs by the normal metabolism of species or by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of the feed) which exerts effect contrary to optimum nutrition (Cheeke et al., 1985). Many plant components have potential to precipitate adverse effects on the productivity of farm livestock. These compounds are present in the foliage and seeds of virtually every plant that is used in practical feeding (D'Mello, 2000). The major factor limiting the wider food nutrient utilization of many plants is the ubiquitous occurrence in them, a diverse range of natural compounds capable of precipitating the deleterious effects on man and animals.

This work will focus on determining the actual quantity of minerals and some anti-nutrient that are present in the sample to that nutrients that are not sufficient or even not available in the grass so that it can be replenished with other alternative. Also this work will help our local villagers who most often solely rely on grasses and hays as the major source of animal feed to know what exactly they are feeding their livestock with, and also to know if there is the need to supplement the unavailable nutrients in the plants, and also to know if there is likely a problem to be encountered if excess of it is given to the animals. Thus, the objective of this study was to determine the nutritional content of *Pennisetum pedicellatum* Trin. grass and quantify their contribution to the animal feed.

MATERIALS AND METHODS

Sample collection and treatments

The samples (*Pennisetum pedicellatum*) were plucked from the outskirt of Gidan Yunfa farms in Wamakko Local Government, Sokoto just before the Usmanu Danfodiyo University main campus (Figures 1 a and b). The samples were washed with clean water and the roots were removed. The sample was identified at the Herbarium of Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto as *Pennisetum pedicellatum*. The sample was then air dried for two (2) weeks. It is then pulverised to powder using mortar and pestle. The powdered sample was sieved and stored properly in an air tight clean polythene bags for further analysis.

Anti-nutrient analysis

Analysis was carried out to determine tannins, phytate, oxalic acid, cyanide and nitrate.

Determination of tannins

The sample (0.5 g each) was weighed into 100 cm^3 flask. 50 cm^3 of distilled water was then added and shaken for 1 hour with a mechanical shaker. This mixture was filtered into a 50 cm^3 volumetric flask and made up to the mark. 5 cm^3 of the filtrate was added into a tube and mixed with 3 cm^3 of 0.1M $FeCl_3$ in 0.1MHCl and 0.008M potassium ferrocyanide. The tube was spinned in a centrifuge; the absorbance was then measured using a spectrophotometer and tannins content was calculated using equation.

$$C_T = \frac{A_T \, X \, C_S}{A_S} \text{---------- (1)}$$

Where: C_T= Tannin concentration in mg %, A_T= Absorbance of the test sample, A_s =Absorbance of the standard and C_S = Concentration of tannin in standard

Determination of phytate

The standard method of Soetan (2012) was used. The *Pennisetum pedicellatum* grasses (4 g) of the sample was soaked in 100 cm^3 of 2% HCl for 3 hours, and then filtered. 25 cm^3 of the filtrate, 5 cm^3 of 0.3% NH_4SCN and 53 cm^3 of distilled water were mixed together. The mixture was then titrated against 0.001M standard $FeCl_3$ solution until a brownish yellow colour persists for 5 second. Phytin phosphorus (1 cm^3 = 1.19mg phytin phosphorus) was determined and the phytic acid content was calculated using:

$$\text{phytate (mg \%)} = \text{TV}(cm^3) \text{ X phytin phosphorous}(1.19g) \text{X } 3.55 \text{----------(2)}$$

Where TV is the volume obtained after colour change.

Determination of oxalic acid

Two grams of the sample were weighed into a 250 cm^3 conical flask containing 190 cm^3 of distilled water and 10 cm^3 of 6M HCl. The mixture was digested for 1 hour in a boiling water bath. After cooling, the mixture was then filtered. 50 cm^3 aliquot of the sample were placed into a beaker and 20 cm^3 of 6M HCl were added. The mixture was evaporated to about half of the volume and then filtered. The residue was washed several times with distilled water and 3 drops of methyl orange indicator was added to 25 cm^3 of the filtrate and titrated against 0.1M $KMnO_4$ solution till a faint pink colour appears and persist for 30 seconds (Day and Underwood, 1986).

