Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v41i1.42693

Protein and carbohydrates fractionation in Paiaguas palisadegrass intercropped with grain sorghum in pasture recovery

Charles Barbosa Santos*, Kátia Aparecida de Pinho Costa, Wender Ferreira de Souza, Victor Costa e Silva, Patrícia Soares Epifanio and Hemylla Sousa Santos

Instituto Federal Goiano, Campus Rio Verde, Rodovia Sul Goiana, Km 01, 75901-970, Rio Verde, Goiás, Brasil. *Author for correspondence.
E-mail: charles.bscarvalho@hotmail.com

ABSTRACT. Paiaguas palisadegrass was released in order to meet the requirements of the different production systems. However, little is known about the quality of the forage. The goal of this study was to evaluate the protein and carbohydrate fractionation of the Paiaguas palisadegrass after intercropping with grain sorghum in the pasture recovery, through the integration of crop and livestock production. The experiment was conducted in the municipality of Rio Verde, State of Goias. The experimental design was a randomized complete block design with four replications. The treatments were composed of the following forage systems: Paiaguas palisadegrass in monocropped, sorghum intercropped with Paiaguas palisadegrass in the row, sorghum intercropped with Paiaguas palisadegrass in the interrow, and sorghum intercropped with oversown Paiaguas palisadegrass. The evaluations were carried out in the four climatic seasons of the year (winter, spring, summer, and fall) in the same plots, over a period of one year, in 16 paddocks under continuous stocking system. The results showed that forage systems did not influence protein and carbohydrate fractionation. The winter season presented higher values of protein and carbohydrate fractionation, negatively affecting the animal performance.

Keywords: *Brachiaria brizantha;* forage; nutritive value; ruminal degradation.

Received on March 5, 2018.
Accepted on June 11, 2018.

Introduction

One of the major challenges faced by man today is the production of food in a sustainable manner, that is, without damage to the environment, especially in the use of soil and water (Cordeiro, Vilela, Marchão, Kluthcouski, & Júnior, 2015). For this reason, the use of integrated systems to diversify production without opening new areas has grown in Brazil (Costa et al., 2016). Thus, the crop-livestock integration system provides, in addition to grain production, an increase in forage availability in the dry period, resulting in sufficient amount of forage for nutritional maintenance of herds, promoting animal weight gain with recovery of pastures degraded at lower costs (Santos et al., 2016).

Among the forages, stands out *Brachiaria brizantha* cv. BRS Paiaguas, which was released in 2013 by Embrapa Gado de Corte, in order to meet the requirements of different production systems, with varying levels of technology. In addition, the great advantage of BRS Paiaguas is during the dry period, when it presents greater accumulation of forage of better nutritive value and consequently higher productivities per year (Costa et al., 2016; Cruvinel, Costa, Silva, Severiano, & Ribeiro, 2017). However, little is known about the quality of the forage. Information on the fractions of proteins and carbohydrates are important. In addition to enabling and estimating animal performance with greater accuracy, estimates of carbohydrate and protein fractions can increase the efficiency of nutrient utilization and also determine the type of supplementation for each season of the year (Fox, Sniffen, O'Connor, Russell, & Van Soest, 1992). In this sense, to evaluate the quality of the forage, new systems and methodologies for evaluation of feed for ruminants are being used to maximize the use of the nutrients by the animals. Cornell Net Carbohydrate and Protein System (CNCPS) is a nutritional model that evaluates the environmental and nutritional resources available in an animal production system and enables the formulation of diets that closely match the predicted animal requirements (Higgs, Chase, Ross, & Van Amburgh, 2015). This system that considers the dynamics of ruminal fermentation and the potential loss of nitrogen, as ammonia, in food evaluation (Sniffen, O'Connor, Van Soest, Fox, & Russell, 1992) and aims to adequate ruminal digestion of carbohydrates and proteins, aiming to maximize microbial production, reduction of nitrogen losses by the animal and to estimate ruminal escape of nutrients (Balsalobre, Corsi, Santos, Vieira, & Cárdenas, 2003).

