



Protein and carbohydrate fractionation of Jiggs Bermudagrass in different seasons and under intermittent grazing by Holstein cows

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ABSTRACT. Jiggs bermudagrass has presented competitive production potential over other forages. However, there is a lack of information about the nutritional value of this forage with important relevance in ruminant nutrition. This study aimed to evaluate the protein and carbohydrate fractionation of Jiggs bermudagrass in different seasons of the year under intermittent grazing by Holstein cows. The experiment was conducted during one year in a completely randomized design with nine replications and treatments consisted of the effect of four seasons: fall, winter, spring and summer. The results showed that there were seasonal variations in the fractions of proteins and carbohydrate, with the exception of the protein fraction B3. The better climatic conditions in spring and summer contributed to an increase in the protein fraction A and carbohydrate A+B1. The winter had a greater fraction C of protein and carbohydrate, reflecting the nutritive value of the forage. The use of irrigation during fall and winter had a positive effect on nitrogen fractions B1 and B2 and on non-fiber carbohydrates.

Keywords: chemical composition; climate conditions; *Cynodon* spp.; ruminal degradability.

Received on June 19, 2018.
Accepted on September 6, 2018.

Introduction

Livestock production in the Brazilian Cerrado is mostly conducted on pasture and this is made possible by the predominant tropical conditions that allows the production of forage (Ferraz & Felício, 2010). The viability of pasture-based animal production systems depends on the use of high-quality forages and management practices that optimize the intake of nutrients by animals, allow the most efficient use of the resources available to the cattle-rancher and reduce their production costs (Maixner et al., 2007). However, the seasonality in forage production, due mainly to absence and/or low rainfall and also low temperatures, may limit the efficiency of livestock activity. In this sense, researchers are constantly working with the genetic improvement of forages, aimed to develop materials that meet the demands of the livestock sector (Kingston-Smith, Marshall, & Moorby, 2013). Based on this, it is required materials that present, in addition to high productive potential, nutritional advantages such as, for example, high protein content and more digestible fiber.

The release of *Cynodon* cultivars, such as the Coastal cultivar, represented a revolution in the southern US cattle ranching, as it evidenced the viability of grasses of this genus as a forage species. Since then, several other hybrid materials have been developed from selection and assessment programs by the Universities of Georgia and Florida in the humid southeast of Florida. Among these, Jiggs bermudagrass (*Cynodon dactylon* cv. Jiggs) was introduced in Brazil by an East Texas producer named J. C. Riggs (Carvalho, Pedreira, & Tonato, 2012). This grass has presented a competitive production potential over other forages, during different seasons of year, surviving and producing under adverse climatic conditions (Brandstetter et al., 2018; Poczynek, Neumann, Horst, Leão, & Ueno, 2016a; Poczynek et al., 2016b; Silva et al., 2016). In addition, it presents a better structural arrangement of the cell wall, with lower fiber concentration, being more interesting for animal nutrition in relation to the known Tifton 85 grass (Rezende et al., 2015). In spite of the diverse studies evaluating the Jiggs bermudagrass, in the literature there is still little knowledge about the protein and carbohydrate fractions of this forage, which has important relevance in ruminant

nutrition. Proteins and carbohydrates are fundamental because of the high impact on the productive system, resulting in different gains in animal performance.

Advances in CNCPS have occurred in recent years by incorporation of new research data and descriptions of rumen function and metabolism, as consequence several updated versions have been released over the last 15 years. The protein A fraction in the CNCPS, formerly classified as no protein nitrogen, was reclassified to ammonia for ease and availability of analysis and to provide a better prediction of the contribution of metabolizable protein from free amino acids (AA) and small peptides. For amino acid, profiles were updated using contemporary data sets and now represent the profile of AA in the whole feed rather than the insoluble residue (Higgs, Chase, Ross, & Van Amburgh, 2015). Therefore, to evaluate the nutritive value of this grass is essential to the ruminal degradation of protein fractions, carbohydrates and microbial growth due to the availability of these nutrients, for high yielding Holstein cows.

This study aimed to evaluate the fractions that compose the protein and the carbohydrates of Jiggs bermudagrass (*Cynodon dactylon* cv. Jiggs) in different seasons of the year under intermittent grazing by Holstein cows.

