Revista Brasileira de Zootecnia

ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.41, n.9, p.1983-1988, 2012

Nitrogen and carbohydrate fractions on Tifton-85 pastures overseeded with annual winter and summer forage species in different seasons[1]

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ABSTRACT - An experiment was conducted during the 2001-2002 winter-spring-summer to determine the nitrogen and carbohydrate fractions in Tifton-85 pastures exclusively or overseeded with oats, millet and sorghum-sudangrass hybrids. The treatments were Tifton-85 overseeded with millet + bristle oat; sorghum-sudangrass + bristle oat, on 06/19/2002 and 07/02/2002, respectively; and Tifton-85 (Control). The experiment was conducted in a randomized block design with three replications. Nitrogen and carbohydrate fractions were affected by the nitrogen and total carbohydrate contents observed in the pasture overseeded at different seeding times, and by the different growth periods. The highest nitrogen fractions (A + B1) were observed in the early growth periods. Overseeding affected the forage nitrogen and carbohydrate fraction contents positively. The high solubility of both carbohydrate and protein from millet + bristle oat and bristle oat + sorghum-sudangrass mixtures indicates the quality of these forages and their potential use as an important supplement in forage systems based on tropical pastures.

Key Words: bristle oat, soluble nitrogen, sorghum-sudangrass, total carbohydrate

Introduction

Forage of better quality is obtained in the South and Southeast regions of Brazil using annual winter species harvested from April to September. Overseeding winter forage species in areas formed by tropical perennial forage species is an alternative for increasing forage production and the seasonal distribution (Roso et al., 1999), particularly due to the high nutritional value of the forage obtained during the dry and cold seasons (Reis et al., 2001).

The Cornell program demonstrated the importance of better characterization of animal feed. Sniffen et al. (1992) divided the nitrogen compounds of forage plants in five fractions: fraction A, constituted by non-protein nitrogen (NPN) that is highly soluble in the rumen; fraction B, divided in a soluble fraction B1, part of the true protein, quickly degraded in the rumen, and fraction B2, insoluble protein of intermediate degradation in the rumen; fraction B3, an insoluble protein, called extensin, which is bound to the cell wall and slowly degraded in the rumen; and fraction C, corresponding to non-available nitrogen, contained in acid detergent fiber (ADF) as the acid detergent insoluble nitrogen (ADIN).

The nitrogen compound fractions can be influenced by the forage species. However, they seem to be more affected by management, physiological stage and nitrogen fertilization (Malafaia et al., 1997; Lima et al., 1999).

Forage carbohydrates (CHO) are also classified into four fractions according to their rumen degradation: fraction A, of fast degradation (CHO with low molecular weight, glucose, saccharose and organic acids); fraction B1 with intermediate degradation kinetics (storage compounds of the plants, such as starch, fructans, and galactans) and carbohydrates from the medium lamella, pectin and β-glycans; fraction B2, potentially digestible low-degradation fibers (polysaccharides that constitute the cell wall, cellulose, hemicellulose); and fraction C, formed by indigestible fibers.

The objective of this study was to fractionate the nitrogen and carbohydrate compounds of Tifton-85 grass exclusively and overseeded with winter and summer forage species in two overseeding periods.

Received December 17, 2010 and accepted May 23, 2012.
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Material and Methods

The experiment was conducted in the Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal of Universidade Estadual de São Paulo/UNESP on a clayey dystrophic red latosol (Andrioli & Centurion, 1999).

The winter and summer forage species overseeded on Tifton-85 (*Cynodon nlemfuensis* Vanderyst × *Cynodon dactylon* (L.) Pers) pasture were: bristle oat (*Avena strigosa* Schreb) cv. IAPAR 61, millet (*Pennisetum americanum* (L.) Leeke) cv. BRS1501 and sorghum-sudangrass hybrid (*Sorghum bicolor* (L.) Moench × *Sorghum sudanense* (Piper) Stapf) cv. AG2501C.

The summer species were chosen based on the hypothesis that seeds germinate and develop at higher temperatures, resulting in a better forage mass distribution during the period of evaluation.

