



# Nutritional composition, nitrate and nitrite levels of Kikuyo grass and its influence on milk production

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## ABSTRACT

**Objective.** Establish variations of nutritional composition and the contents of nitrates and nitrites in the Kikuyo grass (*Cenchrus clandestinus* (Hochst. Ex Chiov.) Morrone) from four veredas in the municipality of San Miguel de Sema: Peña Blanca, Sirigay, Sabaneca, and Quintero, during dry and rainy seasons. **Material and methods.** Nutritional characterization parameters, were associated a two-way ANOVA, using Tukey's as a multiple comparison test. **Results.** The results revealed in dry matter a high average content of total protein (TP) (23.48±3.71%), neutral detergent fiber (60.86±3.03%), with important DM degradability values at different time points (62.97±3.74% after 48 Hours). The report indicated decreased values of lignin (4.25±0.5%), non-structural carbohydrates (11.44±2.43%) and of the ratio of non-structural carbohydrates: rumen degradable protein (NSC:RDP) of 0.84±0.16. The levels of nitrates reached an average of 2977 ppm ±2061, which differed significantly according to the seasons and veredas, for the dry season in the different veredas the level of nitrated remained at 4728 ppm, a value 3.9 higher in comparison to the rainy season. The levels of nitrites showed average contents of 2.97 pm of DM, contents highly decreased to be potentially toxic. **Conclusions.** These results allowed us to establish a difference in the protein:energy relation as the limiting factor for milk production. Nitrate levels indicated as potentially toxic.

**Keywords:** Kikuyo; grass; nutrient; nitrate; nitrite (Source: EcuRed)

## RESUMEN

**Objetivo.** Establecer variaciones de la composición nutricional e incidencia de los contenidos de nitratos y nitritos en el pasto Kikuyo (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone), procedente de cuatro veredas: Peña Blanca, Sirigay, Sabaneca y Quintero, en épocas seca y de lluvia del municipio de San Miguel de Sema – Boyacá. **Materiales y métodos.** Parámetros de caracterización nutricional fueron asociados a un análisis de varianza, usando la prueba de Tukey como comparación

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de medias. **Resultados.** En la materia seca, se destacan altos contenidos promedios de proteína total ( $23.48 \pm 3.71\%$ ), de fibra en detergente neutra ( $60.86 \pm 3.03\%$ ), importantes niveles de degradabilidad de la MS en los diferentes tiempos, indicando  $62.97 \pm 3.74\%$  para el tiempo 48; así como valores disminuidos en niveles de lignina  $4.25 \pm 0.5\%$ ; de carbohidratos no estructurales ( $11.44 \pm 2.43\%$ ) y de la relación carbohidratos no estructurales:proteína degradada en rumen ( $0.84 \pm 0.16\%$ ). Los niveles de nitratos con un promedio de 2977 ppm  $\pm 2061$ , presentaron diferencias significativas para las épocas y veredas, para la época seca en las diferentes veredas el contenido promedio fue de 4728 ppm, siendo 3.9 mayor comparativamente con la época de lluvia. Los niveles de nitritos presentaron contenidos promedios de 2.97 ppm de la MS, contenidos muy disminuidos para ser relacionados como tóxicos. **Conclusiones.** Se mostró un desbalance en la relación proteína:energía como limitante en la zona para la producción de leche. Los niveles de nitratos fueron considerados como potencialmente tóxicos.

**Palabras clave:** Kikuyo; gramínea; nutriente; nitrato; nitrito (Fuente: EcuRed)

## INTRODUCTION

The dairy sector plays an essential role in Colombia's economy because it represents 2.3% of national GDP, 24.3% of agricultural Gross Domestic Product (GDP), generates more than 700.000 direct jobs, and has more than 395.000 small farmers. Most of the milk production takes place in the departments of Antioquía, Boyacá, and Cundinamarca (1). Until May 2018, the national production recorded 303.860,115 liters, a volume that corresponds to a daily average of 9.445.978 liters of milk for the first five months of the year, a value that corresponds to the highest volume in the last ten years (2,3), estimated growth of raw milk production for 2017 of 9%, which is equivalent to 600 million liters, reaching a total production of 7.000 million liters in the year.

