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Effects of nitrogen fertilizer on carbohydrate and protein fractions in pearl millet (*Pennisetum glaucum*) cultivars

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Abstract

Our research characterizes and quantifies carbohydrate and protein fractions of pearl millet (*Pennisetum glaucum*) cultivars at different nitrogen (N) rates and 2 sowing dates in Ceres, Goiás, Brazil. A randomized block design using 3 cultivars (ADR-7010, ADR-500 and BRS-1501), 4 N rates (0, 50, 100 and 200 kg N/ha) and 2 sowing dates (December 2010 and February 2011) was employed. Two harvests were undertaken for each sowing date, when plants were 0.70 m high. There were no significant differences among cultivars in either total carbohydrates or A+B_1, B_2 and C fractions of the carbohydrates. Total carbohydrates and their fractions were not affected by N rate nor sowing date. When protein fractionation was investigated, differences in fraction A were observed among cultivars but not in the B_1, B_2, B_3 and C fractions. Nitrogen rate did not affect protein fractions, but sowing date did affect fractions B_2 and B_3. The significance of these findings in feeding animals is discussed.

Resumen

En Ceres, Goiás, Brasil, se caracterizaron y cuantificaron las fracciones de carbohidratos y proteína en cultivares de millo (*Pennisetum glaucum*) sometidos a diferentes dosis de nitrógeno (N) y fechas de siembra. Se utilizó un diseño de bloques al azar con 3 cultivares (ADR-7010, ADR-500 y BRS-1501), 4 dosis de N (0, 50, 100 y 200 kg N/ha) y 2 fechas de siembra (1 de diciembre de 2010 y 20 de febrero de 2011). Para cada fecha de siembra se efectuaron 2 cosechas cuando las plantas alcanzaron 0.70 m de altura. No se encontraron diferencias significativas entre los cultivares para la concentración total de carbohidratos ni para sus fracciones A + B_1, B_2 y C. La concentración de carbohidratos y sus fracciones no fue afectada por la dosis de N ni por la fecha de siembra. Para la proteína se observaron diferencias entre cultivares solo en la fracción A. La dosis de N no afectó las fracciones de proteína, pero la fecha de siembra sí influyó en las fracciones B_2 y B_3. Se discuten las implicaciones de estos resultados para la alimentación animal.

Introduction

The intensification of ruminant production triggers a growing need for high quality forage products. Nutritional value of tropical forage is influenced by a number of factors, such as soil, climatic conditions, plant characteristics and forage production per unit area. In Central-West Brazil, pearl millet (*Pennisetum glaucum*) is usually used in crop-livestock systems as a ley pasture for grazing, being grown between annual crops due to its high productivity in the cool dry season (May to October) and ability to recycle nutrients (Pereira Filho 2014).

According to Henriques et al. (2007b), forage nutritional value must not be judged merely by its fiber and protein concentrations. Quantification of different forage

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nitrogen (N) compounds and carbohydrates provides important information to develop management strategies for pasture-raised animals to improve livestock production. The determination of the different nitrogenous and carbohydrate fractions helps the formulation of diets to optimize usage by rumen microorganisms. Based on this information, strategies may be developed to improve the efficiency of utilization of ingested N by rumen microorganisms and by the animal (Cabral et al. 2000).

Carbohydrates and proteins have different chemical and physical characteristics, plus ruminal degradation pathways and post-ruminal digestibility. The Cornell Net Carbohydrate and Protein System (CNCPS) aims to optimize digestion of protein and carbohydrate by ruminal microorganisms by reducing nutrient losses in the rumen and by estimating nutrient leakage from the gut (Sniffen et al. 1992).

Nitrogen fertilization usually significantly improves herbage productivity and forage nutritive value (Chagas and Botelho 2005). According to Lupatini et al. (1996), production of pearl millet forage depends on both management and application of fertilizer, with rates of 150 and 300 kg N/ha yielding positive linear increases in dry matter production and crude protein concentration in pearl millet shoots.

This research evaluates the effects of N fertilizer and sowing date on particular carbohydrate and protein fractions of the forage (shoots) of 3 pearl millet cultivars in Ceres, Goiás, Brazil.

Materials and Methods

The experiment was performed on the experimental farm of the Instituto Federal Goiano (IFG), in Ceres, GO, Brazil (15º21' S, 49º36' W; 564 masl). According to the Köppen classification, regional climate is Aw: a warm subhumid climate with 2 well-defined seasons. Average annual rainfall in the region is 1,550 mm, with a rainy season from October to April and a dry season from May to September.