Material and methods

The experiment was conducted on a dairy farm in the municipality of Santa Helena de Goiás, Go, Brazil, from April 2014 to March 2015. The property is part of the “Balde Cheio” project, which seeks to promote the sustainable development of dairy ranching via technology transfer to meet the extension demands of public and private entities and dairy farmers throughout Brazil.

The soil at the experimental site was classified as a dystroferic Red Latosol (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2013) with 530 g kg⁻¹ clay. In May 2014, soil samples were collected at the 0-20-cm layer, and the chemical properties of the experimental site were determined as follows: pH in CaCl₂: 5.8; Ca: 2.55 cmol_c dm⁻³; Mg: 1.09 cmol_c dm⁻³; Al: 0.06 cmol_c dm⁻³; Al + H: 2.6 cmol_c dm⁻³; K: 0.23 cmol_c dm⁻³; cation exchange capacity (CEC): 6.47 cmol_c dm⁻³; P: 5.23 mg dm⁻³; V: 58.9%; and organic matter (OM): 33.50 g dm⁻³.

Phosphorus was applied as single superphosphate (120 kg ha⁻¹ P₂O₅), and potassium was applied as potassium chloride and fritted forms (50 and 20 kg ha⁻¹ K₂O and FTE BR 12, respectively). The fertilizers were disseminated in two applications, October 2014 and December 2014, and nitrogen fertilizer (10 kg ha⁻¹ of N) was applied as urea every 19 days when the animals left the paddocks.

The property had 19 paddocks of 800 m² each. In each paddock, the stocking rate was 6.0 AU ha⁻¹ in the dry season and 12 AU ha⁻¹ in the period of waters of Holstein cows averaging 480 kg body weight. The Jiggs bermudagrass pasture was studied in a mob-stocking grazing system, in which each paddock was stocked at a high rate for one day and then allowed to rest for 19 days.

The experiment was conducted using a completely randomized design with five replicates (the number of forage cuts in each season) and the four seasons (fall, winter, spring and summer) as treatments.

During the dry period, started in May, a sprinkler irrigation system was used with a microsprinkler in each paddock. The irrigation regime was determined according to the plants requirement; irrigation was conducted every four days for a total of 6.5 hours per day from May to October 2014 using a 30mm water blade.

The dry matter (DM) production and the nutritional value of Jiggs bermudagrass were evaluated each season (summer, fall, winter and spring) for a year. The rainfall and mean monthly temperature were also monitored during this period (Figure 1). The grass was evaluated under a successive-cut system, every 19 days, by collecting two forage samples (1m²) per paddock, with 5 cm residual height. Because of the limited areas of the paddocks, two representative points were sampled per paddock to avoid damaging the pasture structure through successive collections.

Fresh material was immediately weighed and sent to laboratory for further DM estimation, consisted of oven-drying and forced air circulation at approximately 55°C. Samples were ground through a 1mm sieve and stored in plastic jars for analysis.

Determination of non-protein nitrogen (NPN), neutral detergent (NIDN) and acid detergent (NIDA) insoluble nitrogen was estimated according to the methodology described by Licitra, Hernandez, and Van Soest (1996). Protein fractionation was calculated by the Cornell Net Carbohydrate and Protein System (CNCPS) system (Sniffen, O'Connor, Van Soest, Fox, and Russell (1992). Protein was analyzed and calculated in five fractions A, B1, B2, B3 and C.