The CNCPS system classifies ruminal microorganisms into two large groups: fiber carbohydrate fermenting bacteria, which use ammonia as the source of N, and non-fiber carbohydrate fermenting bacteria, which use either ammonia or amino acids or peptides as a source of N. This system allows the fractionation of carbohydrates in the fractions A (simple sugars) and B1 (starch and pectin), of fast and medium ruminal degradation, respectively, B2 (available cell wall and according to the rates of passage and degradation) and fraction C (lignin) not degraded by ruminal microorganisms (Sniffen et al., 1992). The CNCPS has been evolving by incorporation of new research data and descriptions of rumen function and metabolism into mathematical equations and quantitative representations with the primary objective of field application and diet formulation. As consequence, several updated versions have been released over the last 15 years. One of the objectives of the CNCPS modeling process has been to incorporate enhanced knowledge in the platform to further explain differences in cattle productivity, for predictions of nutrient requirements and supply (Van Amburgh et al., 2015).

Due to the nutritional variation of the pastures, retention of forage in the rumen-reticulum makes the digestion and fermentation difficult, interfering with the prediction of the animal performance from the diet components and, therefore, for its proper characteristic, the nutrients used in the ruminant feed must be fractionated (Higgs et al., 2015; Sniffen et al., 1992).

In view of this, there was a need to generate more information about the fractionation of protein and carbohydrate, leading to the development of more studies, especially regarding the use of forage. This study aimed to evaluate the protein and carbohydrate fractionation of the Paiaguas palisadegrass after intercropping with grain sorghum in the pasture recovery, through the integration of crop and livestock production.

Material and methods

Characterization of the area and treatments

The experiment was conducted in the cattle sector of the Goiano Federal Institute, Rio Verde Campus, at 748 m altitude, 17°48'S and 50°55'W (Köppen & Geiger, 1928).

The grass used was *Brachiaria brizantha* cv. Marandu pasture, implemented more than 40 years ago and in an advanced stage of degradation.

Soil samples were collected (Dystrophic Red Latosol) (EMBRAPA, 2013) to determine the physicochemical characteristics of the 0-20 cm layer of the soil in the experimental area before the forage systems were implemented, with the following results: clay: 500 g kg^{-1}; silt: 220 g kg^{-1}; sand: 280 g kg^{-1}; pH in $CaCl_2$: 5.51; Ca: 2.20; Mg: 0.91; Al: 0.01; Al + H: 3.30; K_2O: 0.09; CEC: 6.51 $cmol_c$ dm^{-3}; P (Mehlich): 0.34; Cu: 2.2; Zn: 0.4; Fe: 14.4 in mg dm^{-3}; and MO: 28.70 g kg^{-1}. The preparation of the area began with the desiccation of the previous crop by an application of the herbicide (Transorb 3.5 L ha^{1}) at 2.058 g a.i. ha^{1} with a broth volume of 150 L ha^{1}. Thirty days after crop desiccation, disking was performed with a disk harrow, followed by leveling with a disk.

The experimental design was a randomized complete block design with four replications. The treatments were composed of the following forage systems: Paiaguas palisadegrass in monocropped, sorghum intercropped with Paiaguas palisadegrass in the row, sorghum intercropped with Paiaguas palisadegrass in the interrow, and sorghum intercropped with oversown Paiaguas palisadegrass. The evaluations were carried out in the four climatic seasons of the year (winter, spring, summer, and fall) in the same plots, over a period of one year, from July 2015 to August 2016.

Implementation and maintenance of forage systems

The area of each plot was 1042 m^2, divided into 16 enclosures with electric fence. The sorghum used was the Buster hybrid of red grain, without tannin and of low size, and the *Brachiaria* species was BRS cv. Paiaguas.