Determination of hydrocyanic content

The alkaline titration procedure of Anhwange et al. (2006)

Figure 1a. Foliage part of *Pennisetum pedicellatum* grass.

Figure 1b. *Pennisetum pedicellatum* Trin*. grass.*

was adopted. 10g of the grass was soaked in a mixture of 200 cm^3 of distilled water and 10 cm^3 of orthophoshoric acid. The mixture was left overnight to release all bounded hydrocyanic acid. The mixture was distilled until 150 cm^3 of the distillate was collected. 20 cm^3 of distillate was taken into a conical flask and diluted with 40 cm^3 of water, 8 cm^3 of 6M aqueous ammonia (NH_4OH) and 2 cm^3 of 5% potassium iodide (KI) solutions were added. The mixture was titrated with 0.02M silver nitrate ($AgNO_3$) using a micro burette until a faint but permanent turbidity was obtained.

Determination of Nitrate

The method described by IITA (1988) was adopted in which 100 mg of the powdered sample was weighed into a 15 cm^3 centrifuge tube and 10 cm^3 of distilled water was added. The content was incubated in water bath at 45^{o}C for one hour, cooled and centrifuged at 5000 revolution per minute for 15 minutes. The clear supernatant was put into a clean test tube, stoppered and stored in a refrigerator prior to nitrate analysis.

Nitrate stock solution (100 ppm) was prepared by dissolving KNO_3 (1.63g) in distilled water in a 100 cm^3 volumetric flask up to the mark. To prepare series of standard solutions of 0, 1, 2, 3, 4, and 5 ppm, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 cm^3 of the stock solution were added to six 20 cm^3 volumetric flask. Similarly, 0.2 cm^3 of the extract was put into another 20 cm^3 volumetric flask. In the flasks, 0.8 cm3 of 5% (w/v) salicylic acid-sulphuric acid reagent was added and mixed thoroughly. The content was allowed to stand for 20 minutes and followed by the addition of 2M NaOH solution (to raise the pH to above 12) to the mark. The content was cooled to room temperature and its absorbance measured at 410 nm with a spectrophotometer. The calibration curve was plotted from which the concentration of nitrate (X) in the samples was extrapolated and nitrate content in the sample was calculated using the equation below.

$$NO_3(mg/100g) = \frac{\text{Xppm X Vol } (cm^3)}{\text{Aliquot } (cm^3)\text{X weight (g)}}$$

Sample digestion

Two grams of the sample were weighed into a 250 cm^3 digestion flask. 25 cm^3 of concentrated sulphuric acid, nitric acid and perchloric acid ($HClO_4$) in the ratio of 2:1:1 was added to the sample. The flask was fixed to a clamp and kept overnight. When the initial reaction subsided, the temperature of the micro-digestion bench was increased slowly to 180^{o}C to 200^{o}C. The digestion was continued at that temperature until no visible particles are observed, the temperature was raised to 240^{o}C and the digestion acid was evaporated until dense white fume formed within the digestion flask. Then, the content of the flask was filtered. The filtrate was diluted to 50 cm^3 mark. The solution was then used for the mineral analysis.

Determination of mineral contents

The minerals were determined by atomic absorption techniques (Agumuo et al., 2016)

Determination of potassium and sodium

The ash of each sample obtained was diluted by adding 5 cm^3 of 2M HCl to the ash in the crucible. The mixture was heated to dryness on a heating mantle. 5 cm^3 of 2M HCl was added again, heated to boil, and filtered with filter paper (Whatman No 1) into a 100 cm^3 volumetric flask. The filtrate was made up to mark with distilled water and made ready for the reading of concentration of potassium and sodium on Jenway PFP7 Flame Photometer using the filter corresponding to each mineral element.