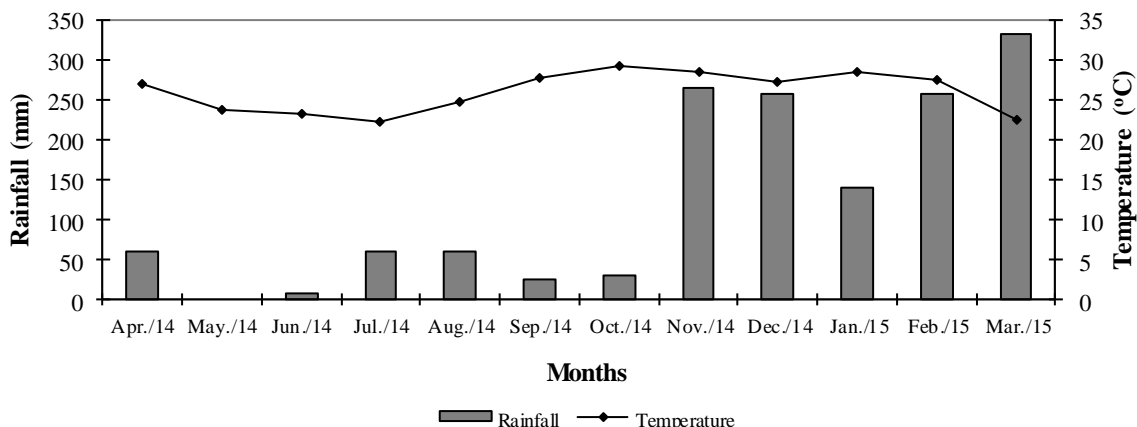


Figure 1. Rainfall (mm) and mean temperature (°C) in Santa Helena de Goiás, GO, Brazil, from April 2014 to March 2015.

Percentage of total carbohydrates (CT) was estimated by the equation (Sniffen et al., 1992): $CT = 100 - (\%CP + \%EE + \%ash)$; fiber carbohydrate (CF), from the NDF corrected for ash and protein content (NDF_{CP}); non-fiber carbohydrates (CNF), which correspond to fractions A+B1, by the difference between total carbohydrates and FDN_{CP} ; and fraction C, by the indigestible NDF after 144 hours of in situ incubation. Fraction B2, which corresponds to the available fraction of the fiber, was calculated by the difference between the NDF_{CP} and fraction C.

Data were tested by analysis of variance using the software R version R-3.1.1 (2014), and ExpDes package (Ferreira, Cavalcanti, & Nogueira, 2014). For the evaluation of the forage in different seasons of year, the analyses were carried out by the split plot model subdivided in time. The means were compared by the Tukey’s test, with a significance level of 5% probability.

Results and discussion

Nitrogen fractions A, B1, B2 and C of Jiggs bermudagrass were influenced by climatic seasons ($p < 0.05$) however, for fraction B3 there was no significant effect ($p > 0.05$) as presented in Table 1.

With better conditions of rainfall and temperature (Figure 1), there was a 20.3% increase in protein fraction A in spring and summer compared to fall followed by winter. The fraction A is considered soluble with rapid ruminal degradation and its presence in grasses is due to the high levels of crude protein under favorable climatic conditions for the development of the forage, thus improving the degradation rate. In the spring and summer, Jiggs bermudagrass presented crude protein contents of 19.68 and 20.04%, respectively, leading to better yields of animals and resulting in higher milk production (Brandstetter et al., 2018).

According to Russell, O’connor, Fox, Van Soest, and Sniffen (1992) fraction A is fundamental for good ruminal functioning, because the ruminal, structural carbohydrate fermenting microorganisms use ammonia as a source of nitrogen. This fraction, formerly classified as no protein nitrogen, was reclassified to ammonia for ease and availability of analysis and to provide a better prediction of the contribution of metabolizable protein from free amino acids (AA) and small peptides (Higgs et al., 2015). These updates improved the capacity of the model to detect the most limiting nutrient, which allows the user to refine diet formulation to improve the productive efficiency of cattle (Van Amburgh et al., 2015). Ribeiro, Pereira, Valadares Filho, Garcia, and Cabral (2001) reported values of nitrogen compounds of fraction A of 22.1% for Tifton 85 grass when cut at 35 regrowth days. These values were higher than those found in this study for Jiggs bermudagrass, thus showing the nutritional nutritive value of this forage when compared to Tifton 85. The best structural arrangement of the cell wall, with lower fiber concentration, makes Jiggs bermudagrass a more interesting option for animal nutrition in relation to Tifton 85 grass (Rezende et al., 2015).

In relation to the fraction B1 (rapid degradation soluble fraction) and B2 (intermediate degradation fraction), Table 1 lists differences ($p < 0.05$) and significant increases in the fractions obtained in fall and winter in reference to spring followed by summer. These results can be explained by the influence of the climatic factors of temperature and radiation on the protein fractions B1 and B2 with increase of these fractions to the detriment of the fraction A. It is worth mentioning that the use of irrigation during fall and

winter had a positive effect on the quality of forage produced in reference to these nitrogen fractions, which were higher than the values found by Ribeiro et al. (2001). Moreover, the high content of fraction A, when compared to the literature data (Table 1), may have contributed to the solubility of B1 and B2 protein in fall and winter.

