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RESEARCH NOTE

Effect of genus and growth stage on the chemical and mineral composition of tropical grasses used to feed dairy cows

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Abstract

G.L. Arzate-Vázquez, F.A. Castrejón-Pineda, R. Rosiles-Martínez, S. Carrillo-Pita, S. Angeles-Campos, and E. Vargas-Bello-Pérez. 2016. Effect of genus and growth stage on the chemical and mineral composition of tropical grasses used to feed dairy cows. Cien. Inv. Agr. 43(3): 476-485. In tropical production systems, dairy cows are rarely supplemented with minerals, which often lead to mineral imbalances. Grasses grown together in the same soil type and sampled at the same growth stage can vary widely in chemical and mineral composition; therefore, the objective of this study was to characterize the chemical and mineral composition of *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. at three different stages of growth in the same soil type. The content of dry matter (DM; g 100 g^{-1}) was higher ($P \leq 0.05$) in *Urochloa* spp. than in *Andropogon* spp. and *Megathyrsus* spp. Hemicellulose, non-fiber carbohydrates and slowly degraded true protein contents were higher ($P \leq 0.05$) in *Andropogon* spp. than in *Urochloa* spp. and *Megathyrsus* spp. Ether extract, crude protein, rapidly degraded true protein and unavailable protein contents were not affected by genus, whereas ether extract, ash, neutral detergent fiber, non-fiber carbohydrates, rapidly degraded true protein and unavailable protein contents were not affected by growth stage. Compared with *Urochloa* spp. and *Andropogon* spp., *Megathyrsus* spp. had higher ($P \leq 0.05$) contents of Na and Mg, intermediate ($P \leq 0.05$) contents of Mn and lower ($P \leq 0.05$) contents of Zn. P, Na, and K contents were affected by growth stage. Compared with *Megathyrsus* spp. and *Andropogon* spp., *Urochloa* spp. was higher ($P \leq 0.05$) in P and Na contents. Results from this study may be useful in predicting the nutrient and mineral supply from forages in dairy production systems in tropical areas.

Key words: Cows, forage, nutritive value, ruminants, trace minerals.

Introduction

An adequate mineral supply guarantees proper function (structural, physiological, catalytic, and regulatory) in animals (Suttle, 2010). Grazing cattle must absorb 30 micronutrients, 7 macro-minerals, 9 trace minerals, 10 water-soluble vitamins and 4 fat-soluble vitamins to remain healthy and productive (Suttle, 2016).

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Tropical grasses are important components of ruminant diets in Mexico (Avilés-Nieto *et al.*, 2013); however, in tropical production systems, animals are rarely supplemented with minerals, and this often leads to mineral imbalance. It has been shown that mineral imbalance affects the health status of dairy cattle; for example, it has been related to the pathogenesis of lameness (Zhao *et al.*, 2015). Additionally, dairy cows consuming inadequate amounts of essential nutrients can suffer different health problems and often have impaired milk production and reproductive efficiency (NRC, 2001).

Grasses mature in response to internal (e.g., genetic) and external (e.g., soil and climate) factors, which can also provoke changes in mineral composition (Suttle, 2010). For example, P concentrations in forage plants decline with advancing maturity, whereas the decline is lower in legumes than in grasses (Coates *et al.*, 1990). Concentrations of many minerals (e.g., Co, Cu, Fe, K, and Mg) also decline, but rarely to the same extent as P, which reflects an increase in the proportion of stem to leaf and old to new leaves ratios (with stems and old leaves having lower mineral concentrations than young leaves) (Minson, 1990). Grasses grown together on the same soil type and sampled at the same growth stage can vary widely in mineral composition (Suttle, 2010).

Since growth stage can affect mineral concentrations in grasses, as well as the lack of information regarding the mineral profile of tropical grasses in Mexico, the objective of this study was to characterize the chemical composition and mineral profile of *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. at three different stages of growth in the same soil type. Data from this study will provide information that could guide farmers in the choice of forage resources for improved dairy performance.

Materials and methods

Cultivation conditions of grasses

This study was conducted at the Centro de Estudios Profesionales del Colegio Superior Agropecuario (CSAEGRO) in Guerrero, Mexico (18°15′52′′N, 99°38′52′′W). The climate is type AWo, which is the driest of the hot subhumid group, with a summer rainy season, an annual mean temperature of 25 °C, and a total annual rainfall of up to 1135 mm.

