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Evaluation of the Mineral and Nitrogen Contents of Three Forage Crops (*Vigna unguiculata* (L.) Walp., *Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf.) Grown in the Boundji area (Republic of Congo)

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Abstract **Original Research Article**

Pennisetum purpureum Schum., *Vigna unguiculata* (L.) Walp. and *Hyparrhenia diplandra* (Hack.) Stapf are fodder plants that can provide quality herbaceous above-ground phytomass for domestic animal feeding. They are used in degraded pasture restoration practices. The objective of this study was to evaluate the mineral elements and nitrogenous matter of these three forage crops. The latter were planted in the pedoclimatic conditions of the Boundji area. Phytomass samples were taken two months after planting by sowing seeds for *Vigna unguiculata* (L.) Walp., by cuttings for *Pennisetum purpureum* Schum. and by splitting the stumps for *Hyparrhenia diplandra* (Hack.) Stapf. The samples were oven-dried at 70 °C to constant weight and chemically analysed. The results obtained show that the mineral elements and nitrogenous matter vary from one forage species to another. *Vigna unguiculata* (L.) Walp. is the richest forage species in total nitrogenous matter (16.43 ± 2.43%) and digestible nitrogenous matter (117.50 ± 22.64 g/k DM). It provides excellent quality fodder capable of meeting the needs of maintenance, growth, reproduction, gestation and milk production in cattle. *Pennisetum purpureum* Schum. is the richest grass in total nitrogenous matter (8.43 ± 1.81%) and digestible nitrogenous matter (43.18 ± 16.83 g/kg DM). *Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf produce good quality fodder to meet the vital needs of cattle. These results could be used for the improvement of degraded pastures in order to sustainably manage the pastoral ecosystem.

Keywords: *Pennisetum purpureum*, *Vigna unguiculata*, *Hyparrhenia diplandra*, mineral elements, nitrogenous matter, Boundji area.

INTRODUCTION

In livestock feeding, grasses and legumes are complementary; hence the idea of combining them on the same pasture. Forage crops grown in association with grasses and legumes aim to limit some of the disadvantages of the two families (César *et al*., 2004). The legume, while improving the fodder ration, provides the necessary nitrogen to the grass which limits the development of weeds. Grasses are the most important and form the basis of the natural herbaceous flora on which wild and domestic herbivores feed. Legumes are particularly interesting in livestock farms because they contribute, through symbiotic nitrogen fixation, to reducing the input of mineral nitrogen in the crop or rotation, and especially because they improve the protein value of the ruminant ration. Legumes produce quality fodder for animals (César *et al*., 2004; Ehouinsou, 2004; Diouf and Rippstein, 2004). The many agronomic, economic and environmental benefits of forage associations are now being rediscovered: possibility of overproduction compared to monocultures (or pure crops); reduced nitrogen fertilisation, which generates financial savings; less fossil energy use and less negative environmental impact (greenhouse gas emissions, nitrate leaching); production of a forage balanced in protein and energy; more regular production during the year (Finn *et* *al*, 2013; Huyghe and Delaby, 2013; Luscher *et al*., 2014; Emile *et al*., 2016; Protin *et al*., 2016). Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume of warm regions of African origin, cultivated as an annual for human and livestock food needs. The mineral content of the leaves of this cultivar can cover the needs of maintenance, growth, reproduction, gestation and milk production of animals during the whole vegetation cycle. Cowpea leaves can therefore be valued in livestock improvement (Yoka *et al.*, 2014a).

Hyparrhenia diplandra (Hack) Stapf dominates the Congolese fodder potential together with *Urochloa maxima* Jacq. It is a tall, erect perennial with fewer clumps. It is predominant in peri-forest zones of tropical Africa on deep clay and sandy-clay soils (Koulengana *et al*., 2017). *Pennisetum purpureum* Schum. is a perennial tropical grass, native to sub-equatorial forest and pre-forest regions, growing in clumps up to 4-7 metres in height. It is a highly resproutable, hardy plant with high biomass production and good food values if harvested at the right stage, but requires establishment by cuttings and a lot of labour to harvest.

In tropical countries in general, and in the Republic of Congo in particular, livestock are largely raised on large areas of savannah (potential pasture), which is often their only source of key nutrients (protein, carbohydrates, minerals, vitamins, etc.).