Determination of Ca, Mg, Mn, Fe, Zn, Pb, Cr, and Cu

The digest of the ash was washed into 100 cm^3 coulometric flask with distilled water and made up to mark. These diluents were aspirated into AA32ON Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

Statistical analysis

The mineral and anti-nutrient compositions were determined by different methods. SPSS version 16 was used and reported as mean of three (3) replicate values.

RESULTS AND DISCUSSION

The mineral composition of *Pennisetum pedicellatum* is represented in Table 1. The concentration of sodium in *P. pedicellatum* grass was found to be 111.67 mg/100 g which is higher than the value (96.56 mg/100 g) reported by Waziri et al. (2013) on the analysis of *Andropogon gayanus* grass and also what was reported by Falola et al. (2013) for vetiver grass (*Chrysopogon zizanioides*). On the other hand, the value is lower than what was reported for *Cassia hirtsutse* (421.05 mg/100 g) as reported by Akpabio et al. (2012). The recommended dietary allowance (RDA) for sodium in sheep is 70mg/100g, which implies that *P. pedicellatum* are high in sodium as compared to the recommended daily allowance of 70 (Hassan et al., 2007) to ruminants (sheep). Feeds containing high amount of sodium is useful to the livestock in that it assists in controlling nerve impulses, normal absorption of sugars and amino acids from the digestive tract (Akpabio et al., 2012).

Potassium happens to be the most abundant element (10850 mg/100 g) in *P. pedicellatum* grass. The value is higher compared to the value (4,953.49 mg/100 g) reported for *Ipomea batatas* leaves (Tayie and Asibey-Berko, 2001; Monamodi et al., 2003). The value is also higher compared to *Celosia Argentea* leaves (5,200 mg/100 g), *Solanuma ethiopicum* leaves (5,000 mg/100 g) and even *Talinum triangulare* (8,000 mg/100 g) (Monamodi et al., 2003). It is also higher compared to that obtained for *Chenopodium album* (6938 mg/ 100 g) and *Solanum nigrum* (3084 mg/100 g) as reported by Afolayan and Jimoh (2009). This shows that the plant is a very good source of potassium. The high value of potassium may be due to its abundance in Nigerian soil (Oshodi et al., 1999). Potassium is involved in acid-base regulation, osmotic pressure maintenance, nerve impulse transmission, muscle contraction, and carbondioxide and oxygen transport (Oshodi et al., 1999).

Table 1. Mineral contents of *Pennisetum pedicellatum* Trin. grass.

Elements	Concentration [mg/100g DW]	RDA Value for Sheep [mg/100g DW]*
Na	111.67 ± 3.82	70
K	10850 ± 229.13	220
Ca	1.083 ± 0.03	260
Mg	0.283 ± 0.03	170
P	3.157 ± 0.03	270
Fe	0.58 ± 0.02	3.5
Cr	5.81 ± 0.12	
Ni	4.07 ± 0.21	
Cu	ND	0.9
Zn	ND	3
Mn	ND	3

The values are mean ± standard deviation (SD) of three replicates; ND = Not Detected; * Hassan et al. (2007a).

Calcium is an essential component of a healthy diet and a mineral necessary for life. It plays a vital role in building healthy and dense bones and teeth, blood clotting and for normal functioning of the heart, nervous system and muscles (Idris et al., 2011). The concentration of calcium in *P. pedicellatum* is 1.083 mg/100 g DW. The value is lower than that obtained for *Vetiver* grass (0.48 mg/100 g) as reported by Falola et al. (2013). The value is lower than the RDA of 260 for sheep, which implies that the grass is a poor source of calcium for ruminants. The value is lower compared to that obtained for *Amaranthus viridis* (110.67±15.51 mg/100 g) as reported by Umar et al. (2007).