The protein fraction B3 has a very slow degradation rate, and is associated with the cell wall of the plant with a slowly available portion (neutral detergent insoluble nitrogen) and another unavailable (acid detergent insoluble nitrogen) (Sniffen et al., 1992). Table 1 lists no significant differences between seasons ($p > 0.05$). The values of the protein fraction B3 found in this study were lower than those found by Ribeiro et al. (2001), who observed Tifton 85 grass, proving once again a positive point in the nutritional quality of Jiggs bermudagrass. Considering the habit of cattle to select leaves, it is important that the fractions A, B1 and B2 represent the highest proportion in this part (Branco et al., 2012).

As grass develops at the expense of non-fiber carbohydrates, the structural components of the cell wall develop thereby increasing the fiber fractions and reducing the proportion of potentially digestible nutrients and impairing the qualitative characteristics of pasture during the dry period (Velásquez et al., 2010). Therefore, fraction C is composed by proteins and nitrogen compounds associated with lignin and highly resistant to the attack of enzymes of microbial origin.

It can be observed in table 1 an increase of 23.56% of the fraction C in winter in relation to the summer and fall, which may be related to the development stages of the plant. Even with the use of irrigation in these seasons, forage production was lower than in the other seasons, producing 49% in relation to summer and 33.2% in relation to spring and fall. Also, in these periods, in the same experimental area, Brandstetter et al. (2018) observed a greater elongation of stems, where the leaf: stem ratio declines rapidly, because in addition to the higher stem growth, leaf appearance (fundamental for dry mass production) was reduced due to the lower quantity and quality of the light that reaches the inside of the canopy. As a result, there was an increase in fractions C, which also reflected lower milk yield (Brandstetter et al., 2018). The results found for fraction C herein (Table 1) are lower than those observed by Branco et al. (2012), who evaluated the chemical composition and fractionation of crude protein of the Coastcross grass, and reported values of 14.73, 14.98 and 15.01% for winter, spring and summer, respectively. The lower values of fraction C for Jiggs bermudagrass when compared to Coastcross grass, also a species of *Cynodon dactylon*, reinforces the fact that this forage has high potential for use in pasture systems for dairy cattle.

The explanation for the increase of total carbohydrates (CHT) of Jiggs bermudagrass in winter in relation to the spring, summer and fall (Table 2), is associated with the increase in structural carbohydrates and lignin, which reduces the proportion of potentially digestible nutrients, impairing the qualitative characteristics of the pasture (Velásquez et al., 2010). Further, it is important to note that the fiber present in tropical grasses represents most of the total carbohydrates of the pasture, thus the highest proportion of fibers in Jiggs bermudagrass during winter contributed to the greater content of CHT (80.10%) in this season.

Table 1. Protein fractions A, B1, B2, B3 and C (%) of Jiggs bermudagrass, in different seasons of the year.

Protein fractions	Season				CV (%)
	Fall	Winter	Spring	Summer	
Fraction A	43.00 b	40.78 b	50.03 a	55.00 a	13.86
Fraction B1	18.39 a	17.19 a	13.84 b	14.40 b	15.93
Fraction B2	15.36 a	15.69 a	12.67 b	10.05 b	24.46
Fraction B3	13.66 a	14.82 a	12.50 a	12.52 a	29.36
Fraction C	9.57 b	11.50 a	10.93 a	8.02 b	18.89

Means values followed by different letters, in the same row, are significantly different by Tukey's test at the 5% probability level.

Table 2. Total carbohydrate (CHT), non-fiber carbohydrate (NFC), fractions A+B1, B2 and C (%) of Jiggs bermudagrass in different seasons of the year.

Carbohydrate	Season				CV (%)
	Fall	Winter	Spring	Summer	
CHT	76.32 b	80.10 a	76.19 b	75.54 b	1.66
NFC	53.71 b	58.85 a	53.21 b	53.09 b	1.98
Fraction A+B1	32.70 b	24.06 c	25.72 c	37.61 a	13.48
Fraction B2	57.99 b	63.06 a	64.98 a	53.88 b	7.5
Fraction C	9.30 b	12.87 a	9.29 b	8.49 b	16.65

Means values followed by different letters, in the same row, are significantly different by Tukey's test at the 5% probability level.