The protein-calorie deficiency of these pastures is often blamed for the low productivity of grazing animals, but it has been found that they also decline in spite of an adequate dietary supply of protein and energy, due to deficiencies in several essential mineral elements. It is therefore essential, in these hot climate areas, to assess these deficiencies by chemical analysis of the grass in order to introduce adequate prophylaxis by means of appropriate supplementation and thereby increase the profitability of the farms.

Natural tropical forages are nitrogen deficient. The value of grasses in particular, whether spontaneous or cultivated, decreases rapidly with the age of the regrowth (Cesar *et al*., 2004). In the Boundji area, extensive cattle breeding is developing, resulting in the overexploitation of pastures (Yoka *et al*., 2011). In order to qualitatively solve the problem of animal feeding, trials of *Pennisetum purpureum* Schum., *Vigna unguiculata* (L.) Walp. and *Hyparrhenia diplandra* (Hack.) Stapf were conducted. The aerial parts of plants harvested at two months after sowing and cutting are used as fodder for animals. However, little is known about the mineral content of fodder from these three forage species grown at the two-month stage in the study area. Knowledge of the mineral content of fodder is therefore necessary, as their deficiencies can cause serious health problems in domestic animals (IEMVT, 1988). It is in this perspective that the present study was conducted to improve the feed quality of domestic herbivores.

MATERIALS AND METHODS

Study Environment

The study was carried out in the south-western part of the Congolese basin, in the north of the Republic of Congo, specifically in the Boundji area (Cuvette department). The selected site is the Essimbi ranch, located about 3 km from the town of Boundji, and managed by the Centre d'Appui Technique de Boundji (CATB). This study area is located between 0 and 2º south latitude and between 15° and 16º east longitude.

The climate of the study area is subequatorial (Samba-Kimbata, 1991). Annual minimum temperatures in northern Congo as well as in the Boundji area fluctuate between 19.5o C and 21.9^{o} C while maxima vary between 29.9^{o} C and 31.9^{o} C (Moutsamboté, 2012). The average annual temperature is 25.5^{o} C and the average annual rainfall is 1657mm (Yoka, 2009). Ecologically, there is no dry period in the study area, as rainfall is almost permanent. There is only a three-month rainy period (June to August) called the "dry season" (Yoka, 2009). Relative humidity is quite high in the Congolese Cuvette (95-100%).

The Congolese Cuvette has two geological formations, the Batéké sands and the alluvium (ORSTOM, 1969; Bouka-Biona *et al*., 2001). There are mainly highly desaturated and impoverished ferrallitic soils, represented by the sandy-clay series formed on sandy or weakly clayey materials, poor in bases and very permeable, and hydromorphic soils occupying large areas, especially in the centre. In addition, these soils are sandy (86-96%), rich in fine sand (62-73%), and clay (0-85.5%). Their pH varies between 5.2 and 5.9 and the C/N ratio varies between 13 and 20 (Yoka, 2009).

The Congolese Basin is dominated by semi-caducified forests and savannas. The savannahs are of four types (IUCN, 1990 and Yoka, 2009): *Hyparrhenia diplandra* (Hack) Stapf savannah, *Trychypogon spicatus* (L.f.) Kuntze savannah, *Andropogon schirensis* Hochst. Ex A. Rich, *Loudetia simplex* (Nees) C.E Hubbard savannah or "lousséké" steppe. The forests are also of several types (IUCN, 1990): dense dryland rainforest, open dryland rainforest, dryland evergreen forest, flooded and swampy evergreen forest, mesophilic deciduous forest of central Congo, flooded forest, the Raphiales in flooded areas in pure or almost pure stands of *Raphia* sp.

Methods

Experimental set-up: Grid of agronomic plots

In open scrubby pasture dominated by *Annona senegalensis* Pers., four (4) experimental plots of 25 m x 25 m (i.e. 625 m²) each were selected for a fodder crop trial. Each plot is subdivided into two plots of 25 m x 12.5 m (312.5 m²) each, in which measurements were made. Therefore an area of 0.25 ha (2500 m²) was sampled.

Planting of fodder crops

Soil preparation

After clearing the shrubs and squaring the agronomic plots, soil preparation was done manually during the dry season using machetes, shovels, hoes, forks and rakes. Plots were made for the establishment of fodder crops. These planks are on average 10.25 m long and 1.5 m wide each. The spacing between two plots is 0.5 m, between two plots 1 m and between two plots 3.5 m. Each plot has 22 plots (11 per plot). Therefore a number of 88 plots were tested. Crop establishment was done in rows.