Given that importance, this sector largely depends on the quality of the materials used as a food for dairy cattle, and any irregularities would bring negative economic consequences for this group and ultimately for the country (3), also registered an increase of 11% in the milk production in May 2018. However, the report presented from the beginning of January 2017, showed that more than 2.000 animals died due to poisoning with nitrates in Boyacá and Cundinamarca (4).

The reason for that was the prolonged periods of summer that caused the soil to lose moisture and concentrate nitrates, so the pastures accumulated considerable amounts of nitrate after the beginning of the rainy season, which ultimately caused the poisoning; other climatic factors such as frost, hailstorms, cloudy days, alter the plant growth and can generate nitrate accumulation (5).

In the Colombian Andean area, Kikuyo grass (*Cenchrus clandestinus* (Hochst. Ex Chiov.) Morrone) is the most widely used grass in milk production systems. Its nutritional characteristics affect both milk's production and quality. The most important limitations are the high content of total protein (TP) ( $20 \pm 3.26\%$  from the dry matter, DM), non-protein nitrogen ( $>90\%$  of the TP'S, soluble fraction), potassium ( $3.69 \pm 0.77\%$  in DM), and fiber in neutral detergent ( $58.1 \pm 3.91\%$  in DM) as well as the low content of sodium ( $0.02 \pm 0.01\%$  in DM) and non-structural carbohydrates ( $13.4 \pm 2.51\%$  in DM). The high nitrate content ( $5250 \pm 3153$  ppm) may cause various reproductive and health disorders in animals. These characteristics put at risk the competitiveness of milk production systems based on this grass (6,7,8).

Nitrates are not always toxic to animals; most pastures contain nitrates, and the microorganisms present in the rumen reduce nitrates to nitrites via the nitrate reductase enzyme and nitrites are reduced to ammonia via the nitrite reductase. However, the toxicity of nitrites depends on the amount and proportion of nitrate consumption, and under certain conditions may limit the conversion rate of nitrites to ammonia, and its accumulation begins (9).

Once nitrite accumulates, the clinical signs of nitrite poisoning in ruminants appear as the formation of methemoglobin due to the oxidation of iron from hemoglobin, which brings difficulty in transporting oxygen in the blood. Clinical signs become evident when methemoglobinemia levels reach 30-40%, and ultimately, death occurs at levels above 80% (9).

Nitrate and nitrite poisoning in ruminants are detected by the sudden appearance of severe signs of dyspnea, brownish mucus and blood staining, terminal seizures, and high mortality. Plants with levels of more to 10.000 ppm of nitrates in the dry matter are considered lethal. Moreover, the contribution of nitrates during water consumption should also be considered (10).

Based on that, the present study aimed to Establish variations of nutritional composition and the contents of nitrates and nitrites in the Kikuyo grass (*Cenchrus clandestinus* (Hochst. Ex Chiov.) Morrone) from four veredas in the municipality of San Miguel de Sema: Peña Blanca, Sirigay, Sabaneca, and Quintoque, during dry and rainy seasons.

## MATERIALS AND METHODS

**Study Location.** The current study was carried out in the municipality of San Miguel de Sema, department of Boyacá, located at 5°31' north latitude and 73°43' west longitude, within an area of approximately 90 km<sup>2</sup> and an average altitude of 2650 meters above sea level, average annual rainfall of 1097 mm and average annual temperature of 14.2°C.

Considering that the grass of widespread use in the area is Kikuyo grass (*Cenchrus clandestinus* (Hochst. Ex Chiov.) Morrone), forage samples were taken in four representative veredas. The veredas were Peña Blanca, Consuelo farm; Sirigay, New Zealand farm; Sabaneca, Media Luna farm, and Quintoque, El Recuerdo farm. The sampling was performed at two different periods, the dry season (42.6 mm of average monthly rainfall), and the rainy season (154.8 mm average monthly rainfall) (11).