A total of six treatments were tested: treatment 1 - millet + bristle oat overseeded on June 19; treatment 2 - sorghum-sudangrass + bristle oat overseeded on June 19; treatment 3 - control: exclusive Tifton-85, grazed on June 19; treatment 4 - millet + bristle oat overseeded on July 2; treatment 5 - sorghum-sudangrass + bristle oat overseeded on July 2; and treatment 6 - control: exclusive Tifton-85, grazed on July 2.

The entire area with Tifton-85 was grazed to 5 cm on June 18 and July 1, so that overseeding could be performed on June 19 and July 2, respectively. The areas with exclusive Tifton-86 were grazed on the same dates as the control for comparison with the respective overseeding treatments.

Overseeding was performed with a no-tillage seeder with 22.5 cm distance between rows using the following amounts of seeds: 60 kg/ha of bristle oat and 16 kg of millet or sorghum-sudangrass. Initial fertilization was performed with 30 kg/ha of nitrogen (N) using urea, 60 kg/ha of P_2O_5 with simple superphosphate, and 60 g/ha of K_2O with potassium chloride, and the fertilizer was mixed during seeding.

On July 19, 2002, (period 1) and on August 2, 2002 (period 2), 30 days after overseeding, top-dressing fertilization was performed with 40 kg/ha of N and 40 kg/ha of K_2O, using urea and potassium chloride mixed and distributed by hand.

Sprinkler irrigation was performed at an application rate of 60 mm of water per month from the seeding dates until August to ensure the adequate establishment of the forage species. Irrigation was quantified based on previous experimental data from the Forage Sector of UNESP/Jaboticabal.

All pastures were managed in a rotational grazing system. The forage mass was evaluated and grazing was started when the plants reached 55-60 cm high in the areas with winter forage species (first, second and third evaluation cycles). After the disappearance of the annual species, the evaluation of the exclusive Tifton-85 pastures (fourth and fifth evaluations), and in the exclusive Tifton 85 pasture, grazing was done when the plants were 35-40 cm high in all grazing cycles.

Before the animals were let into the area, sampling was performed by hand plucking an area of 1 m^2 at 20 cm height in the annual species areas (first, second, and third production cycles), and at 10 cm, in areas of exclusive Tifton 85 (fourth and fifth production cycles and control).

After sampling, Holstein dairy cows were put to graze until the target residue heights of 20 and 10 cm were reached in evaluations 1 to 3 and 4 to 5 and control, respectively. After grazing, top-dressing fertilization was applied using 40 kg/ha of urea N in all cycles.

The following cycles of production (C) were evaluated in the two seeding periods (E): E1C1: Jun. 19 to Aug. 06 (49 days); E1C2: Aug. 07 to Sept. 13 (38 days); E1C3: Sept. 14 to Oct. 25 (42 days); E1C4: Oct. 26 to Dec. 09 (45 days); E1C5: Dec. 10 to Jan. 15 (37 days); E2C1: Jul. 02 to Aug. 19 (49 days); E2C2: Aug. 20 to Oct. 01 (41 days); E2C3: Oct. 02 to Nov. 11 (41 days); E2C4: Nov. 12 to Jan. 07 (58 days); and E2C5: Jan. 08 to Feb. 11 (35 days).

Forage samples were sent to the Forage Laboratory for drying in an air circulation oven at 60 °C, after which the samples were ground in a Willey-type mill and sieved through 40 mesh.

The crude protein (CP) contents were estimated by the total nitrogen result multiplied by factor 6.25, according to the AOAC (1995), and the nitrogen compounds of the samples were fractionated following Licitra et al. (1996).

Total carbohydrate content (TC) was estimated after the determination of the contents of total nitrogen, ether extract and mineral matter (AOAC, 1995), following Sniffen et al. (1992). The non-fibrous carbohydrates (fractions A + B1) were calculated according to Valadares Filho (2000). The B2 and C fractions, which correspond to the neutral detergent fiber (NDF) potentially digestible and indigestible fractions, respectively, were determined according to Sniffen et al. (1992).

The nutrient content of the total mass of all grazing areas was estimated using the proportion of winter and summer forages and Tifton-85 times the nutrient content, in which % total nutrient = (% winter forage × % nutrient) + (% summer forage × % nutrient) + Tifton-85 × % nutrient).