Sowing and cuttings

Crop establishment was done either by sowing (*Vigna unguiculata* (L.) Walp.) and by cuttings (*Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf) in the rainy period on beds with 50 cm x 50 cm spacing between plants. For *Vigna unguiculata* (L.) Walp. planting, three seeds were sown per stake (Yoka *et al*., 2014b) and four lines were used. For the establishment of *Pennisetum purpureum* Schum. young cuttings (cane 4-6 months old) were preferred, as beyond that, drier canes have a lower establishment capacity. Each cutting of 40-60 cm with at least 4 nodes was used. The cuttings were planted in a line, with 50 cm on the line. Only one line was used for the cuttings of *Pennisetum purpureum* Schum. Two 45 cm seedlings of *Hyparrhenia diplandra* (Hack.) Stapf were transplanted per planting point. The planting was done in rows of 50 cm by 50 cm on beds by stump burst. Four lines were used on each bed. The planting of the crops was done manually.

Forage sampling and chemical analysis

For each forage species, forage samples were taken at two months after establishment in a 1 m^2 plot with four replicates. These samples were weighed and oven-dried at 70º C to constant weight. After drying, samples of 300 g per forage plant were made up. The resulting samples were packed in plastic bags and sent to the Laboratoire de Chimie Analytique of the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) in Pointe-Noire, Republic of Congo, for chemical analysis. These analyses focused on the determination of nitrogen, phosphorus and calcium. Nitrogen was determined by the Kjeldahl method, phosphorus by the cold colorimetric method, and calcium by the ICP-Radial method (Masson *et al*., 1999). The ash was obtained by calcination in a muffle furnace at 480° C for 5 hours. The nitrogen contents were used to calculate total nitrogenous matter or total or crude protein and digestible nitrogenous matter by the Demarquilly (1968) method as follows:

DNM (g/kg DM) = 9.29 x MAT (%) -35.2

TNM (%) = N (%) x 6.25

With: DNM: digestible nitrogenous matter; TNM: total nitrogenous matter

N (%): percentage of nitrogen; 6.25: the conventional accepted coefficient.

Table I shows the recommended mineral intake in ruminant rations.

Table I: Recommended mineral intakes in ruminant feed rations: case of cattle

Recommandation	**Major elements (g/kg DM)**				
	P	**Ca**	**Mg**	**K**	**Na**
Maintenance	1,2	1,8	-	-	-
100g/d of ADG	1,7	2,4	-	-	-
Breeding cow of 300kg live weight	2,1	3,1	1	5	1,2
Pregnant sows	2,5	3,4	1,5	4,2	1
7kg of milk per day	3,5	6,2	1,6	6,7	1,5
Adapted from	Underwood (1981)				

Legend: ADG: average daily gain; P: phosphorus; Ca: calcium; Mg: magnesium; K: potassium; Na; sodium.

Table II shows the characteristic digestible nitrogen values for the different forage categories.

Table II: Characteristic values of digestible nitrogenous matter (DNM) for the different forage categories (Boudet & Rivière, 1968)

DNM **(g/kg DM)**	**Fodder quality**
DNM < 25	**Poor:** fodder that does not support the maintenance and small movements of the tropical cattle unit (TCU)
25 < DNM < 34	**Poor:** maintains TCU, liveweight gain 100g/d
34 < DNM < 53	**Good:** the TCU produces 1 to 3 litres of milk per day and gains 100 to 300 g of live weight per day
53 < DNM	**Excellent:** production of 3 litres of milk per day; gain of over 300 g of live weight per day

Legend: TCU: tropical cattle unit

The values in these two tables serve as a basis for comparing the data obtained in this study.

Statistical analysis of the data

All data collected in this study were processed in R software, version i386 4.1.0. and Microsoft Excel spreadsheet version 2016. Normality and Student's test were performed (significance level = 5%) to differentiate between elemental and protein contents of the forage crops two months after establishment. Means and standard errors were calculated using Microsoft Excel version 2016. The means are presented in the text together with their standard error to express the standard deviation of the sampling distribution of the different parameters studied (Dagnelie, 2007). The standard error expresses the precision of the measurement made on the sample (Altman and Bland, 2005). The upper limit for the probability of first order risk α is 5%. P-values calculated for statistical inference tests are said to be significant ($0.01<P<0.05$), highly significant ($0.001<P<0.01$) or very highly significant ($p<0.001$). Correlations between the mineral contents of these forages were carried out in order to find out the relationship between these elements.