Soil of the experimental area is a dystrophic Oxisol (Embrapa 2006). Samples were collected at 0–20 cm depth and analyzed, with the following results: Ca: 24.0 $mmol_c/dm^3$; Mg: 13.0 $mmol_c/dm^3$; CEC: 76.7 $mmol_c/dm^3$; Al: 0.0 $mmol_c/dm^3$; H: 37.0 $mmol_c/dm^3$; P (Mehlich) 5.0 mg/dm^3; K: 101 mg/dm^3; pH ($CaCl_2$): 5.6; base saturation: 51.8%; organic matter: 1.5%; sand: 39%; clay: 50%. Conventional tillage was performed with 2 passes of harrows and the soil was leveled with a disc harrow the day before seeding.

A 3 x 4 x 2 randomized block design was employed, with 3 replications, giving 72 experimental units. Treatments comprised 3 millet cultivars (ADR-7010, ADR-500 and BRS-1501), 4 nitrogen rates (0, 50, 100 and 200 kg N/ha) and 2 sowing dates (1 December 2010 and 20 February 2011).

Each experimental unit was 6.0 m^2 and contained four 5 m rows of plants with 0.3 m between rows and 1.0 m between plots. Seeds were sown at 2 cm depth, with 20 pure viable seeds per linear meter.

The whole area was fertilized with simple phosphate (20 kg P/ha) at sowing. Potassium chloride (30 kg K_2O/ha) was applied 10 days after germination. The N fertilizer (urea) was applied to the various treatments as split dressings, namely: 60% applied 10 days after germination and 40% on the day after the first harvest.

Harvest cuts at a height of 20 cm were performed when at least 50% of plants reached 0.70 m height. In plots sown in December the first harvest occurred on January 5 (35 days after sowing) and the second on January 27 (22 days regrowth). In plots sown on February 20 the first harvest occurred on March 27 (35 days after sowing) and the second on April 21 (25 days regrowth). No flowering was observed at any harvest.

Material from the central 2 rows (minus 0.5 m at each end) was harvested on each occasion and weighed. Representative samples of the fresh material, totaling 500 g, were removed for laboratory testing and dried at 60 ºC for 72 h in a forced-air oven to determine percentage dry matter (DM). The samples were then ground in a Willey mill with a 1 mm sieve. Samples from the 2 harvests within the respective sowing time were mixed (50% from each harvest) and homogenized for subsequent laboratory analysis. Concentrations of DM, crude protein (CP) and mineral matter were determined according to Silva and Queiroz (2002), while neutral detergent fiber (NDF), ether extract (EE) and lignin were calculated according to the protocols of Van Soest (1994).

Total carbohydrate and protein were evaluated following Sniffen et al. (1992). Further, non-protein nitrogen (NPN), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996), whereas soluble nitrogen followed Krishnamoorthy et al. (1982).

The total carbohydrates (tCHO) were determined by the expression: tCHO = [100 - (CP % + EE % + ash %)], where tCHO = total carbohydrates; CP = crude protein; EE = ether extract. Total carbohydrates can be divided into fractions A + B_1, B_2 and C, which were determined by the following expressions: A + B_1 = 100 - (C + B_2);

B_2 = {100 * NDF (DM %) - NDIP (CP %) * 0.01 * CP (DM %) - [NDF (DM %) * 0.01 * Lignin (NDF %) * 2.4]}/tCHO (DM %), where NDIP = neutral detergent insoluble protein; and C = {[100 * NDF (DM %) * 0.01 * Lignin (NDF %) * 2.4]/TC (DM %)}.

The protein fractions were determined as follows:

Fraction "A", consisting of non-protein nitrogen (NPN), was determined by the difference between total N and trichloracetic acid (TCA)-insoluble N, according to the following formula: A (tN %) = (tN - N1)/tN x 100, where tN = total nitrogen in the sample and N1 = trichloracetic acid-insoluble nitrogen. Fraction "B_1", composed of soluble and rapidly rumen-degraded proteins, was obtained by the difference between the borate-phosphate buffer-insoluble nitrogen and the NPN, and calculated as follows: B_1 (tN %) = (N1 - N2)/tN x 100, where N2 = borate-phosphate buffer-insoluble nitrogen. The "B_2" fraction is formed by intermediate rumen degradation rate, insoluble but digestible protein, and was determined by the formula: B_2 (tN %) = (N2 - NDIN)/tN x 100, where NDIN = neutral detergent-insoluble nitrogen. The "B_3" fraction is composed of slow rumen degradation rate, insoluble but digestible protein, and was determined by the formula: B3 (tN %) = (NDIN - ADIN)/tN x 100, where ADIN = acid detergent-insoluble nitrogen. The fraction "C", consisting of insoluble and indigestible proteins, was determined by the equation C = ADIN/tN.