The experiment was conducted in a completely randomized block design with three repetitions and the experimental data were submitted to statistical analysis by the MIXED procedure and repeated measures in time, following Littell et al. (1998) using software SAS (Statistical Analysis System, version 6.4). The means were obtained by the minimum square method and compared by the F test at 10% significance.

Results and Discussion

There was a significant interaction (P<0.01) between the CP of the forage mass of the treatments and the production cycles (Table 1). The greatest CP contents were observed in the first cycle of E2 in the bristle oat + millet pasture (17.2%) (P<0.01), which was not significantly different from treatments E1 bristle oat + millet (16.2%) and E1 sorghum-sudangrass (16.2%), respectively. The greatest crude protein (CP) contents observed in the first evaluation were due to the bristle oat in the pastures, as also reported by Moreira et al. (2006a).

In the following evaluations, a decrease in the CP content during the blooming of the annual species, associated with a low occurrence of bristle oat, was noticed. These results are coherent, as the change in the plant structure, such as the leaf/stem ratio, resulted in a change in the CP content observed during the study.

It is also worth noting that the CP contents observed in the total mass of pastures at different times and growth periods are within the minimum value of 7% recommended by Minson (1990), thus providing appropriate nitrogen supply to ruminal microorganisms and ensuring the maintenance of the animals.

Table 1 - Crude protein content (%) of total mass of exclusive Tifton-85 and Tifton-85 overseeded with winter and summer species at different seeding times (E) and production cycles (C)

Treatments	Seeding time	Production cycle				
		1st C	2nd C	3rd C	4th C	5th C
BO+M	1	16.2ABa	9.1Ab	7.7Ac	7.6BCc	7.9Bc
BO+SS	1	16.2ABa	9.9Ab	7.4Ac	7.0Cc	7.3BCc
T-85	1	14.2Ba	10.4Ab	7.0Ac	7.7BCc	7.4Bc
BO+M	2	17.2Aa	11.1Ab	7.7Ad	10.1Abc	9.0Ac
BO+SS	2	10.8Ca	10.7Aa	7.5Ab	9.7Aa	8.9Aa
T-85	2	14.4Ba	10.3Ab	7.2Ac	8.3Bc	8.0Abc

BO - bristle oat; M - millet; SS - sorghum-sudangrass; T-85 - Tifton-85 grass.
1st C: E1 from June 19 to August 06; E2 from July 02 to August 19.
2nd C: E1 from August 07 to September13; E2 from August 20 to October 01.
3rd C: E1 from September14 to October 25, E2 from October 02 to November 11.
4th C: E1 from October 26 to December 09, E2 from November 12 to January 07.
5th C: E1 from December 10 to January 15, E2 from January 08 to February 11. 2.
Means followed by the same lowercase letter in rows and capital letter in columns do not differ (P>0.1).

In all the evaluations and pastures, the nitrogen fractions varied greatly and were influenced by the nitrogen content of each pasture, the botanic composition and the leaf/stem ratio (Moreira et al., 2006a).

The non-protein nitrogen (NPN) content, fraction A, in all of the production cycles varied from 22.6% (E2 - Tifton-85, second cycle) to 35.0% (E1 - BO + SS, first and second cycles) (Table 2).

The high proportion of soluble nitrogen compounds (fraction A) can result in great losses of nitrogen (N) through ammonia, which can be recycled in the rumen. However, a large part must be metabolized. The consumption of energy

Table 2 - Nitrogen fractions as percentage of total nitrogen of the total mass in exclusive Tifton-85 pasture and Tifton-85 overseeded with winter and summer species at different seeding times (E) and production cycles (C)

