



Protein Content of *Brachiaria ruziziensis* (Poaceae) under the Direct and Residual Effects of Fertilization with Hen Droppings

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GJA, FT and DFW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VFN and ETP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: A study was conducted at the Research and Experimental Farm (REF) of the University of Dschang between March 2015 and December 2016, and then at the Animal Nutrition Laboratory of the Faculty of Agronomy and Agricultural Sciences. The objective of this study was to evaluate the direct and residual effects of fertilization with hen droppings on the protein content of *Brachiaria ruziziensis* at different phenological stages.

Methodology: A factorial design comprising five levels of fertilization in terms of nitrogen in the form of hen droppings (0, 25, 50, 75 and 100 kg N/ha), and three phenological stages of cutting (bolting, flowering and after seed set) on 6 m² plots (3 m x 2 m) in four replicates, i.e. a total of 60 experimental plots was used. Fertilization with hen droppings was done one month after the stump chips were grown in the first year of cultivation (direct effects). In the second year of cultivation (residual effects), no fertilization was applied. The total nitrogen content of the plant samples was

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determined by the Kjeldhal method and the crude protein contents were obtained by multiplying the nitrogen contents by the forage-specific coefficient of 6.25.

Results: This study showed that protein contents obtained under the direct effect were significantly higher than those obtained under the residual effect of fertilization. Fertilization at 100 kg N/ha resulted in the highest protein contents under direct and residual fertilization.

Conclusion: In view of the results obtained, fertilization with hen droppings at a dose of 100 kg N/ha would be recommended for the cultivation of *B. ruziziensis* in order to limit mineral fertilizer inputs and improve its protein content.

Keywords: *Brachiaria ruziziensis*; hen droppings; proteins; Dschang; Cameroon.

1. INTRODUCTION

The necessity to feed an increasing population in developing countries pushes farmers to increasingly exploit land to the detriment of pastoral areas [1,2,3]. It is therefore becoming necessary to develop livestock farming in a farm system. In such a context, good quality fodder should be provided to meet the nutritional needs of the animals [4]. Forage cultivation can be a solution to obtaining the forage. This means of fodder supply would ensure year-round availability of fodder [5,6]. Among the fodder plants introduced in Cameroon is *Brachiaria ruziziensis*. It is a plant of the Poaceae family that has good nutritional value and is well eaten by animals [7,8]. Its cultivation and use can provide a solution to forage supply in a farm system [6]. When cultivated, plants of the genus *Brachiaria* can be able to extract all the few nutrients that remain in poor soils [9]. As a result, their cultivation requires fertilizer to improve their biomass and chemical composition yields. Several studies have shown that chemical fertilizers have improved fodder production through nitrogen, which is the principal limiting factor in tropical rangelands [1,10]. However, their prolonged use often leads to environmental pollution, hence the need to seek more environmentally friendly solutions such as composts and animal manures [11,12]. In addition, in a farm system, the use of animal feed waste (droppings, manure, dung) could be a solution to the fertilization of forage plants. Several studies have indeed shown the importance of organic fertilization in the growth and production of forage plants [2,3,4,12]. To date, few studies have been done on the use of organic fertilizers such as hen droppings in improving the protein content of forage plants. It is in this perspective that this study is aimed at finding the optimal doses of fertilization with hen droppings, which would allow to obtain the best protein contents on *Brachiaria ruziziensis*.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the Research and Experimental Farm (REF) of the University of Dschang between March 2015 and December 2016, then at the Animal Nutrition Laboratory of the Faculty of Agronomy and Agricultural Sciences (FASA). The REF is located in the West Cameroon Region, in the Menoua Division. The mean geographical coordinates are 05°20' North latitude and 10°03' East longitude [13]. The climate of the West Cameroon Region is an equatorial Cameroonian climate with mountainous facies determined by altitude, the average being 1400 m. It is characterized by a rainy season from mid-March to mid-November and a dry season from mid-November to mid-March [14]. The average temperature of the hottest month (February) is 25°C and the average temperature of the coolest month is 19°C and is in July or August. The annual insolation is 1800 hours, with relative humidity varying between 33 and 98% during the year. Precipitation varies between 1200 and 1800 mm per year [14].