**Nutrition Characterization.** The nutritional composition was carried out in the animal nutrition laboratory –Faculty of Agricultural Sciences of the University of Applied and environmental Sciences U.D.C.A.

The variables evaluated were: Dry matter (DM), Total protein (TP), Ethereal extract (EE), Ash (C), Organic matter (OM) (100-C), Cell content (CC) (100- NDF) (12).

Plant fiber was quantified in neutral detergent (NDF) and acid detergent (ADF), the Lignin (LIG) (13), and non-structural carbohydrates (NSC) (14).

In vitro degradability: The degradability of dry matter (DIVDM) and ruminally degraded protein (RDP) was evaluated according to the in vitro method of Tilley and Terry (15).

Nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) evaluations were performed by UV-Vis spectrophotometry (16).

**Statistical analysis.** In the experimental design, the four veredas and two seasons (dry and rainy seasons) were considered as treatments, in which 15 variables were evaluated. Each treatment had three replicates, resulting in a total of 24 experimental units. The data was analyzed using the programming language R. The performed analysis of variance to determine the significance of the vereda and season on each variable (two-way ANOVA), and for the multiple comparisons, was selected the Tukey's test (Honest Significance Difference Tukey: HSD Tukey).

The generic model used for the analysis of variance was the 2-way without interaction as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

where

$\mu$  is the global mean,  
 $\alpha_i$  or level  $i$  of the epoch factor,  
 $\beta_j$  or level  $j$  of the location factor, and  
 $\epsilon_{ij}$  the random deviations around the means, which are also assumed to be normally distributed, independent and have mean 0 and variance  $s^2$ .

## RESULTS

Table 1 shows the nutritional differences of Kikuyo grass in the four representative veredas of San Miguel de Sema.

**Table 1.** Chemical composition of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four veredas of the San Miguel de Sema - Boyacá Municipality.

NUTRIENTS (%)	SEASON	VEREDA			
		PEÑA BLANCA	SIRIGAY	SABANECA	QUINTOQUE
DM	Dry	19.38 ± 1*Ab	20.43±1*Ab	25.47±1*Aa	17.78±1*Ac
	Rain	18.34±1*Aa	18.86±1*Aa	19.00±1*Ba	18.95±1*Aa
TP	Dry	26.34±0.28*Ab	26.25±0.03Ab	25.62±0.23*Ac	28.68±0.12*Aa
	Rain	22.68±0.13*Ba	21.36±0.13*Bb	17.37±0.35*Bd	19.57±0.25*Bc
EE	Dry	1.73±0.06*Bc	3.37±0.03*Aa	3.03±0*Ab	3.39±0.03*Aa
	Rain	2.32±0.05*Ab	2.61±0.05*Ba	2.06±0*Bc	2.19±0.08*Bb
C	Dry	7.69±0.02*Bc	10.79±0.1*Aa	7.34±0.02*Bd	10.51±0.01*Bb
	Rain	9.14±0.01*Ad	10.24±0.03*Bc	11.17±0.09*Ab	12.77±0.08*Aa
OM	Dry	92.31±0.02*Ab	89.21±0.1*Bd	92.66±0.02*Aa	89.49±0.01*Ac
	Rain	90.86±0.01*Ba	89.76±0.03*Ab	88.83±0.09*Bc	87.23±0.08*Bd

DM = Dry matter; TP = Total protein; EE = Ethereal Extract; C = Ash; OM = Organic matter. \* =  $\bar{x} \pm s$  (Standard deviation). Comparison of means via Tukey (HSD Tukey). Capital letters indicate significant differences between epochs; Lowercase letters indicate significant differences between locations.

The different pastures studied presented, in general, an average TP level of 23.48±3.71%, with significant differences between the seasons ( $p<0.05$ ). During the dry season, TP values increased by 6.48% when compared to the rainy season (20.24%).

For this forage, the contents of EE are diminished and in the case of the pastures studied the EE showed an average of 2.59±0.59%.