Experimental plots were 40.5 m wide and 6 m long, and the row spacing was 75 cm (54 rows per plot); the plots had been sown with *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. since 2009. Soil characteristics were as follows: colluvial and *in situ* soil, shallow (0 - 25 cm), sandy clay texture, moderate medium sub angular block structure, dark brown color, intermediate drainage, furrow erosion, and a pH of 6.6. Soil mineral contents of macro-minerals (g 100 g^{-1}) were 4.3 for Ca, 0.0008 for P, 0.03 for Na, 0.12 for K and 0.42 for Mg; the contents of microminerals (mg kg^{-1}) were 3904 for Fe, 4 for Cu, 235 for Mn and 101 for Zn. At the onset of the study, grasses from all plots were cut to homogenize growth. Forage samples were obtained at 4, 6 and 8 weeks of re-growth.

Chemical composition

Forage samples (leaves and stems) were clipped to a height of 15 cm and stored in paper bags at room temperature. Forage samples of 250 g were oven-dried at 60 °C for 24 h, ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and then analyzed for dry matter (DM) (967.03), ash (923.03) ether extract (EE; 920.29), and crude protein (CP: N × 6.25; 981.10) using standard procedures (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined (Van

Soest *et al*., 1991). Hemicellulose was calculated as NDF-ADF, and non-fiber carbohydrates (NSC) were calculated by the following formula: NSC = 100 – (CP + EE + NDF + ash) (DM basis). The CP from grasses was partitioned into five fractions (A, B1, B2, B3, and C) according to the Cornell Net Carbohydrate and Protein System. Another sample (0.5 g in duplicate) of forage material was incinerated in a muffle furnace at 500 °C for 16 h and stored at room temperature for mineral analysis.

Mineral profile analysis

The nutrient and mineral concentrations of forages were determined by wet ashing, using a digestion method. Forage samples of approximately 0.5 g were weighed into Teflon-lined digestion vessels to which 10 ml of HCL was added and were allowed to digest for 25 min before being heated at 250 °C for 20 min. After cooling, the samples were filtered through an ash-free filter paper and diluted in ion-exchanged distilled water to a final volume of 50 ml. The contents of Ca, Mg, Cu, Fe, Mn, and Zn were detected by atomic absorption spectroscopy, whereas the contents of Na and K were analyzed by atomic emission spectrometry. Both analyses were performed using a 3110 Perkin-Elmer Atomic Absorption Spectrometer (The Perkin-Elmer Corporation, Norwalk, CT, USA) with standard conditions for each of the elements as described by the instrument manufacturer. P was measured by the molybdate-vanadate method with UV spectrophotometry (965.17-1966, AOAC, 1995).

Statistical analysis

Statistical analysis was performed using the SAS Statistical Package (SAS Institute Inc., Cary, NC). The statistical design was a split-plot in time analysis, where the week of growth was the fixed effect. Data on the chemical and mineral compositions of *Urochloa* spp., *Megathyrsus* spp. and *Andropogon* spp. at 4, 6 and 8 weeks of growth were analyzed as repeated measurements using the General Lineal Models procedure of SAS. When significant treatment effects were detected, the means were separated using Tukey's test. Significance was declared at $P \leq 0.05$.

Results

Chemical composition

The chemical composition of grasses varied according to genus and weeks of growth (Table 1). The contents of DM (g 100 g^{-1}) were higher ($P \leq 0.05$) in *Urochloa* spp., than those found in *Andropogon* spp. and *Megathyrsus* spp. Hemicellulose and non-fiber carbohydrates contents were higher ($P \leq 0.05$) in *Andropogon* spp. than those observed in *Urochloa* spp. and *Megathyrsus* spp. The slowly degraded true protein contents were higher in *Megathyrsus* spp. than in *Urochloa* spp. and *Andropogon* spp.

Ether extract, crude protein, rapidly degraded true protein and unavailable protein contents were not affected by genus; ether extract, ash, NDF, non-fiber carbohydrates, rapidly degraded true protein and unavailable protein contents were not affected by growth stage. Dry matter, ADF and hemicellulose contents increased ($P \leq 0.05$) from week 4 to week 6. Crude protein contents decreased ($P \leq 0.05$) from week 4 to week 8. Slowly degraded true protein increased from week 4 to week 6 (Table 1).