The concentration of magnesium in the sample is 0.283 ± 0.023 mg/100 g DW. The value obtained is lower than what was obtained in *Andropogon gayanus* (2.74 mg/100 g DW) as reported by Waziri et al. (2013). Magnesium is a constituent of bone and teeth and is closely associated with calcium and phosphorus. Magnesium is necessary for the release of parathyroid hormone and for its action in the backbone, kidney and intestine and for the reactions involved in converting vitamin D to its active form (Waziri et al., 2013). Magnesium is important in tissue respiration, especially in oxidative phosphorylation leading to formation of Adenosine triphosphate (ATP). It is also

possible for nutrients to cross the cell membrane (Guthrie, 1989). The concept of Ca/P ratio as introduced by Shills and Young (1988) as cited in Oko et al. (2012) takes into consideration that diet rich in proteins and phosphorus, promote the loss of calcium in urine, resulting in decrease of calcium levels in bones (Hemen and Lalita 2012). To predict the bioavailability of some divalent elements specifically calcium, magnesium, zinc and iron, anti-nutrients to nutrients molar ratios were calculated and the results represented in the Table 3.

The result of the anti-nutritive composition of *P. pedicellatum* is represented in Table 2. The result indicates high concentration of phytate (3474 ± 0.00223 mg/100g) while oxalates concentration records lowest (1.00 ± 0.0008mg/100g). The concentration of oxalate in *P. pedicellatum* grass is 1.00 ± 0.0008 mg/100 g DW. The value is higher compared to the value obtained for *Pennisetum purpureum* (0.159 ± 0.010 mg/100 g DW) as reported by Okaraonye and Ikewuchi (2009) and also higher than what was obtained for *Vetiver* grass (0.25 ± 0.03 mg/100 g DW) reported by Falola et al. (2013). High oxalate content causes irritation in the mouth and interfere with the absorption of divalent minerals particularly calcium by forming insoluble salts with them leading to kidney stone which may eventually lead to death (Hassan et al., 2007).

The phytate content of *P. pedicellatum* (3474 ± 0.02 mg/100 g DW) is higher than that of *P. purpureum* (0.006 ± 0.001 mg/100 g DW) as reported by Okaraonye and Ikewuchi (2009), the value is also higher than (0.56 ± 0.02 mg/100 g DW) obtained for *Vetiver* grass reported by Falola et al. (2013). The phytate in food can bind some essential mineral elements such as Ca, Mg, Zn and Fe in the digestive tract and render them not bioavailable (Bello, 2008). Consumption of this grass may have effect on the bioavailability of minerals in monogastric animals (Thompson, 1993). Protein and starch solubility digestion was also reported to be affected by phytate. Nevertheless, phytate was known to be a potent anti-carcinogen that protects against colon- cancer and it is known to be a potent antioxidant that inhibits Fenton reactions leading to lipid peroxidation and inhibition of polyphenol oxidase (Agte et al., 1999).

The concentration of saponins in *P. pedicellatum* (56.00 ± 0.0035 mg/100 g DW) is higher compared to 0.85 ± 0.03 mg/100 g DW reported for *P. purpureum* as reported by Okaraonye and Ikewuchi, 2009, and higher than what was reported for *Panicum maximum* (0.44± 13 mg/100 g) (Ajayi, 2007). Saponin reduces intake of feed and uptake of certain nutrients including glucose and cholesterol. From the level obtained in this study, the saponin contents of *P. pedicellatum* may not affect its nutritional potentials.

