Animal Feed Science and Technology 183 (2013) 17–21

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

# Fiber monosaccharides and digestibility of Milenio grass under N fertilization

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ARTICLE INFO

*Article history:*
Received 23 March 2012
Received in revised form 31 January 2013
Accepted 22 April 2013

*Keywords:*
Carbohydrate
Cell wall
Digestibility
*Panicum maximum*
Pasture

ABSTRACT

Nutritive value of tropical forages is extremely dependent on management strategies adopted. The objective of this study was to evaluate the effects of N fertilization doses (0, 150, 300, or 450 kg N/ha/year) on chemical composition, *in vitro* digestibility, and neutral monosaccharides from cell walls of *Panicum maximum* Jacq. cv. IPR-86 Milenio. Grass samples were collected after 35 d of regrowth from paddocks managed under rotational stocking (35-d resting and 5-d occupation). All nutrient concentrations were affected by N fertilization, except ether extract. Although crude protein (CP) increased linearly with N dose, most nutrients were altered according to a quadratic trend. Each kilogram of N resulted in a 0.13 percentage unit increase in CP content ($P < 0.01$). Nitrogen fertilization led to a quadratic increase of cellulose ($P < 0.01$) and lignin (sa) ($P < 0.01$) and, thus, ADF content ($P < 0.01$). In contrast, hemicellulose was quadratically decreased ($P < 0.01$), resulting in lower contents of NDF with N application rates higher than 89.3 kg/ha/year ($P < 0.01$). For fiber monosaccharides, arabinose decreased quadratically ($P < 0.01$), following hemicellulose decrease. Hemicellulose is mainly composed of arabinose and xylose. Glucose content increased linearly with the N supply ($P < 0.01$), and hence, cellulose accumulation. The xylose content was unaffected by treatments. Glucose and xylose were quantitatively more important than arabinose in cell walls of Milenio grass. *In vitro* digestibilities of DM, OM, and ADF increased linearly with N fertilization ($P < 0.01$), whereas NDF digestibility followed a quadratic trend ($P < 0.01$), with a maximum response at 349 kg N/ha. Improvements in digestibility were also observed for neutral monosaccharides ($P < 0.01$). For all treatments, xylose was less digestible than arabinose and glucose. In conclusion, N fertilization increased CP content, stimulated the formation of glucose and arabinose in cell walls, leading to a higher forage quality. Forage quality was enhanced with N doses up to 300 kg/ha/year.



*Abbreviations:* DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber assayed without a heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber expressed inclusive of residual ash; CEL, cellulose; HEM, hemicellulose; NFC, nonfiber carbohydrates; IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; NDFD, neutral detergent fiber digestibility; ADFD, acid detergent fiber digestibility.

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http://dx.doi.org/10.1016/j.anifeedsci.2013.04.014

## 1. Introduction

Nutritional value of forages is extremely dependent on management adopted. Growth of tropical grasses is mainly affected by such factors as age of crop, light intensity, water, temperature, and level of fertilization. In the 1960s, Dienum et al. conducted several experiments to evaluate the effects of temperature, light intensity, and nitrogen fertilization on nutritive value of grasses. In a trial involving tropical grasses, high temperatures promoted dry matter (DM) production, but an adverse effect on digestibility was reported (Dienum and Dirven, 1972). Higher DM yields were achieved at 35 d of regrowth and were associated with N fertilization (Dienum and Dirven, 1975). These authors speculated that high temperature in tropical areas could be a plausible explanation for the poor quality of tropical grasses. Despite Wilson and Minson (1980) confirmed that the temperature is an important factor for the low quality of tropical species, other factors were also important, such as the plant growth potential and nitrogen utilization, leaf morphology, stem development, and sward structure.

Wilson (1973) reported that an increase in N application from low to moderate rates increased the digestibility by 3–5 percentage units and had a small positive effect on the percentage of water-soluble carbohydrates in a tropical grass (*Panicum maximum* Jacq. var. trichoglume). Overall, N supply is often considered the most important limiting factor for biomass accumulation, followed by water availability (Lemaire et al., 2008). Because the climate is variable, large amounts of N fertilizer have been frequently applied in efforts to obtain higher DM yields.

Protein accumulation in the cell due N fertilization dilutes cell wall components, leading to a high forage digestibility (Van Soest, 1994). Furthermore, protein accumulation in fertilized crops may improve the ruminal environment and increase microbial growth and fiber digestion due to increases in rumen $NH_3$ (Hoover and Miller-Webster, 2000). In grazing systems, forage prehension might be additionally facilitated by N fertilization, mainly due to the higher green leaf mass per unit area (Peyraud and Astigarraga, 1998).