The analysis of the total mineral (C) state of the forages represented by the ash levels presented an average of 9.95±1.74% and differed ( $p<0.05$ )

according to the seasons. During the rainy season, recorded the highest average content (10.83%).

The OM also plays a crucial role in an efficient ruminal degradability. The results showed an average of 90.05±1.74%.

The table 2 shows, the characteristics of the cell wall, represented by the levels of NDF, and the lignocellulose fraction represented by the contents of the ADF, the contents of CC and LIG, differed significantly ( $p<0.05$ ) between veredas and seasons.

**Table 2.** Content of Neutral Detergent Fiber, Acid Detergent Fiber, Cell Content and Lignin (% of DM) of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four paths of the San Miguel de Sema - Boyacá Municipality.

Fractions (%)	Season	Vereda			
		Peña Blanca	Sirigay	Sabaneca	Quintoque
NDF	Dry	61.58±0.64*Aa	58.71±0.31*Bb	59.66±0.78*Bb	55.68±0.14*Bc
	Rainy	59.91±0.47*Bc	62.50±0.22*Ab	66.22±1.01*Aa	62.63±0.25*Ab
ADF	Dry	25.98±0.33*Aa	25.28±0.32*Bab	23.99±0.48*Bc	24.75±0.35*Bb
	Rainy	24.73±0.18*Bd	26.37±0.43*Ac	28.75±0.38*Ab	30.38±0.54*Aa
CC	Dry	38.42±0.64*Bc	41.29±0.31*Ab	40.34±0.78*Ab	44.32±0.14*Aa
	Rainy	40.09±0.47*Aa	37.50±0.22*Bb	33.78±1.01*Bc	37.37±0.25*Bb
LIG	Dry	3.98±0.23*Aa	3.80±0.92*Aa	4.57±0.22*Ba	4.46±0.24*Aa
	Rainy	3.74±0.33*Ac	3.83±0.13*Ab	5.22±0.05*Aa	4.42±0.27*Ab

NDF = neutral detergent fiber; ADF = Acid detergent fiber; CC = Cell content; LIG = Lignin. \* =  $\bar{x} \pm s$  (Standard deviation). Comparison of means via Tukey (HSD Tukey). Capital letters indicate significant differences between seasons; Lowercase letters indicate significant differences between locations.

The NDF showed an average of  $60.86 \pm 3.03\%$ , with a reduction of 3.92% during the dry season.

Regarding the ADF, was found an average value of  $26.28 \pm 2.13\%$ . ADF also showed a reduction of 2.5% during the dry season, which differed significantly ( $p < 0.05$ ) from the rainy season.

The CC indicated by the soluble fraction in neutral detergent solution provides information on the highly digestible nutrients the analysis. The analysis showed an average of  $39.14 \pm 3.03\%$ ,

in addition, the results suggest that the dry season increases the CC values for three of the four samples analyzed.

Analysis of lignin, an indigestible element by animal species that interferes in the absorption of nutrients, presented an average of  $4.25 \pm 0.5$  in the forages supplied to animals in the paths and seasons of the year.

Table 3. shows the percentages of the in vitro degradability (DIVDM) for the Kikuyo.

**Table 3.** In-vitro degradability of dry matter (DIVDM) of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov,) Morrone), collected in four veredas of the San Miguel de Sema Boyacá Municipality.

