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Nutrient digestibility in horses of tropical grasses found in semi-arid areas of the Brazilian Northeast region assessed using mobile bags

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ABSTRACT - The present study used mobile bags to estimate horse nutrient digestibility of tropical grasses found in semi-arid areas of the Brazilian Northeast region. Five female mixed-breed horses with a mean weight of 400±23 kg were assigned to a 5×5 Latin square design with five periods of seven days and five grasses: Tifton 85 hay (*Cynodon spp.*), sixweeks threeawn (*Aristida adscensionis* Linn.), Alexandergrass (*Brachiaria plantaginea* (Link) Hitchc), capim-de-raiz (*Chloris orthonoton* Doell), and Sabi grass (*Urochloa mosambicensis*). The nutrient content of forages was determined prior to inoculation in horses and after recovery of mobile bags from feces. The digestibility coefficients were determined from the difference between the inoculated and recovered material. The dry matter, organic matter, mineral matter, crude protein, neutral detergent fiber, and acid detergent fiber contents of the grass species were analyzed. Digestibility data were subjected to analysis of variance using the Statistical Analysis System (SAS, version 9.0) software. Higher dry matter digestibility coefficients were observed in Tifton 85 (74.61%), Alexandergrass (74.30%), and capim-de-raiz (68.88%) than in sixweeks threeawn (48.40%) and Sabi grass (52.89%). The highest crude protein digestibility coefficients were found for Alexandergrass (95.70%), Tifton 85 (93.50%), and sixweeks threeawn (93.35%). Sixweeks threeawn had lower apparent mineral matter digestibility than the other grasses. The digestibility coefficients of Alexandergrass and capim-de-raiz indicate that those grasses have potential to be used in equine feed.

Keywords: equine, forage intake, nutritional value

1. Introduction

Some grass species found in the Brazilian Caatinga biome are part of the roughage feed of grazing horses, especially during the rainy season. However, very little is known about the physicochemical characteristics and digestibility of those grasses in horses.

Capim-de-raiz (*Chloris orthonoton* Doell) is an endemic species in the Caatinga biome most often found in the semi-arid agreste and sertão regions of the state of Pernambuco. It is a perennial stoloniferous

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grass with long-sheathed leaves and fasciculate palmate inflorescence formed by numerous terminal spikes. Capim-de-raiz is widely consumed by horses in the Northeast region of Brazil due to its palatability and low availability of forages with similar characteristics.

Another species native to the region is sixweeks threeawn (*Aristida adscensionis* Linn.), which has an annual cycle and features thin, straight stalks. This species has been used as sheep feed during the dry season, which increases the possibility of being used in horse diets (Silva et al., 2000).

Although not native to the Brazilian semi-arid region, Alexandergrass (*Brachiaria plantaginea* (Link) Hitchc) is well adapted to the region and thrives with the seasonal rainfall. As an annual species, it has high potential for forage production during periods of greater rainfall (Lançanova et al., 1988). Its stalks are sturdy, erect, and branched, reaching up to 1 m tall, and it can shoot roots from lower nodes or stalks lying on the ground (Moreira and Bragança, 2011).

Apart from Alexandergrass, one of the most commonly used exotic species in the Brazilian semi-arid region is Sabi grass (*Urochloa mosambicensis*). Originally from southern Africa, it is a perennial species that is adapted to the tropical climate and has good tolerance to drought. The species exhibits varied growth, prefers slightly loamy soils, and requires 500 to 1,000 mm of annual rainfall. Sabi grass has stolons, smooth stems that can reach 1 m in length, and leaves 15 cm long and 1.5 cm wide with hair on both faces, and produces 4 to 12 spikelets.

Although some grass species found in the region have been studied for their agronomic potential, the capacity of horses to digest their nutrients is still unknown. Therefore, in the present study, we used mobile bags to estimate the nutrient digestibility in horses of tropical grasses found in semi-arid areas of the Brazilian Northeast region.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (11/2017).

2.1. Site

The trial was carried out on an experimental farm located in the municipality of Garanhuns, PE, Brazil (8°53'27" S and 36°29'48" W).

2.2. Animals

We used five female mixed-breed horses with a mean body weight of 400±23 kg and a mean age of 8.6 years. Before the experimental period, the animals were dewormed with a broad-spectrum vermifuge primarily composed of ivermectin and pyrantel (Piraverme®) and were spray-treated against ectoparasites. Horses were housed in individual masonry stalls (16 m^2) with sand bedding and had *ad libitum* access to water and a mineral lick. For exercise, the animals were walked for about 20 min every day in a round pen with a sand floor.