Mineral profile

Compared with *Urochloa* spp. and *Andropogon* spp., *Megathyrsus* spp. had higher ($P \leq 0.05$) contents of P, Na, and Mg, intermediate ($P \leq 0.05$) contents of Mn and lower ($P \leq 0.05$) contents of Zn (Table 2). Due to their low contents, Cu was not detected in either of the three grass genera.

Contents of P, Na, and K (g 100 g^{-1}), were affected by growth stage (Figure 1). Na contents increased from week 4 to week 6 in *Megathyrsus* spp. and *Andropogon* spp. and then decreased in week 8. Compared with *Megathyrsus* spp. and *Andropogon* spp., *Urochloa* spp. had higher ($P \leq 0.05$) Na contents.

Discussion

Chemical composition

Forage is the most abundant and economical source of feed for cattle in tropical and subtropical grazing systems in Mexico (Juárez *et al*., 2005). In

Table 1. Chemical composition (g 100 g^{-1} DM) of *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. at 4, 6 and 8 weeks of growth.

Chemical analysis	Genus			SED[3]	P-value
	Andropogon	*Urochloa*	*Megathyrsus*		
Dry matter	27.24 b	29.49 a	25.65 c	1.06	<0.001
Ether extract	5.42	5.86	5.53	0.271	0.204
Ash	7.63b	10.90 a	11.39 a	0.302	<0.001
Neutral detergent fiber	72.39 a	70.15 b	70.87 b	0.866	0.046
Acid detergent fiber	42.63 b	42.38 b	44.04 a	0.742	0.042
Hemicellulose[1]	29.76 a	27.77 b	26.84 b	0.700	<0.001
Non-fiber carbohydrates[2]	12.86 a	10.64 b	9.45 b	0.834	0.001
Crude protein	5.58	5.47	6.05	0.316	0.120
Protein fractions					
A: non-protein N	0.58 c	1.06 b	1.15 a	0.146	0.002
B1: rapidly degraded true protein	0.51	0.49	0.52	0.029	0.641
B2: slowly degraded true protein	0.70 c	0.92 b	1.10 a	0.127	0.015
B3: by pass true protein	1.87 a	1.03 c	1.30 a	0.168	<0.001
C: unavailable protein	2.02	1.82	1.99	0.109	0.752
	Week				
	4 wk	6 wk	8 wk	SED	P-value
Dry matter	24.89 b	29.48 a	28.76 a	1.02	<0.001
Ether extract	5.44	5.55	5.97	0.261	0.108
Ash	10.47	10.23	10.30	0.291	0.708
Neutral detergent fiber	70.68	70.49	71.50	0.834	0.441
Acid detergent fiber	41.60 b	43.05 a	44.31 a	0.715	0.001
Hemicellulose	29.08 a	27.44 b	27.19 b	0.674	0.012
Non-fiber carbohydrates	10.59	11.28	10.34	0.804	0.485
Crude protein	6.56 a	5.61 b	4.88 c	0.305	<0.001
Protein fractions					
A: non-protein N	1.04 a	1.22 a	0.63 b	0.141	<0.001
B1: rapidly degraded true protein	0.52	0.51	0.47	0.028	0.175
B2: slowly degraded true protein	1.18 a	0.78 b	0.83 b	0.123	0.003
B3: by pass true protein	1.78 a	1.16 b	1.05 b	0.162	<0.001
C: unavailable protein	1.96	2.01	1.95	0.105	0.843

Means in the same row with different superscripts differ ($P \leq 0.05$); [1]Hemicellulose = NDF-ADF; [2]Non-fiber carbohydrates = 100 – (CP + EE + NDF + ash); [3]SED = Standard error of the difference.

Table 2. Mineral contents of *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. at 4, 6 and 8 weeks of growth.