The planting of the forage systems was performed mechanically on January 24, 2015, with the application of 240 kg ha^{-1} of P_2O_5 and 20 kg ha^{-1} of FTE BR 20, in the sources of simple superphosphate and frits, respectively.

In monocropped and intercropping, the sorghum was sown at a depth of 3 cm. In the Paiaguas palisadegrass monocropped, the grass was sown at a depth of 3 cm; in the row, it was sown at a depth of 6 cm; in the interrow, it was sown at a depth of 3 cm and 0.25 m from the sorghum row; and in the oversown treatment, it was sown at a depth of 6 cm and 0.25 m from the sorghum row, 15 days after the sorghum had been sown.

At 15 DAS (days after sowing), 80 kg ha^{-1}of nitrogen and 40 kg ha^{-1}of K_2O, in the form of urea and potassium chloride, were applied. On 05/04/2015, all forage systems were harvested for silage by a row forage harvester at 20 cm height above the soil.

After the plants were harvested from the forage systems, cover fertilization was conducted in all systems, with the application of 80 kg ha^{-1}of nitrogen and 30 kg ha^{-1}of K_2O. The Paiaguas palisadegrass remained at rest for 94 days to allow regrowth and development. The long rest period was due to the low precipitation obtained during this period, which limited development of the grass.

On 08/25/2015, the animals entered the area for evaluation of animal performance and forage production. The grazing method was continuous stocking, with a variable stocking rate. The animals used were of the Nellore breed and included 32 females, with a mean age of 12 months (heifers) and mean initial weight of 180 kg. Evaluations were performed over a year, for all seasons of the year, with winter being the first season evaluated. During this period, eight cuts were performed: two cuts per season, with a 28-day interval and eight grazing cycles. All animals received water and a complete mineral mixture *ad libitum* in the area of each enclosure, in addition to sanitary management (vaccination and deworming).

Due to the low availability of forage, in December 2015, 80 kg ha^{-1} nitrogen and 50 kg ha^{-1} of K_2O were applied in the form of urea and potassium chloride. After fertilization, the *Paiaguas palisadegrass* was not grazed for 28 days for better forage development.

Over the course of the experiment, rainfall and mean monthly temperature data were monitored daily (Figure 1).

Evaluation the fractionation of protein and carbohydrate

To evaluate the fractionation of protein and carbohydrate, the forage was collected by means of two samplings per paddock using square frame with 0.50 x 0.50 m (0.25 m^2), cutting the forage contained inside the square at 20 cm from the ground, which was placed in a paper bag and dried in a forced circulation oven at 55°C for 72 hours. Subsequently, the samples were ground for analysis.

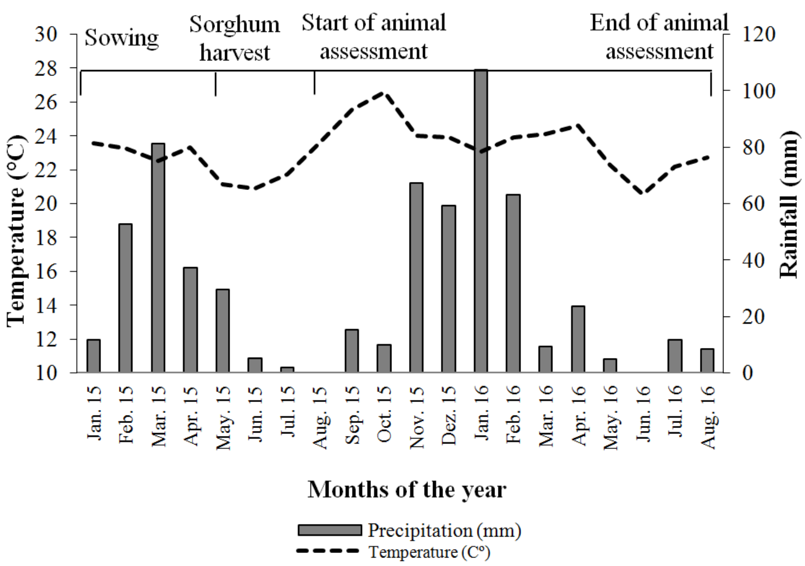

Figure 1. Rainfall values (mm) and mean temperature (°C) in Rio Verde, Goiás, Brazil, from January 2015 to August 2016.