Data from proteins and carbohydrates were submitted to joint analysis of variance, including the 2 sowing dates, and means were compared by Tukey´s test at 5% significance level. Analyses were made using the R software package (R Development Core Team 2010).

Results

Forage production

While there was no triple interaction (P>0.05) for sowing date, cultivar and N rate for fresh or dry matter (DM) production, sowing date did influence (P<0.05) both fresh and DM production; mean fresh matter production was 34.2 and 26.4 t/ha for plants sown in December and January, respectively, while corresponding mean DM production was 3.4 and 2.7 t/ha.

Table 1 shows mean fresh and DM production of cultivars according to N rate. Average DM concentrations for the 3 cultivars were: 10.4% (ADR-500), 10.3% (ADR-7010) and 10.2% (BRS-1501). Both fresh and DM production followed similar patterns, with production increases (P<0.05) with N application. The cultivars responded differently to N fertilizer, with maximum yields for ADR-500 and ADR-7010 being reached at 100 kg N/ha, while yields of BRS-1501 were still increasing at 200 kg N/ha. Yields of the 3 cultivars did not differ at N rates up to 100 kg N/ha but BRS-1501 produced more forage than ADR-7010 at 200 kg N/ha (P<0.05), with ADR-500 intermediate.

The average composition of all cultivars was (DM basis): CP = 21.7%; NDF = 57.9%; ADF = 31.0%; lignin = 4.73%; EE = 1.94%; and mineral matter = 6.32%.

Carbohydrate

There were no significant main effects or interactions (P>0.05) on total carbohydrate (tCHO) and carbohydrate fractions due to cultivar, N rate or sowing date (Table 2).

A lower tCHO concentration (69.2%) for the December sowing was associated with the highest CP concentration (22.5%), whereas the higher tCHO concentration for the February sowing (70.9%) was associated with a lower CP of 20.9%.

B_2 fraction levels were approximately 65.5%, while mean value for fraction C was 16.2%.

Protein

Crude protein concentration in forage did not differ between cultivars sown in December but application of

Table 1. Effects of N fertilizer on fresh and dry matter yields (mean of 2 sowing dates) of 3 pearl millet cultivars.

Cultivar	N rate (kg/ha)			
	0	50	100	200
		Fresh matter (t/ha)		
ADR-500	26.89aB[1]	28.28aAB	33.24aA	33.78abA
ADR-7010	26.65aC	27.55aBC	34.83aA	32.01bAB
BRS-1501	21.37aC	29.06aB	35.29aB	38.49aA
		Dry matter (t/ha)		
ADR-500	2.79aB	2.93aAB	3.44aA	3.50abA
ADR-7010	2.73aC	2.82aBC	3.52aA	3.28bAB
BRS-1501	2.18aC	2.96aB	3.60aB	3.93aA

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ significantly (P>0.05).

Table 2. Effects of N fertilizer and sowing date on total carbohydrate (tCHO, % DM) and A+B_1, B_2 and C fraction levels (% of tCHO) in pearl millet cultivars.

Cultivar	CHO fraction	December 2010				February 2011			
		N rate (kg/ha)				N rate (kg/ha)			
		0	50	100	200	0	50	100	200
ADR-500	A+B_1	12.9	21.2	20.5	15.2	15.3	19.1	22.7	19.5
	B_2	66.6	60.1	63.5	64.5	65.9	61.9	63.6	64.8
	C	20.5	18.6	16.0	20.3	18.8	19.0	13.6	15.5
	tCHO	70.8	70.1	69.4	67.9	71.3	69.9	72.5	71.9
ADR-7010	A+B_1	16.6	18.4	16.8	15.2	17.7	21.0	18.2	20.0
	B_2	65.9	65.6	64.7	71.0	70.4	68.0	67.0	63.3
	C	17.4	15.9	18.4	13.7	11.9	10.9	14.7	16.5
	tCHO	71.4	68.1	67.6	68.4	71.6	69.3	70.5	70.1
BRS-1501	A+B_1	18.7	22.2	14.7	14.1	17.6	18.1	20.7	21.5
	B_2	63.5	62.6	73.4	67.2	64.1	67.7	63.7	62.3
	C	17.7	15.2	12.0	18.7	18.3	14.1	15.5	16.2
	tCHO	71.5	69.3	68.2	67.9	71.6	70.2	70.4	71.8