	Genus				
Minerals	*Urochloa*	*Megathyrsus*	*Andropogon*	SED[1]	P-value
Ca, g 100 g^{-1} DM	0.37	0.32	0.43	0.03	0.138
P, g 100 g^{-1} DM	0.08	0.13	0.10	0.01	0.059
Na, g 100 g^{-1} DM	0.04 b	0.10 a	0.04 b	0.01	0.013
K, g 100 g^{-1} DM	1.9	2.24	1.55	0.17	0.125
Mg, g 100 g^{-1} DM	0.22 b	0.26 a	0.20 c	0.01	0.041
Fe, mg kg^{-1} DM	232	199	207	19.9	0.545
Mn, mg kg^{-1} DM	42 a	25 b	20 c	2.12	0.010
Zn, mg kg^{-1} DM	16 a	11 b	15 a	0.42	0.006
	Week				
	4 wk	6 wk	8 wk	SED	P-value
Ca, g 100 g^{-1} DM	0.37	0.37	0.37	0.02	0.937
P, g 100 g^{-1} DM	0.13 a	0.10 b	0.09 c	0.01	0.028
Na, g 100 g^{-1} DM	0.06 b	0.08 a	0.05 c	0.01	0.031
K, g 100 g^{-1} DM	2.2 a	1.8 b	1.69 c	0.12	0.003
Mg, g 100 g^{-1} DM	0.23	0.22	0.21	0.01	0.388
Fe, mg kg^{-1} DM	246	227	164	29.1	0.201
Mn, mg kg^{-1} DM	30	29	28	2.21	0.710
Zn, mg kg^{-1} DM	14	14	14	0.75	0.947

Means in the same row with different superscripts differ (P≤0.05), [1]SED = Standard error of the difference.

terms of nutritional value, tropical grasses can be affected by their growth stage; therefore, maturity is considered the most important factor affecting the chemical composition and nutritional quality of forages (Ribeiro *et al*., 2014). The nutritional value of forages linearly declines with increasing physiological maturity (Santos *et al*., 2014). As observed in this study, the increase in DM content with increasing maturity was expected. The DM content of forages is important in calculating feed intake and improving milk production, but it is also important when farmers are interested in conserving this resource (i.e., silages). A DM content of 25% is recommended for minimizing effluent loss in silos and the preservation of nutrients in silages (Santos *et al*., 2014). In this study, grasses showed similar DM contents, showing their suitability for silage conservation.

Ruminants feeding on tropical forages obtain their energy mainly from the rumen microbial fermentation of structurally complex carbohydrates within plant cell walls (Van Soest *et al*., 1991). According to the NDF contents of the grasses evaluated in this study, forage intake would likely be reduced as NDF (approximately 71 g kg^{-1} DM) is negatively correlated with voluntary intake. As hemicellulose constitutes a portion of NDF, it was expected that NDF would also decrease from week 4 to week 8, but this relationship was not observed in the present work. Juárez *et al*. (2005) noted that the major changes in the cell wall carbohydrates occurred before week 4 of re-growth.

In general, chemical compositions were different between genera and growth stage. NDF, ADF, and hemicellulose contents were similar to those previously reported for *Adropogon gayanus* (Olafadehan, 2013). Similarly, the NDF, ADF and CP contents found for *Andropogon* spp. were similar to those reported by Ribeiro *et al*. (2014) in *Adropogon gayanus* at 7 weeks of re-growth. The contents of DM, CP, NDF and ADF were