Fractions (%)	Seeding time (E1)			Seeding time (E2)		
	BO+M	BO+SS	T-85	BO+M	BO+SS	T-85
	1st production cycle					
A	34.8a	35.0a	30.8b	30.2b	32.0ab	23.2c
B1	13.4a	11.3ab	13.4a	12.4ab	15.2a	9.2b
B2	22.9b	26.0a	23.5b	28.6a	23.1b	19.6c
B3	16.7b	13.8b	13.7b	16.9b	14.9b	34.8a
C	12.2b	13.9b	18.6a	11.9b	14.8b	13.2b
	2nd production cycle					
A	34.8a	35.0a	30.8b	30.2b	32.0ab	22.6c
B1	11.4a	11.3a	12.4a	11.4a	12.2a	8.5b
B2	25.9ab	27.0ab	28.5a	28.6a	28.1a	25.1ab
B3	16.7b	13.8c	13.7c	16.9b	14.9bc	26.9a
C	11.2c	12.9bc	14.6ab	12.9bc	12.8bc	16.9a
	3rd production cycle					
A	26.6b	26.3b	26.6b	23.3c	26.2b	29.8a
B1	11.1b	11.5b	12.4ab	13.4a	13.6a	13.9a
B2	28.7b	28.9b	28.8b	32.2a	29.4b	23.6c
B3	14.5c	18.9ab	18.4ab	18.2ab	19.4a	21.2a
C	19.1a	14.4b	13.8b	12.9bc	11.4c	11.5c
	4th production cycle					
A	29.8a	25.2b	26.7ab	28.6a	28.4a	27.7ab
B1	10.6b	11.5ab	12.9a	12.1a	10.5b	12.2a
B2	24.7c	29.4b	29.7b	30.9b	38.0a	36.5a
B3	15.0b	18.6a	15.3b	15.6b	11.7c	8.0d
C	19.9a	15.3b	15.4b	12.8c	11.4c	15.6b
	5th production cycle					
A	26.0b	28.9a	27.1a	25.2b	28.6a	25.7b
B1	8.8b	8.7b	9.8a	9.9a	8.5b	8.5b
B2	29.7a	22.3c	25.0b	25.2b	26.0b	28.9a
B3	18.8b	21.7ab	21.9ab	23.6a	18.9b	22.8a
C	16.7b	18.4a	16.2b	16.1b	18.0a	14.1c

BO - bristle oat; M - millet; SS - sorghum-sudangrass; T-85 - Tifton-85 grass; A - non protein nitrogen; B1 - soluble fraction of rapid degradation; B2 - insoluble fraction of intermediate degradation fraction; B3 - insoluble fraction of slow degradation; C - non-degradable insoluble fraction.
1st C: E1 from June 19 to August 06; E2 from July 02 to August 19.
2nd C: E1 from August 07 to September13; E2 from August 20 to October 01.
3rd C: E1 from September14 to October 25, E2 from October 02 to November 11.
4th C: E1 from October 26 to December 09, E2 from November 12 to January 07.
5th C: E1 from December 10 to January 15, E2 from January 08 to February 11. 2.
Means followed by the same lowercase letter in rows and capital letter in columns do not differ (P>0.1).

of this process is called urea cost (Owens & Zinn, 1988). Thus, the use of this nitrogen fraction can be optimized by making energy readily available for the increase of the microbial protein synthesis.

The proportion of rapid degradable soluble protein in the rumen (fraction B1) in all of the production cycles (Table 2) ranged from 8.5% (E2 - Tifton-85 and E2 - BO + SS, both in the fifth production cycle) to 15.2% (E2 - BO + SS in the first production cycle).

The higher the contents of fractions A and B1 (Table 2), the greater the requirement for rapid degradable carbohydrate supply for the appropriate synchronism of carbohydrate and protein fermentation in the rumen. Thus, the sum of these fractions in pastures in the different periods of growth were higher than that of 19.9% for Tifton-85 reported by Malafaia & Vieira (1997), but lower than those from 33.1 to 53.8% for Tanzania grass in different growth periods reported by Balsalobre et al. (2003).

The proportions of insoluble N with intermediate rumen degradability (fraction B2) in the production cycles varied from 19.6% (E2 - Tifton-85 in the first cycle) to 38.0% (E2 - BO + SS in the fourth cycle, Table 2), while the proportion of insoluble protein of slow degradability (fraction B3) in the production cycles ranged from 8.0% (E2 - Tifton-85 in the fourth cycle) to 34.8% (E2 - Tifton-85 in the first cycle).