Throughout the experimental period, horses were fed exclusively Tifton 85 hay (*Cynodon* spp.) at 2.0% of their live weight per day on a dry matter (DM) basis according to recommendations of NRC (2007) for adult animals in maintenance. The daily roughage was split into two meals provided at 6:00 and 17:00 h.

2.3. Experimental design

The experiment employed a 5×5 Latin square experimental design comprising five periods of seven days and five grasses: Tifton 85 hay (*Cynodon* spp.), sixweeks threeawn, Alexandergrass, capim-de-raiz, and Sabi grass.

Tifton 85 hay was purchased locally, Alexandergrass and capim-de-raiz were collected in the *agreste* region of Pernambuco state (municipality of Garanhuns), sixweeks threeawn was collected in the *sertão* region of the state (municipality of Serra Talhada), and Sabi grass was obtained from the metropolitan area of the state capital, Recife. The grasses were collected early in the rainy season and before their reproductive stage since water availability may have a greater impact than cutting age on forage species in the semi-arid region of Brazil.

2.4. Sample processing

During the pre-experimental phase, the grasses were pre-dried in a forced-air oven (55 °C) for 72 h and then ground in a Wiley mill. The material captured by the 2-mm sieve was used for intubations, whereas the material captured by the 1-mm sieve was used for chemical composition determination.

The laboratory determined the contents of DM (930.15, AOAC, 1995), organic matter (OM), mineral matter (MM) (942.05, AOAC, 1990), crude protein (CP) (954.01, AOAC, 1990), and ether extract (EE) (920.39, AOAC, 1990) in the grasses. The analyses of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed according to the methodology of Senger et al. (2008).

Hemicellulose (HEM), cellulose (CEL), and lignin (LIG) fractions were determined using the equations HEM = NDF − ADF and CEL = ADF − LIG, while lignin content was determined through the solubilization of cellulose with 72% sulfuric acid, based on the methodology proposed by Van Soest et al. (1991).

Total carbohydrate (TC) content was estimated using the equation TC = 100 − (CP + EE + MM) described by Sniffen et al. (1992). Non-fiber carbohydrates (NFC), corresponding to the A + B1 fractions, were estimated using the equation NFC = TC − NDFap, in which NDFap is NDF corrected for ash and protein. The indigestible fiber fraction (C) was estimated using the equation C = NDF × 0.01 × LIG × 2.4, while the digestible fiber fraction (B2) was obtained from the difference between NDFap and C (Sniffen et al., 1992).

2.5. Mobile bag preparation

The mobile bags were made of polyester fabric (Oxfordgold®) with 45 μm porosity and dimensions of 7.5 × 2 × 2 cm and were heat-sealed (Araújo et al., 1996). Bags were later individually identified, washed in distilled water, and dried in a forced-air oven at 55 °C for 24 h. Next, each bag was filled with approximately 510 mg of the sample according to the recommendation by Vanzant et al. (1998), who established a ratio of between 10 and 20 mg of sample per cm^2 of bag (i.e., 17 mg DM per cm^2). Bags were then weighed on a precision scale to determine their empty weight, and their total weights were subtracted from the sample weight.

2.6. Intubations

Bags were introduced into the animals' stomachs using a silicone nasogastric probe with a 15-mm inner diameter. The probe was externally lubricated with liquid petroleum jelly, and bags were carried through the probe to the horses' stomachs with the aid of human blowing. Each horse received 35 bags during each probing for a total of 140 bags per animal per period.

2.7. Experiment duration

The experiment lasted 63 days; the first 28 of which were used to adapt the horses to their confinement and diet, followed by 35 days of the experimental phase, which was split into five periods of seven days. On the first and second days of each period, mobile bag intubations were performed twice a day at intervals of 12 h for a total of four intubations per period. Mobile bags were recovered from feces between the second and fifth days. To allow horses to rest between intubations, thus providing greater welfare, no activities were conducted on the sixth and seventh days of each period.

2.8. Collections

Collections were carried out starting on the second day of each period and lasted for four days, beginning 18 h after the first intubation (Silva et al., 2009). Bags were collected immediately after they were excreted in feces, the recovery time was recorded, and they were then stored in a freezer at −18 °C. The chemical analyses of the residue considered only the bags recovered after 48±12 h of retention so as to approximate the mean retention time of fibrous food in the gastrointestinal tract of horses.

By the end of the experiment, all bags selected were manually washed in running water until clean. Next, the samples were kept in a forced-air oven (55 °C) for 48 h. After drying, bags were weighed to determine DM losses, and a compound sample was obtained from the bags selected from each animal in each period.