Few studies have evaluated in detail the nutritional quality of *Panicum maximum* Jacq. cv Milenio in rotational stocking systems under N fertilization. Thus, the objective of this study was to evaluate the effects of N application rates (0, 150, 300, or 450 kg N/ha/year) on NDF monosacharides composition and *in vitro* digestibility of Milenio grass. We hypothesized that intermediate doses of N could maximize grass quality and production, avoiding N excess in tropical grazing systems. Grazing study data has been previously published (Sarmento et al., 2005; Lugão et al., 2001).

## 2. Materials and methods

### 2.1. Location, treatments and sampling

The experiment was conducted at the Agronomic Institute of Paraná, in Paranavaí, PR, Brazil. Soil was classified as a dystrophic Red-Yellow Argisol with a sandy texture; with composition 89% sand, 10% clay, and 1% silt, and 69.2% base saturation and 10.7 mg/dm$^3$ phosphorus.

A total of 32 paddocks (varying from 839 m$^2$ to 1687 m$^2$ each) cultivated with *Panicum maximum* Jacq. cv. Milenio grass (IPR 86) randomly received one of the following N fertilization treatments: 0, 150, 300, and 450 kg N/ha/year as ammonium nitrate (eight paddocks per treatment). The paddocks were managed with rotational stocking (35-d resting and 5-d occupation periods). Grass samples were collected at 35 d of regrowth in one grazing cycle during the summer (December 2001). Five different points of the paddock were randomly sampled immediately before animal occupation. Samples were cut at a 5 cm height from the soil surface and composed by paddock.

### 2.2. Sample analysis

After collection, samples were manually chopped, dried in a forced-air oven at 60 °C for 72 h and ground using a Wiley mill with a 1 mm sieve. Sub-samples were analyzed for dry matter at 105 °C (DM; index no. 934.01), ash (index no. 942.05), and ether extract (index no. 920.39; AOAC, 1990), crude protein (CP; Wiles et al., 1998), neutral detergent fiber (NDF; without sodium sulfite and alpha amylase, expressed inclusive of the residual ash) and acid detergent fiber sequentially (ADF; expressed inclusive of the residual ash), according to Van Soest et al. (1991), and lignin (sa) (Robertson and Van Soest, 1981). Organic matter (OM) was calculated as the loss in sample weight upon ashing. Nonfiber carbohydrates was computed as OM – CP – NDF – ether extract. Hemicellulose (HEM) was estimated as NDF minus ADF and cellulose as ADF minus lignin (sa).

*In vitro* true digestibility of DM (IVDMD), OM (IVOMD), NDF (NDFD), and ADF (ADFD) were measured according to Goering and Van Soest (1970). Briefly, 100 mL tubes containing 500 mg sample (dry and 1 mm milled), 10 mL of buffer solution, and 12 mL of rumen fluid (from a non-lactating cow fed hay) were sealed and incubated for 48 h at 39 °C. The true digestibility was obtained after washing the residuals with neutral detergent solution (Goering and Van Soest, 1970).

Concentrations of neutral monosaccharides (arabinose, xylose, and glucose) were analyzed both in the forage NDF and in the residual NDF after *in vitro* digestibility incubation. Shortly, after NDF isolation (Van Soest et al., 1991), cell walls were hydrolyzed with sulfuric acid 72% (w/w) and the solution was neutralized with ammonia hydroxide. Monosaccharides were reduced by sodium borohydride and converted into alditol acetate by adding 1-methylimidazole and acetic anhydride (Harris et al., 1988). Monosaccharides were determined by gas-liquid chromatography (Hewlett-Packard 5890 Series II using a 12 m × 0.32 mm capillary column SGE-ID-BPX70 0.25 [S/N 4010A53 and P/N 054605]). The operational parameters of the

**Table 1**
Influence of N fertilization on chemical composition of Milenio grass at 35 d regrowth (g/kg dry matter or as stated).