The cynogenic glycoside (hydrocyanic acid) content of *P. pedicellatum* is (11.00 ± 0.004 mg/100 g DW) is higher compared to 2.83 ± 0.04 mg/100 g DW for *P. purpureum* as reported by Okaraonye and Ikewuchi (2009). Badifu (2001) reported HCN content in some raw leaves such as *Celosia argentea* (20 mg/100 g DW) *Tralinum triangulare* (75 mg/100 g DW) and *Celosia laxa* (30 mg/100 g DW) which all contain higher concentration of cyanide when compared with *P. pedicellatum*. Consumption of high levels of cyanide is associated with a serious health problem, spastic paraparetis known as *Konzo*. In Nigeria, a neurological disease known as Tropical Ataxic Neuropathy (TAN) was also linked to consumption of high level of cyanide in cassava-based diet (Hassan and Umar, 2004). Only plants with more than 200 mg of HCN equivalent per 100 mg fresh weight are considered dangerous (Betancur-Ancona et al., 2008). This shows that *P. pedicellatum* leaves are safe for consumption as far as HCN is concerned.

The concentration of tannins in *P. pedicellatum* is (10.00 ± 0.002 mg/100 g DW). The value is higher compared to 0.58 ±0.02 mg/100 g DW in *Vetiver* grass as reported by Falola et al. (2013) and lower than (28.64 ± 0.00 mg/100 g DW) in *P purpureum* as reported Okaraonye and Ikewuchi (2009). Tannins in food impose an astringent taste affecting palatability; reduces the intake of the food and consequently body growth. Tannins can bind to both exogenous and endogenous proteins including enzymes of the digestive tract, thereby affecting the utilization of protein by their ability to complex and precipitate proteins (Umar, 2007). Tannins are also known to inhibit the activities of enzymes such as trypsin, chemotrypsin, amylase and lipase and also interfere with iron absorption and growth in general (Umar et al., 2007). The level of tannin which adversely affect digestibility in sheep and cattle is between 2 and 5 mg/100 g DW (Diagayette and

Table 2. Anti-nutritive value of *Pennisetum pedicellatum* Trin. grass (mg/100 g DW).

Parameters	Composition
Oxalate	1.00± 0.0008
Phytate	3474 ± 0.0223
Saponins	56.00 ± 0.0035
Cyanide	11.00 ± 0.0014
Tannins	10.00 ± 0. 0025

The values are the mean ± standard deviation of three replicates.

Table 3. Anti-nutrient to nutrient molar ratio of *Pennisetum pedicellatum* grass.

Anti-nutrient to nutrient	Molar ratio	Critical value
[Oxalate]/[Ca]	4.07 X 10^{-3}	2.5*
[Oxalate]/[Ca+Mg]	3.06 X 10^{-3}	2.5*
[Phytate]/[Ca]	1.95	0.2*
[Phytate]/[Fe]	9.03	0.4***
[Phytate]/[Zn]	-	1.5**

Source: *Umar (2005); **Frontela et al. (2008);***Mitchikpe et al. (2008).

Huss, 1981), which indicate that the concentration of tannins in the *P. pedicellatum* grass may have adverse effects on the nutritional quality of the feed.

To evaluate the bioavailability of some mineral elements specifically Ca, Mg, Fe, and Zn, the anti-nutrients to nutrients molar ratio was calculated, and the result presented in Table 3. From Table 3, it was observed that, [Oxalate]/ [Ca], [Oxalate] / [Ca + Mg] ratio are all below the critical level known to impair calcium and magnesium bioavailability (Umar, 2005). [Phytate] / [Ca] ratio when compared to the critical level would be observed to be higher known to cause calcium deficiency by the phytates. The [Phytate] / [Fe] ratio is higher than the critical value known to impair the iron bioavailability and thus indicate poor iron bioavailability due to phytates as reported by Mitchikpe et al. (2008).

Conclusion

This study revealed that *Pennisetum pedicellatum* grass is rich in sodium, potassium, phosphorus and calcium but contains low amount of magnesium. Also, the heavy metals analysis reveals that the grass contains high quantity of chromium but low amount of iron. The predicted bioavailability of calcium and iron is high. The anti-nutritional analysis reveals that the grass contains high amount of phytate which is a strong chelator of important minerals like calcium, magnesium, iron etc therefore contribute to non-bioavailability of these minerals and low oxalate content which is far below the critical level and as such will not pose health threat to the animals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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