2.2 Experimental Design

The experimental design is a factorial (5 x 3) design comparing five levels of fertilization in terms of nitrogen in hen droppings (0, 25, 50, 75 and 100 kg N/ha), and three phenological stages of cutting (bolting, flowering and after seed set) on 6 m² (3 m x 2 m) plots in four replicates, i.e. a total of 60 experimental plots.

2.3 Origin and Nitrogen Content of the Fertilizer

The organic fertilizer used in this experiment is hen droppings. It was obtained from a farmer in the Menoua Division (Dschang). Its nitrogen

content was analysed at the Laboratory of Soil Analysis and Environmental Chemistry of the University of Dschang according to the method described by Pauwels et al. [15]. The result obtained showed that the nitrogen content of the hen droppings was 2600 mg/kg of hen droppings, and its moisture content was 14.8%. With this result it was possible to calculate the quantities of hen droppings to be applied in terms of nitrogen doses.

Fertilization levels were defined in terms of the amount of nitrogen contained in hen droppings because nitrogen is a factor in yield and sometimes quality. It increases the protein content in plants [16]. Thus, five nitrogen rates have been defined (0, 25, 50, 75 and 100 kg N/ha) for the cultivation of *B. ruziziensis*. For each of the defined rates of hen droppings, there was a specific amount of hen droppings as presented in Table 1.

2.4 Soil Preparation, Cultivation of Plants and Fertilization

During the establishment of the experimental plot, soil samples were taken from the trial site in the 0 - 20 cm horizon. These samples were analyzed at the Laboratory for Soil Analysis and Environmental Chemistry of the University of Dschang according to the method described by Pauwels et al. [15]. The results of the analysis of the soil samples showed that the mean textural class of the experimental soil is silty. This soil is slightly acidic with an average pH of 5.5. This excludes the risk of toxicity due to excess aluminium and manganese. Total nitrogen levels are low (0.6 g/kg soil). In fact, for most Cameroonian soils, contents of 1 g/kg already allow to conduct a crop cycle in traditional agriculture. However, for intensive cultivation, these levels must be increased to 2 g/kg. It is therefore necessary to provide a nitrogen supplement. The rate of organic matter (with a C/N ratio = 11.66) is within the ideal range (8-12), which reflects good mineralization of organic matter. This gives this soil a higher retention capacity, allowing it to withstand massive fertilization. With regard to exchangeable bases, this soil has low levels of magnesium, phosphorus, potassium, calcium (respectively 0.43 Cmol/kg, 0.5 mg/kg, 2.06 Cmol/kg and 0.2 Cmol/kg). The absorbent complex in this soil has a high cation exchange capacity (7.84 Cmol/kg soil) due to its high organic matter content. The characteristics of this soil therefore make it possible to apply fertilization with high doses without fear.

The 6 m² (3 m x 2 m) seedbeds were prepared manually. Stump chips with several *B. ruziziensis* plants were taken from the REF course. Several tillers from one to two shoots obtained by manual division were dressed (reduction of leaf and root size) and transplanted on each plot at a depth of 4 cm, with a spacing of 20 cm x 15 cm intra and inter rows, respectively. One month after the stump chips were grown, the plots were fertilized with hen droppings once in the quantities indicated in Table 1. In the second year of cultivation (under the residual effect of the fertilization), a regulating mowing was carried out at 5 cm above the ground, but no fertilization was carried out after the regulating mowing.

2.5 Data Collection and Analysis

2.5.1 Determination of protein content

At each phenological stage, 20 beds (four replicates per treatment) were mowed using a cutting table, 5 cm above the ground on a 2 m² (2 m x 1 m) area in the centre of the bed. A sample of 0.5 kg of *B. ruziziensis* fodder harvested in this way was taken from each block per level of fertilization. Each sample was then separated into leaves and stems, which were cut and dried at 60°C in a ventilated Gallekamp oven to constant weight. Each sample of the different parts (leaves, stems) of *B. ruziziensis* dried as previously described was crushed using a 1 mm mesh hammer mill and then stored at room temperature in plastic bags for analysis of the protein content by the Kjeldhal method [17], which involves successive mineralization, distillation and titration. A sample of 0.5 g of fodder and 0.2 g of catalyst (selenium) is mixed and wrapped in Whatman N°1 paper. The whole is introduced into a mineralization flask (Pyrex) with 10 ml of concentrated sulphuric acid, and brought to high temperature on a mineralization ramp placed under a ventilated hood. After 3 hours of mineralization, a light green solution reflecting the conversion of organic nitrogen to ammonium sulphate is obtained. The flask is cooled under the hood and its contents transferred to a 100 ml volumetric flask and the volume is made up to the mark with distilled water. Then 10 ml of the mineralised solution is drawn off with a syringe and introduced into the distillation tube. The whole is then mounted on the distiller Kjeldhal, and 20 ml of NaOH (40%) is added. The cooling end of the distiller is immersed in 20 ml of a mixture of boric acid (40%) and coloured indicators (bromocresol green and methyl red) contained in a 250 ml