| | Nitrogen application rate (kg/ha/year) | | | | | Probability[a] | |
|---|---|---|---|---|---|---|---|
| Chemical component | 0 | 150 | 300 | 450 | SEM | L | Q |
| Dry matter, g/kg as fed | 232 | 199 | 173 | 158 | 0.32 | <0.01 | <0.01 |
| Organic matter | 871 | 838 | 832 | 828 | 6.80 | <0.01 | 0.06 |
| Crude protein | 50.6 | 62.0 | 98.4 | 104 | 1.48 | <0.01 | 0.07 |
| Ether extract | 14.9 | 15.8 | 14.7 | 14.3 | 0.78 | 0.42 | 0.43 |
| Nonfiber carbohydrates | 128 | 100 | 85.1 | 78.2 | 2.35 | <0.01 | <0.01 |
| CP:NFC ratio[b] | 3.95 | 6.22 | 11.6 | 13.3 | 0.45 | <0.01 | 0.54 |
| Neutral detergent fiber | 717 | 730 | 705 | 702 | 1.88 | <0.01 | <0.01 |
| Acid detergent fiber | 373 | 400 | 396 | 395 | 1.10 | <0.01 | <0.01 |
| Hemicellulose | 333 | 326 | 306 | 307 | 1.09 | <0.01 | <0.01 |
| Cellulose | 342 | 364 | 358 | 359 | 0.89 | <0.01 | <0.01 |
| Lignin (sa) | 31.3 | 35.9 | 38.0 | 35.8 | 0.49 | <0.01 | <0.01 |
| Fiber monosaccharides, g/kg NDF | | | | | | | |
| Arabinose | 79.0 | 67.7 | 57.6 | 52.6 | 2.70 | <0.01 | <0.01 |
| Xylose | 339 | 331 | 330 | 321 | 10.8 | 0.29 | 0.96 |
| Glucose | 573 | 584 | 604 | 612 | 13.2 | 0.03 | 0.92 |

[a] L: linear effect; Q: quadratic effect.
[b] Crude protein: nonfiber carbohydrates ratio.

chromatography were as follows: a 185 °C initial oven temperature with an elevation rate of 10 °C per minute up to 230 °C; an injector temperature of 250 °C; a detector temperature of 270 °C; a helium flow in the column under working conditions (hot) of 1.6 mL/min; a hydrogen flow of 66.5 mL/min; a synthetic air flow of 412 mL/min; and a total gas flow of 459 mL/min for 7.5 min, which included the collection and column cleaning time after reading the final peak. Digestibility of each neutral monosaccharide was calculated using the concentrations of monosaccharides in the forage cell wall and in the residue after *in vitro* incubation.

### 2.3. Statistical analysis

Data were analyzed as a completely randomized design with four nitrogen doses (0, 150, 300, and 450 kg N/ha/year) and eight independent replicates using the GLM procedure of SAS (2003). The total number of observations was 4 (N doses) × 8 (replicates) = 32 (paddocks). The model used was the following:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where $Y_{ij}$ = observation, $\mu$ = overall mean, $T_i$ = nitrogen dose effect ($i$ = 1–4), and $\varepsilon_{ij}$ = residual error ($j$ = 1–8). Linear and quadratic effects due to nitrogen fertilization were determined, and correlations were established using the CORR procedure of SAS. Differences were accepted if $P \leq 0.05$.

## 3. Results

The chemical composition of Milenio grass is shown in Table 1. All nutrients were affected by N fertilization, except ether extract content. Although CP increased linearly with the N dose (CP = 49.38 + 0.130 × N), most of nutrients were altered in a quadratic trend. Each kilogram of N resulted in a 0.13 percentage unit increase in CP content, which occurred concomitantly with a quadratic reduction in the NFC level. Thus, the CP:NFC ratio increased linearly as a function of N application.

Nitrogen fertilization led to a quadratic increase of CEL and lignin (sa) and, thus, the ADF content. Conversely, HEM was quadratically decreased, resulting in lower NDF contents with application rates higher than 89.3 kg/ha/year ($NDF = 721.9 + 0.0299 \times N - 0.1676 \times 10^{-3} \times N^2$). For the fiber monosaccharides, arabinose decreased quadratically, following HEM decrease, as hemicellulose is mainly composed of arabinose and xylose. The glucose content increased linearly with N supply, supporting the accumulation of its polymer, CEL. The xylose content was unaffected by treatments. Glucose and xylose were quantitatively more important than arabinose in the cell walls of Milenio grass (Table 1).

Digestibilities of DM, OM, and ADF increased linearly with N fertilization ($IVDMD = 0.567 + 0.111 \times 10^{-3} \times N$; $OMD = 0.594 + 0.114 \times 10^{-3} \times N$; $ADFD = 0.220 + 0.306 \times 10^{-4} \times N$), whereas NDF digestibility followed a quadratic trend ($NDFD = 0.419 + 0.399 \times 10^{-3} \times N - 0.570 \times 10^{-6} \times N^2$) with a maximum response at 349 kg N/ha. Improvements in digestibility were also observed for neutral monosaccharides. A quadratic increase was observed for arabinose digestibility, though glucose and xylose digestibilities were linearly enhanced. For all treatments, xylose was less digestible than arabinose and glucose (Table 2).

As expected, IVDMD and IVOMD were highly correlated with NDFD ($r$ = 0.97, $P < 0.01$). Because the cellular content is almost completely digestible, the DM digestibility is primarily a function of the concentration and digestibility of NDF. As NDF decreased and CP increased with N fertilization, IVDMD and IVOMD were also correlated with CP ($r$ = 0.82, $P < 0.01$).