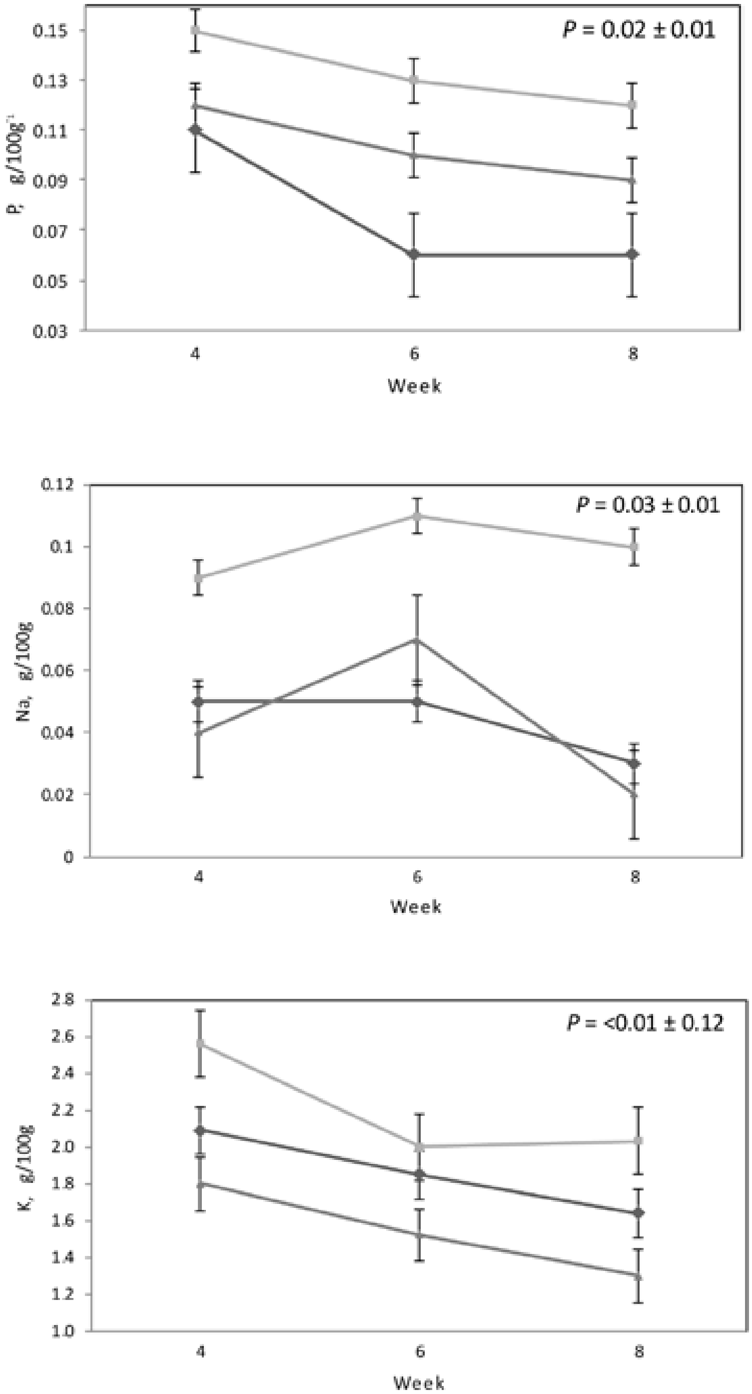

Figure 1. The contents of P, Na, and K in (◆) *Urochloa* spp., (■) *Megathyrsus* spp., and (▲) *Andropogon* spp. at different growth stages.

in agreement with those reported for *Panicum maximum* at 55 days of re-growth. *Megathyrsus* spp. contents of CP were similar to those reported by Aganga and Tshwenyane (2004), who found that the CP from this grass ranges from 5.5 to 20.5% of DM. In *Urochloa* spp., the contents of DM, NDF and EE were similar to those reported previously (de Oliveira *et al*., 2015) for *Brachiaria brizantha* harvested at 61 d of growth.

Mineral profile

Despite the expected high variability within species, this was not evaluated in this study; instead, we studied only mineral profile differences between genera in colluvial and *in situ* soil. However, variability among species in terms of mineral bioavailability must be considered, as some minerals vary considerably (Kambashi *et al*., 2014).

Under the conditions of this study, Cu (≤0.002 absorbance units) was below the absorbance limit of detection of our equipment (0.003 absorbance units). Diets with as little as 0.4% S (i.e., 2 times the requirement) can have detrimental effects on cattle, ranging from secondary Cu and Se deficiencies to polioencephalomalacia (NRC, 2005).

Consumption of Ca is important during lactation because the amount of Ca per kg of milk produced varies slightly with the amount of protein in the milk, which also varies with breed; for example, the absorbed Ca required per kg of milk produced is 1.22 g in Holstein cows and 1.45 g in Jersey cows (NRC, 2001). In this study, grasses had between 0.32 to 0.43% Ca content, which may not be enough for dairy cows in lactation, and supplementation would be highly recommended. For grazing cattle not fed with supplements, it is necessary to rely on both indirect and direct methods for providing minerals. The self-feeding of 'free-choice' mineral supplements is widely used for grazing cattle. Grazing animals pose a problem, and the provision of minerals and other supplements on a free-choice or free access basis is often the only practical method (Chládek and Zapletal, 2007).

Na was affected by growth stage, which is important to consider when designing strategies for rotational grazing systems because if cows are lactating, they will require mineral supplementation to avoid mineral imbalance and health problems. The Na, K, and P contents in *Urochloa* spp. and *Megathyrsus* spp. were similar to those reported by Kambashi *et al*. (2014) in tropical forages in the Democratic Republic of the Congo. The Na concentration in milk is 0.63 g kg^{-1}, and for the NRC (2001), this is considered the requirement for absorbed Na for milk yield. During lactation, K in milk is fairly constant, even under different intakes, and the NRC (2001) requirement for absorbed K is 1.5 kg of milk produced. Based on the NRC (2001) recommendations for dairy cattle, a concentration within the range of 0.32 to 0.42% of P for the entire lactation period will be sufficient; however, this will depend on the milk production potential of the cows and the feed supplied.