The determination of non-protein nitrogen (NNP), neutral detergent (NIDN) and acid detergent (NIDA) insoluble nitrogen was performed according to the methodology described by Licitra, Hernandez, and Van Soest (1996). The determination of soluble nitrogen (NS) was performed according to Krishnamoorthy, Sniffen, Stern, and Van Soest (1983). Protein fractionation was calculated by the CNCPS system (Sniffen et al., 1992). Protein was analyzed and calculated in five fractions A, B1, B2, B3 and C. Fraction A, composed of non-nitrogen compounds (NNP), was determined by the difference between the total nitrogen (total N) and the trichloroacetic acid-insoluble N (TCA). Fraction B1 relative to soluble proteins, rapidly degraded in the rumen, was obtained by the difference between the nitrogen soluble in borate phosphate buffer (TBF) minus NNP. The fractions B2 and B3, consisting of the insoluble proteins with intermediate and slow degradation in the rumen respectively, were determined by the difference between the TBF-insoluble fraction and the NIDN fraction (B2 fraction), and NIDN minus NIDA (B3 fraction). Fraction C, consisting of proteins insoluble and indigestible in the rumen, was determined by the residual nitrogen content of the sample after treated with acid detergent (NIDA) and expressed as a percentage of the total N of the sample. The percentage of total carbohydrates (CT) was obtained by the equation (Sniffen et al., 1992): CT = 100 – (% CP + % EE + % ash); the 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} (Hall, 2003); and fraction C, by the indigestible NDF after 144 hours of in situ incubation (Cabral et al., 2004). Fraction B2, which corresponds to the available fraction of the fiber, was obtained by the difference between the NDF_{CP} and fraction C.

Statistical analysis

The variables were tested by analysis of variance, through the software R version R-3.1.1 (R Development Core Team, 2014), using the ExpDes package (Ferreira, Cavalcanti, & Nogueira, 2014). The means were compared by the Tukey test at 5% probability. For the evaluation of the forage in the different seasons of the year, the analyses were carried out by the model of repeated measures in time.

Results and discussion

Forage systems had no influence ($p>0.05$) on protein fractionation (A, B1, B2, B3 and C), total carbohydrates and carbohydrate fractionation (A + B1, B2 and C), showing similar results. However, there was an effect ($p<0.05$) between the seasons of the year (Tables 1 and 2). The highest values of A and B1 protein fractions, obtained in summer and fall (Table 1), may be associated with a higher occurrence of rainfall in these periods (Figure 1), with favorable climatic conditions for forage development, improving the quality of forage, with higher protein value and lower fiber fractions, resulting in a high rate of ruminal degradation. A quality forage provides greater fraction A, as it is more soluble and has rapid ruminal degradation (Epifanio et al., 2014). It is worth noting that in the summer and fall seasons, the highest daily gain (0.34 and 0.48 kg day^{-1}) and the stocking rate (9.3 and 10.1 AU ha^{-1}) were also found and it is possible to observe the superiority of the carrying capacity in pastures formed under a crop-livestock integration system, showing the advantages of using this system under real livestock conditions in Brazil, the stocking rate is usually less than 1 AU ha^{-1}, compromising the animal indices of animals.