Table 1. Levels of fertilization and quantities of hen droppings which is spread [3]

Fertilization levels	Quantity of droppings applied (kg/plot)	Doses of nitrogen equivalent (kg N/ha)
T ₀	0	0
T ₁	5.77	25
T ₂	11.53	50
T ₃	17.30	75
T ₄	23.07	100

Erlenmeyer flask. During distillation, the ammonium hydroxide dissociates, vaporizes, and then is liquefied in the refrigerant and recovered in boric acid which turns from red to green. At the end of the distillation, 150 ml of distillate is recovered and titrated in the presence of HCl (0.01 N). The nitrogen (N) content is then calculated according to the formula below, where: 1 ml of HCl (0,01 N) neutralises $14 \times 10^{-3} \times 10^{-2}$ g of nitrogen.

$$N (\%D) = \frac{(V - V_0) \times 100 \times 0.14 \times 10^{-3}}{m \times V_e} \times 100$$

Where V = Volume of HCl used for the titration of the sample,

V₀ = Volume of HCl used for white titration,

V_e = Volume of mineralization used for distillation,

m = Weight of the mineralized sample.

The crude protein (CP) contents were obtained by multiplying the nitrogen contents by the forage-specific coefficient of 6.25.

$$CP (\%DM) = N (\%DM) \times 6.25$$

2.5.2 Statistical analyses

Multi-factor analysis of variance (ANOVA) according to the General Linear Model (GLM)

using SPSS version 20.0 software was used to analyze protein content data as a function of different fertilization levels. Where differences existed between the different treatments, the means were separated by Duncan's test at the 5% significance level. Comparison of data by mowing year was done using Student's "t" test.

3. RESULTS AND DISCUSSION

3.1 Direct and Residual Effects of Different Levels of Fertilization with hen Droppings on the Protein Contents of *Brachiaria ruziziensis* During the Bolting

The variations in the protein content of leaves, stems and whole plants of *B. ruziziensis* under the direct and residual effects of fertilization with hen droppings at the bolting are presented in Tables 2 and 3.

It can be seen from the Table 2 that under the direct and residual effect of fertilization, protein contents in the leaves of plants in the plots fertilized at the 75 and 100 kg N/ha doses were comparable to, and significantly higher ($P < .05$) than those in the leaves of plants in the plots fertilized at the 25 and 50 kg N/ha

Table 2. Direct and residual effects of hen droppings fertilization on the protein content (% DM) of leaves and stems of *B. ruziziensis* during the bolting

Fertilization levels	Leaves				Stems			
	Direct effect	Residual effect	SEM	P	Direct effect	Residual effect	SEM	P
T ₀	11.1 ^{a**}	5.9 ^{a*}	0.32	0.0001	6.2 ^{a**}	2.4 ^{a*}	0.49	0.001
T ₁	11.1 ^{a**}	6.1 ^{ab*}	0.21	0.0001	6.4 ^{a**}	2.4 ^{a*}	0.42	0.0001
T ₂	11.2 ^{a**}	6.4 ^{abc*}	0.24	0.0001	6.5 ^{a**}	2.4 ^{a*}	0.23	0.0001
T ₃	12.2 ^{b**}	6.6 ^{bc*}	0.18	0.0001	7.0 ^{a**}	2.8 ^{a*}	0.26	0.0001
T ₄	12.4 ^{b**}	6.9 ^{c*}	0.34	0.0001	7.1 ^{a**}	3.3 ^{b*}	0.16	0.0001
SEM	0.15	0.10			0.16	1.93		
P	0.001	0.011			0.310	0.001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*}, ^{**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