The proportion of insoluble non-digestible proteins in the rumen and the intestines (fraction C) evaluated in the production cycles ranged from 11.2% (E1 - BO + M, in the second cycle) to 19.9% (E1 - BO + M, in the fourth cycle, Table 2). The increase in the unavailability of part of the CP is one of the most negative effects of the physiological aging of the plant, from the nutritional viewpoint. However, Van Soest (1994) reported that from 5 to 15% of the total N of forage species is bound to lignin and is totally unavailable.

Depending on the passage rate of the solid phase through the rumen, as fraction B3 degrades slowly, it may have a low degradation rate. Thus, pasture treatments E2 - Tifton-85, first cycle (71.2%) and E1 - BO + SS, fifth cycle (69.0%), which presented fractions A, B3, and C, representing around 70% of the nitrogen content, may result in a lower use of nitrogen in ruminants. This means that despite the high crude protein contents of these pastures, there may be a protein deficit in the rumen some time after the consumption of the forage by the animals.

The total carbohydrate content (TC, Table 3) increased in all production cycles, ranging from 55.3 to 88.0%, as a result of the botanical composition of each plant during evaluation (Moreira et al., 2006a). Higher values of TC were observed in the fifth growth period as a result of the increase in the stem fraction, and also in the cell wall content (Moreira et al., 2006b) and the decrease in the CP content (Table 1).

The carbohydrate fractions (CHO; Table 4) of all the production cycles showed a great variation under the influence of the TC content of each forage species (Table 3), botanic composition during evaluation and the leaf/stem ratio of the investigated pastures (Moreira et al., 2006a).

The values of fractions A+B1 (% TC) of all the production cycles ranged from 6.8% (E1 - Tifton-85, fifth cycle) to 26.7% (E1 - BO + M, third cycle), which are higher than the values from 0.74 to 11.62% of the proportion of TC reported by Malafaia et al. (1998) in tropical grasses, while Vieira et al. (2000) found 11.0 and 15.1% of TC for A+B1 in native pastures in the dry and rain periods, respectively.

Valadares Filho (2000) pointed out that when the A+B1 fraction constitutes the main fraction of carbohydrates in the diet, it is necessary to include protein sources of fast and intermediate degradability in the rumen for the synchronization between the energy and the nitrogen releases.

Concerning the potentially digestible CHO of the cell walls (B2 fraction), the investigated pastures had higher values in the first and the fifth production cycles. In contrast, in the second and the third cycles, there was a decrease in the B2 fraction (Table 4). Throughout the course of evaluation, it was observed that fraction B2 represents the greatest part of the carbohydrate fraction, and its availability in the rumen is associated with its rumen degradation rate.

It must be noted that forages with high NDF have a higher proportion of fraction B2 of CHO. These types of feed deliver energy to the rumen more slowly, which may affect the microbial synthesis and the animal performance.

Table 3 - Protein fractions as percentage of total nitrogen of the total mass in exclusive Tifton-85 pasture and Tifton-85 overseeded with winter and summer species at different seeding times (E) and production cycles (C)

Treatments	Time	Production cycle				
		1st C	2nd C	3rd C	4th C	5th C
BO+M	1	72.8ABc	77.9ABab	73.6Dc	74.3Bbc	80.2Ba
BO+SS	1	75.1ABbc	77.6ABab	72.3Ec	73.1Bc	80.2Ba
T-85	1	76.6Ab	74.8Bb	70.8Cc	73.4Bbc	81.0Ba
BO+M	2	73.0ABc	79.5Ab	88.0Aa	82.6Ab	81.7ABb
BO+SS	2	55.3Cd	65.0Cc	86.8Ba	80.1Ab	81.2ABb
T-85	2	71.1Bd	79.3Ac	85.6Ca	82.3Abc	82.8Ab

BO - bristle oat; M - millet; SS - sorghum-sudangrass; T-85 - Tifton-85 grass.
1st C: E1 from June 19 to August 06; E2 from July 02 to August 19.
2nd C: E1 from August 07 to September13; E2 from August 20 to October 01.
3rd C: E1 from September14 to October 25, E2 from October 02 to November 11.
4th C: E1 from October 26 to December 09, E2 from November 12 to January 07.
5th C: E1 from December 10 to January 15, E2 from January 08 to February 11. 2.
Means followed by the same lowercase letter in rows and capital letter in columns do not differ (P>0.1).