**Table 2**
*In vitro* digestibility coefficients of dry matter (IVDMD), organic matter (IVOMD), neutral detergent fiber (NDFD), acid detergent fiber (ADFD), and NDF monosaccharides of Milenio grass at 35 d regrowth.

| Item | Nitrogen application rate (kg/ha/year) | | | | | Probability[a] | |
|---|---|---|---|---|---|---|---|
| | 0 | 150 | 300 | 450 | SEM | L | Q |
| IVDMD | 0.569 | 0.588 | 0.616 | 0.614 | 0.007 | <0.01 | 0.16 |
| IVOMD | 0.590 | 0.611 | 0.642 | 0.637 | 0.006 | <0.01 | 0.06 |
| NDFD | 0.420 | 0.464 | 0.490 | 0.482 | 0.009 | <0.01 | <0.01 |
| ADFD | 0.222 | 0.219 | 0.234 | 0.232 | 0.003 | <0.01 | 0.88 |
| Fiber monosaccharide digestibility | | | | | | | |
| Arabinose | 0.542 | 0.649 | 0.734 | 0.698 | 0.014 | <0.01 | <0.01 |
| Xylose | 0.498 | 0.540 | 0.591 | 0.574 | 0.010 | <0.01 | 0.09 |
| Glucose | 0.609 | 0.628 | 0.663 | 0.665 | 0.005 | <0.01 | 0.07 |

[a] L: linear effect; Q: quadratic effect.

## 4. Discussion

Most of the nutrients were affected by N fertilization. As anticipated, N application induced higher moisture and CP contents because of larger absorption and accumulation of water and nitrogen compounds in the plant cells. The NFC concentrations, however, were decreased, mainly due to the synthesis of cell wall, which drained the cytoplasmic nutrients. Although data regarding plant growth were not shown, biomass accumulation was greatly stimulated by N application (see Sarmento et al., 2005). Schmidt and Blaser (1969) also reported an inverse relationship between N dose and NFC concentration. Under intense growth, the NFC reserves are mostly consumed for fiber synthesis (Trenholm et al., 1998).

In this way, an increase in CEL and lignin (sa) (*i.e.*, ADF) was noted, which was likely due to the structural arrangement of the plant tissues to support the accumulation of biomass. The increase in biomass and accumulation of fibrous parts of forage (*e.g.*, stems and leaf sheaths) with N fertilization are well established for tropical grasses (Sarmento et al., 2005; Lugão et al., 2001). According to Wilson (1976), under conditions of rapid growth, the stem tracheids mature rapidly, even with thin walls, because the very active meristematic regions drain most of the available assimilate. Thus, less carbohydrate is available for secondary thickening and lignification of the cell walls before the tracheids mature and lose their cytoplasm. This process most likely occurred in our trial, as suggested by chemical composition and digestibilities of DM and fiber components.

In the current study, the increase in glucose proportion in cell walls was in agreement with its polymer, CEL. Hemicellulose is mainly composed of pentoses: xylose and arabinose. Although the xylose proportion was unaffected by treatments, the content of hemicellulose and proportion of arabinose in the NDF fraction were decreased by N fertilization. Indeed, glucose and xylose were quantitatively more important than arabinose in cell walls of Milenio grass, as has been reported for other grasses (Dien et al., 2006).

Due to the almost complete digestibility of nonfiber organic matter (Van Soest, 1994), DM digestibility is primarily a function of NDF concentration and digestibility. In addition to the higher ADF content, a lower NDF was observed for the high N application rates. Furthermore, NDFD increased quadratically with N fertilization (maximum at 349 kg N/ha/year). Digestibilities of DM and OM were improved by 8 and 9%, respectively, with higher N dosages. In addition, the increase in CP may favor forage digestion (Brito et al., 2003). In the present study, CP was positively correlated with NDFD, IVDMD, and IVOMD.

The alteration in neutral monosaccharides may also have contributed to the fiber digestibility enhancement. Because arabinose and glucose were increased without alterations in xylose, N fertilization induced a higher (arabinose + glucose): xylose ratio. Arabinose and glucose were always more digestible than xylose, which is commonly digested to a lesser extent, likely due to lignin having a greater protective effect on xylose than on the other monosaccharide units (Ford, 1978).

## 5. Conclusions

Nitrogen fertilization increased CP content and stimulated the formation of neutral monosaccharides (glucose and arabinose) in cell walls, resulting in better-quality fiber and higher forage digestibility. Greater improvements in the forage quality were obtained with N doses up to 300 kg/ha/year.

## Acknowledgement

The authors are grateful to the financial support provided by the FAPESP foundation.

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