Table 3. Direct and residual effects of hen droppings fertilization on the protein contents (% DM) of whole plants of *B. ruziziensis* during the bolting

Fertilization levels	Direct effect	Residual effect	SEM	P
T ₀	17.3 ^{a**}	8.4 ^{a*}	0.65	0.0001
T ₁	17.5 ^{a**}	8.5 ^{a*}	0.58	0.0001
T ₂	17.7 ^{ab**}	8.9 ^{ab*}	0.40	0.0001
T ₃	19.3 ^{bc**}	9.4 ^{bc*}	0.40	0.0001
T ₄	19.6 ^{c**}	10.3 ^{c*}	0.43	0.0001
SEM	0.28	3.81		
P	0.011	0.001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*,**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

Table 4. Direct and residual effects of hen droppings fertilization on the protein content (% DM) of leaves and stems of *B. ruziziensis* at flowering

Fertilization levels	Leaves				Stems			
	Direct effect	Residual effect	SEM	P	Direct effect	Residual effect	SEM	P
T ₀	11.6 ^{a**}	6.2 ^{a*}	0.24	0.0001	5.4 ^{a**}	1.6 ^{a*}	0.28	0.0001
T ₁	12.7 ^{b**}	6.2 ^{a*}	0.28	0.0001	5.4 ^{a**}	1.6 ^{a*}	0.20	0.0001
T ₂	13.5 ^{c**}	7.1 ^{b*}	0.21	0.0001	5.9 ^{a**}	2.3 ^{b*}	0.41	0.001
T ₃	13.4 ^{c**}	7.3 ^{b*}	0.20	0.0001	6.3 ^{a**}	2.7 ^{b*}	0.36	0.0001
T ₄	13.6 ^{c**}	8.3 ^{c*}	0.20	0.0001	7.6 ^{b**}	3.2 ^{c*}	0.15	0.0001
SEM	0.17	0.20			0.22	0.15		
P	0.0001	0.0001			0.001	0.0001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*,**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

doses and in the control plots. All protein contents obtained in the leaves under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization, regardless of the different levels of fertilization.

Under the direct effect of fertilization, protein contents in the stems of the fertilized plots were comparable to those of the control plots (Table 2). Under the residual effect, fertilization at the 100 kg N/ha dose resulted in a significantly higher protein content than at the lower fertilization rates.

Protein contents in stems obtained under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization.

Protein contents of whole plants from plots fertilized at 75 and 100 kg N/ha were statistically comparable ($P < .05$) and significantly higher ($P < .05$) than those of whole plants from plots

fertilized at the lower rates, under the direct and residual effect of fertilization (Table 3). Protein contents of whole plants obtained under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization.

3.2 Direct and Residual Effects of Different Levels of Hen Droppings Fertilization on the Protein Content of *Brachiaria ruziziensis* at Flowering

Under the direct effect of fertilization, the protein contents obtained in the leaves of the fertilized plots were significantly higher ($P < .05$) than those of the control plots (Table 4). Under the residual effect of fertilization at flowering, the highest protein content in the leaves was obtained with fertilization at the 100 kg N/ha rate. This was significantly higher ($P < .05$) than the leaves fertilized at the lower fertilizer rates. Leaf protein contents obtained under the direct effect of fertilization at flowering were significantly

higher ($P < .05$) than those obtained under the residual effect of fertilization.

Under the direct effect of fertilization at flowering, protein contents obtained in plant stems from plots fertilized at the dose of 100 kg N/ha were significantly higher ($P < .05$) than those obtained at lower fertilization doses (Table 4). The stem protein content obtained from the residual effect of fertilization at the dose of 100 kg N/ha was significantly higher than those obtained from the residual effect of fertilization at the doses of 50 and 75 kg N/ha. The latter were statistically comparable and significantly higher ($P < .05$) than the residual effect of 25 kg N/ha and the control plots. Protein contents in stems obtained under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization at flowering.

As a direct and residual effect of fertilization, whole plant protein contents in the plots fertilized at 50, 75 and 100 kg N/ha were significantly

higher ($P < .05$) than in the control plots (Table 5). The highest protein content was obtained with the 100 kg N/ha dose. Protein contents of whole plants obtained under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization.