RESULTS

Data on the mineral (ash, nitrogen, phosphorus and calcium) and protein contents of the forages from the improved pastures during a two-month trial are presented in Table III.

The ash or mineral matter (MM) content is higher in the legume (*Vigna unguiculata* (L.) Walp.) at two months after sowing (100.4 ± 10.4 g/kg) than in the grasses *Pennisetum purpureum* Schum. (86.2 ± 35.9 g/kg) and *Hyparrhenia diplandra* (Hack.) Stapf. Of the two forage grasses studied, *Pennisetum purpureum* Schum. is the grass with the higher ash content. There is a highly significant difference ($P < 0.05$) between the ash contents of these three forage crops. *Vigna unguiculata* (L.) Walp. is the forage species with the highest nitrogen content (26.2 ± 3.9 g/kg), followed by *Pennisetum purpureum* Schum. (13.5 ± 2.9 g/kg). The phosphorus contents of *Vigna unguiculata* (L.) Walp., *Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf. are 4.4 ± 0.6 g/kg; 1.5 ± 0.4 g/kg and 1.3 ± 0.2 g/kg respectively. *Vigna unguiculata* (L.) Walp. is the forage species with the highest phosphorus content. For calcium, *Vigna unguiculata* (L.) Walp. is the forage species with the highest content (13.2 ± 2 g/kg) followed by *Pennisetum purpureum* Schum (5.2 ± 3.5 g/kg) after two months of sowing.

Taking into account the recommended intakes of minerals in feed rations for cattle, the phosphorus contents (≥1.2 g/kg DM) in the three planted forages can ensure maintenance requirements in cattle. However, only the phosphorus content in Vigna unguiculata (L.) Walp. can additionally provide the requirements for growth (≥1.7 g/kg DM), reproduction (≥2.1 g/kg DM), gestation (≥2.5 g/kg DM) and milk production (≥3.5 g/kg DM).

The calcium contents (≥1.8 g/kg DM) of these three forage plants can provide maintenance and growth requirements in cattle. In contrast, only the calcium contents of *Vigna unguiculata* (L.) Walp. can provide the requirements for growth (≥2.4 g/kg DM), reproduction (≥3.1 g/kg DM), gestation (≥3.4 g/kg DM) and milk production (≥6.2 g/kg DM). The calcium content of *Pennisetum purpureum* Schum. can provide for growth (≥2.4 g/kg DM), reproduction (≥3.1 g/kg DM), management (≥3.4 g/kg DM) and not for milk production (≤6.2 g/kg DM). In contrast, the calcium content of *Hyparrhenia diplandra* (Hack.) Stapf. can only satisfy growth requirements.

Overall, in terms of phosphorus and calcium content, *Vigna unguiculata* (L.) Walp. is the richest forage plant, followed by *Pennisetum purpureum* Schum.

The total nitrogen content and consequently the digestible nitrogen content is higher in *Vigna unguiculata* (L.) Walp. (117.50 ± 22.64 g/kg DM). The differences in nitrogen, phosphorus, calcium, total nitrogen and digestible nitrogen contents of these three forage crops were not statistically significant ($P > 0.05$).

Figure 1 shows the different correlations between nitrogen and phosphorus (a), nitrogen and calcium (b), and between phosphorus and calcium (c). There are positive correlations between nitrogen and phosphorus ($r = 0.96$), nitrogen and calcium ($r = 0.77$), and between phosphorus and calcium ($r = 0.81$). These results show that the presence of one could explain the presence of the other mineral element in the plant, and therefore the deficiency of one mineral element could be compensated by the presence of the other.

Table III: Data on protein and mineral content of the three fodder crops of the improved pastures of the Essimbi ranch in Boundji after two months of establishment

Fodder crops	MM(g/kg)	N(g/kg)	P(g/kg)	Ca(g/kg)	TNM(%)	DNM(g/kg DM)
Vigna unguiculata (L.) Walp.	100,4 ± 10,4	26,3 ± 3,9	4,4 ± 0,6	13,2 ± 2	16,43 ± 2,43	117,50 ± 22,64
Pennisetum purpureum	86,2 ± 35,9	13,5 ± 2,9	1,5 ± 0,4	5,2 ± 3,5	8,43 ± 1,81	43,18 ± 16,83