With respect to non-fiber carbohydrates (NFC), there was an increase of 9.38% in winter compared to the other seasons. Among the probable determinants of this increase in winter is the irrigation that provided adequate water conditions for forage development during the experimental period (Brandstetter et al., 2018). Importantly, the grazing system adopted - Mob Stocking - works with high animal load for a short time. Thus, it favors the quality of the forage under grazing, since in these situations there is a greater removal of pseudostems.

Regarding the fraction A + B1, there were significant differences between the seasons (Table 2). Favorable climatic conditions in the summer (Figure 1) promoted a reduction in the fiber fraction and an increase in the proportion of the potentially digestible nutrients (soluble carbohydrates, proteins, minerals and vitamins) of the grass, which explains the greater fraction A+B1 in this season. According to the Cornell Net Carbohydrate and Protein System model definitions, fraction A consists of sugars and fraction B1 consists of starch, pectin and glucans (Sniffen et al., 1992). These fractions represent carbohydrates of rapid ruminal degradation and when they are the main carbohydrate of the diet, it is necessary to include nitrogen compounds A and B1 to maintain adequate protein and carbohydrate timing, leading to better ruminal energetic adequacy, promoting better microbial growth (Sniffen et al., 1992). As Jiggs bermudagrass showed greater protein soluble fraction A and B1 when compared to other species and cultivars of the genus *Cynodon* (Branco et al., 2012; Ribeiro et al., 2001), there will be a synchrony between the degradation of nitrogen and protein compounds in the rumen.

For the carbohydrate fraction B2 (potentially digestible fiber), greater values were obtained in winter and spring ($p < 0.05$) (Table 2). As for the fraction C (unavailable fiber), the winter presented a greater value ($p < 0.05$) than spring, summer and fall seasons (Table 2). These results are due to the greater proportion of fibers obtained in this period, due to the lower leaf: stem ratio, resulting in a negative effect on the utilization and nutritive value of the forage produced in the dry period, even with the use of the irrigation system (Brandstetter et al., 2018). According to Rodrigues, Rodrigues, Reis, and Soares Filho (2006), the quantity and quality of forage produced is associated not only with humidity but also with other factors that influence and drive vegetative development and maturity, such as light and temperature. The carbohydrate compounds are attached to the fiber fractions, becoming potentially digestible (B2) and unavailable (C). Sá et al. (2010) reported that the high content for the fraction C found in the winter is also attributed to the increase in the fraction of indigestible hemicellulose and cellulose of the cell wall. Forages with high NDF content have a higher proportion of the fraction B2, and the increase in the fraction C can be partly attributed to the increased lignin concentration in NDF (Silva & Silva, 2013). It is worth mentioning that one of the most important information provided by carbohydrate fractionation is in the evaluation of the content of fraction C considered unavailable in the digestive behavior of ruminants. The high content of this component can result in a lower potentially degradable fiber fraction (B2), besides inhibiting the intake by the rumen-fill effect (Favoreto, Deresz, Fernandes, Vieira, & Fontes, 2008).

The increase of fraction C and reduction of fractions A+B1 obtained in winter (Table 2) implies a reduction in energy availability for the microorganisms that ferment fiber and non-fiber carbohydrates (Silva & Silva, 2013); thus, reducing milk yield, which was $13.62 \text{ kg day}^{-1}$ when compared to the average of the other seasons, which was $17.74 \text{ kg day}^{-1}$. In view of these results, it is recommended that in this period animals be supplemented with energetic sources of rapid availability in the rumen, when there is no protein limitation, in quantity and quality.

Conclusion

Better climatic conditions in spring and summer contributed to an increase in the protein fraction A and carbohydrate A+B1. The winter had a greater fraction C of protein and carbohydrate, reflecting the nutritive value of the forage. The use of irrigation during fall and winter had a positive effect on nitrogen fractions B1 and B2 and on non-fiber carbohydrates.

Acknowledgements

The authors would like to thank the Federal Institute Goiano for supporting this study.

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