3.3 Direct and Residual Effects of Different Levels of Hen Droppings Fertilization on the Protein Content of *Brachiaria ruziziensis* after Seed Set

Fertilization resulted in significantly higher protein contents ($P < .05$) in the leaves after seed set than those obtained in the leaves of the control plots (Table 6) under the direct effect of fertilization. Under the residual effect of fertilization, protein contents of plants in the plots fertilized at 50, 75 and 100 kg N/ha were significantly higher than in the control plots. Fertilization at the 100 kg N/ha rate resulted in the highest protein contents under the direct and

Table 5. Direct and residual effects of hen droppings fertilization on the protein contents (% DM) of whole plants of *B. ruziziensis* during the flowering

Fertilization levels	Direct effect	Residual effect	SEM	P
T ₀	17.1 ^{a**}	7.8 ^{a*}	0.43	0.0001
T ₁	18.1 ^{a**}	7.8 ^{a*}	0.39	0.0001
T ₂	19.5 ^{b**}	9.5 ^{b*}	0.60	0.0001
T ₃	19.7 ^{b**}	10.1 ^{b*}	0.27	0.0001
T ₄	21.2 ^{c**}	11.5 ^{c*}	0.27	0.0001
SEM	0.35	0.34		
P	0.0001	0.0001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*,**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

Table 6. Direct and residual effects of hen droppings fertilization on the protein content (% DM) of leaves and stems of *B. ruziziensis* after seed set

Fertilization levels	Leaves				Stems			
	Direct effect	Residual effect	SEM	P	Direct effect	Residual effect	SEM	P
T ₀	6.3 ^{a*}	5.6 ^{a*}	0.28	0.061	3.9 ^{a**}	1.4 ^{a*}	0.11	0.0001
T ₁	7.7 ^{b**}	5.6 ^{a*}	0.17	0.0001	3.9 ^{a**}	1.4 ^{a*}	0.36	0.0001
T ₂	9.1 ^{c**}	6.5 ^{b*}	0.28	0.0001	4.4 ^{a**}	1.6 ^{ab*}	0.30	0.0001
T ₃	9.1 ^{c**}	6.7 ^{b*}	0.16	0.0001	5.3 ^{b**}	2.0 ^{b*}	0.35	0.0001
T ₄	9.8 ^{d**}	7.3 ^{c*}	0.27	0.0001	6.3 ^{c**}	3.5 ^{c*}	0.24	0.0001
SEM	0.29	0.17			0.23	0.18		
P	0.0001	0.0001			0.0001	0.0001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*,**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

residual effect of fertilization. Leaf protein contents obtained under the direct effect of fertilization after seed set were significantly higher than those obtained under the residual effect of fertilization.

At the stem level, fertilization at 75 and 100 kg N/ha resulted in significantly higher protein contents ($P < .05$) than those of stems from the control plots (Table 6). Fertilization at the dose of 100 kg N/ha resulted in the highest protein levels. Protein contents in the stems obtained as a direct effect of fertilization were significantly higher than those obtained under the residual effect of the fertilization.

As a direct effect of the fertilization after seed set, protein contents obtained in whole plants from the fertilized plots were significantly higher ($P < .05$) than those from the control plots. Under the residual effect of fertilization after seed set, protein contents in whole plants from plots fertilized at 50, 75 and 100 kg N/ha were significantly higher ($P < .05$) than those in whole plants from control plots (Table 7). Fertilization at the dose of 100 kg N/ha resulted in the highest protein contents as a direct and residual effect of fertilization. Protein contents of whole plants obtained under the direct effect of fertilization were significantly higher ($P < .05$) than those obtained under the residual effect of fertilization.

3.4 Discussion

Fertilization with hen droppings positively influenced the protein content of the whole plant of *B. ruziziensis* and its individual compartments. Thus, during the bolting, flowering and after seed set, the protein contents obtained on the fertilized plots were higher than those on the unfertilized