In-Vitro Degradability (%)	Season	Vereda			
		Peña Blanca	Sirigay	Sabaneca	Quintoque
Solubility Rate	Dry	$37.00 \pm 0.78^{*Bc}$	$38.28 \pm 1.46^{*Ab}$	$42.91 \pm 1.32^{*Aa}$	$40.31 \pm 0.17^{*Aa}$
	Rainy	$43.06 \pm 0.65^{*Aa}$	$37.80 \pm 1.21^{*Ab}$	$36.42 \pm 0.59^{*Bb}$	$36.42 \pm 1.08^{*Bb}$
Time 24 H	Dry	$53.42 \pm 0.74^{*Bb}$	$63.29 \pm 0.53^{*Aa}$	$49.24 \pm 2.65^{*Ab}$	$51.54 \pm 1.89^{*Bb}$
	Rainy	$57.17 \pm 0.68^{*Aa}$	$52.65 \pm 2.04^{*Bb}$	$53.56 \pm 0.72^{*Ab}$	$57.03 \pm 1.12^{*Aa}$
Time 48 H	Dry	$62.86 \pm 0.87^{*Ba}$	$63.71 \pm 0.78^{*Aa}$	$59.79 \pm 1.82^{*Ab}$	$61.25 \pm 0.54^{*Aa}$
	Rainy	$71.69 \pm 1.32^{*Aa}$	$62.45 \pm 1.69^{*Ab}$	$59.87 \pm 0.46^{*Ab}$	$62.13 \pm 1.02^{*Ab}$

H = Hour; \* =  $\bar{x} \pm s$  (Standard deviation). Comparison of means via Tukey (HSD Tukey). Capital letters indicate significant differences between seasons; Lowercase letters indicate significant differences between locations.

The results showed an increase in the usability of the DM following the time of permanence in the rumen reticulum. First, the solubility rate (zero time) indicated that the rapidly soluble fraction of the DM averaged in  $39.03 \pm 2.75\%$ .

The literature indicates that good quality forage has a degradability equal to or greater than 40% after 24 hours (17). In the case of the materials evaluated in the different veredas, was report an average of  $54.74 \pm 4.33\%$ .

Likewise, evaluating the materials after 48 hours of fermentation, revealed an average DIVMS of  $62.97 \pm 3.74\%$ .

The NSC present in Kikuyo's forages are the readily available source of energy for efficient ruminal microbial fermentation (8). The analysis (Table 4) showed an average of  $10.44 \pm 2.44\%$  of NSC, with a significant difference ( $p < 0.05$ ) between seasons and veredas studied.

**Table 4.** List of non-structural carbohydrates and protein degraded in the rumen (NSC:RDP) in samples of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov,) Morrone), collected in four veredas of the Municipality of San Miguel de Sema - Boyacá.

Parameter (%)	Season	Vereda			
		Peña Blanca	Sirigay	Sabaneca	Quintoque
Nsc	Dry	$12.01 \pm 0.24^{*Bb}$	$10.84 \pm 0.47^{*Ac}$	$14.47 \pm 0.61^{*Aa}$	$11.29 \pm 0.20^{*Ab}$
	Rainy	$15.09 \pm 0.36^{*Aa}$	$11.31 \pm 0.36^{*Ab}$	$8.28 \pm 1.21^{*Bc}$	$8.21 \pm 0.50^{*Bc}$
Rdp	Dry	$15.03 \pm 0.43^{*Ab}$	$14.89 \pm 0.26^{*Ab}$	$15.24 \pm 0.20^{*Ab}$	$16.38 \pm 0.30^{*Aa}$
	Rainy	$13.80 \pm 0.27^{*Ba}$	$11.49 \pm 0.12^{*Bc}$	$10.13 \pm 0.49^{*Bd}$	$12.55 \pm 0.26^{*Bb}$
Nsc:rdp	Dry	$0.80 \pm 0.01^{*Bb}$	$0.73 \pm 0.02^{*Bb}$	$0.95 \pm 0.05^{*Aa}$	$0.69 \pm 0.02^{*Ac}$
	Rainy	$1.09 \pm 0.01^{*Aa}$	$0.98 \pm 0.03^{*Aa}$	$0.82 \pm 0.16^{*Ab}$	$0.65 \pm 0.05^{*Ac}$