Nutrient losses were expressed as a digestibility coefficient (DC) of DM, CP, NDG, ADF, OM, and MM according to equation 1:

$$DC\ (\%) = ((I - F)/I) \times 100 \tag{1}$$

in which I is the amount of nutrient inserted into each bag and F is the amount of nutrient left after bags were recovered from feces (Moore-Colyer et al., 2002).

The CP content in the residue was corrected for microbial impregnations according to the methodology described by Mass et al. (1999), in which the total nitrogen analysis is performed based on the residue of NDF analysis and the microbial protein present in the sample is disregarded.

2.9. Statistical analyses

The data were initially subjected to the Shapiro-Wilk normality test. Only the DM results required logarithmic transformation. Next, the results were subjected to analysis of variance, and the means were compared using Tukey's test at 5% significance. These calculations were made using the GLM procedure of the SAS (Statistical Analysis System, version 9.0) software. Data were analyzed according to the following model:

$$Y_{ijkl} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}, \tag{2}$$

in which Y_{ijkl} is the dependent variable, μ is the overall mean, T_i is the effect of the treatment (fixed effect), P_j is the effect of the period (random effect), A_k is the effect of animal (random effect), and ε_{ijk} is the random error associated with each observation.

3. Results

According to the chemical composition analysis, Tifton 85 hay, which was used as a reference, had the highest DM content (870.8 g kg^{-1} DM) when compared with Alexandergrass (197.6 g kg^{-1} DM), capim-de-raiz (293.3 g kg^{-1} DM), Sabi grass (318.3 g kg^{-1} DM), and sixweeks threeawn (440.6 g kg^{-1} DM) (Table 1).

An overall comparison of the grasses showed that Alexandergrass had good CP content (124.0 g kg^{-1} DM), even higher than that of Tifton 85 hay (85.1 g kg^{-1} DM), which is a grass considered to have high nutritional value for horses. However, Alexandergrass had lower DM content (197.6 g kg^{-1} DM).

Capim-de-raiz stood out with its EE content of 15.2 g kg^{-1} DM, which was higher than the mean content of 5.9 g kg^{-1} DM observed in Tifton 85, Alexandergrass, and sixweeks threeawn. However, capim-de-raiz had the lowest CP percentage among the five species of the study. Notably, its content of indigestible protein in neutral detergent (IPND) was 825.8 g kg^{-1} CP, which is a relatively high value when compared with sixweeks threeawn at only 266.8 g kg^{-1} CP.

The fibrous fraction of capim-de-raiz had lower lignin content (37.1 g kg^{-1} DM) than the same fraction in the other grasses. The same behavior was observed for the C fraction of TC, which, at around 67.5 g kg^{-1} TC, was much lower than in the other species.

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Similar to capim-de-raiz, Sabi grass had high EE content (11.6 g kg^{-1} DM) when compared with Tifton 85, Alexandergrass, and sixweeks threeawn.

Sixweeks threeawn had the second highest CP content (96.8 g kg^{-1} DM) but only 266.8 g kg^{-1} CP of IPND. However, it showed the highest concentrations of C fraction when compared with Alexandergrass, capim-de-raiz, and Sabi grass.

Tifton 85 hay and Alexandergrass had the highest (P<0.05) nutrient digestibility coefficients for all nutrients in comparison with the other forage plants (Table 2).

The DM digestibility value of capim-de-raiz was similar to those of Alexandergrass and Tifton 85 hay and higher (P<0.05) than those of Sabi grass and sixweeks threeawn. Its CP digestibility (88.92%) was found to be lower than that of sixweeks threeawn (93.35%, P<0.05).

Sabi grass had lower DM digestibility than Alexandergrass, capim-de-raiz, and Tifton 85 hay (P<0.05). Its protein digestibility was found to be 85.89%, which was lower than that of Alexandergrass (95.70%), Tifton 85 hay (93.50%), and sixweeks threeawn (93.35%). In addition, the ADF digestibility of Sabi grass was the lowest among all the grasses assessed.