plots. These observations are consistent with the work of Tendonkeng [6] who observed an increase in protein content as a result of nitrogen fertilization of *B. ruziziensis* with urea. Fertilization at the 100 kg N/ha rate resulted in the highest protein contents compared to the lower rates at all phenological stages. Indeed, in the absence of nitrogen fertilization, the crude protein content of the plant depends essentially on the nitrogen availability of the soil [18]. Fertilization with doses that exceed the plant's growth requirements considerably increases its nitrogen content [19,20], this is due to the fact that the entry of nitrogen into the plant, which is essentially in the form of nitrate in the soil, increases rapidly with fertilization, leading initially to the accumulation of non-protein nitrogen, then nitrate ($N-NO_3^-$) for high fertilization levels [6,18,21,22,23]. Nitric nitrogen can then represent 10 to 15% of total nitrogen, or even 20% for fertilizations of more than 100 kg N/cycle. The increase in the protein content of *B. ruziziensis* due to increasing levels of hen droppings fertilization observed in this study are comparable to the results of the work of other authors such as Guerin [20] and Tendonkeng [6], who observed in their work on forage plants that the higher the nitrogen doses applied to the plants, the higher the crude protein content of the plants. Protein contents obtained from the residual effect of hen droppings fertilization at 25 kg N/ha on all parts of the plant were comparable to those obtained in the control plots regardless of phenological stages. This is because the amount of additional nitrogen supplied one year rather by fertilization at this rate was fully utilized by the plants in the same year. However, leaf and whole plant protein levels obtained from the residual effect of fertilization at 50, 75 and 100 kg N/ha were significantly higher than those of the control plots. This shows that the nutrients

Table 7. Direct and residual effects of hen droppings fertilization levels on the protein content (% DM) of whole plants of *B. ruziziensis* after seed set

Fertilization levels	Direct effect	Residual effect	SEM	P
T ₀	10.2 ^{a**}	7.1 ^{a*}	0.38	0.0001
T ₁	11.6 ^{b**}	7.1 ^{a*}	0.27	0.0001
T ₂	13.5 ^{c**}	8.2 ^{b*}	0.38	0.0001
T ₃	14.4 ^{c**}	8.7 ^{b*}	0.42	0.0001
T ₄	16.2 ^{d**}	10.9 ^{c*}	0.27	0.0001
SEM	0.49	0.33		
P	0.0001	0.0001		

^{a,b,c}: Averages with the same letters on the same column are not significant at the 5% threshold.

^{*,**}: Averages with the same number of asterisks on the same line are not significant at the 5% threshold.

T₀= control plots, T₁= 25 kg N/ha, T₂= 50 kg N/ha, T₃= 75 kg N/ha, T₄= 100 kg N/ha

SEM: standard error of means

provided under the direct effect of these levels of fertilization were not fully utilized and allowed the plants to have significantly different protein contents than the control plots under the residual effect. This confirms, on the one hand, Delaby's [18] observations that, in the absence of fertilization, the plant protein content depends on the soil nitrogen availability, and on the other hand, Morot-Gaudry's [21] observations that nitrogen is a growth factor and a quality factor that influences the plant protein rate.

The protein contents of the plant and its various parts obtained under the direct effect of fertilization were significantly higher than those obtained under the residual effect of fertilization. This is explained by the fact that the hen droppings introduced during the first year of cultivation is a fertilizer that contains urea and ammonia [24]. The latter are forms of nitrogen that can be assimilated by the plant because they are rapidly converted into nitrate by soil microorganisms [16]. Thus, these forms of nitrogen were absorbed by the plant roots, which significantly increased protein levels in the first year of cultivation (direct effect of fertilization). The decrease in protein content observed in the second year of cultivation (under the residual effect of fertilization) can therefore be interpreted as being related to the fact that the plants took up these forms of rapidly assimilable nitrogen from the soil in the first year of cultivation. In the second cropping year, in the absence of fertilization, the plants had to use only the small amounts of residual nitrogen in the soil, hence the relatively low protein contents obtained from the plants in the second year of mowing. This confirms the observations of Husson et al. [9] that the use of *Brachiaria* as a fodder crop without fertilization may lead to a depletion of the soils that will become uncultivated.

4. CONCLUSION

The objective of this work was to evaluate the direct and residual effects of different levels of fertilization with hen droppings on the protein content of *Brachiaria ruziziensis*. It was found that the direct effect of fertilization resulted in significantly higher protein contents than the residual effect of fertilization on *B. ruziziensis* and its different compartments. Fertilization at the dose of 100 kg N/ha resulted in the highest protein contents in the whole plant, leaves and stems of *B. ruziziensis*. This leads to the conclusion that fertilization with hen droppings at the dose of 100 kg N/ha would be the

recommended fertilization rate over a two-year period for the *B. ruziziensis* crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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