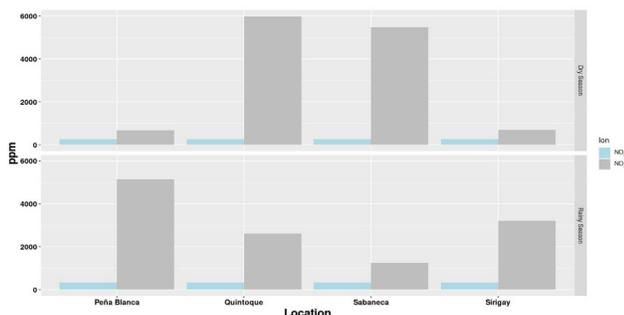
NSC = Non-structural carbohydrates (NSC =  $100 - (TP + EE + NDF + C) + PCIDN$ ; NRC 2001); RDP = ruminally degraded protein; NSC:RDP = Non-structural carbohydrate ratio:ruminally degraded protein. \* =  $\bar{x} \pm s$  (Standard deviation). Comparison of means via Tukey (HSD Tukey). Capital letters indicate significant differences between seasons; Lowercase letters indicate significant differences between locations ( $p < 0.05$ ).

The effect of seasonality was heterogeneous as indicated by the results in Peña Blanca and Sirigay with an average 13.2% NSC in the rainy season, whereas the highest concentration of carbohydrates non structural, in the locations of Sabaneca and Quintoque occurred during the dry season (12.88%), so a direct correlation between seasonality and the site cannot be established, probably due to other variables or correlations not studied in this work.

The ruminally degraded protein (RDP) is related to the fraction of soluble nitrogen usable by microorganisms to produce microbial protein, which in the case of forages such as Kikuyo and Raygrass is highly degradable (8,18,19). The current study showed an RDP average of  $13.69 \pm 2.05\%$ .

Another indication of greater efficiency and optimal microbial growth is the NSC:RDP in a ratio of 3: 2 and 3.5 (8,20,21). In the study, was found this relationship with an average value of 0.84, which can become smaller during the dry season (0.79), especially when compared to the rainy season (0.89).

Figure 1 shows the nitrates ( $\text{NO}_3^-$ ) and nitrites ( $\text{NO}_2^-$ ) contents found in the DM of the Kikuyo grass for the different seasons and locations.



**Figure 1.** Nitratos ( $\text{NO}_3^-$ ) and nitritos ( $\text{NO}_2^-$ ) levels of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four paths of the San Miguel de Sema - Boyacá Municipality. Dry season (ES) and Rainy season (ELL).

The  $\text{NO}_3^-$  values with an average of 2977 ppm  $\pm$  2061 showed a significant difference ( $p < 0.05$ ) between seasons, where the dry season (4728 ppm) showed a concentration 3.9 times higher than during the rainy season. This difference directly related to the TP contents, which at the same time showed values 6.8% higher, indicating

higher contents of soluble nitrogen in the rumen possibly in the form of  $\text{NO}_3^-$ .

The  $\text{NO}_2^-$  contents presented in the dry season (2.55 ppm) showed no significant difference when compared against the values during the rainy season (3.38 ppm).

## DISCUSSION

**Total Protein.** Once again, the contents found in the current study agree with the previous reports for Kikuyo's TP, maximum of 25.12% during the dry season, and an average of 19.24% for the rainy season in the area.

This increase in TP to values above 25% of the DM may occur due to grasslands fertilization with NNP, or due to grazing in short vegetative states (7,21,22). In general, the pastures showed TP levels higher than the 20.3% recommended for Holstein cows, which produce 40-liter of milk with 3.5% fat and 3.0% protein (22).

**Ethereal Extract.** The results obtained in the 4 veredas show values between 0.56-5.81 of the DM (8), which do not represent a toxic level for ruminal bacteria, which occurs when the values are higher than 14%.

**Total Mineral.** The ash content of the kikuyo grass ranges between 11.1 and 24.6% of the DM, and these total minerals are important due to the relationship they present with the energetic content, because their increase reduces the amount of available energy (23). In this work, the ashes presented a value like previous analysis of Kikuyo grasses from that municipality during both dry and rainy seasons (minimum 8.21% and maximum 11.64%). The veredas differ statistically from each other, where was observed that Sabaneca ( $11.17 \pm 0.09\%$ ) and Quintoque ( $12.77 \pm 0.08\%$ ) showed the highest value during the rainy season. Therefore, the values obtained are within the percentages already evaluated by other authors (24).