Schum.						
Hyparrhenia diplandra (Hack.) Stapf	74,4 ± 21	12,5 ± 1,9	1,3 ± 0,2	2,8 ± 0,8	7,81 ± 1,18	37,37 ± 11,03
Tests	Normality test (0,40); Student test (0.0007)	Normality tyest (0,02); Wilcoxon test (0,12)	Normality test (0,007); Wilcoxon test (0,09)	Normality test (0,14); Student test (0,06)	Normality test (0,02); Wilcoxon test (0,12)	Normality test (0,02); Wilcoxon test (0,12)

Legend: MM : mineral matter; N: Nitrogen; P: Phosphorus; Ca: Calcium; TNM: Total total nitrogenous Matter; DNM: digestible nitrogenous matter

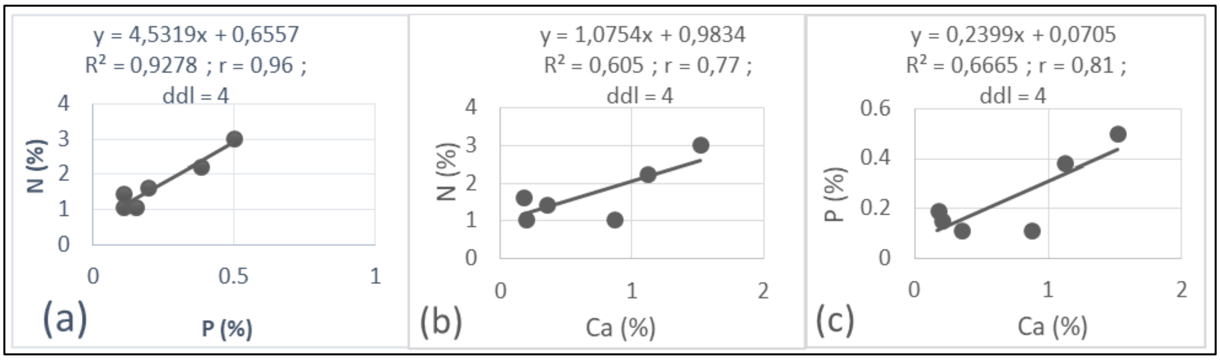

Figure 1: Different correlations between nitrogen, phosphorus and calcium contents in forage plants

DISCUSSION

The contents of mineral elements, total nitrogenous matter (TNM) and digestible nitrogenous matter (DNM) of the forage species studied show the variability of the chemical constituents of forages. This variability would be of genetic origin because for the same vegetation and for the forage species, the differences in mineral elements are clear, as shown by the values obtained for the forage of the crops planted.

The mineral content at two months is high in *Vigna unguiculata* (L.) Walp. (100.4 ± 10.4% DM), medium in *Pennisetum purpureum* Schum. (86.2 ± 35.9% DM) and low in *Hyparrhenia diplandra* (Hack.) Stapf. (74.4 ± 21% DM). *Vigna unguiculata* (L.) Walp. is the most nitrogen and calcium rich forage species. Total nitrogenous matter is higher in *Vigna unguiculata* (L.) Walp. (16.43 ± 0.34 % DM) than in *Pennisetum purpureum* Schum. (8.43 ± 1.81% DM) and *Hyparrhenia diplandra* (Hack.) Stapf de (7.81 ± 1.18 % DM). According to some nutritionists (Milford and Minson, 1966; Minson, 1990), a minimum TNM content (8% DM) is required for adequate rumen microorganism function in polygastrics. Forages with less than 8% TNM in the older stages of *Hyparrhenia diplandra* (Hack.) Stapf are in the critical zone where TNM levels are limiting for proper rumen function. In view of this constraint, polygastrics will not be able to ingest significant amounts of *Hyparrhenia diplandra* (Hack.) Stapf forage at the two-month stage after implantation. The digestible nitrogen content of *Vigna unguiculata* (L.) Walp. (117.50 ± 22.64 g/kg DM) is much higher than that of *Pennisetum purpureum* Schum. (43.18 ± 16.83 g/kg DM) and *Hyparrhenia diplandra* (Hack.) Stapf. (37.37 ± 11.03 g/kg DM). *Vigna unguiculata* (L.) Walp. provides excellent quality fodder, taking into account its DNM, and can provide a production of milk per day and achieve a gain of more than 300 g live weight per day, according to Underwood (1981). *Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf. provide good quality fodder allowing an animal weighing 250 kg or LU to produce 1 to 3 litres of milk per day and gain 100 to 300 g of live weight per day based on the work of Boudet and Rivière (1968). These fodder crops can meet the needs of maintenance, growth, reproduction, gestation and milk production of herbivores, based on the work of Underwood (1981). These three fodder crops could be a real solution for the qualitative improvement of degraded pastures. The results show a significant amount of digestible nitrogenous matter, especially that provided by the legume. The same observation was made by Granier (1972), Ahamat (2008) and Ado (2014). This may be related to the forage species used, the cultivation techniques and the soil and climatic conditions of the study area. The results of our work are similar to those of César *et al*., (2004) and Yoka (2009). Legume-rich fodder (high nitrogen content) can solve milk production needs. Legumes produce quality fodder for animals (César *et al*., 2004; Diouf and Rippstein, 2004; Ehouinsou, 2004). The work of César *et al*., (2004) shows that the digestible nitrogen content of legumes is higher than 200 g/kg DM and that of grasses does not reach 125 g/kg DM and can drop to zero.