nitrogen increased CP percentage for all 3 cultivars, with no increase above 50 kg N/ha (Table 3). When cultivars were sown in February, CP concentration presented quadratic response to N fertilization (Table 4). While significant differences (P<0.05) in protein A fraction were observed among cultivars and between fertilizer levels (Tables 3 and 4), results were generally inconsistent. B_1, B_2, B_3 and C fractions did not differ (P>0.05) among cultivars or N rates (Tables 3 and 4). There were no significant interactions (P>0.05) between cultivars, N rates and/or sowing dates for the various nitrogenous fractions.

Table 3. Effects of N fertilizer on mean levels of crude protein (CP, % DM) and A, B_1, B_2, B_3 and C fractions (% CP) in pearl millet cultivars sown in December 2010.

Cultivar	CP fraction	N rate (kg/ha)			
		0	50	100	200
ADR-500	CP	20.88B[1]	22.20A	22.94A	22.83A
	A	21.70a	17.28b	26.13a	14.85b
	B_1	1.47	3.85	3.14	3.03
	B_2	27.60	27.79	26.10	24.13
	B_3	47.75	48.83	42.60	56.54
	C	1.50	2.25	2.04	1.45
ADR-7010	CP	20.13B	22.84A	23.68A	23.33A
	A	21.72a	21.72a	19.36b	21.40a
	B_1	3.39	2.64	3.41	2.28
	B_2	27.73	25.78	24.47	23.46
	B_3	43.79	47.81	50.33	49.96
	C	2.51	2.05	2.43	2.91
BRS-1501	CP	20.84B	23.14A	23.42A	23.21A
	A	11.79b	20.94a	15.57c	16.83b
	B_1	2.91	3.08	2.05	3.86
	B_2	26.05	24.72	23.65	26.46
	B_3	58.00	49.60	56.71	50.46
	C	1.24	1.66	2.01	2.39

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ significantly (P>0.05).

Table 4. Effects of N fertilizer on mean levels of crude protein (CP, % DM) and A, B_1, B_2, B_3 and C fractions (% CP) in pearl millet cultivars sown in February 2011.

Cultivar	CP fraction	N rate (kg/ha)			
		0	50	100	200
ADR-500	CP	19.74B[1]	20.72AB	21.27A	20.57B
	A	16.52c	22.42a	19.99c	19.63c
	B_1	5.38	4.85	4.78	4.12
	B_2	25.29	24.84	23.44	26.47
	B_3	49.84	45.81	50.30	47.88
	C	2.97	2.08	1.49	1.89
ADR-7010	CP	20.05B	21.32A	21.94A	20.72B
	A	25.19a	20.96b	21.20b	24.50a
	B_1	3.13	6.03	5.03	4.23
	B_2	23.75	23.52	25.33	24.25
	B_3	45.55	46.05	45.40	44.54
	C	2.37	3.45	3.05	2.48
BRS-1501	CP	20.44B	21.15A	21.86A	20.53B
	A	21.81b	19.07c	23.15a	21.37b
	B_1	3.74	3.69	4.76	4.40
	B_2	24.53	26.70	26.35	26.56
	B_3	48.00	48.98	43.51	44.86
	C	1.93	1.56	2.22	2.80

[1]Means followed by the same upper-case letter in rows and by the same lower-case letter in columns do not differ (P>0.05).

When sowing date effects were isolated, differences were found for B_1 (made up of true and rapidly degraded protein) (2.92 vs. 4.51%, P<0.05) and B_3 (cell wall-bound protein, featuring slow degradation by digestion in the gut) (50.2 vs. 46.7%, P<0.05) fractions, for sowings made in December 2010 and February 2011, respectively.

Discussion

Carbohydrate

The treatment receiving no N (rate 0) presented the highest tCHO concentration (71.4%). Nitrogen fertilization promoted DM production with corresponding decreases in tCHO levels.

Carbohydrates are the main energy reserve of plants, varying between 50 and 80%, and are extremely important for animal nutrition, being the primary energy source for rumen microorganisms (Van Soest 1994). Carbohydrates act as an energy source for animals, with most digestion occurring in the rumen in the case of ruminants. Total CHO levels in our current research (70.1%) fell within this range.