Table 1 - Mean values of chemical composition of the grasses

Composition (g kg^{-1} DM)	Grass				
	Tifton 85	Alexandergrass	Capim-de-raiz	Sabi grass	Sixweeks threeawn
Dry matter (DM)	870.8	197.6	293.3	318.3	440.6
Crude protein (CP)	85.1	124.0	68.2	77.6	96.8
Ether extract	6.0	6.3	15.2	11.6	5.4
Neutral detergent fiber	750.9	631.0	609.2	689.6	752.1
Acid detergent fiber	355.8	286.9	300.1	376.9	380.7
Organic matter	909.1	890.9	887.1	934.6	951.9
Mineral matter	91.0	109.0	112.9	65.3	48.0
Hemicellulose	395.1	344.1	309.1	312.7	371.4
Cellulose	298.9	242.5	263.0	324.5	305.3
Lignin	56.9	44.4	37.1	52.4	75.4
IPND (g kg^{-1} CP)	678.6	343.4	825.8	371.8	266.8
Total carbohydrates (TC)	817.9	756.6	803.7	845.5	849.7
A+B1 (g kg^{-1} TC)	189.1	268.2	336.3	226.6	148.0
B2 (g kg^{-1} TC)	685.4	642.9	596.2	670.8	691.9
C (g kg^{-1} TC)	125.5	88.9	67.5	102.5	160.0

IPND - indigestible protein in neutral detergent; A+B1 - soluble carbohydrates; B2 - potentially digestible fiber; C - indigestible fiber.

Table 2 - Mean values of horse apparent digestibility coefficient of nutrients in tropical grasses found in the Brazilian Northeast semi-arid region estimated by the mobile bags technique

Treatment	Apparent digestibility coefficient (%)					
	DM	CP	NDF	ADF	OM	MM
Tifton 85	74.61a±14.69	93.50a±3.68	70.31a±16.94	65.32a±20.88	73.42a±15.39	86.57a±7.76
Alexandergrass	74.30a±2.90	95.70a±0.36	66.05a±4.05	55.34a±6.36	73.29a±2.76	82.48a±4.50
Capim-de-raiz	68.88a±1.47	88.92b±0.47	63.76a±20.25	49.95ab±2.97	66.95a±1.41	84.04a±3.22
Sabi grass	52.89b±3.26	85.89b±1.94	39.61b±4.94	31.29c±2.46	47.35b±3.07	81.57a±7.36
Sixweeks threeawn	48.40b±2.83	93.35a±0.67	38.22b±3.78	34.19bc±9.02	50.88b±3.66	61.86b±12.77
P-value	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
CV (%)	9.4	2.1	15.4	17.9	10.1	6.9

DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; OM - organic matter; MM - mineral matter.
Means followed by different letters in the columns indicate difference among the grasses according to Tukey's test (P<0.05).

Similar to Sabi grass, sixweeks threeawn had a lower (P<0.05) DM digestibility coefficient (48.40%) than Tifton 85 hay (74.61%), Alexandergrass (74.30%), and capim-de-raiz (68.88%). However, it showed high CP digestibility (93.35%), at the same level as Alexandergrass (95.70%) and Tifton 85 hay (93.50%).

Concerning MM digestibility, only sixweeks threeawn differed from the other treatments, with the lowest coefficient among the five forages tested.

4. Discussion

The superiority of Tifton 85 hay in terms of DM content could be attributed to the haying process, which consists of rapidly dehydrating the forage plant to allow for long-term storage and achieve a product with good nutritional value and no loss of nutrients (Evangelista and Lima, 2013). Furthermore, according to Paz et al. (2000), DM content below 80% may inhibit hay conservation.

Similar to the current findings of protein content in Alexandergrass (124.0 g kg^{-1} DM), Alencar et al. (2010) reported values between 110 and 130 g kg^{-1} DM in six grass species from the Caatinga biome subjected to different irrigation depths over four seasons, which could indicate the potential of Alexandergrass as a good protein source even under dry farming conditions.

However, Alexandergrass exhibited lower DM content compared with the other treatments, which can potentially be explained by its higher leaf:stem ratio, which results in lower concentrations of dry material (Deminicis et al., 2010). Moreover, annual plants, such as Alexandergrass, accumulate less senescent material, unlike perennial species, which live for long periods in the soil. Alexandergrass resprouts from seeds, which enter dormancy and germinate upon adequate levels of water, oxygen, temperature, and luminosity for the species (Castro and Vieira, 2001).

Fiber is a key component in diets for horses. According to Braga et al. (2008), diets with NDF levels below 350.0 g kg^{-1} DM may leave horses prone to laminitis and colic. Therefore, Alexandergrass shows potential for use in horse feed given its content of NDF of 631.0 g kg^{-1} DM.