In Niger, in the comparison between the different varieties of *Vigna unguiculata* (L.) Walp. studied, Ado (2014) shows the variability of the chemical constituents of the haulms, but especially their digestible nitrogen matter (DNM) and energy content

(UF). This variability is of genetic origin because for the same vegetation period, the differences in DM and UF are clear, as shown by the values obtained for the varieties IN92E-26 and TN3-78. This author obtains the mineral matter (MM) or ash rate within the accepted reference range of less than 10% for all varieties used. In Chad, a study with a cowpea variety gave the following results: 16% of TNM; 10.8% of MM and 58.2 g/kg DM of DNM (Ahamat, 2008). In Madagascar, Granier (1972) for a new variety of *Pennisetum purpureum* Schum. during the months of January found 18.96% DM of TNM in Tananarive and, 16.16% DM of TNM in Kianjosoa; this yield is considerable. This fodder may allow some milk production. In the dry season, the content is dependent on the richness of the soil, the method of exploitation (height of the plant), the water reserves of the soil and can in no way characterise the plant (Granier 1972). He points out that legumes generally contain 5 to 10 times more calcium than phosphorus, and the Kisozi variety seems to have a particular physiology relating to phosphorus, which plays an important role in synthesis, and that by favouring root development it is a factor in precocity. The results on mineral element and protein content provided by Ahamat (2008) and Granier (1972) are lower than ours ; this could be explained by the forage crop varieties used, the production techniques, the harvesting date and the soil and climatic conditions in the study area.

In the savannahs of the Congolese basin, Yoka *et al*., (2012) report that the phosphorus and calcium contents of different forage species, depending on the type of forage and the place of harvest, can only cover the maintenance needs of cattle ; those of potassium and magnesium can cover the growth and reproduction needs of cattle. The levels of all these major elements cannot, on the whole, cover the needs of milk production. The work of Crespo *et al*., (2016), reports that the grass-legume mixture can both produce protein-rich fodder and ensure nitrogen supply to the grasses. Our results confirm the work of the latter authors. These three fodder crops could contribute to the improvement of pastures, not only to meet the needs of maintenance, growth, reproduction and gestation, but especially those of milk production of cattle.

CONCLUSION

The study showed that the mineral and protein contents of the three forage plants (*Pennisetum purpureum* Schum., *Vigna unguiculata* (L.) Walp. and *Hyparrhenia diplandra* (Hack.) Stapf.) vary between species. In the vast majority of comparisons made, the presence of the legume significantly improves the nitrogen value of the forage. The legume is particularly interesting in livestock farms because it improves the ration of herbivores. *Vigna unguiculata* (L.) Walp. is the forage species with the highest mineral and protein content. *Pennisetum purpureum* Schum. is a grass that is richer in total nitrogenous matter and therefore in digestible nitrogenous matter than *Hyparrhenia diplandra* (Hack.) Stapf.

At two months after sowing, *Vigna unguiculata* (L.) Walp. provides excellent quality fodder for good milk production. *Pennisetum purpureum* Schum. and *Hyparrhenia diplandra* (Hack.) Stapf provide good quality fodder that can also meet the vital needs of herbivores. These three fodder crops could be a real solution for the qualitative improvement of degraded pastures and the productivity of livestock. The evaluation of the trace element content of the fodder of these three plants will be the subject of our next studies in the Congolese basin, with a view to enhancing the value of the cultivation of these species in the context of pasture improvement.

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