The values for mean (A+B_1) fractions for the pearl millet cultivars, which represent carbohydrates and starch, were about 18.3%, and were within the range often reported in the literature (Cabral et al. 2000; Clipes et al. 2006; Henriques et al. 2007a; Sá et al. 2010). Vieira et al. (2000) reported that tropical grasses rarely present levels much above 20% for these fractions.

According to Nocek and Russel (1988), when the availability of rapidly degraded carbohydrate was high, an adequate supply of rapidly degraded protein was needed to maintain an appropriate energy:nitrogen balance in the fermentation process. It is significant that levels of the A+B_1 fraction varied between 16.5 and 20.0% of tCHO and remained below those found for protein fraction A. Since both fractions (protein A and carbohydrate A+B_1) present similar degradation rates, a considerable part of the protein fraction may be used as an energy source by rumen microorganisms. The B_2 fraction is composed of cell wall carbohydrates with slow ruminal availability and, therefore, susceptible to effects of rate of passage of the ingesta.

Lima et al. (2008) evaluated elephant grass (*Pennisetum purpureum*) fertilized with 100 kg N/ha and harvested at 56 days regrowth and found 82.0% for the B_2 fraction, well above the 65.5% in our study. Since the B_2 carbohydrate fraction is related to fiber content, this suggests a lower impact of fiber concentrations on total CHO of pearl millet cultivars than elephant grass. Forage with high NDF levels presents elevated proportions of the B_2 fraction and is more slowly degraded in the rumen, with effects on microbial synthesis and animal performance (Ribeiro et al. 2001).

In a study with elephant grass, Lista et al. (2007) found CHO fraction C values of 9.6%. The lower level in their study compared with our 16.2% may be due to the fact that their samples were collected as a grazing simulation, so that a high proportion of young leaves was collected, with a relatively low fiber concentration.

Protein

Nitrogen fertilization increased CP concentration, corroborating findings of other authors, e.g. Van Soest (1994).

The low concentration of A fraction (non-protein nitrogen, NPN) found in cultivar BRS-1501 may be justified by the cultivar's early flowering, so that it was physiologically more mature at sampling.

Crude protein concentrations were higher than those found by Lupatini et al. (1996) (6.9, 12.2 and 14.3% for N doses of 0, 150 and 300 kg/ha, respectively) and were at least equal to the critical level (7%), below which a reduction in intake and digestibility often occurs due to a limitation on development of the microbial population in the rumen-reticulum, which modifies ruminal fermentation (Van Soest 1994).

The fractionation of N compounds showed that soluble portions of CP were high, indicating that the plant, cultivated in fertile soil, may be considered a good supplementary protein source. Rapid breakdown in the rumen would provide the NPN sources, which are critical for proper rumen functioning, since structural carbohydrate-fermenting microorganisms from the rumen use ammonia as an N source (Russel et al. 1992). However, if levels of ammonia produced in the rumen are in excess of the requirements of the microflora, utilization of the protein in the forage would be less efficient.

The C fraction of CP is the protein recovered in the acid detergent fiber and is highly resistant to microbial and enzymatic degradation. Thus, its degradation rate is considered zero (Rodrigues and Vieira 2011). This fraction represented 2.11% of total protein (Tables 3 and 4).

According to Sniffen et al. (1992), the B_1 fraction is composed of albumin and globulin and is completely degraded in the rumen. Balsalobre et al. (2003) stated that the B_1 fraction is only slightly relevant for forage, since it usually comprises less than 10% of total protein. The levels of this fraction observed in our study (from 1.47 to 6.03%) would provide an additional N supply for the rumen microorganisms but much less than B_2.

About 5 to 15% of total forage N is bound to lignin, or rather, is unavailable to ruminal microorganisms (Van Soest 1994). Mean levels ranging between 1.87 and 2.40% obtained in this study remained below the above levels, probably due to the plants being harvested at a height of 0.70 m, which provides a leaf:stem ratio highly favorable for grazing. The lignin level of cultivars in our study was unaffected by N fertilizer level or sowing date, with a mean concentration of 4.73%.

In this study, pearl millet cultivars displayed the ability to grow rapidly and produce good yields of high quality forage. These findings may help farmers, who generally supplement their herds with concentrate during the dry season, to formulate more accurate diets for ruminants by determining the appropriate forage-to-concentrate ratios. This could lead to more precise production systems, even for dairy or beef cattle, which would reduce costs per unit of product by enhancing animal performance.

Additional studies incorporating different fertilizer levels and forage-to-concentrate ratios, which evaluate animal performance (milk production and liveweight gains) and include economic assessments, are needed to build on these preliminary findings.

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