The EE content of 15.2 g kg^{-1} DM found in capim-de-raiz could be considered useful for equine diets as it represents a major source of energy for horses, thus safely increasing the energy density of the diet and reducing the risk of gastrointestinal disorders often caused by rapidly fermentable carbohydrates (Pastori et al., 2009). The relatively low CP content of capim-de-raiz in comparison with the other species studied can be explained by the different types of soil in which the samples were grown. In addition, CP content is directly affected by plant age (Cedeño et al., 2003) and fertilization, particularly with nitrogen (Bernardi et al., 2018).

Although Tifton 85 hay had relatively higher lignin and indigestible fiber content than the other grasses, it was found to have the highest nutrient digestibility. Since most forage digestion takes place through microbial fermentation and given that the diversity and proportion of microorganisms in the cecum and colon of horses are directly affected by diet (Garber et al., 2020), the higher digestion of Tifton 85 hay may have been because the diet provided to the horses throughout the experiment was made up exclusively of this forage plant.

Alexandergrass had lower indigestible fractions, including lower lignin content, than the other grasses, which may have caused it to be more digestible than Sabi grass and sixweeks threeawn. According to Fukushima and Hatifield (2004), lignin content is correlated with forage digestibility. Likewise, CP digestibility of Alexandergrass was higher due to its lower content of fiber-bound protein compared with capim-de-raiz and Sabi grass.

The lower protein digestibility observed in capim-de-raiz may be related to its high content of fiber-bound protein (i.e., IPND content of 825.8 g kg^{-1} CP), which hinders the digestibility of the nutrient. Prior research suggests that, under semi-arid climate conditions, where temperatures remain high for

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longer, some forage species undergo reactions that may decrease CP availability via the formation of complexes between crude proteins and carbohydrates, such as Maillard reactions (Berchielli et al., 2006).

The DM disappearance rate in sixweeks threeawn was highly correlated with NDF digestibility ($R^2 = 0.89$). Neutral detergent fiber was the nutrient present at the highest level in the DM of sixweeks threeawn, and its low digestibility negatively impacted the DM disappearance rate. However, this grass is a potential source of protein for horses during drought since its CP digestibility was higher than 90%. This result is likely related to the lower content of IPND of sixweeks threeawn (Table 1).

The lignin content of forage plants could be considered the main factor involved in reducing fiber digestibility (Van Soest, 1981). Therefore, the lower NDF and ADF digestibility of sixweeks threeawn may have been due to its higher lignin and indigestible carbohydrate content (Table 1), which makes it the least digestible grass in the present study. Concurrent with this result, Silva et al. (2009) assessed the digestibility of forage plants in horses using the mobile bags technique and also found lower fiber digestibility in feed with higher lignin content.

According to Reis et al. (2005), higher cellulose, hemicellulose, and lignin fractions decrease digestible nutrient levels and, consequently, feed digestibility.

Although sixweeks threeawn and Sabi grass showed lower digestibility in comparison with Tifton 85 hay, those grasses may be an alternative source of roughage for horses, particularly in the dry season, when pastures with exotic grasses become senescent and unavailable to the animals. According to Brandi and Furtado (2009), structural carbohydrates are important sources of energy for horses since the production of short-chain fatty acids through fiber fermentation in the cecum and colon can provide 30% of maintenance energy.

Only the MM digestibility of sixweeks threeawn was lower than that of the other grasses. This finding can potentially be explained by the fact that many forage plants native to the Caatinga have secondary compounds whose synthesis and storage are associated with the adaptation of those plants to the edaphoclimatic variation of the habitat (Andrade et al., 2010). In some cases, those compounds are complexed with minerals, which makes them unavailable to animals.

In Brazil, pastures with tropical characteristics tend to have low mineral concentrations, which results in a diet deficient in those nutrients for horses that graze in those pastures. However, the use of supplements can, in most cases, prevent the occurrence of disorders linked to a lack of minerals in equine diets, especially those related to bone dystrophies (Wajnsztejn, 2010).

5. Conclusions

The digestibility coefficients of Alexandergrass and capim-de-raiz indicate that these grasses have potential to be used in equine feed.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Data curation: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Formal analysis: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Funding acquisition: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Investigation: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Methodology: A.H. Silva, J.E.C. Lucena, J.M. Santiago, D.A.S. Melo, D.P.S.S. Silva, H.J.X. Assis, D.S.A. Pinto and V.N. Maia. Project administration: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Resources: J.E.C. Lucena and J.M. Santiago. Supervision: J.E.C. Lucena and J.M. Santiago. Writing-original draft: A.H. Silva, J.E.C. Lucena and J.M. Santiago. Writing-review & editing: A.H. Silva, J.E.C. Lucena and J.M. Santiago.

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