**Organic Matter.** Once again, the values remained within the ranges indicated for Kikuyo in the localities of the department of Antioquia (25), which vary between 86.1 and 91.35%, with smaller differences between seasons and veredas, but with a negative linear relationship with ash; indicating that the increase in OM content decreases the ash levels.

Content of Neutral Detergent Fiber, Acid Detergent Fiber, Cell Content and Lignin (% of DM) of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four paths of the San Miguel de Sema - Boyacá Municipality. (Table 2).

**Neutral Detergent Fiber.** The Peña Blanca vereda showed the smallest seasonality effect (1.67%), whereas Quintoque presented the highest difference between seasons (6.95%). The values obtained from NDF in the different sites and seasons remained within the values reported as maximum, 63.52% for rainy seasons and 55.93% for drought (20).

**Acid Detergent Fiber.** Sabaneca villages with  $28.75 \pm 0.38\%$  and Quintoque with  $30.38 \pm 0.54\%$ , presented the highest ADF-contents in the rainy season, likely indicating a decrease in the nutritional quality of the forages offered to the Animals at that period. The veredas significantly influenced ( $p < 0.05$ ) the ADF fraction, and the differences became bigger during the rainy season. The lowest nutritional quality represented by higher ADF content,  $30.38 \pm 0.54\%$ , was found in the materials of the Quintoque village in the rainy season, and the best quality with a value of  $23.99 \pm 0.48\%$ , is related to the materials of the Sabaneca village in the dry season. The average value of ADF  $26.28 \pm 2.13\%$ , presented by the paths in the two seasons is related to the minimum value indicated for Kikuyo grass in the rainy season (26.29%) in the municipality of San Miguel of Sema (20). The results suggest that the dry season favors the nutritional quality of forages since they present a decrease in ADF values; increasing ADF intake does not affect milk production, but some authors (26) have mentioned an increase in fat concentration and longer chewing and rumination times.

**Cell Content.** Similar results of CC to those reported for the Kikuyo in the municipality of San Miguel de Sema during the rainy season (36.48-52.51%) and within the interval reported for Kikuyo grass from Antioquia (33.10-48.30%) (21). The seasons also played a significant influence on this variable ( $p < 0.05\%$ ), where was found that during the dry season, the values were 3.90% higher than in the rainy season. Besides, the veredas also showed significant differences ( $p < 0.05\%$ ). Quintoque had the highest value,  $44.32 \pm 0.14\%$ , during the dry season, whereas Sabaneca showed the lowest value,  $33.78 \pm 1.01\%$  during the rainy season.

**Lignine.** This result was close to the average reported previously for that area during the dry (3.43%) and rainy (4.06%) season (21). Altogether, the results indicate that in general, the nutritional quality of forages in this area was not affected by the contents of lignin.

In vitro degradability of dry matter (DIVDM) of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four veredas of the San Miguel de Sema Boyacá Municipality. (Table 3).

**Solubility rate.** a value 19.9% higher than the previous report for Kikuyo as a soluble fraction 20% (20). This result suggests that a higher amount of soluble nutrients is quickly available to the animals.

**In vitro degradability at 24 hours.** the average of 54.74% thus demonstrating that Kikuyo grass presents good nutritional qualities that can be efficiently consumed by the animals (24).

**In vitro degradability at 48 hours.** This result allowed us to establish that, depending on the time of fermentation, the grass can generally be appropriately used with an increase of 9.2% degradability compared to the first time point (24 hours). The observed values are within the previous report of Kikuyo's digestibility values, which ranged between 50% and 72% (6,7,8). Therefore, was found that despite the numerical difference between seasons in the different veredas, these differences did not compromise the DIVMS.

Carbohydrates and protein degraded in the rumen (NSC:RDP) in samples of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four veredas of the Municipality of San Miguel de Sema - Boyacá. (Table 4).