Table 4 - Carbohydrate composition as percentage of total carbohydrates in the dry mass of exclusive Tifton-85 pasture and Tifton-85 overseeded with winter and summer species at different seeding times (E) and production cycles (C)

Fractions (%)	E1			E2		
	BO+M	BO+SS	T-85	BO+M	BO+SS	T-85
			1st production cycle			
A + B1	9.3b	6.9c	7.2c	13.4a	11.8a	9.9ab
B2	75.1a	77.4a	74.3a	69.1b	63.7c	69.6b
C	15.6d	15.7d	18.5c	17.5c	24.5a	20.5b
			2nd production cycle			
A + B1	12.8ab	12.9ab	13.6a	13.0a	11.6b	9.1c
B2	68.4b	69.9ab	69.3ab	72.8a	68.6b	73.7a
C	18.8a	17.2b	17.1b	14.2c	19.8a	17.2b
			3rd production cycle			
A + B1	26.7a	26.2a	25.9ab	25.4ab	19.7c	18.9c
B2	55.8c	56.5bc	55.8c	59.7b	65.5a	65.2a
C	17.5b	17.3b	18.3a	14.9c	14.8c	15.9c
			4th production cycle			
A + B1	13.7bc	15.9a	14.8b	13.4	8.1d	12.5c
B2	66.8c	65.9c	67.0c	72.8b	78.4a	71.2b
C	19.5a	18.2ab	18.2ab	13.8c	13.5c	16.3b
			5th production cycle			
A + B1	9.6b	8.4b	6.8c	9.7b	11.9a	11.3a
B2	74.1ab	73.2b	75.4ab	73.9ab	73.0b	77.8a
C	16.3ab	18.4a	17.8a	16.4ab	15.1b	10.9c

BO - bristle oat; M - millet; SS - sorghum-sudangrass; T-85 - Tifton-85 grass; A + B1 - soluble fraction of rapid degradation; B2 - insoluble fraction of intermediate degradation fraction; C - non-degradable insoluble fraction.
1st C: E1 from June 19 to August 06; E2 from July 02 to August 19.
2nd C: E1 from August 07 to September13; E2 from August 20 to October 01.
3rd C: E1 from September14 to October 25, E2 from October 02 to November 11.
4th C: E1 from October 26 to December 09, E2 from November 12 to January 07.
5th C: E1 from December 10 to January 15, E2 from January 08 to February 11. 2.
Means followed by the same letter in rows do not differ (P>0.1).

In addition, forage intake may be limited by the high indigestible fraction (fraction C) of these feeds, as described by Malafaia et al. (1998) and Cabral et al. (2000).

The proportion of indigestible cell wall (fraction C) in each production cycle (Table 4) ranged from 15.6 to 24.5, 14.2 to 19.8, 14.8 to 18.3, 13.5 to 19.5 and 10.9 to 18.4 of the proportion of TC of the first to the fifth cycles, respectively. Thus, the variation of the indigestible portion of the cell wall was small (Moreira et al., 2006b). Fraction C consists basically of the cell wall associated with lignin, which exerts a negative effect from the nutritional viewpoint.

Cabral et al. (2000) observed that the Tifton-85 pasture harvested at 50 cm height had a greater proportion of indigestible cell wall (fraction C) than when harvested at 30 cm (19.3 vs. 16.6% C). Malafaia et al. (1998) observed fraction C values between 15.8 and 25.2% in grasses, pointing out that this fraction is related to the indigestibility of CHO.

Conclusions

The nitrogen and carbohydrate fractions were influenced by the total nitrogen and carbohydrate contents of each pasture, the seeding time and the production cycle. Greater fractions of soluble nitrogen compounds (A + B1) were obtained in the first growth periods. Overseeding influenced the quantity of nitrogen and carbohydrate fractions positively in the pastures evaluated. The high solubility of carbohydrates and nitrogen of both millet and bristle oat, and sorghum-sudangrass and bristle oat combinations are indicative of the quality of these forages and their potential use as forage supplements in tropical pasture systems.

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