Fraction A together with the fraction B1 of protein are considered soluble with rapid ruminal degradation. According to Sniffen et al. (1992), this fraction is essential for good ruminal functioning, because the ruminal microorganisms, structural carbohydrate fermenting bacteria, use ammonia as a source of nitrogen. However, high proportions of non-protein nitrogen may result in higher nitrogen losses due to the lack of the readily available carbon skeleton for the synthesis of microbial protein, denoting the importance of carbohydrate and protein balance for ruminal microorganisms (Queiroz et al., 2011). It is worth noting that ruminants that consume forage, whose protein fractions supply the lowest values of the B1 fraction, may decrease the escape of ammonia nitrogen by the ruminal epithelium (Carvalho et al., 2008). Considering the intake habit of cattle to select the leaves, it is important that the fractions A, B1, B2 and B3 represent the highest proportion in this part (Henriques et al., 2007).

Higher values of fractions B2, B3 and C, obtained in winter and spring, are due to the lower quality of forage in these seasons, this affirmation is possibly related to the slowest tissue replenishment during this period and flowering tillers accumulation. The fraction B2 corresponds to the intermediate degradation rate in the rumen (potentially degradable fiber with slower degradation rate) (Sniffen et al., 1992). The fraction B3 presents a very slow degradation rate, since it is associated to the cell wall of the plant, which is more lignified. The protein fraction (B3), although digestible, has ruminal degradation rate of 0.02 to 1.0% h^{-1} (Pires et al., 2009). However, the fraction C corresponds to the unavailable nitrogen and consists of proteins and nitrogen compounds associated with lignin, tannin-protein complexes and Maillard products, which are highly resistant to the attack of enzymes of microbial and host origin (Sniffen et al., 1992; Van Soest, 1994).

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 of proteins and nitrogen compounds associated with lignin and highly resistant to the attack of enzymes of microbial origin. It is observed an increase by 13.4% of this fraction in the winter in relation to the spring and an increase of 45.8% in relation to the summer and fall. It is noteworthy that this (indigestible) fraction had lower values in relation to the other nitrogen compounds, it is interesting from the nutritional point of view (Henriques et al., 2007). Greater fractions C in relation to this study were obtained by Velásquez et al. (2010), who evaluated the protein fractionation of tropical forages (Marandu palisadegrass, Tifton 85 grass and Tanzania guineagrass) at different cutting ages, verified a C fraction from 19.66 to 27.04%, higher than those obtained in this study.

Regarding total carbohydrate, the highest values were obtained in winter (Table 2). This is due to the increase of structural carbohydrates and lignin, which reduces the proportion of potentially digestible nutrients compromising the qualitative characteristics of the pasture (Velásquez et al., 2010). Additionally, it is important to note that the fiber present in tropical grasses represents most of the total carbohydrates of the pasture (Favoreto, Deresz, Fernandes, Vieira, & Fontes, 2008); thus the highest proportion of fibers in Paiaguas palisadegrass during winter contributed to the higher content of total carbohydrate in this season.

The mean value of total carbohydrates of the forage systems was 84.8%, being above those reported by Van Soest (1994), in which they constitute 50 to 80% of the dry matter of the forage plants. Moreover, according to the authors mentioned above, the nutritive characteristics of forage carbohydrates depend on the sugars that compose them, the bonds established between them and other factors of a physical and chemical nature.

The carbohydrate fractions A + B1 corresponds to readily degraded soluble sugars and non-fiber carbohydrate (starch and pectin). The lowest fraction carbohydrate A + B1 obtained in spring, summer and fall is due to the better climatic conditions in these periods, with a mean rainfall in the summer of 230 mm (Figure 1), favoring a higher production of leaves in relation to stem, resulting in a reduction in fiber fractions and an increase in the proportion of potentially digestible nutrients (soluble carbohydrates, proteins, minerals and vitamins) of Paiaguas palisadegrass, and explains the increase of this fraction in these seasons (Table 2). 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 an adequate protein and carbohydrate synchrony, leading to a better energy adequacy in the rumen, promoting better microbial growth (Pereira et al., 2010; Ribeiro, Macedo Júnior, & Silva, 2014; Sniffen et al., 1992). It is important to note that the fiber present in tropical grasses represents most of the total carbohydrates of the pasture (Vieira, Tedeschi, & Cannas, 2008).