**Non-structural Carbohydrates.** The Peña Blanca location had a higher average content of 13.55% for both seasons. The values found in the study corroborate with the information already available in the literature (7,8,26) which suggests a nutritional limitation of Kikuyo grasses because the values of NSC are lower than those recommended for dairy cattle (35-40% of the DM) (22).

**Protein degraded in the rumen.** indicated, therefore, in the rumen, 58.3% of the forage

protein is usable by the animal. This variable also showed changes influenced by seasonality ( $p < 0.05$ ) with a higher amount of degraded protein (15.38%) during the dry season. The different locations also influenced the RDP ( $p < 0.05$ ), where was observed that Quintoque had the higher content of RDP,  $16.38 \pm 0.30\%$  (dry season).

**The NSC:RDP.** ratio also differed between the locations. Despite that, when was compared the data to the available literature, was noticed that this relationship is lower than previous reports for the Kikuyo grasses from Antioquia, which had values of 1.28 with a minimum of 0.89 (21) indicating a greater deficiency between 65 to 95% comparatively with Antioquia.

Therefore, a deficiency in protein and energy balance could limit the use of Kikuyo forages in areas of milk production (21,25,27).

Nitrate and Nitrite levels of Kikuyo grass (*Cenchrus clandestinus* (Hochst ex Chiov.) Morrone), collected in four paths of the San Miguel de Sema - Boyacá Municipality. Dry season (ES) and Rainy season (ELL). Figure 1.

**Nitrate Levels.** the microorganisms present in the rumen did not completely reduce ammonium, forming nitrites that are the cause of poisoning. In the Sirigay village, the grass had the lowest contents, 2781 ppm in the dry season and 639 ppm in the rainy season, considered diminished values for possible poisoning. The other three locations presented an average of 5377 ppm during the dry season, values classified according to the literature as high nitrate content ( $5250.9 \pm 3153.7$  ppm) (10,28) and considered potentially toxic. However, the literature reports as tolerable values of  $\text{NO}_3^-$  a content between 4000 ppm (29) and 9300 ppm as safe for non-pregnant animals. In general, it is important to indicate and especially for the Quintoque vereda, the highest  $\text{NO}_3^-$  contents - in both dry and rainy seasons, 5787 ppm and 2517 ppm, respectively, that the forage should be managed with caution and observation of animal behavior. Likewise, it is also possible to control the nitrogen fertilization or use of crops with a higher vegetative state since the forage maturity has an inverse relationship with nitrate levels.

**Nitrite levels:** The values were very similar in the paths studied except for the Sabaneca location that indicated a value for nitrites  $\text{NO}_2^-$  in the rainy season of 7.92 ppm. Was classified

the amounts of nitrites as very low to potentially cause of poison intoxication in the animal. The values also were very similar to those previously reported for Kikuyo (2.028 ppm) and much lower than those indicated by the literature as a deleterious concentration in the rumen (8,30,31).

Finally, the results of the study allow us to conclude:

The pastures used for feeding milk cattle on the locations within the municipality of San Miguel de Sema (Boyacá) showed a statistically significant difference in nutritional values influenced by seasonality, where the materials offered during the dry season presented better nutritional composition with higher dry matter content (DM), higher total protein level (TP), lower cell wall level, and lower lignin content. The low levels of non-structural carbohydrates and deficiency in protein balance in the two seasons was a limited that presented of Kikuyo grass in this municipality.

The results also evidenced that the materials had higher nitrate contents in the dry season compared to the rainy season, reaching potentially toxic levels. Therefore, it is important to recommend to the farmers to control and review of the urea fertilization for their pastures or offer forage to the animal with a more prolonged vegetative state or maturity time. Therefore, was recommended to monitor the forage's levels of the protein (nitrogen) and carbohydrate, determine the relationship with the established nitrate and nitrite contents, and evaluate the degraded and non-degraded amounts of protein and cell wall before indicating the potential use of these nutrients by the animal.

### Conflict of interests

The authors declare that they have no competing interests.

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