In this study, the Cu content was not high enough to be detected. The Mn content was the highest in *Andropogon* spp., whereas the Zn content was higher in *Urochloa* spp. and *Andropogon* spp. These minerals are involved in numerous biological pathways during horn production, while Mg and P can improve the hardness and density of the hoof by speeding up the rate of formation and regeneration (Zhao *et al*., 2015).

Many feeds, especially forages, have extremely high concentrations of Fe, but because of the low bioavailability of that Fe, it is not an animal toxicity issue (NRC, 2005). According to the NRC (2001), a cow producing 25 kg of milk d^{-1} at 205 days of gestation and consuming 20 kg d^{-1} DM requires 24 mg of Fe kg^{-1}. The Cu requirement for dairy cows (NRC, 2001) varies according to days of gestation: 0.5 mg day^{-1} at 100 days of gestation, 1.5 mg day^{-1} if the gestation stage is between 100 and 225 days and 2.0 mg day^{-1} if the gestation stage is over 225 days.

In this study, micro-mineral (trace mineral) contents, such as Mn and Zn, were affected by grass genus, but Fe was not affected. This is important to consider since they are directly associated with reproductive performance and health problems such as mastitis. Trace minerals play an important role in dairy cow immune function, fertility, and growth (Machado *et al*., 2013).

The main conclusions are as follows. The contents of DM and ADF increased from week 4 to week 6 in *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp., whereas CP decreased. Under the conditions of this study, Ca, Mg, Fe, Mn and Zn contents were not affected by growth stage, whereas the Na content was reduced by growth stage in *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp. Data from this study may be useful in calculating or predicting the intake of minerals in dairy production systems in tropical areas.

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Resumen

G.L. Arzate-Vázquez, F.A. Castrejón-Pineda, R. Rosiles-Martínez, S. Carrillo-Pita, S. Angeles-Campos y E. Vargas-Bello-Pérez. 2016. Efecto del género y etapa de crecimiento sobre la composición química y mineral de pastos tropicales utilizados para alimentar vacas lecheras. Cien. Inv. Agr. 43(3): 476-485. En los sistemas de producción tropical, las vacas lecheras rara vez se suplementan con minerales y frecuentemente esto provoca un desequilibrio mineral. Los pastos que crecen en las mismas condiciones de suelo y que son muestreados en la misma etapa de crecimiento pueden variar en su composición química y mineral, por lo tanto el objetivo de este estudio es caracterizar la composición química y mineral de *Urochloa* spp., *Megathyrsus* spp., and *Andropogon* spp., en tres etapas distintas de crecimiento con las mismas condiciones de suelo. El contenido de materia seca (g 100 g^{-1}) fue mayor ($P\leq0.05$) en *Urochloa* spp., que en *Andropogon* spp., y *Megathyrsus* spp. Los contenidos de hemicelulosa, carbohidratos no estructurales y proteína verdadera de degradación lenta, fueron mayores en ($P\leq0.05$) *Andropogon* spp., que en *Urochloa* spp., y *Megathyrsus* spp. Los contenidos de extracto etéreo, proteína cruda, proteína verdadera de rápida degradación no fueron afectados por el género del pasto; mientras que el extracto etéreo, ceniza, fibra detergente neutra, contenido celular, carbohidratos no estructurales, proteína verdadera de rápida degradación y proteína no degradables tampoco fueron afectados por la etapa de crecimiento de los pastos. En comparación con *Urochloa* spp., y *Andropogon* spp., *Megathyrsus* spp., tuvo mayor ($P\leq0.05$) cantidad de P, Na y Mg, valores intermedios ($P\leq0.05$) para Mn y valores menores ($P\leq0.05$) para Zn. P, Na y K fueron afectados por la etapa de crecimiento de los pastos. Comparado con *Megathyrsus* spp., y *Andropogon* spp., *Urochloa* spp., presentaron contenidos más altos ($P\leq0.05$) de P y Na. Los contenidos de K fueron menores ($P\leq0.05$) en *Andropogon* spp., que aquellos observados en *Megathyrsus* spp., y *Urochloa* spp. Los resultados de este estudio podrían ser utilizados para calcular o predecir el consumo de nutrientes y minerales de vacas lecheras en sistemas de pastoreo tropical.

Palabras clave: Forraje, minerales, rumiantes, vacas, valor nutricional.

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