Table 1. Protein fractionation (%) of Paiaguas palisadegrass in different forage systems and seasons of the year.

Forage systems	A	B1	B2	B3	C
Paiaguas palisadegrass in monocropped	46.37	16.94	14.52	11.47	10.81
Sorghum x Paiaguas palisadegrass in the interrow	46.92	16.90	13.68	11.36	11.13
Sorghum x Paiaguas palisadegrass in the row	46.71	17.30	13.34	11.40	11.23
Sorghum x Paiaguas palisadegrass oversown	46.42	17.20	13.01	12.00	11.47
P-value	0.43	0.60	0.42	0.23	0.12
CV (%)	2.30	5.80	9.15	8.47	6.90
Season of the year					
Spring	43.52 b	15.42 b	15.86 a	13.10 a	12.10 b
Summer	50.69 a	17.23 a	12.72 b	10.44 b	9.02 c
Fall	49.44 a	17.55 a	12.82 b	10.50 b	9.80 c
Winter	42.77 b	16.16 b	14.18 a	13.17 a	13.72 a
P-value	0.002	0.001	0.003	0.001	0.001
CV (%)	2.30	5.80	9.15	8.47	6.90

Mean values followed by different letters are significantly different by Tukey's test at 5% probability.

Table 2. Total carbohydrate (CHOT) (%), fractions carbohydrate (%) of *Paiaguas palisadegrass* in different forage systems and seasons of the year.

Forage systems	CHOT	A+B1	B2	C
Paiaguas palisadegrass monocropped	84.74	57.32	20.86	21.82
Sorghum x Paiaguas palisadegrass in the row	85.11	57.52	20.69	21.77
Sorghum x Paiaguas palisadegrass in the interrow	84.60	57.56	20.81	21.61
Sorghum x Paiaguas palisadegrass in the oversown	84.62	57.82	20.61	21.55
P-value	0.55	0.96	0.98	0.97
CV (%)	1.33	4.72	9.24	8.09
Season of the year				
Spring	85.48 b	63.19 a	19.42 b	17.37 b
Summer	81.81 c	61.31 a	20.45 b	15.60 b
Fall	81.11 c	62.28 a	20.02 b	17.70 b
Winter	90.67 a	48.44 c	23.08 a	28.48 a
P-value	0.001	0.001	0.002	0.001
CV (%)	1.33	4.72	9.24	8.09

Mean values followed by different letters are significantly different by Tukey's test at 5% probability.

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). Foods with higher concentrations of fraction A and B1 are considered good energy sources, consequently raising the content of ruminal microorganisms. In addition, they are important to maintain adequate protein and carbohydrate timing (Mizubuti et al., 2014).

Higher values of fraction B2 and C obtained in the winter are due to the higher accumulation of fibers fraction (NDF and ADF), due to the low rainfall (Figure 1), which impaired forage development in this period. Besides that, the increase of these fractions, provided lower average daily gain, stocking rate and body weight/area. In these periods, the structural components of greater interest, that is, the leaves reduce and occur increase of dead material and fibers. Sá et al. (2010) reported that the high content for the fraction C found in winter, is also attributed to the increase in the indigestible fraction of hemicellulose and cellulose of the cell wall.

It is known that the association between lignin and protein is an inherent factor to the constitution of the plant and can be formed in a greater or smaller scale depending on morphogenic characteristics of the plant, in which they are influenced by climatic seasons. Therefore, the variations in forage composition observed in the present study demonstrate that Paiaguas palisadegrass in winter with higher concentration of fraction C possibly affects animal intake (filling effect), directly reducing animal performance (Mertens, 1987).

Conclusion

Forage systems have no influence on protein and carbohydrate fractionation. The winter season presents higher values for protein and carbohydrate fractionation, directly reflecting the reduction of animal performance.

Acknowledgements

We would like to thank the Goiano Federal Institute for supporting this study and the CNPq e FAPEG/CAPES for funding the project.

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